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ABSTRACTS

Abstracts of 51st EASD Annual Meeting

OP 01 Insulin analogues: Is newer always better?

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Basal Insulin peglispro (BIL) demonstrates hepato-preferential action vs insulin Glargine (GL) in patients with type 1 diabetes mellitus

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Background and aims: BIL, a novel, long-acting basal insulin analog, has previously been shown to demonstrate hepato-preferential action in healthy subjects. This analysis evaluated the effects of therapeutic concentrations of BIL and GL and supratherapeutic concentrations of BIL on liver (endogenous glucose production [EGP]) and peripheral tissues (glucose disposal rate [GDR] and lipolysis) in patients with T1DM.
Materials and methods: This was a randomised, open-label, 4-period, crossover study in patients with T1DM (n=14; 22 - 48 years). Each patient had 4 euglycemic clamp assessments of 8-10 hours duration with primed, continuous infusions of BIL (15.3 and 74.1 mU/min) and GL (10 and 20 mU/m^2/min). D-[3-^{3}H]-glucose infusion was administered to assess EGP and GDR.To correct for differences in insulin receptor binding, equivalent human insulin concentrations (EHIC) were calculated by dividing each insulin concentration by its Ki (binding constant) and multiplying by the Ki of human insulin.
Results: At both low and high doses, BIL showed similar effects on EGP suppression (endpoint EGP) compared to GL, but had an attenuated effect on GDR (endpoint GDR) (Table). Lipolysis, assessed by serum concentrations of free fatty acids (FFA) and glycerol, was suppressed at low and high GL doses but only at the high BIL dose (Table).
Conclusion: BIL has similar hepatic activity (EGP suppression) but less peripheral activity (GDR stimulation, lipolysis suppression) at clinically relevant concentrations compared to GL, confirming hepato-preferential action.

Parameter (Units)	"Low" Doses		"High" Doses	
	GL (10 mU/m^2/min) N=13	BIL (15.3 mU/min) N=14	GL (20 mU/m^2/min) N=13	BIL (74.1 mU/min) N=13
	Arithmetic Mean ± Standard Deviation			
Insulin Concentration (pmol/L)	125 ± 40.5	2577 ± 740	235 ± 131	17603 ± 7170
Corrected Insulin Concentration (EHIC) (pmol/L)	134 ± 43.4	197 ± 56.5	252 ± 141	1345 ± 548
	Least Squares Mean ± Standard Error (p-value)[d]			
Baseline EGP (=Baseline GDR)[a] (mg/kg/min)	1.88 ± 0.136	1.88 ± 0.136 (p=0.9724)	1.88 ± 0.144[g]	2.15 ± 0.135 (p=0.0767)
Endpoint EGP[b] (mg/min/kg)	0.455 ± 0.0931	0.650 ± 0.0928 (p=0.0546)	0.0508 ± 0.0983[g]	0.0427 ± 0.0925 (p=0.9373)
Endpoint EGP/EHIC[b] (µg/kg/min/pmol/L)	3.64 ± 0.634	3.53 ± 0.634 (p=0.8772)	0.118 ± 0.674[g]	0.049 ± 0.631 (p=0.9263)
Endpoint GDR[b] (mg/kg/min)	1.73 ± 0.246	1.20 ± 0.245 (p=0.0459)	3.61 ± 0.259[g]	3.06 ± 0.244 (p=0.0449)
Endpoint GDR/EHIC[b] (µg/kg/min/pmol/L)	12.6 ± 2.84	5.21 ± 2.83 (p=0.0205)	22.0 ± 3.00[g]	5.06 ± 2.82 (p<0.0001)
Baseline FFA[a] (mmol/L)	0.394 ± 0.108	0.511 ± 0.107 (p=0.3406)	0.536 ± 0.109	0.682 ± 0.107 (p=0.2300)
Endpoint FFA[c] (mmol/L)	0.290 ± 0.072	0.809 ± 0.072 (p<0.0001)	0.131 ± 0.073	0.157 ± 0.071 (p=0.7553)
Baseline Glycerol[a] (mmol/L)	0.371 ± 0.130	0.468 ± 0.130[f] (p=0.4913)	0.533 ± 0.131	0.641 ± 0.129 (p=0.4420)
Endpoint Glycerol[c] (mmol/L)	0.128 ± 0.074[e]	0.530 ± 0.065 (p<0.0001)	0.111 ± 0.082[e]	0.150 ± 0.065 (p=0.6030)

a. Baseline was the 0-hour time point (start of infusion).
b. Endpoint for EGP and GDR was the average of the last 2 timepoints of the infusion period.
c. Endpoint for FFA and glycerol was the last timepoint of the infusion period (8 or 10 hours).
d. p-value for comparison of BIL vs GL, comparing low dose vs low dose and high dose vs high dose.
e. N=8
f. N=13
g. N=12
Model: Parameter = Treatment + Period + Sequence + Subject + Error, subject was fitted as a random effect.

Clinical Trial Registration Number: NCT01654380
Supported by: Eli Lilly and Company

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Reduced intra-subject variability of Basal Insulin peglispro (BIL) compared to insulin Glargine (GL) in patients with type 1 diabetes mellitus

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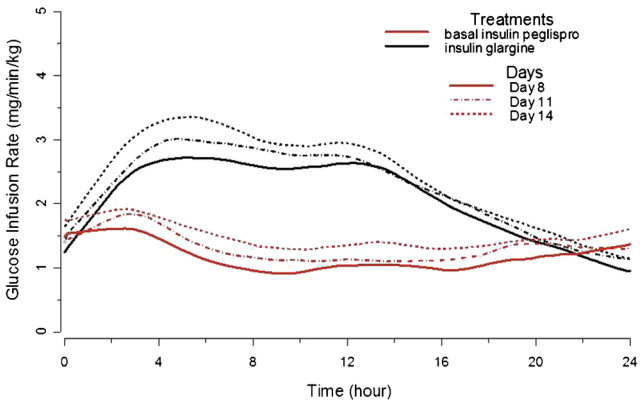
Background and aims: BIL is a novel PEGylated basal insulin that has a flat pharmacokinetic (PK) and glucodynamic (GD) profile which has a hepato-preferential action resulting from reduced peripheral effects. This study compared the variability in the PK and GD of BIL and GL at steady state.
Materials and methods: In this randomised, open-label, parallel study, 75 patients received either 0.5 U/kg BIL or GL subcutaneously once daily after a transition from pre-study basal insulin. On Days 8, 11 and 14, serial blood samples were collected to assess PK and 24-hour automated euglycaemic clamps were performed to assess GD. Intra-subject variability, expressed as CV, was estimated using a linear mixed model on log-

transformed PK and GD parameters and compared using an F-test at 10% significance level.

Results: BIL showed a flatter time-action profile than GL (figure) with a more even distribution of the GD effect over the 1st and 2nd 12 hours of the clamp (total amount of glucose infused [G_{tot}] during the 1st 12 hours/ G_{tot} during the 2nd 12 hours, expressed as percentage of G_{tot} during the whole clamp: 47%/53% for BIL, 61%/39% for GL at Day 8; 55%/45% for BIL, 61%/39% for GL at Day 11; and 57%/43% for BIL, 63%/37% for GL at Day 14). Intra-subject CV was statistically significantly lower by 41% (11% vs 19%), 55% (13% vs 28%) and 35% (41% vs 64%) with BIL vs GL for PK and GD parameters $AUC_{0\text{-}24,ss}$, $C_{max,ss}$ and G_{tot}, respectively.

Conclusion: BIL has both a flatter and more predictable time-action profile with less day-to-day intra-subject PK and GD variability compared to GL. This may result in improved glycaemic control and reduced hypoglycaemia.



Clinical Trial Registration Number: NCT01784211
Supported by: Eli Lilly and Company

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Greater HbA_{1c} reduction with basal insulin peglispro (BIL) vs insulin glargine (GL) in an open-label, randomised study in type 1 diabetic patients: IMAGINE 1

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Background and aims: Basal insulin peglispro (BIL) is a novel basal insulin with a flat pharmacokinetic profile which has a hepato-preferential action resulting from reduced peripheral effects.

Materials and methods: We compared BIL with GL in a Phase 3, 78-week study (primary endpoint 26 weeks) in 455 patients (51% female) with T1D (HbA1c <12% [<108 mmol/mol]). Patients were randomised to bedtime BIL (N=295) or GL (N=160) with prandial insulin lispro. An electronic diary was used for data capture and insulin dosing decisions.

Results: At Week 26, HbA1c reduction was greater with BIL compared with GL (baseline: 7.8% [62 mmol/mol]; treatment difference -0.37% [-4.0 mmol/mol]; 95% CI: -0.50%, -0.23% [-5.5, -2.5 mmol/mol]). More patients taking BIL reached HbA1c targets. Glucose variability was reduced in the BIL group, and nocturnal hypoglycaemia rate was 36% lower. Total hypoglycaemia rate was 29% higher with BIL, and severe hypoglycaemia rate was also higher. Basal insulin dose was higher, but bolus and total insulin dosages were lower in the BIL group. Patients on BIL had a significant weight loss. Triglycerides (TG) increased from baseline with BIL; no treatment differences were seen in LDL-C or HDL-C. In the BIL group, ALT increased from baseline. More patients taking BIL had ALT ≥3× ULN (4.5% vs 0.7%, p=.041) elevations, with no cases of Hy's law. Liver fat content (LFC), assessed by MRI in a subset of T1D patients in 2 studies of BIL vs GL, also increased with BIL. More patients taking BIL had injection site reactions (25% vs 0%, p<.001).

Conclusion: Treatment with BIL compared with GL resulted in lower HbA1c, reduced nocturnal hypoglycaemia, and weight loss, with higher levels of TG, ALT, and LFC, and injection site reactions in patients with T1D.

Outcomes	26 Wks			78 Wks		
	GL	BIL	p-value[a]	GL	BIL	p-value[a]
HbA1c (%)[b]	7.4 ± 0.1	7.1 ± 0.0	<.001	7.7 ± 0.1	7.4 ± 0.1	.002
HbA1c <7% (% of pts)	27.5	44.9	<.001	22.9	34.5	.002
HbA1c ≤6.5% (% of pts)	13.7	28.6	<.001	13.1	20.2	.013
FSG (mmol/l)[b]	8.9 ± 0.3	7.7 ± 0.2	<.001	9.2 ± 0.3	8.0 ± 0.3	.007
Body weight change (kg)[b]	0.7 ± 0.3	-1.2 ± 0.2	<.001	1.0 ± 0.4	-0.9 ± 0.3	<.001
Nocturnal hypoglycaemia[c,d]	2.7 ± 0.2	1.7 ± 0.1	RR 0.64***	2.3 ± 0.2	1.6 ± 0.1	RR 0.69***
Total hypoglycaemia[d]	12.4 ± 0.6	16.0 ± 0.4	RR 1.29***	11.4 ± 0.6	14.3 ± 0.4	RR 1.25***
Severe hypoglycaemia[e]	16.2 ± 5.9	39.0 ± 7.4	RR 2.41*	9.5 ± 2.9	23.8 ± 3.9	RR 2.50**
Basal daily insulin (U)[b]	25.9 ± 0.8	29.2 ± 0.7	<.001	26.6 ± 0.9	30.5 ± 0.8	<.001
Bolus daily insulin (U)[b]	35.0 ± 1.4	26.9 ± 1.1	<.001	36.4 ± 1.7	28.1 ± 1.4	<.001
Total daily insulin (U)[b]	59.7 ± 1.9	53.3 ± 1.6	.002	61.1 ± 2.4	55.9 ± 1.9	.044
Between-day glucose variability (mmol/l)[b,f]	3.3 ± 0.1	2.9 ± 0.1	.026	3.4 ± 0.2	3.1 ± 0.1	.105
Between-day glucose variability (mg/dl)[b,f]	59.3 ± 2.4	52.5 ± 1.8	.026	60.7 ± 2.8	54.9 ± 2.2	.105
ALT (IU/l)[b]	21.0 ± 1.3	29.4 ± 0.9	<.001	21.3 ± 1.3	28.3 ± 1.0	<.001
Triglycerides (mmol/l)[b]	0.96 ± 0.06	1.24 ± 0.04	<.001	1.00 ± 0.07	1.25 ± 0.05	.005
LFC (N) (2 studies)	(64)	(118)		(11)	(26)	
LFC (%)	3.0 ± 0.3	5.4 ± 0.2	<.001	2.9 ± 0.8	6.1 ± 0.5	.001

[a]Between treatments; [b]LSM ± SE; [c]Bedtime to waking,10 PM to 10 AM; [d]Events/pt/30d (Group mean ± SE); [e]Events/100 pt yrs (Aggregated rate ± SD); [f]SD of 7d FBG; ALT, alanine aminotransferase; LSM, least squares mean; RR, relative rate BIL/GL; ULN, upper limit of normal. *p<.05, **p<.01, ***p<.001

Clinical Trial Registration Number: NCT01481779
Supported by: Eli Lilly and Company

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Sustained glycaemic control and less nocturnal hypoglycaemia with new insulin glargine 300 U/ml versus glargine 100 U/ml over 1 year in Japanese people with type 1 diabetes mellitus (EDITION JP 1)

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Background and aims: In EDITION JP 1, Japanese people with T1DM receiving new insulin glargine 300 U/ml (Gla-300, n=122) showed comparable glycaemic control and less hypoglycaemia over 6 months compared with glargine 100 U/mL (Gla-100, n=121).

Materials and methods: Participants continued to receive Gla-300 or Gla-100 for an additional 6 months.

Results: The 12-month study period was completed by 228 participants; 114 (93.4%) receiving Gla-300 and 114 (94.2%) receiving Gla-100. HbA_{1c} and FPG levels decreased from baseline to month 12 with Gla-300 (mean [SD] change −0.20 [0.80] % and −0.8 [4.8] mmol/l) and Gla-100 (mean [SD] change −0.25 [0.72] % and −0.4 [5.2] mmol/l). At month 12, mean daily Gla-300 dose was 24 U/day (0.36 U/kg/day) and Gla-100 dose was 18 U/day (0.28 U/kg/day), with little change in dose observed between months 6 and 12. Daily mealtime insulin doses were comparable in the Gla-300 (29 U/day [0.45 U/kg/day]) and Gla-100 (29 U/day [0.47 U/kg/day]) groups at the end of the study period. Over 12 months, rates (events per participant-year) and percentage of participants experiencing ≥1 nocturnal confirmed (≤3.9 mmol/l [≤70 mg/dl]) or severe hypoglycaemic event were comparable between groups. At the lower (<3.0 mmol/l [<54 mg/dl]) threshold, a reduction in event rate was observed with Gla-300 compared with Gla-100 during the night (2.39 vs 3.85; rate ratio 0.62; 95% CI: 0.39 to 0.97). Consistently, the percentage of participants experiencing ≥1 nocturnal event at this threshold was also reduced with Gla-300 compared with Gla-100 (52.5 vs 66.1; relative risk 0.79; 95% CI: 0.64 to 0.98). Severe hypoglycaemia occurred in 12 and 11 participants receiving Gla-300 and Gla-100, respectively. Similar numbers of adverse events were reported for each treatment group.

Conclusion: Japanese people with T1DM achieved sustained glycaemic control with less nocturnal confirmed (<3.0 mmol/l [<54 mg/dl]) or severe hypoglycaemia over 12 months with Gla-300 vs Gla-100. Gla-300 was well tolerated over the 12-month study perio.
Clinical Trial Registration Number: NCT01689129
Supported by: Sanofi

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Switching basal insulin treatment to insulin degludec in patients with type 1 diabetes having an unsatisfactory HbA_{1c}: a clinical follow-up
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Background and aims: Insulin degludec, a basal insulin, with an ultra-long duration of action became available in Sweden from July 2013. The diabetes team at Danderyd Hospital decided to conduct a simple, prospective, clinical follow-up of patients with type 1 diabetes who switched basal insulin to degludec. Since this evaluation was a part of the quality assurance regarding routine patient care, permission from the ethics committee was not necessary and no written consent was obtained but all patients were informed about the follow-up and agreed to be part of it.
Materials and methods: From August 2013 all consecutive patients with type 1 diabetes, who switched to insulin degludec, were registered in a simple form which included data on (1) most recent HbA1c, (2) type of insulins before switching, (3) insulin doses, (4) self-reported hypoglycaemias - defined as hypoglycaemic symptoms ameliorated by carbohydrate treatment or a self-measured plasma glucose <3.5 mmol/L - during the previous four weeks and (5) the reason/indications for switching. Four major indications were predefined: (i) currently administering basal insulin twice daily; (ii) having unacceptable HbA1c (in relation to the patient's individual goal); (iii) experiencing repeated hypoglycaemic events and/or unstable glucose; and (iv) having an irregular daily schedule making fixed-time administration difficult. For an individual patient one or more of these indications could be present. The switch was decided in conjunction with professional judgment by the healthcare professional. After 4-6 months data on HbA1c, insulin doses and self-reported hypoglycaemias were collected again. Around one year after the change in basal treatment a second HbA1c was retrieved from medical records in order to assess the long-term effect of the switch on glycaemic control as it was recognized that an initial improvement in HbA1c could be due to factors such as patients increasing their self-monitoring of blood glucose when switching to a novel insulin and/or being more conscientious about their diabetes management. In 60% of all patients who switched basal insulin to insulin degludec an unacceptable HbA1c was given as one of the indications for changing treatment. Data from the follow-up in this subgroup are presented here.
Results: At the end of March 2015 we had follow-up data on 239 patients in this poorly controlled subgroup. Median time to follow-up was 21 weeks [IQR 18 to 24] and mean [SD] HbA1c decreased from 78.0 [13.3] to 72.2 [13.7] mmol/mol, $p<0.0001$. In 138 patients a second HbA1c had been retrieved after median 50 weeks [IQR 44 to 54] showing a sustained improvement in HbA1c. This was achieved using lower doses of insulin, median reduction of basal dose was 13% (IQR -22% to -0%) while total dose (basal + prandial) was reduced by 11% (IQR -19% to -1%). The total number of self-reported hypoglycaemic events was unchanged but there was a reduction in nocturnal events, from 1.3 [2.3] at baseline to 0.6 [2.0] at follow-up, $p<0.0001$. No validated measurement of patient satisfaction was used. However, spontaneous comments made by patients suggested that they felt as though blood glucose was much more stable with insulin degludec but also that their prandial insulin seemed to have a better effect.
Conclusion: Due to the improvement in glycaemic control, reduction of insulin dose and less nocturnal hypoglycaemias, we concluded that insulin degludec was clinically useful for our patients with type 1 diabetes.

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BIOD-531 demonstrates superior prandial glucose control, post-meal dosing flexibility, and less insulin "stacking" compared to marketed prandial/basal insulins
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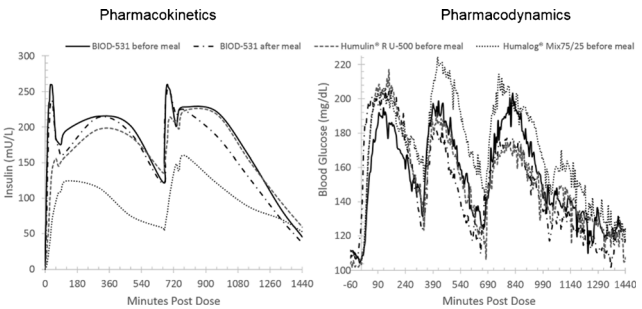
Background and aims: Subcutaneous (SC) injection of BIOD-531, a 400 U/ml formulation of human insulin, EDTA, citrate and magnesium sulfate is associated with ultra-rapid absorption and a basal duration of glucose lowering action. This study was designed to assess the pharmacokinetic (PK) and postprandial glycemia (PD) profiles associated with breakfast and dinner time doses of BIOD-531 vs. marketed comparators in patients with insulin resistant diabetes.
Materials and methods: Twelve patients with type 1 or type 2 diabetes who use insulin at doses of≥150 U/day or≥100 U/injection were randomized into this 4-period crossover trial. Each subject underwent in random order one of the following treatments on separate days: (a) BIOD-531 immediately before meals (pre-meal); (b) Humalog® Mix 75/25 (HMix) pre-meal; (c) Humulin® R U-500 (U-500) pre-meal; and (d) BIOD-531, 20 min after the start of the meals (post-meal). After baseline glucose normalization, test insulins (1.2 U/kg) were administered by SC injection with a standardized breakfast (921 kcal) followed by a second dose (0.8 U/kg) 11 hours later with a standardized dinner (963 kcal). Subjects also received a standardized lunch (669 kcal) 5.5 hours after breakfast without additional insulin. The primary endpoint was average glucose concentration in the breakfast to lunch interval.
Results: Mean baseline characteristics for the patients evaluated were as follows: insulin dose 205 U/day, 55.4 years old, 117 kg, 25% female. The Figure shows PK and PD profiles. The absorptive phase of BIOD-531 was significantly more rapid than either HMix or U-500 (time to half-maximal insulin concentrations (11.7±1.2, 42.2±2.6, and 41.7±9.8 min, respectively, $p<0.001$ for both comparisons). Peak insulin levels after BIOD-531 injections were similar after breakfast and dinner. The post-dinner:post-breakfast peak insulin concentration ratio was significantly higher for HMix (1.21) and U-500 (1.19) compared to BIOD-531 (0.97, $p<0.05$ for both comparisons). Pre-meal BIOD-531 resulted in mean post-breakfast period glucose concentration (mg/dl) of 164.6± 11.8 compared to 179.9±10.0 with HMix ($p=0.009$) and 178.0±7.3 with U-500 ($p=0.019$). Over the entire 24 hours of observation, pre- and post-meal BIOD-531 resulted in lower glucose concentrations compared to pre-meal HMix and comparable to pre-meal U-500. The frequency of adverse events was similar between treatments.
Conclusion: Ultra-rapid absorption of BIOD-531 was associated with superior prandial control compared to HMix and U-500 in insulin-resistant diabetes patients. Post-meal dosing of BIOD-531 resulted in superior or comparable overall glycemic control compared to pre-meal treatment with HMix and U-500. The duration of exposure of BIOD-531 is suitable for twice-daily dosing with evidence for less insulin "stacking" after a second dose.



Clinical Trial Registration Number: NCT02324309

OP 02 Sledge hammers to crack nuts: cutting-edge cardiometabolic epidemiology

7

Diabetes and cause-specific mortality: evidence from 55 000 deaths in 700 000 adults in 44 studies from the Prospective Studies Collaboration

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Background and aims: While the association of diabetes with macrovascular mortality has been well-studied, evidence for the effect of diabetes on mortality from other causes is more equivocal.
Materials and methods: Information from 44 prospective studies was obtained on 54,855 deaths among 690,700 adults with no previous vascular or other chronic disease recorded at baseline. We assessed the associations of DM with cause-specific mortality using Cox proportional-hazards models adjusted for age at risk, gender, smoking, body mass index and cohort.
Results: During 13 million person-years of follow-up there were 21 762 ischaemic, 5 386 non-ischaemic vascular, 18 658 neoplastic and 9 048 other deaths between ages 35-89. Diabetes was associated with about a 2.5-fold increased risk of death from ischaemic heart disease (HR 2.37; 95%CI 2.23-2.52), ischaemic stroke (2.75; 2.29-3.31) and cardiac arrhythmia (2.47; 1.80-3.38), but was more weakly associated with non-ischaemic vascular mortality (1.57; 1.34-1.85). There was about a 2-fold increased risk of renal (1.90; 1.40-2.58) and hepatic (1.75; 1.36-2.26) mortality among people with diabetes, while the increased risk for mortality from any cancer was small (1.13; 1.06-1.21), and was largely driven by cancer of the mouth (1.81; 1.17-2.81), liver (1.80; 1.26-2.58) and pancreas (1.57; 1.21-2.03).
Conclusion: Diabetes was associated with a substantial increased risk of mortality from selected ischaemic and non-ischaemic diseases. Understanding these associations should help reduce the excess mortality among people with diabetes.

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Causal effects of cardiometabolic traits on cardiovascular and total mortality: a Mendelian randomisation study

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Background and aims: Epidemiological studies have shown associations between cardio-metabolic traits and increased risk of mortality, yet the underlying causal relationships remain unclear. We aimed to understand the causal nature of the associations of common cardio-metabolic traits with cardiovascular (CVD) mortality and/or all-cause total mortality using Mendelian randomization analyses.
Materials and methods: Our study included 28589 participants from the population based Malmö Diet and Cancer study with a mean follow-up period of 15.4 years. We conducted Mendelian randomization analyses by using trait specific weighted genetic risk scores (GRS) for BMI (comprising of 31 SNPs), systolic blood pressure (SBP;29 SNPs), HDL-cholesterol (HDL-C; 41 SNPs), LDL-cholesterol (LDL-C; 32 SNPs), triglycerides (TG; 26 SNPs) and fasting plasma glucose (FPG; 15 SNPs) as instrumental variables to investigate the causal role of these traits in CVD-mortality (1913 deaths) and total mortality (5708 deaths). We performed a 2-stage regression instrumental variable analysis. First, each cardio-metabolic trait was regressed on its respective GRS creating instrumental variables and second, the instrumental variables were used as predictor variables for CVD- and total mortality in Cox proportional hazards models. The analyses were adjusted for age and sex. Lipid-lowering and antihypertensive medications were added to the model when lipid traits or SBP were used as outcome or predictor variables, respectively. Finally, we performed multivariable Mendelian randomization analyses using the effect estimates of each SNP first on all cardiometabolic traits and second, on CVD-mortality and total mortality to adjust for potential pleiotropic effects of the SNPs.
Results: Higher baseline levels of SBP and FPG, and lower levels of HDL-C associated with increased risk of both CVD- and total mortality while higher BMI and TG associated only with increased risk of CVD-mortality. The variances explained by GRSs for BMI, SBP, LDL-C, HDL-C, TG and FPG were 0.8, 0.5, 7.2, 5.7, 4.3 and 2.2%, respectively, in association with their respective traits ($P<10-15$ for all). The instrumental variable analyses indicated that aone standard deviation (1SD) of genetically elevated BMI (HR: 1.82, 95% CI: 1.09-3.03; P: 0.02), LDL-C (1.20, 1.02-1.42; 0.03) and TG (1.28, 1.05-1.57; 0.02) associated with increased risk of CVD-mortality and a 1SD of genetically elevated HDL-C (0.78, 0.65-0.93; 0.006) associated with decreased risk of CVD-mortality. Additionally, a 1SD of genetically elevated TG associated with increased risk of total mortality (1.18, 1.05-1.33, 0.005) and 1SD of genetically elevated HDL-C associated with decreased risk of total mortality (0.89, 0.80-0.99; 0.03). Adjusting for pleiotropic effects in the multivariable Mendelian randomization analyses proposed a direct causal association of TG ($P=0.02$) and an inverse association of HDL-C ($P=0.035$) with CVD-mortality.
Conclusion: While randomized clinical trials and Mendelian randomization studies have only provided evidence for causality of higher LDL-C and TG but not of lower HDL-C in coronary disease development, our results suggest a causal role of HDL-C in addition to TG in CVD-mortality. However, whether other cardiometabolic traits causally impact mortality requires further evidence.
Supported by: VR, HLF, NNF, SDF, PF, KAWF, LF

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Value of genetic risk score in personalised risk prediction of type 2 diabetes: analysis of 10273 individuals of the Estonian Biobank Cohort

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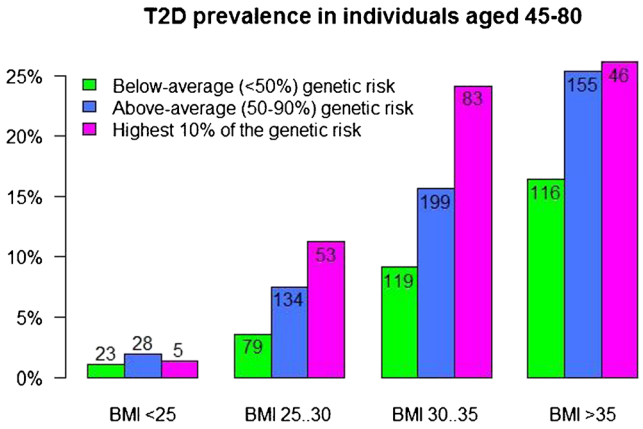
Background and aims: Large-scale genome-wide association studies (GWAS) have revealed more than 80 geneic loci associated with prevalent Type 2 Diabetes (T2D), improving the understanding of molecular pathways leading to the disease. However, practical usefulness of such results in T2D prevention is still heavily disputed. Our aim is to assess the association of the genetic risk score (GRS) for T2D with both prevalent and incident T2D and cardiovascular mortality in a large population-based cohort of European Origin.
Materials and methods: The GRS is computed as a weighted sum of allele counts of 7500 markers identified by genome-wide genotyping of the cohort. The selection of markers and their weights is based on a large-scale GWAS meta-analysis (Morris et al, 2012). Predictive ability of the GRS is validated in 10,273 individuals of the Estonian Biobank cohort (1181 prevalent and 386 incident T2D cases, median follow-up time 5.5 years).
Results: The odds for T2D prevalence are 2.20 times higher (95% CI 1.82-2.65) in the highest GRS quintile compared to the rest of the cohort and 3.66 times higher (95% CI 2.72-4.91) compared to the lowest GRS quintile. For individuals in the highest GRS quintile, the estimated hazard of developing T2D during the follow-up is 1.78 times higher (95% CI 1.41-2.24) compared to the rest of the cohort and 2.72 times higher

(95%CI 1.94-3.81) compared to the lowest GRS quintile. From the available risk factors, GRS was the second strongest predictor for T2D (after BMI), increasing the area under the receiver operator characteristic curve for overweight individuals from 0.706 to 0.756 (p=5.4*10-13). In addition, we detected that individuals in the highest GRS quintile for T2D have significantly higher risk for cardiovascular mortality (adjusted hazard ratio=1.25, 95%CI 1.06-1.48, p=0.009).

Conclusion: Our results indicate that implementation of genetic testing in routine risk assessment could greatly improve the accuracy of T2D risk assessment in general population.



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Glycaemic control and incidence of dementia in 363,573 patients with type 2 diabetes: an observational study

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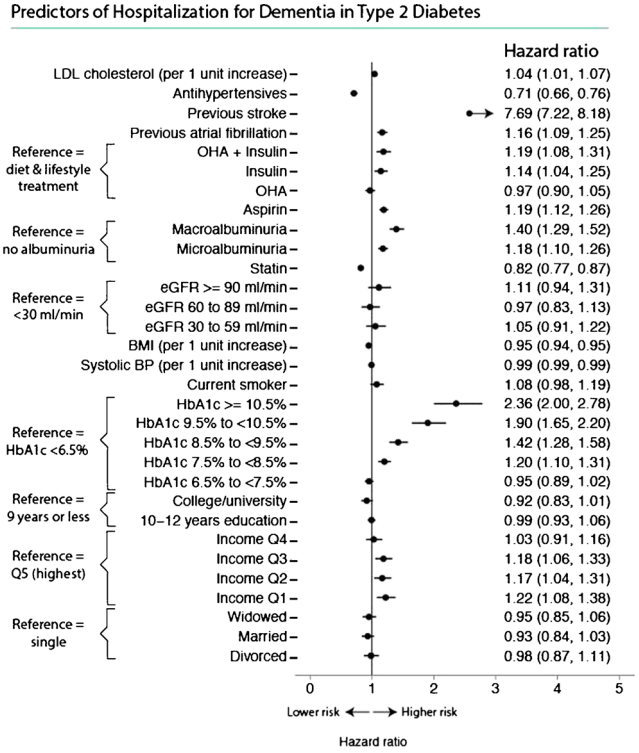
Background and aims: A growing body of evidence indicates that diabetes increases the risk of cognitive impairment. This has prompted interest in delineating predictors of cognitive decline and dementia in diabetes. Cohort studies relating glycated haemoglobin (HbA1c) to risk of dementia are lacking. We evaluated extensive clinical data to explore the association between HbA1c and the risk of hospitalization for dementia among persons with type 2 diabetes.

Materials and methods: We identified all patients with type 2 diabetes and no known hospitalization for dementia who were registered in the Swedish National Diabetes Registry between January, 2004, and December, 2012. These patients were followed up until hospital admission for dementia, death, or end of follow-up on Dec 31, 2012. We used Cox regression to assess the association between patients' characteristics, including HbA1c, and dementia. All covariates were modelled as time-dependent predictors.

Results: In a cohort of 395,173 patients (contributing 2,981,247 registrations) with mean age of 64.6 years (SD 12.5) at baseline, 9,175 patients (2.3%) were admitted to hospital with a primary or secondary diagnosis of dementia during a mean (SD) follow-up of 4.6 (2.5) years. In a Cox regression analysis - with adjustment for age, sex, duration of diabetes, marital status, income, education, smoking status, systolic blood pressure, body mass index, estimated glomerular filtration rate, statins, albuminuria, type of glucose lowering treatment, atrial fibrillation, stroke and antihypertensive medications - the hazard ratio for hospitalization for dementia was 2.36 (95% CI 2.00-2.78) in patients with HbA1c of 10.5% or higher, compared with a reference group of patients with HbA1c of less than 6.5%. Statins and antihypertensive medications appeared to be protective from hospitalization for dementia. Stroke was associated with a hazard ratio of 7.69 (95% CI 7.22-8.18) for developing dementia. Albuminuria was also associated with increased risk of dementia. Refer to Figure 1.

Conclusion: The positive association between HbA1c and risk of dementia in fairly young patients with type 2 diabetes indicates a potential for prevention of dementia with improved glycaemic control.



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The risk of developing diabetes and other diseases 10-17 years after pregnancy

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Background and aims: A high pre pregnancy BMI and excessive maternal weight gain has negative implications for pregnancy and delivery but has also negative long term effects since these conditions have been acknowledged as risk factors for post gestational weight retention and obesity later in life. The aim of this study was to analyze if a high maternal BMI at start of pregnancy and a high or low maternal weight gain during pregnancy were associated with diseases later in life.

Materials and methods: We used a population based cohort of 23,524 women from southern Sweden. Data regarding these women were merged with the Swedish Medical Birth Register (SMBR). 15014 of theses women were found to have at least one registered delivery at least 10 years before answering a health questionnaire. The women were divided into groups based on their BMI (≤25 and >25; n=2415 and 347) and weight gain (≤15 and >15 kg; n=2228 and 1302).

Results: The mean pre pregnancy BMI was 21.84±2.9 kg/m^2 and the mean weight gain was 14.5±4.6 kg. There was a positive association between BMI >25 and the risk of developing obesity, diabetes, cardiac disease, endocrine diseases and overall/other morbidity (OR 21.9, 6.1, 2.6, 2.1, 1.2; p-value <0.001, <0.001, 0.001, <0.001, 0.012 respectivly). There was a positive correlation between low maternal weight gain and the risk of developing psychiatric diseases (OR 1.6; p<0.001). There was however no significant association between a high weight gain and the risk of developing diabetes, cardiac or endocrine diseases 10-17 years after delivery.

Conclusion: A high pre pregnancy BMI significantly increases the risk of several diseases later in life, including diabetes and cardiac disease. A high weight gain during pregnancy did not have any implications on metabolic diseases within ten years in our study.

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Use of antibiotics and risk of type 2 diabetes: a population-based case control study

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Background and aims: There is accumulating evidence that bacteria in the human gut may influence nutrient metabolism. In rodent models, it is possible to manipulate insulin sensitivity, glucose tolerance and nutrient deposition through antibiotics-induced alterations in the gut microbiota. Further, associations between use of antibiotics and obesity have been reported in recent observational studies. We investigated whether use of antibiotics influences the risk of developing type 2 diabetes, and if so, if the effect can be attributed to specific types of antibiotics.
Materials and methods: The analysis was conducted as a population-based case-control study of incident type 2 diabetes cases in Denmark (population 5.5 million) during the period January 1st 2000 to December 31st 2012. Data from the Danish National Registry of Patients, the Danish National Prescription Registry and the Danish Person Registry were combined. The crude and adjusted odds ratios for the association between antibiotic exposure and risk of developing type 2 diabetes were estimated using conditional logistic regression, controlling for potential confounders.
Results: The study included 170,504 patients with type 2 diabetes and 1,364,008 matched controls. An increased odds ratio for risk of type 2 diabetes was seen with increasing exposure to antibiotics of any type with an odds ratio of 1.53 (95% CI 1.50-1.55) with redemption of 5+ vs 0-1 prescriptions. While no individual group of antibiotics was specifically associated with type 2 diabetes risk, slightly higher odds ratios for type 2 diabetes were seen with narrow-spectrum and bactericidal antibiotics compared to broad-spectrum and bacteriostatic types of antibiotics. The increased use of antibiotics in patients with type 2 diabetes was found up till 15 years before diagnosis of type 2 diabetes, as well as after the diagnosis.
Conclusion: These findings may represent an increased demand for antibiotics due to increased risk of infections in patients with yet undiagnosed diabetes, pre-diabetes or manifest type 2 diabetes. However, the possibility that antibiotics exposure increases diabetes risk cannot be excluded.

OP 03 Incretin-based therapy: on and off conventional targets

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Treatment with liraglutide leads to an important reduction in liver fat content, assessed by magnetic resonance spectroscopy, in people with type 2 diabetes

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Background and aims: Most studies found that 60-75% of patients with type 2 diabetes have Non-Alcoholic Fatty Liver Disease. Unfortunately, there is no treatment of NAFLD in people with diabetes besides weight loss and increased physical activity. Some animal studies have shown that GLP-1 receptor agonists may reduce liver lipogenesis. The effect of the GLP-1 receptor agonist, liraglutide on liver fat remain unknown in patients with type 2 diabetes. This prompted us to perform a prospective study on the effect of liraglutide on liver fat content, in type 2 diabetes.
Materials and methods: A total of 43 patients with type 2 diabetes mellitus were included in this study. Liver fat was measured by 1H-magnetic resonance spectroscopy (gold-standard method for liver fat measurement) before and after 6 months of liraglutide therapy (1.2 mg/j).
Results: Thirty-five (81.3%) patients had steatosis at baseline (hepatic triglyceride content greater than 5.5%). At inclusion, liver fat content was correlated with fasting serum triglycerides ($r=0.33$; $p=0.02$) and alanine aminotransferase (ALAT) ($r=0.46$; $p=0.002$). Treatment with liraglutide was associated with a mean weight loss of 4.4 kg ($p<0.001$), a mean HbA1C reduction of 2.6% ($p<0.001$) and an important reduction in liver fat content (from 19.1% to 12.7%, $p<0.001$) corresponding to a relative decrease of 33.3%. Reduction in liver fat was correlated with weight reduction ($r=0.49$, $p=0.001$), ALAT reduction ($r=0.49$, $p=0.001$), baseline liver fat content ($r=0.33$, $p=0.031$) and borderline significant with HA1c reduction ($r=0.29$, $p=0.057$). In multivariate analysis, reduction in liver fat was independently associated with weight reduction ($p<0.0001$) and baseline liver fat ($p<0.0001$) ($r2=0.62$). In the subgroup of patient who showed a relative weight reduction below the median value (3.3%), a significant 19.6% decrease was observed ($p=0.029$) whereas the mean body weight remained unchanged.
Conclusion: Our data indicate that liraglutide is an effective treatment to decrease liver fat content, in patients with type 2 diabetes. Although weight reduction induced by liraglutide appears to be an important factor promoting reduction in liver fat content, we cannot exclude a direct action of liraglutide on lipogenesis since a significant decrease in liver fat was observed in patients without significant weight reduction. Further studies are needed to explore the mechanisms involved in liver fat content reduction linked with liraglutide treatment.
Clinical Trial Registration Number: EudraCT: 2012-000375-16
Supported by: NovoNordisk Grant

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Adipose tissue response to GLP-1 analogue-induced weight loss

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Background and aims: Glucagon like peptide-1 (GLP-1) analogues in the treatment of Type 2 diabetes are used for the control of HbA1c and

have the additional benefit of weight loss. Weight loss is considered to reduce obesity-associated adipose tissue inflammation, however, little is known about the effect of GLP-1 on adipose tissue, which is known to express GLP-1 receptors. The aim of the study was to assess the effect of weight loss on adipose tissue inflammatory and fibrotic responses achieved either by 1) treatment with the GLP-1 analogue Liraglutide 2) sustainable calorie reduction and 3) very low calorie dieting.

Materials and methods: Age and BMI matched subjects with well controlled Type 2 diabetes were randomised toLiraglutide (at a dose of 0.6 mg for the first 4 weeks and if tolerated continued at 1.2 mg), or to dietetic counselling and follow up to allow for similar weight loss. Independently, subjects with and without insulin resistance underwent a very low calorie diet (VLCD) of 450 kcal/ day to achieve best possible non-surgical weight loss. Assessments included a subcutaneous abdominal adipose tissue biopsy at baseline and after 4 months. Fat samples were analysed for inflammatory and profibrotic markers by microarray and Taqman low density array based analysis. For statistical comparison Wilcoxon Signed Rank Test for paired analysis was used.

Results: Subjects who completed 4 months treatment with Liraglutide lost 3.2±1.08 kg (mean ± SD, age: 61.4±5.48 years, baseline HbA1c: 59.6±5.28 mmol/mol, BMI: 30.06±4.85 kg/m^2, n=9) and lost 5.84±3.27 kg in the diet arm (age: 57.0±9.6 years, baseline HbA1c: 56.4±6.54 mmol/mol; BMI: 32.4±3.67 kg/m^2, n=6). The mean weight loss after VLCD was 28±3.7 kg (baseline HOMA-IR: 4.4±2.7, baseline BMI: 37.6±4.9 kg/m^2, BMI at 4 months: 28.6±4.1 kg/m^2, n=24). After Liraglutide treatment, adipose tissue expression increased for the chemoattractant monocyte chemotactic protein-1 (1.01±0.13 AU (mean ± SE) versus 1.54±0.24 AU, $p<0.05$), the inflammatory adipokine tumor necrosis factor alpha (1.05±0.16 AU versus 1.63±0.21 AU, p=0.01) and the fibrotic cytokine transforming growth factor (TGF) beta-1 (0.91±0.07 AU versus 1.25±0.11 AU, $p<0.05$); whereas expression of these genes did not change in the diet arm. In contrast, we observed a trend in lower macrophage marker CD14 expression with a diet only induced weight loss of 5.84±3.27 kg (1.1± 0.18 AU versus 0.67±0.11 AU, p=0.08). Unlike with Liraglutide treatment and similar to sustained dieting we observed no change in TGFbeta-1 expression with VLCD, whereas there was a tendency for increased expression 2 weeks after refeeding (0.83± 0.04 AU versus 0.86±0.05 AU at 4 months and 0.97±0.07 AU with refeeding p=0.1).

Conclusion: Unlike with calorie induced dieting, Liraglutide treatment appears to induce an inflammatory and fibrotic response in adipose tissue with increased expression of inflammatory markers and fibrotic factors not otherwise found with dieting and typical of overfeeding. It remains to be shown whether Liraglutide treatment could accelerate adipose tissue dysfunction in the long term.

Supported by: EFSD/GlaxoSmithKline

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Upper and/or lower GI adverse events with long- vs short-acting GLP-1 receptor agonists: incidence, co-incidence, effects on HbA_{1c} and weight

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Background and aims: In direct comparative clinical trials, long- and short-acting glucagon-like peptide-1 receptor agonists (GLP-1RA) for T2DM appeared to have different efficacy and tolerability profiles. Early transient gastrointestinal adverse events (GI AE) are characteristic of GLP-1RA, but may differ in type and frequency for long- vs short-acting GLP-1RA. Our objective was to evaluate differences in GI tolerability with long- versus short-acting GLP-1 RA (specifically exenatide once weekly [ExQW], exenatide twice daily [ExBID] or liraglutide once daily [LiraQD]) according to location (upper, lower) and co-incidence. We also investigated whether GI AE were associated with differences in HbA1c or weight reductions.

Materials and methods: Intent to treat analyses of patient-level data from direct comparative trials of 2 GLP-1RA were conducted. Data from 3 studies of ExQW vs ExBID were pooled. Data comparing ExQW with LiraQD (DURATION-6) were analysed separately. GI events were identified according to MedDRA terminology and classified as upper or lower GI. Incidences of upper GI AE, lower GI AE, and combinations of upper and lower GI AE were determined for individual GLP-1RA. Changes in HbA1C and body weight from baseline at study endpoint were assessed according to occurrence of GI AE.

Results: The most common upper GI AE were nausea or vomiting; the most common lower GI AE were diarrhoea or constipation. Incidences of upper GI AE appeared less for ExQW than short-acting GLP-1RA (Table), while lower GI AE appeared comparable across GLP-1RA. Diarrhoea was found to be accompanied by upper GI AE, and mainly occurred within 10 days of an upper GI AE for all GLP-1RA. Although 55% of patients overall were male in both analyses, the majority with upper GI AE were female (57-58%). Few patients discontinued GLP-1RA due to GI AE, but discontinuation was more frequent with short-acting GLP-1 RA than ExQW (Table). Reductions from baseline in HbA1C were similar for patients with no GI AE and those with upper, lower, or combined GI AE for short or long-acting GLP-1RA. Reductions in body weight from baseline were observed in patients without GI AE (-2.0, -2.0 kg for ExQW, ExBID; -2.4, -3.5 kg for ExQW, LiraQD) but reductions were greater for those with upper + lower GI AE compared with no GI AE (-3.5 vs -2.0 kg) for ExBID, and for upper GI AE compared with no GI AE for ExQW (-3.9 vs -2.4 kg) in DURATION-6.

Conclusion: Pooled patient-level data from direct comparative trials indicate that upper GI AE occur more frequently with short- vs long-acting GLP-1RA. Lower GI AE are less common and do not differ between GLP-1 RA. Upper GI AE and diarrhoea were often reported by the same patients, suggesting some may be susceptible to upper and lower GI AE with GLP-1 RA. Presence or absence of GI AE did not affect HbA1c reductions but may influence weight reductions in some patients.

Table

	Pooled Data		DURATION-6	
	ExQW	ExBID	ExQW	LiraQD
Patient population (N)	617	606	461	450
Any upper GI (%)	24	36	16	31
Upper GI only (%)	15	27	11	19
Upper + Lower GI (%)	10	11	7	14
Upper GI + diarrhoea (%)	6	5	3	10
Upper GI + constipation (%)	3	4	2	2
Diarrhoea only (%)	5	3	3	4
Constipation only (%)	3	2	3	3
Discontinued due to GI AE (%)	1	6	1	4

Supported by: AstraZeneca

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Differential effects of GLP-1 receptor agonists on 24-hour averaged heart rate in healthy volunteers and patients with type 2 diabetes

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Background and aims: Heart rate (HR) increases have been reported in studies of glucagon-like peptide-1 receptor agonists (GLP-1 RAs). The present analysis compares data from 24 h time-averaged HR monitoring with GLP-1 RAs.
Materials and methods: A review of studies that report 24 h time-averaged HR monitoring with GLP-1 RAs, including an unpublished QT/QTc study of lixisenatide in healthy subjects, published data for lixisenatide, exenatide, liraglutide, albiglutide and dulaglutide, and a head-to-head comparison of lixisenatide and liraglutide.
Results: Shorter-acting GLP-1 RAs, such as exenatide BID and lixisenatide QD, are associated with a smaller and shorter increase in HR (1-3 bpm, 1-12 h) compared with longer-acting GLP-1 RAs, such as liraglutide, albiglutide and exenatide QW, which induce a greater and more prolonged diurnal and nocturnal effect (6-9 bpm). The HR increase with dulaglutide is relatively small but persists at night (Table). In the head-to-head comparison, smaller LS mean HR increases from baseline were observed with shorter-acting lixisenatide versus longer-acting liraglutide, and the treatment difference was significantly more pronounced at night (Table).
Conclusion: Shorter-acting GLP-1 RAs induce a smaller and transient HR increase; most longer-acting GLP-1 RAs are associated with a more pronounced diurnal and nocturnal HR increase. The clinical consequences of these differential effects are not known.

	Drug	Population; study duration	Regimen	Mean heart rate increase from baseline to study end	Source
Placebo-controlled studies	Lixisenatide	Healthy; 28 days	QD dosing: 10 µg Week 1, 15 µg Week 2, 20 µg Weeks 3–4	1.3 bpm*	Previously unpublished
	Exenatide BID	T2DM; 12 weeks	BID dosing: 5 ug Weeks 1–4, 10 µg Weeks 5–12	3 bpm*	AstraZeneca Product Monograph 2013, Canada
	Exenatide QW	T2DM; 30 weeks	QW dosing: 2 mg	3.5 bpm†	Sager et al, ADA 2011
	Liraglutide	Healthy; 3 weeks	QD dosing: 0.6 mg Week 1, 1.2 mg Week 2, 1.8 mg Week 3	7–8 bpm*	Novo Nordisk Product Monograph 2011, Canada
		T2DM, obese patients; 5 weeks	QD dosing: titrated from 0.6 mg, increased by 0.6 mg weekly to 1.8 mg or 3 mg end dose	Overall: 6.0–7.0 bpm‡ daytime: 4.3–4.6 bpm‡ sleeping: 7.0–8.9 bpm‡	FDA briefing document, 3 mg Liraglutide
	Albiglutide	Healthy; 6 weeks	QW dosing: 30 mg for 2 weeks then 50 mg for 4 weeks	30 mg: ≥3 bpm* 50 mg: 6–8 bpm*	Darpo et al. *Diabetes Ther* 2014;5:141–1543
	Dulaglutide	T2DM; 26 weeks	QW dosing: 0.75 mg or 1.5 mg	0.75 mg:1.3 bpm* 1.5 mg: 3.5 bpm*	Ferdinand et al. *Hypertension* 2014;64:731–7
Head-to-head study	Lixisenatide vs liraglutide	T2DM; 8 weeks	Lixisenatide QD dosing: 10 µg Weeks 1–2, 20 µg Weeks 3–8	Overall: 3.3 bpm† Daytime: 3.7 bpm† Nighttime: 2.2 bpm†	Meier. ADA 2014; P1017 and previously unpublished data
			Liraglutide QD dosing: 0.6 mg Week 1, 1.2 mg Week 2, 1.2 or 1.8 mg Weeks 3–8	1.2 mg overall: 9.3 bpm† 1.2 mg daytime: 9.4 bpm† 1.2 mg nighttime: 10.0 bpm† 1.8 mg overall: 9.2 bpm† 1.8 mg daytime: 9.1 bpm† 1.8 mg nighttime: 10.1 bpm†	

*Data are changes from baseline in the placebo-adjusted mean HR.
†Data are changes from baseline values (not placebo-adjusted).
‡Data are differences from placebo in mean HR at end of study.
BID, twice daily; bpm, beats per minute; T2DM, type 2 diabetes mellitus; QD, once daily; QW, once weekly.

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Risk of pancreatic cancer associated with use of incretin-based therapy and other glucose-lowering agents: a nationwide case-control study in Denmark

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Background and aims: Incretin-based drugs (glucagon-like peptide 1 (GLP-1) mimetics and dipeptidyl peptidase 4 (DPP-4) inhibitors) have been suspected to increase risk of pancreatic cancer. We examined if use of incretin-based drugs was associated with risk of pancreatic cancer and compared with other glucose-lowering agents.
Materials and methods: Using population-based medical databases this analysis was carried out as an age-, gender- and residence-matched (1:10) case-control study from 2005 to 2012 (6,036 pancreatic cancer cases and 60,360 controls). Odds ratios (ORs) for pancreatic cancer associated with use of incretin-based drugs and other glucose-lowering agents were computed, using conditional logistic regression, and we adjusted for other pancreatic cancer risk factors.
Results: A total of 122 incident pancreatic cancer patients (2.0%) vs. 400 controls (0.7%) had used incretin-based drugs at least once, whereas 20.8% cases vs. 8.6% controls had used any glucose-lowering agent. Patients with pancreatic cancer more often than controls had a hospital

history of chronic pancreatitis (3.6% vs. 0.3%), gallstone (10.7% vs. 5.3%), obesity (4.0% vs. 2.9%), alcoholism (6.5% vs. 3.9%), and chronic pulmonary disease (21.2% vs. 17.9%). Compared with non-users of any glucose-lowering agents, the adjusted risk of pancreatic cancer was increased for DPP-4 inhibitor users (adjusted OR=3.87, 95% CI 3.06-4.89) and GLP-1 mimetic users (adjusted OR=2.70, 95% CI 1.82-4.00), and furthermore for ever users of metformin (adjusted OR=2.65, 95% CI 2.44-2.88), sulfonylureas 2.65 (2.41-2.91) and insulin (adjusted OR= 3.61, 95% CI 3.24-4.03). The highest cancer ORs were observed among new initiators of each glucose-lowering agent and in those with fewer than 3 prescriptions filled, suggesting a non-causal or reverse causal association.

Conclusion: The risk of pancreatic cancer was increased to similar levels for all glucose-lowering agents. This suggests that diabetes is a risk factor for pancreatic cancer independent of a specific drug effect, yet warrants further investigation.

Supported by: the Clinical Epidemiological Research Foundation, Denmark

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DPP4 inhibitor can protect TZD induced bone loss via sclerostin expression in obese diabetic rat

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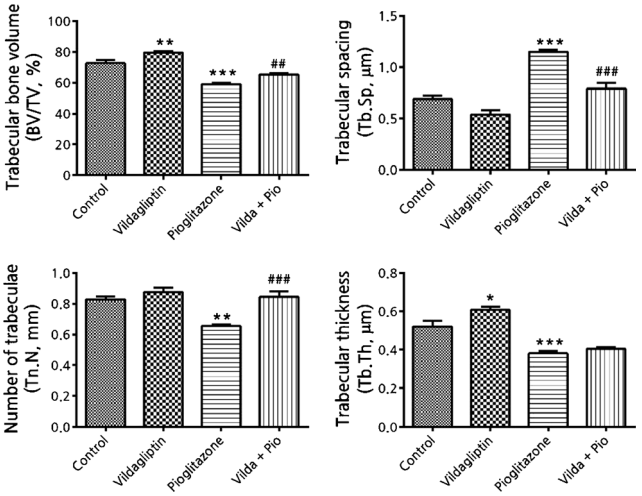
Background and aims: Thiazolidinediones (TZDs) are widely prescribed drugs in T2DM. However, recent studies, including large-scale randomized controlled trials and observational studies, suggests that long-term use of thiazolidinediones is associated with bone loss and an increased risk of fractures in women with Type 2 diabetes. Incretin based drugs (glucagon-like-peptide-1 agonist and dipeptidylpeptidase-4 inhibitor) have been added to a new treatment option for glucose control in T2DM and many beneficial effects on beta cell including insulin secretion and beta cell mass. Beyond pancreas, Incretins affect bone mass through stimulating bone remodeling. We already reported exendin-4 might increase BMD by decreasing the expression of SOST/sclerostin in osteocytes in T2DM animal model. This experiment, we evaluate protective effects of DPP-4 inhibitor on TZD induced bone loss in T2DM animal model.

Materials and methods: 12 week old male Zucker Diabetic Fatty (ZDF) rats were randomized with control, vildagliptin, pioglitazone, vildagliptin and pioglitazone treated group and treated for 5 weeks. We performed IPGTT, ITT before and after treatment and measure bone mineral density (BMD) by DEXA and trabecular volume by micro CT at the end of treatment. Also, we assayed circulating levels of GLP-1, GIP, sclerostin, osteocalcin, and tartrate-resistant alkaline phosphatase (TRAP) 5b by ELISA.

Results: Pioglitazone treated group decreased blood glucose and increased body weight. Also, this group decreased BMD in DEXA and decreased trabecular bone volume and thickness in micro CT. Vildagliptin, DPP4 inhibitor, treatment increased BMD and trabecular bone volume and thickness compared with control. Also, combination treated group showed significant increase in BMD and trabecular thickness compared to pioglitazone group. Pioglitazone treatment increased sclerostin, but add vildagliptin to pioglitazone suppressed sclerostin.

Conclusion: Vildagliptin, DPP4 inhibitor, increased bone mass in type 2 diabetes animal model. Also, Vildagliptin can protect bone loss in TZD treated Type 2 diabetes through reducing sclerostin from osteocyte at least in T2DM animal model.



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OP 04 The heat is on

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Postprandial brown adipose tissue fatty acid metabolism in lean and obese subjects

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Background and aims: Brown adipose tissue (BAT) is activated by cold exposure, increasing its glucose uptake, blood flow and fatty acid uptake (FAU). In animals BAT has thermogenic activity also in pursuance of eating (meal-induced thermogenesis) but this is poorly studied in humans. As fatty acids are the main fuel source for BAT we aimed to quantify FAU in lean and obese subjects during cold exposure and after eating, in postprandial state.
Materials and methods: FAU was quantified in lean (n=11, 8 F/3 M, age 33.4±11.8 years, BMI 25.2±1.5 kg/m2) and obese subjects (n=6, 5 F/1 M, age: 41.5±11.0 years, BMI 31.5±2.7 kg/m2) using 18 F-FTHA, a palmitate analog and PET/CT. Tissue specific perfusion was measured with 15O-H2O-PET/CT. Each subject was imaged twice, once during cold exposure in fasting conditions and once in ambient temperature postprandially. Tissue specific NEFA uptake and perfusion was calculated for supraclavicular adipose deposits, deltoid muscles and subcutaneous white adipose tissue (WAT).
Results: In lean subjects cold-induced FAU in BAT was 3-fold higher than postprandial FAU (2.05±1.66 vs. 0.66±0.64 μmol/100 g/min, P=0.004). Cold stimulated FAU also in WAT 2-fold (0.42±0.12 vs. 0.19±0.13 μmol/100 g/min, P=0.003) and in muscle 3-fold (1.06±0.40 vs. 0.32±0.20, P=0.0005) when compared to postprandial FAU, however, these FAU rates were clearly lower than in BAT. In obese subjects, both cold-stimulated and postprandial FAU rates were lower than in lean subjects but FAU was higher in cold than after meal (1.19±0.60 vs. 0.38± 0.40 μmol/100 g/min, P=0.001, cold vs postprandial). Muscle FAU rate was similar in obese and lean subjects being 2-fold higher in cold than postprandial state (0.98±025 vs. 0.38±0.31 μmol/100 g/min, P=0.02) in obese subjects. Postprandial BAT perfusion was higher in lean than in obese subjects (20.00±16.74 vs. 4.85±1.63 ml/100 g/min, P=0.03). BAT FAU in cold correlated with postprandial BAT FAU (r=0.84, P=0.00002) but in WAT or muscle no such correlation was found. Serum FFA levels were similar in lean and obese subjects during cold exposure (0.89±0.18 vs 0.89±0.15 mmol/L) and after meal (0.29±0.22 vs 0.43±0.37 mmol/L).
Conclusion: Fatty acid uptake rate after meal in BAT is lower than during cold stimulus both in lean and obese subjects. However, obesity seems to be related with lowered FAU and perfusion in BAT. This data suggest that in addition to blunted cold-induced FAU obese subjects have impaired postprandial lipid metabolism in the supraclavicular area.
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Cold increases brown fat temperature and decreases fat content: measured using MRS in humans

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Background and aims: The role of brown adipose tissue (BAT) is to dissipate stored energy as heat. The aim of this study was to investigate whether the temperature and the proportion of fat (expressed as the proton density fat fraction (PDFF)) of brown and white adipose tissue (WAT) is related to BAT metabolic activity.
Materials and methods: Magnetic resonance spectroscopy (MRS) and 18 F-FDG PET in BAT and subcutaneous WAT were performed for 9 healthy subjects (4 F/5 M, ages 26 - 44 years, BMI 24.6±2.2 kg/m2) using Philips Ingenuity 3 T PET/MR. MRS was obtained with PRESS sequence (TR/TE=2000/35 ms, NSA=72, voxel volume: 1.0 cm3). The voxel was placed in the supraclavicular and subcutaneous adipose tissue. The measurements were obtained during 2-hour non-shivering cold exposure and in ambient room temperature (RT). The chemical shift difference between the CH2 peak of the lipid chain and H2O peak was determined with iNMR software (Mestrelab Research, 2014) and converted to temperature. The proton density fat fraction (PDFF) was calculated by dividing the sum of all LCModel fitted fat peaks by the sum of both water and fat peaks. Fat and water were corrected for T2 and T1 relaxation effects. The dynamic 18 F-FDG PET data was analyzed using graphical analyses with Carimas 2.8.
Results: All study subjects had functionally active BAT based on cold-induced glucose uptake (17.7±8.4 μmol/100 g/min). The PDFF of BAT in cold and RT were 68±12% and 80±14% (P=0.03), respectively, and in WAT 76±18% and 86±6% (NS). The temperature of BAT was higher during cold exposure (34.1±3.0°C in cold and 32.6±4.7°C in RT, P=0.03) while in WAT there was no difference in temperature between cold and RT (31.1±3.8°C and 29.8±2.8°C, NS). All subjects had normal plasma lipid profiles ((HDL 1.94±0.50, and LDL 2.23±0.66 mmol/l) and whole-body insulin sensitivity (M-value 44.4±10.5 μmol/kg/min. BAT temperature in RT correlated strongly with M-value (r=0.86, p=0.0027) and in cold BAT temperature was associated with BAT glucose uptake (r=0.69, p=0.041). In cold, PDFF of BAT correlated significantly with plasma LDL (r=0.76, p=0.016) and inversely with plasma HDL (r=-0.69, p=0.040).
Conclusion: The increment of BAT temperature during cold exposure can be measured with MRS during cold exposure in humans. BAT temperature in room temperature associates with M-value. Further, MRI-derived PDFF during cold associates with plasma lipid values. Our findings suggest that BAT has an important role in whole-body insulin sensitivity and lipid metabolism.
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Impact of caloric restriction on initial age-associated metabolic alterations and browning effect in mice

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Background and aims: Changes in the distribution and function of different deposits of white adipose tissue (WAT) and brown adipose tissue (BAT) occur during ageing. These changes are usually associated to metabolic alterations like Insulin Resistance (IR) and Metabolic Syndrome. It is also known that caloric restriction (CR) reduces the metabolic changes associated with age. Nevertheless, it is difficult to establish when age-associated alterations start. Similarly, the severity and the time-extent to CR are variables under debate. The aim of this work is to investigate the impact of the CR iniciated sind 3 months of age and maintained until 12 months of age, in the development of IR and other metabolic disorders.
Materials and methods: 3 and 12-month-old male mice fed ad libitum and 12-month-old mice under CR (20%) from 3 months of age were used (n=10-12 animals/group). For in vivo studies, we performed glucose (1 g/kg body weight) and insulin (0.75 U/kg body weight) tolerance test in mice. Serum concentration values of glucose, insulin and different cytokines were quantified by Bio-plex ProTM Diabetes Assays. Gen expression involved in lipid metabolism was measured in BAT and

epididimal (eWAT) and subcutaneous (scWAT) WAT. Immunohistochemistry and mRNA expression of UCP-1 was also detected. Total triiodothyronine (T3) and thyroxine (T4) concentrations were determined by radioimmunoassay (RIA) in serum and BAT. Protein levels of lipogenic enzymes in the Central Nervous System (CNS) were also measured.

Results: Our data showed age-associated IR significantly ($p<0.05$; t-Student) appeared at maturity and is prevented with CR (AUC mean +-SE: 1536.64+-115.94 in 12-month old mice compared to 1587+-151.23 in animals fed with CR). These findings were in agreement with serum concentration values quantified. Cytokines serum levels were measured. Adiponectin level significantly ($p<0.05$) decreased with ageing (5.57+-0.47 ug/ml) and increased with CR (13.62+-2.53 ug/ml), while the leptin serum levels significantly ($p<0.05$) increased with ageing (10.5+-3.095 ng/ml) but decreased with CR (2.61+-0.74 ng/ml). Furthermore, CR restored initial age-associated alterations in lipid metabolism and markers of macrophage infiltration, such as MCP-1 (2.00+-0.38 with ageing; 1.32+-0.16 with CR), only in scWAT. Remarkably, brown like adipocytes or browning effect was detected in scWAT in old animals subject to CR. The lower browning process associated to ageing was accompanied by a significant ($p<0.05$) decrease in the expression of some brown fat-selective genes (such as UCP-1, PRDM16, FGF21), but restored with CR. T3 and T4 levels in BAT were significantly ($p<0.05$) decreased with ageing but restored by CR. In addition, serum T3 levels were significantly ($p<0.05$) decreased with ageing. Finally, we also found significantly ($p<0.05$) changes in lipogenic enzymes such as ACC and AMPK in the hypothalamic AMPK pathway during the first stages of agein.

Conclusion: Long-term CR prevents the morphological and initial aged-related metabolic changes in WAT and BAT. The browning effect observed in scWAT and the activation of BAT could be explained by improved thyroid hormones status and function of CNS.

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Identification of compounds inducing UCP1 in differentiating human white preadipocytes

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Background and aims: While white adipose tissue is the major energy storage tissue in the body, brown adipose tissue dissipates energy in the form of heat, due to the unique presence of the mitochondrial uncoupler protein UCP1. Converting white into brown-like adipocytes, expressing high levels of UCP1 with increased oxidative and energy wasting capacity, is a promising therapeutic strategy to counter insulin resistance and type 2 diabetes. Our goal is to identify small molecules that increase UCP1 expression and function, and induce the "browning" of white fat.

Materials and methods: We developed a screening strategy using human adipose derived stem cells (hASCs) isolated from subcutaneous fat. hACSs were differentiated for up to 15 days in the presence or absence of compounds and UCP1 mRNA levels were measured. UCP1 qPCR assay was run in a 384 well format, and compounds were tested at 0.5 and 10 μM. We tested ~3000 small molecules covering more than a thousand targets.

Results: We identified small molecule compounds that robustly induce UCP1 mRNA in hASCs (3% hit rate). Top UCP1 inducers were confirmed by measuring both UCP1 protein and β-adrenergic/free fatty acid stimulated uncoupled respiration using the Seahorse XF Analyzer. High content imaging demonstrated that UCP1 protein is evenly distributed in the lipid droplet-containing adipocytes and co-localizes with mitochondria. In addition to UCP1, other brown fat genes such as Dio2, PGC1α and PRDM16 or beige fat markers (TMEM26), were also increased in treated cells, while white fat markers leptin and Hoxc8 were decreased, confirming the switch of white adipocytes to a brown-like phenotype. PPARγ agonists, such as rosiglitazone, are currently among the most potent inducers of a brown-like phenotype in cultured primary adipocytes. The metabolic modulators described here were increasing UCP1 protein levels to a greater extent than rosiglitazone and other PPAR agonists.

Conclusion: We have identified novel and potent UCP1 inducers using a qPCR based screening assay in human preadipocytes. The UCP1 increase was confirmed at the protein level, and drives uncoupled respiration after activation. Hence, the observed step jump in UCP1 expression level induced by these novel small molecules opens the prospect of bringing white precursor cells one step closer to the true brown adipocyte phenotype.

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The β3-adrenoceptor agonist CL316,243 increases in vivo free fatty acid uptake and utilisation in interscapular and perirenal brown fat but not inguinal fat in high-fat fed rats

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Background and aims: Activation of brown adipose tissue (BAT) and browning of white adipose tissue present potential new therapies for metabolic diseases such as Type 2 diabetes and obesity. Through the partitioning of substrates for mitochondrial uncoupling, energy is used for the production of heat rather than ATP. Here we investigate the effects of β3-adrenergic stimulation on glucose tolerance and whole-body metabolic parameters, and for the first time determine tissue-specific uptake and storage of free fatty acids (FFA) and its implications for whole-body FFA metabolism using a multi-radiotracer technique.

Materials and methods: For whole-body energy metabolism, male C57BL/6 mice were fed 60% (kcal) high fat diet for a total of 20 weeks and during the last 4 weeks administered β3-agonist CL316,243 (1 mg/kg/day) or saline via osmotic minipumps. After 14 days an oral glucose tolerance test was performed. After 21 days the mice were recorded in metabolic cages for 24 hours at ambient room temperature (22±0.2°C), and 24 hours at thermoneutrality (30±0.2°C). For the study of tissue-specific FFA metabolism, male Wistar rats were fed 45% (kcal) high fat diet for 12 weeks in total and during the last 3 weeks administered β3-agonist CL316,243 (1 mg/kg/day) or saline via osmotic minipumps. At the end of the study, the rats were fasted and acutely infused with a tracer mixture (^{14}C-palmitate and the partially-metabolised ^{3}H-R-bromopalmitate) under anaesthesia.

Results: In mice, CL316,243 (CL) infusion decreased body weight (BW change, CL=-1.80±0.63 g vs. saline 1.27±0.45 g, $p<0.01$, t-test), improved glucose tolerance (glucose AUC CL=1110.0±100.1 vs. saline 1459.0±106.9, $p<0.05$; insulin AUC CL=427.4±46.1 vs. saline=762.5±78.0, $p<0.01$, t-tests), and attenuated the reduction of oxygen consumption seen in the transition of environmental temperature from 22 to 30°C (thermoneutrality) seen in saline controls ($p<0.0001$ for infusion, two-way ANOVA). In the rats, CL infusion decreased body weight and fasting plasma glucose levels (BW change CL=17.3±7.3 vs. saline=34.9±4.3 g, $p<0.05$, t-test; glucose CL=4.53±0.09 vs. saline=5.13±0.20 mM, $p<0.05$), while there was no effect on food intake. Using infrared thermo-imaging, an increase in tail heat dissipation was seen in the CL-infused rats (CL=33.6±0.6 vs. saline=29.8±0.3°C, $p<0.01$, t-test). Whole-body FFA clearance and appearance were unchanged while CL administration markedly increased both FFA storage ($p<0.0001$, t-

test) and utilisation ($p<0.0001$, t-test) in the interscapular and perirenal BAT. Surprisingly, CL did not influence FFA uptake or utilisation in the inguinal fat depot.

Conclusion: In summary, β3-agonism improved glucose tolerance and increased oxygen consumption in mice. In rats, β3-agonism robustly increased FFA flux to BAT coupled with an enhanced utilisation, which was not the case in inguinal fat. Enhanced BAT activation was most likely driving the increased heat dissipation through the tail in order to maintain thermostasis. Our results emphasise the quantitative role of brown fat as the functional target of β3-agonism.

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Effect of metformin on glucose and fatty acid utilisation in brown adipose tissue

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Background and aims: Metformin is the first-line drug for the treatment of type 2 diabetes. Its main action is the inhibition of glucose synthesis and gluconeogenesis in the liver. In addition to its antihyperglycemic properties, growing evidence indicates that metformin also affects lipid metabolism and reduces body weight and abdominal fat mass. However, the mechanisms are still unclear. These effects might be mediated by lowered production of VLDL-TG in the liver or by increase in VLDL-TG clearance in peripheral tissues. Recently, brown adipose tissue (BAT) was identified as an important organ involved in clearing plasmatic VLDL-TG for thermoregulation and thus could prevent obesity, ectopic fat accumulation and associated metabolic disturbances. The aim of this study was to investigate if metformin increases glucose and fatty acid utilization in BAT, and ameliorate related parameters of metabolic syndrome.

Materials and methods: Male Wistar rats (age: 8 months) were fed a standard diet (SD) or SD enriched by metformin in the following dose: 300 mg/kg b.wt./day for 4 weeks. Plasma levels of selected parameters were measured using commercially available kits: triglycerides, insulin, high molecular weight (HMW) adiponectin and C-reactive protein (CRP). BAT activity was determined *ex vivo* by oxidation of ^{14}C-U-palmitic acid and ^{14}C-U-glucose into CO_2 in BAT and incorporation of ^{14}C-U-palmitic acid and ^{14}C-U-glucose into BAT lipids after 2-hour incubation at 37°C.

Results: Metformin treatment significantly decreased body weight from 527±20 g at the beginning to 431±16 g at the end of treatment ($p<0.001$), whereas body weight of controls was not significantly affected (559±16 vs. 575±22 g; N.S.). Compared to controls, metformin decreased the weight of visceral adipose tissue: epididymal adipose tissue (0.422±0.025 vs. 1.087±0.078 g/100 g b.wt; $p<0.002$) and perirenal adipose tissue (0.073±0.009 vs. 0.616±0.092 g/100 g b.wt; $p<0.01$). Plasma levels of triglycerides were decreased by metformin treatment (0.560±0.060 vs. 1.360±0.180 mmol/l; $p<0.01$). Metformin also reduced ectopic fat accumulation in selected tissues: the liver (2.718±0.201 vs. 9.594±1.564 μmol/g; $p<0.02$), heart (0.989±0.276 vs. 2.880 ±0.533 μmol/g; $p<0.03$), aorta (4.482±0.866 vs. 8.659±0.434 μmol/g; $p<0.01$) and diaphragm (3.551±1.100 vs. 17.360±2.769 μmol/g; $p<0.01$). Metformin increased palmitic acid oxidation in BAT (106±15 vs. 58±10 nmol palm./g/2 h; $p<0.03$) and palmitic acid incorporation into BAT lipids (2443±129 vs. 1797±111 nmol palm./g/2 h; $p<0.01$). Glucose incorporation into BAT lipids were higher after metformin treatment (basal: 1141±117 vs. 448±61 nmol gl./g/2 h; $p<0.01$ and insulin-stimulated: 4876±345 vs. 1945±583 mnol gl./g/2 h; $p<0.01$), whereas basal and insulin-stimulated glucose oxidation were not influenced. In addition, metformin also reduced plasma insulin levels (0.182±0.008 vs. 0.469±0.003 nmol/l; $p<0.001$), increased HMW adiponectin plasma levels (1.365±0.105 vs. 0.952±127 μg/ml; $p<0.04$) and decreased CRP plasma concentration (238±33 vs. 502±127 μg/ml; $p<0.015$).

Conclusion: Results indicate that metformin-induced increase of lipid and glucose utilization in BAT may participate in the mechanism of improving metabolic syndrome and related disorders.

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OP 05 Mechanisms of diabetic nephropathy

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The peripherally restricted CB1 receptor antagonist AM6545 reverts experimental diabetic nephropathy

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Background and aims: Diabetic nephropathy (DN) is characterised by increased glomerular permeability to proteins and excessive extracellular matrix accumulation in the mesangium. The endocannabinoid system has been implicated in the pathogenesis of DN as the cannabinoid receptor of type 1 (CB1R) is overexpressed by podocytes and CB1R blockade with AM6545 prevents the onset of both albuminuria and renal structural abnormalities in an animal model of streptozotocin-induced diabetes. AM6545, a peripherally restricted CB1R antagonist, is devoid of behavioural side effects as it does not across the blood-brain barrier and may thus represent a novel tool for DN treatment. Our aim was to perform a reversal study to establish if AM6545, either alone or in combination with a ACE-inhibitor, reverts charactheristic features of DN in diabetic mice with established proteinuria.
Materials and methods: Male C57Bl6 mice were made diabetic by intraperitoneal (i.p.) injection of streptozotocin (55 mg/kg in citrate buffer delivered in 5 consecutive days). Control mice (ND) were injected with citrate buffer alone. Eight weeks after diabetes onset, diabetic mice (DM) were further randomised to receive treatment with either vehicle (n=4), AM6545 (DM-A: 10 mg/kg i.p. daily; n=4), enalapril (DM-E: 2 mg/kg gavage daily; n=4) or both (DM-A+E, n=4). After six weeks of treatment, mice were individually placed in metabolic cages for urine collections and blood samples taken for blood glucose and glycated haemoglobin measurements. Then, mice were sacrificed, kidneys removed, weighed, and analysed. Urinary albumin excretion was measured by enzyme-linked immunosorbent assay. Expression of slit-diaphragm proteins (nephrin and podocin) was assessed by immunofluorescence. Markers of fibrosis (fibronectin, collagen I) were assessed by immunofluorescence and/or real-time PCR on total renal cortex.
Results: Diabetes was associated with reduced body weight and an increase in both blood glucose and glycated haemoglobin levels. Systolic blood pressure was unaltered by AM6545, but reduced by enalapril. Albuminuria was significantly ($p<0.001$) increased in the diabetic [DM:429.9 (382.7-513.7) μg/18 hrs, geometric mean (25th-75th percentile)] as compared to ND mice [ND:118.4 (110.7-124.6)] and ameliorated by treatment with either AM6545 [DM-A:242.6 (213.2-297.9); $p<0.05$ DM-AM6545 vs DM] or enalapril [DM-E: 235.1 (217.4-267.9); $p<0.05$ vs DM]. Combination therapy with AM6545 and enalapril further reduced albuminuria and the difference as compared to ND mice was no longer statistically significant [DM-A+E: 144.8 (112.3-204.5); $p<0.05$ DM-A+E vs DM-A and DM-E]. In DM mice the increase in albuminuria was paralleled a significant reduction in both podocin and nephrin expression. This was significantly reduced by treatment with either AM6545 or enalapril and completely prevented by the combination therapy. Diabetes-induced overexpression of fibronectin and collagen was abolished by single therapy and hence administration of both compounds did not result in further benefit.
Conclusion: Treatment with AM6545 ameliorated experimental DN and combination therapy with AM6545 and enalapril abolished diabetes-induced both albuminuria and slit diaphragm protein loss.
Supported by: EFSD/Sanofi

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Age-related carbonylation in glomeruli of diabetic mice influences anti-oxidative defense mechanisms

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Background and aims: Increased levels of reactive oxygen species (ROS) greatly contribute to the development of diabetic complications such as nephropathy. They induce carbonylation of proteins, which in high amounts impedes normal cell function. However, physiological amounts of ROS might have protective effects as they are involved in cellular signaling pathways. This study aims to analyze the influence of age-dependent protein carbonylation on the expression and activity of the cytosolic anti-oxidative selenoenzyme GPx in the context of diabetic nephropathy in mice.
Materials and methods: In addition to standard methods, dinitrophenylhydrazine (DNPH) derivatization has been used to detect carbonylated proteins in kidney homogenates and tissue sections of diabetic Ins2Akita mice of different ages and 18 week old db/db mice. GPx activity was determined using a luminescent activity assay.
Results: Protein carbonylation was found to be increased in the kidneys of both db/db and Ins2Akita mice compared to non-diabetic control mice. Carbonylation increased with age in Ins2Akita mice and carbonylated proteins co-localized with podocytes and mesangial cells in glomeruli. In line, GPx1 and GPx4 expression were found to be increased in those cell types in diabetic Ins2Akita mice compared to non-diabetic animals. In addition, GPx activity was augmented in kidneys of Ins2Akita mice, especially in young (1 month) diabetic animals that did not show a simultaneous upregulation of GPx on the protein level. In these young animals, immunoprecipitated GPx1 was found to be carbonylated. Using a recombinant GPx1 protein, an increased activity after mild in vitro carbonylation could be proved. In podocytes cultured under high glucose conditions (25 mM for 7 days), GPx protein expression was increased as well, whereas its activity was found to be decreased.
Conclusion: GPx carbonylation in the kidney is associated with an increase in its activity at early stages of diabetes whereas this effect was absent in animals with end-stage disease and cultured podocytes demonstrating the role of protein carbonylation in the mechanism of ROS signaling. These results indicate that there might be a threshold for beneficial carbonylation-dependent redox signaling during the progression of diabetic nephropathy.
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Loss of ALCAM/CD166 partially protects against diabetic nephropathy

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Background and aims: Activated leukocyte cell adhesion molecule (ALCAM/CD166) has been shown to function as a pattern recognition receptor, analogue to the receptor of advanced glycation endproducts, and has been implicated in the development and progression of diabetic nephropathy (DN). In this study the role of ALCAM in DN was investigated in patients with type 2 diabetes, suffering from DN, and further investigated in vitro and in vivo using homozygous knock-out mice for ALCAM (ALCAM-/-).
Materials and methods: Serum concentration of soluble ALCAM (sALCAM) and the expression of ALCAM and its ligand S100B in the kidney of patients with type 2 diabetes were determined by ELISA and histology, respectively. In vivo, diabetes was induced by low-dose streptozotocin in wild-type and ALCAM-/- mice. Diabetes was maintained for six months, after which time, the mice were assessed for symptoms of DN, as well expression of ALCAM. In vitro isolated tubular epithelial cells from wild-type and ALCAM-/- mice were studied.
Results: Serum concentration of sALCAM was significantly increased in patients with type 2 diabetes (59.85±14.99 ng/ml vs. 126.88± 66.45 ng/ml, $P<0.0001$), and correlated positively with HbA1c ($R=0.45$, $P<0.001$), as well as the extent of chronic kidney disease but negatively correlated with estimated GFR ($R=-0.3$, $P<0.01$). In the kidneys of patients with type 2 diabetes, the expression of ALCAM was significantly up-regulated in both the glomeruli and proximal and distal tubules. In contrast, the expression of S100B was significantly increased in the glomeruli, especially in the podocytes, but not in the tubules. In mice, the expression of ALCAM was also localized to the glomeruli and tubules. However, expression of S100B was only localized to the tubules. It was found that diabetic ALCAM-/- mice had no increase in albuminuria as well as no changes in either creatinine/body weight ratio or kidney-to-body weight ratio. Furthermore, glomerular and tubular damage were significantly lower than in the wild-type diabetic mice. In vitro it was found that S100B-induced expression of TGF-beta was dependent on ALCAM under diabetes conditions.
Conclusion: This study identifies ALCAM as a novel mediator in the late complications of diabetes in the kidney.
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Soluble Nogo-B ameliorate angiogenesis in vitro in an experimental model of diabetic nephropathy

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Background and aims: We recently explored the role of neurite outgrowth inhibitor (Nogo)-B, in the pathophysiology of diabetic nephropathy (DN). Nogo-B and its soluble form (sNogo-B) have been implicated in the regulation of cell survival, cell migration and vascular remodelling - known to be altered in diabetic glomerulopathy. Both Nogo-B (at the cellular level) and sNogo-B (systemically) bind to NgBR (expressed mainly in endothelial cells), and promote vascular remodelling. In earlier work we have demonstrated that Nogo-B and NgBR are expressed in glomeruli and that Nogo-B is downregulated in glomeruli of experimental animal model of diabetes. The role of Nogo-B and sNogo-B in diabetic glomerulopathy is unknown, therefore our aims were to investigate: i) the role of high glucose and VEGF-A on Nogo-B and sNogo-B expression in human glomerular endothelial cells (GECs) in vitro; ii) in vitro, whether sera obtained from patients with type-1 diabetes and DN (T1DM/DN+) is paralleled by altered angiogenesis when compared to sera from T1DM without DN (T1DM/DN-); iii) whether overexpression of sNogo-B could modulate angiogenesis.
Materials and methods: Conditionally immortalised GECs were incubated with high (HG: 25 mM) or normal (NG: 5 mM+20 mM mannitol) glucose, with or without VEGF-A (50 ng/ml), for 72 h. Serum was obtained from patients with T1DM/DN+ ($n=18$) or T1DM/DN- ($n=17$); the two groups had similar age, sex, HbA1c, duration of diabetes, BP, lipid profile, renal function, urine albumin/creatinine ratio, sNogo-B serum levels and only differed in that the DN+ had history of albuminuria and were all treated with inhibitors of the renin-angiotensin-system. Angiogenesis assay with human umbilical vein endothelial cells (HUVEC) was conducted in the presence of the patient sera (4%vol/vol) on Matrigel and tube formation assessed after 24 h under light microscopy and quantified with image analysis. sNogo-B overexpression was achieved by transfection with adenoviral vector. ELISA was utilised to quantify sNogo-B levels in cell supernatant and patients' sera.
Results: HG and VEGF-A resulted in Nogo-B downregulation in GEC ($p<0.05$), no additive effect was seen with their combination. The downregulation of full length Nogo-B was always paralleled by an increase of sNogo-B in the cells supernatant ($p<0.05$). HUVEC cultured in T1DM/DN+ sera displayed a 50% reduction in tube formation (total loop number), when compared to cells cultured in T1DM/DN- sera ($p<0.05$). HUVEC transfection with sNogo-B vector achieved a 4-5 folds increase in sNogo-B in the cell supernatant when compared to control vector ($p<0.001$). Overexpression of sNogo-B improved the impaired tube formation observed in cells incubated with T1DM/DN+ sera ($p<0.05$). No significant effect of the overexpression was seen within the group incubated with T1DM/DN- sera.
Conclusion: High glucose and VEGF-A downregulate Nogo-B, an event paralleled by upregulation of sNogo-B. Overexpression of sNogo-B improves the impaired angiogenesis seen in cells incubated with sera from patients with T1DM/DN+. Circulating sNogo levels may influence the progression of kidney disease in diabetes and future work will address this question in experimental model of diabetes and in the clinical setting.
Supported by: Heart Research UK

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The renal protective effects of induction of heme oxygenase-1 combined with increased adiponectin on the glomerular VEGF-NO axis in obese rats

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Background and aims: The uncoupling of glomerular vascular endothelial growth factor (VEGF) - nitric oxide (NO) axis is an important mechanism of obesity-related nephropathy. The beneficial effects of heme oxygenase-1(HO-1) induction in metabolic disease are mediated via its effects on adiponectin-dependent pathways, which is called the HO-1/adiponectin axis This study aimed to investigate whether induction of

heme oxygenase-1 (HO-1) can reduce microalbuminuria and provide renal protective effects by improving endothelial dysfunction and uncoupling of glomerular VEGF-NO axis through increasing the adiponectin levels in obese rats.

Materials and methods: Rats received high-fat diets and were injected with either cobalt protoporphyrin (CoPP) to induce HO-1, or stannous protoporphyrin to inhibit HO-1. Blood and urine samples were collected. Endothelial function was determined by measuring endothelium-dependent vasodilatation of the aorta. Renal tissues were collected for CD34 immunohistochemistry. Glomerular NO levels were measured by the Griess reaction. Glomerular AMPK, Akt and eNOS protein were measured by western blot.

Results: Obese rats exhibited a significant increase in body weight, visceral fat, albumin-to-creatinine, plasma FFA levels, and serum levels of triglyceride, hs-CRP, TNF-α and malondialdehyde when compared with the control rats ($P<0.01$). Induction of HO-1 by CoPP resulted in a significant decrease in the above index in obese rats when compared with untreated obese rats (body weight, 579.7±66.5 g vs 702.3±94.4 g; visceral fat, 24.8±10.3 g vs 54.90±14.18 g; albumin-to-creatinine, 49.25±14.93 mg/g vs 94.1±16.27 mg/g; FFA, 0.74±0.31 mmol/L vs 1.29±0.36 mmol/L; hs-CRP, 1.04±0.41 mg/L vs 2.28±0.61 mg/L; TNF-α, 56.8±8.9 pg/ml vs 92.5±0.2 pg/ml; malondialdehyde, 2.28±0.51 umol/L vs 3.92±0.51 umol/L, $P<0.01$). Serum adiponectin levels were reduced in obese rats compared with control rats(12.77±2.77 mg/g vs 20.34±2.76 mg/g, $P<0.01$), which increased after induction of HO-1 (19.35±2.67 mg/g vs 12.77±2.77 mg/g, $P<0.01$). Severe endothelium-dependent vasodilatation impairment was observed in the obese rats, which was partially improved by HO-1 induction. CD34 expression in the glomeruli was also enhanced in obese rats, indicating increased proliferation of glomerular endothelial cells [mean optical density (IOD/area): 0.123±0.015 vs. 0.094±0.010, $P<0.05$]. However, Induction of HO-1 improved the increased proliferation of endothelial cells in glomeruli in obese rats (IOD/area: 0.101±0.009 vs. 0.123±0.015, $P<0.05$). Obese rats showed increased glomerular VEGF expression and reduced NO levels ($P<0.05$). This uncoupling of the VEGF-NO axis was partially improved by induction of HO-1, with enhancement of phosphorylation of glomerular AMPK, AKT and eNOS in obese rats ($P<0.05$)

Conclusion: Induction of HO-1 with CoPP, paralleled by an increase in serum adiponectin levels, reduces the degree of microalbuminuria and has renal protective effects by improving endothelial dysfunction and regulating the uncoupled glomerular VEGF-NO axis in diet-induced obese rats. The mechanism may be related to increased activation of the HO-1/adiponectin axis.

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Cellular hypoxia and mitochondrial reactive oxygen species may promote hyperglycaemic damage in a coordinated manner

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Background and aims: We previously proposed that hyperglycemia-induced mitochondrial reactive oxygen species (mtROS) production is a key event in the development of diabetic complications. However, "legacy effect" and "metabolic memory", which are reported in DCCT/EDIC and UKPDS, respectively, cannot be explained by the theory. Interestingly, there are some common points between hyperglycemia and hypoxia-induced phenomena such as apoptosis and fibronectin production. Therefore, we suspect that hyperglycemia may induce cellular hypoxia, which can produce mtROS without hyperglycemia.

Materials and methods: Bovine aortic endothelial cells (BAEC), C57BL6 mice, and eMnSOD-Tg mice (which express manganese superoxide dismutase (MnSOD) specifically in vascular endothelial cells) were used for analysis. Cellular hypoxia was evaluated by hypoxia-sensing probes, pimonitazol and LOX-1. mtROS was measured by the reduced MitoTracker Red probe. Apoptosis was evaluated by TUNEL assay. Fibronectin mRNA was measured by quantitative RT-PCR.

Results: 1) In BAEC, cellular hypoxia evaluated by pimonitazol and LOX-1 increased 1.9 and 2.0 times, respectively, after 3 hours incubation with hyperglycemic condition (25 mM glucose) compared to that with normoglycemic condition (5.5 mM glucose), and this phenomenon was also observed after 24, 72 or 168 hours incubation with hyperglycemic condition. Hypoxia was also observed in the glomeruli of STZ-induced diabetic mice compared to those of control non-diabetic mice. 2) In BAEC, mtROS production was increased in both hyperglycemic and hypoxic condition (1, 3, 5 or 10% oxygen). The hypoxia-induced mtROS production was reduced by Bis-2-(5-phenylacetamido-1, 3, 4-thiadiazol-2-yl) ethyl sulfide (BPTES), which can inhibit glutamine incorporation into mitochondria, whereas hyperglycemia-induced mtROS production was not reduced by BPTES. 3) Rotenone and antimycin, both of which can reduce oxygen consumption in mitochondria, ameliorated hyperglycemia-induced cellular hypoxia. Interestingly, the hyperglycemia-induced cellular hypoxia was also reduced by the overexpression of MnSOD in BAEC and in eMnSOD-Tg mice. 4) The overexpression of aquaporin-1, which is a water and gas channel, ameliorated hyperglycemia-induced cellular hypoxia, apoptosis and fibronectin mRNA induction in BAEC.

Conclusion: The findings of this study demonstrate that hyperglycemia can induce cellular hypoxia, and the hypoxia can increase mtROS production without hyperglycemia. In addition, increased oxygen consumption and overproduction of mtROS are involved in hyperglycemia-induced cellular hypoxia. Ameliorating cellular hypoxia by the overexpression of aquaporin-1 can inhibit apoptosis and fibronectin production by hyperglycemia. Therefore, hyperglycemia-induced mtROS production and cellular hypoxia may develop diabetic complications in a coordinated manner, and these phenomenna may be one of the mechanisms of "legacy effect" and "metabolic memory".

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OP 06 Is muscle still the heart of diabetes?

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Weight loss improves impaired muscle capillary recruitment in overweight/obese men: effects on metabolic insulin resistance

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Background and aims: Overweight and obesity are associated with metabolic insulin resistance, and an increased risk of type 2 diabetes (T2DM) and cardiovascular diseases (CVD). In skeletal muscle, insulin normally increases the number of perfused capillaries, enhancing its own delivery and that of glucose to myocytes. Impairments in insulin-induced muscle capillary recruitment may be responsible for the metabolic insulin resistance in obese individuals. It is, however, unknown whether weight loss can restore capillary recruitment and whether this improves metabolic insulin sensitivity. Therefore, we compared healthy lean men to overweight/obese men and subsequently studied the effect of weight loss in the latter.
Materials and methods: In a randomized controlled trial with blinded analyses, 53 non-smoking, overweight/obese men (waist circumference 102-110 cm; aged 18-65; no CVD or T2DM) underwent either an 8-week weight loss program (6 weeks low calorie diet (LCD), 2 weeks weight stable) or maintained their usual diet for 8 weeks and were compared to 25 lean men (waist circumference <94 cm). During a 1 mU/kg/min euglycaemic insulin clamp, we estimated metabolic insulin sensitivity from the glucose infusion rate (GIR) and forearm muscle capillary recruitment as the change in microvascular blood volume (ΔMBV) using contrast-enhanced ultrasound. Overweight/obese men were studied at baseline and after completion of their 8-week program, whereas lean men were studied at baseline only.
Results: All 25 lean men and 50 overweight/obese men completed the study. Baseline BMI was 23.3±1.8 kg/m2 in lean men, 29.9±2.5 kg/m2 in the usual-diet group, and 30.0±1.7 kg/m2 in the LCD group. LCD reduced BMI by 3.0±0.8 kg/m2 ($p<0.001$), whereas BMI in the usual-diet group did not change (+0.1±0.4 kg/m2). Insulin-induced ΔMBV at baseline was 44.4±41.2% in lean men; in both the usual-diet (0.7±27.6%) and the LCD group (-5.6±26.2%) capillary recruitment was blunted ($p<0.001$). LCD was able to restore part of this response, to 33.4±39.6% ($p<0.001$), whereas ΔMBV remained unchanged in the usual-diet group (0.4±25.4%). Baseline metabolic insulin sensitivity was 6.76±1.79 mg/kg/min in lean men, 4.03±1.39 mg/kg/min in the usual-diet group, and 4.06±1.28 mg/kg/min in the LCD group. LCD increased insulin sensitivity by 1.31±1.19 mg/kg/min ($p<0.001$), whereas insulin sensitivity remained unaltered in the usual-diet group. At baseline, ΔMBV was associated with both BMI ($r=-0.414$; $p<0.001$) and GIR ($r=0.577$; $p<0.001$). Moreover, changes in ΔMBV were accompanied by changes in GIR ($r=0.499$; $p<0.001$).
Conclusion: We conclude that both insulin-induced muscle capillary recruitment and metabolic insulin sensitivity are impaired in overweight/obese men. LCD-induced weight loss was associated with improved insulin-induced muscle capillary recruitment and insulin sensitivity. Furthermore, these changes were related to one another. These findings support the hypotheses that impaired vascular actions of insulin contribute to metabolic insulin resistance in overweight/obese individuals and that weight loss improves both.
Clinical Trial Registration Number: NCT01675401

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Impaired metabolism and transcriptional profile in cultured muscle stem cells from individuals born with low birth weight

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Background and aims: Individuals born with low birth weight (LBW) have an increased risk of developing type 2 diabetes (T2D) later in life. We hypothesized that immature muscle stem cell functions including abnormal differentiation potential and metabolic function could link LBW with risk of developing T2D.
Materials and methods: We recruited 23 young men with LBW (mean birth weight 2.7±0.2 kg) and 16 age-matched control subjects (mean birth weight 3.7±0.2 kg) with normal birth weight. Biopsies were obtained from vastus lateralis and muscle stem cells were isolated and cultured into fully differentiated myotubes. We studied glucose uptake, insulin signaling, myokine secretion, selected site specific DNA methylation and key transcriptional markers of cell maturity as well as mitochondrial gene expression throughout cell differentiation.
Results: In the cultured LBW cells, we found significantly reduced glucose uptake, decreased glucose transporter-4 gene expression and decreased levels of Akt substrate of 160 kDa compared to NBW cells. Additionally, interleukine-6 release was increased during myoblast differentiation and the expression levels of a selected set of mitochondrial OXPHOS genes including the peroxisome proliferator-activated receptor-γ coactivator-1α (PGC-1α), as well as the sarcomere gene myosin heavy chain 2, were down regulated in LBW cells. Decreased gene expression was not explained by changes in DNA methylation levels in gene promoter regions.
Conclusion: Our findings of transcriptional and metabolic changes in cultured muscle stem cells from LBW subjects support the idea of altered muscle stem cells function potentially contributing to increased risk of developing T2D in these subjects.
Clinical Trial Registration Number: H-A-2009-040 and H-D-2008-127
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Maternal obesity and telomere length associate with skeletal muscle insulin resistance which is reversed by exercise training in elderly womenM. Bucci

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Background and aims: Maternal obesity during pregnancy predisposes for subsequent morbidities in the offspring, including type 2 diabetes. T2DM and insulin resistance have been associated with shortening of leucocyte telomere length (LTL). Effects of resistance training (RT) on whole body and skeletal muscle glucose disposal are not known in elderly. The aims of this study were to test insulin sensitivity in elderly frail

women with maternal obesity and test the effects of RT on muscle insulin sensitivity and masses.
Materials and methods: We recruited 48 women with the mean age of 72 years, of which 11 were non-frail controls (CTR), 20 frail offspring of lean/normal weight mothers (OLM) (BMI≤26.3 kg/m^2) and 17 frail offspring of obese mothers (OOM) (BMI≥28.1 kg/m^2). The frail women were studied before and after a 4 mo RT intervention, and controls once using FDG-PET during hyperinsulinemic euglycaemic clamp and MRI for the measurement of skeletal muscle mass. Glucose uptake (GU/kg) and accounting for masses (GU(depot)) were calculated for the skeletal muscles of the thigh. LTL was assessed from peripheral blood mononuclear cells.
Results: The OOM group had a lower thigh muscle insulin sensitivity compared to the OLM group (p=0.048). Significant positive correlations were found in the OOM group between LTL and thigh muscle GU(/kg and depot) (R≥0.53, p≤0.05). Training increased whole body (p=0.004) and skeletal muscle insulin sensitivity both per volume and per total muscle mass (p=0.013 and p=0.004, respectively). Quadriceps muscle mass increased by intervention in both groups (p=0.008 and p=0.001, OLM and OOM respectively).
Conclusion: Maternal obesity during pregnancy and telemere length are associated with insulin resistance in the adult offspring. RT helps to reverse the insulin resistance in elderly women with maternal obesity. RT is equally effective in increasing muscle mass in elderly women regardless of their maternal BMI.
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Impaired PI 3-kinase signalling in a mouse model of SHORT syndrome

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Background and aims: The phosphatidylinositol 3-kinase (PI3K) pathway regulates a range of cellular functions involving glucose metabolism, cell growth, differentiation and apoptosis. A key factor regulating this pathway is the p85α regulatory subunit of PI3K, encoded by the PIK3R1 gene. We recently identified a heterozygous missense mutation in PIK3R1 resulting in an Arg649Trp substitution in patients with SHORT syndrome, characterized by short stature, partial lipodystrophy and insulin resistance. To investigate whether the Arg649Trp mutation alone is sufficient to replicate characteristic features of SHORT syndrome in vivo and to explore the function of the mutant protein at a mechanistic level, we generated knock-in mice harboring this mutation.
Materials and methods: Body weight was assessed longitudinally at the indicated time-points. Body composition, length, blood glucose, serum insulin insulin- and glucose tolerance tests were performed on animals at the indicated ages. Insulin signaling was assessed in adipose tissue, liver and skeletal muscle, snap frozen ten minutes following intra venous administration of vehicle or insulin. Western blotting was performed on whole cell lysates isolated from brown preadipocyte cell lines or primary hepatocytes.
Results: Heterozygous knock-in mice demonstrated multiple features of SHORT syndrome including a reduction in body weight and length, a Rieger like abnormality in the iris and a reduction in subcutaneous adipose tissue. By 8 weeks, knock-in mice were hyperglycemic in the fed state compared to controls (17.7±1.4 vs. 11.8±0.9 mmol/l). An assessment of insulin levels at the same age revealed marked hyperinsulinemia in mutant animals (fed insulin 107.9±12.9 vs. 8.9±1.2 pmol/l). Mutant mice also demonstrated a clear impairment in glucose tolerance and a reduced response to exogenous insulin. At a molecular level, this was caused by reduced activation of the PI 3-kinase pathway in adipose tissue and liver in response to insulin assessed by the phosphorylation of AKT. Brown preadipocyte cell lines and primary hepatocytes from control and heterozygous mutant mice showed a distinct reduction in insulin-dependent phosphorylation of AKT. Primary hepatocytes demonstrated a reduction in phosphorylation of AKT upon stimulation with additional growth factors including IGF-1 and EGF, suggesting resistance to multiple growth factors. The reduced capacity of the mutant protein to link the upstream activation of the insulin signaling pathway with the downstream activation of AKT is due to a failure of the mutant protein to bind to IRS-1 following insulin stimulation.
Conclusion: Mice heterozygous for the Arg649Trp mutant allele phenocopy multiple aspects of the human SHORT syndrome, including a reduction in weight and length, partial lipodystrophy associated with a selective reduction in subcutaneous adipose tissue and insulin resistance. At a mechanistic level, we have demonstrated that the mutant protein fails to interact with IRS-1 in response to insulin, leading to impaired activation of AKT.
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HIF-1alpha transcription factor: a molecular link between exercise and insulin action

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Background and aims: Skeletal muscle insulin resistance is the hallmark of type 2 diabetes, and develops long before the onset of the disease. It is well accepted that regular physical activity improves glycemic control but the knowledge on underlying mechanisms mediating the protective effects remains incomplete. Exercise is accompanied by a reduction in intra-muscular oxygen tension resulting in enhanced HIF-1α protein expression. HIF-1α is a master regulator of gene expression and might play an important role in skeletal muscle function and metabolism. Thus, we examined the role of oxygen tension in insulin- and contraction-stimulated glucose metabolism in primary human skeletal muscle cells (HSkMC). Furthermore, we analyzed the impact of acute and long-term exercise on skeletal muscle HIF-1α expression in healthy control and dysglycemic subjects.
Materials and methods: HSkMC were in vitro differentiated and electrical pulse stimulation (EPS) was applied at different oxygen tensions. Insulin action was measured by the phosphorylation of kinases of the insulin signaling pathway and glucose uptake. Sedentary individuals for the human study were divided in two groups; controls (n=13) with normal glucose metabolism and dysglycemic subjects (pT2D; n=11). All participants were subjected to a training program for 12 weeks. An acute exercise session was performed before and after the 12 weeks of training intervention. Muscle biopsies were taken before, directly after, and 2 h after the acute exercise before as well as after 12 weeks of training. Whole body insulin sensitivity was assessed by euglycemic-hyperinsulinemic clamp.
Results: Reduced O2 tension in combination with EPS strongly enhanced the insulin action at the level of Akt and AS160 phosphorylation as well as glucose uptake. Furthermore, the combination of 7% O2 and EPS resulted in an enhanced IL-6 production and secretion as well as HIF-1α and GLUT4 protein expression. In contrast, knockdown of HIF-1α totally inhibits the insulin- and contraction-induced glucose uptake as well as insulin-stimulated signaling. Furthermore, GLUT4 and GLUT1 protein expression were decreased. In addition, human skeletal muscle HIF-1α mRNA expression was enhanced after acute and long-term exercise, and positively correlated with glucose infusion rate (p=

0.03; r=0.47) and negatively with fasting glucose (p=0.02; r=-0.49). Interestingly, the induction of exercise-mediated HIF-1α mRNA expression was reduced in the pT2D group.
Conclusion: We have demonstrated that the reduction of oxygen to 7% and the combination with muscle contraction improved insulin action in primary human skeletal muscle cells. Furthermore, we showed that HIF-1α is an important determinant in insulin- and contraction-induced glucose uptake. Muscle HIF-1α mRNA expression was up-regulated by acute and long-term exercise, and positively correlated with whole body insulin sensitivity in subjects with normal glucose metabolism. Thus, we suggest that muscle HIF-1α is a key regulator of insulin- and exercise-stimulated glucose metabolism in human skeletal muscle.

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The influence of hyperinsulinaemia and circulating NEFA elevation on sirtuin 1 expression in human skeletal muscle
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Background and aims: Sirtuin 1 (SIRT1), belonging to the family of sirtuins (Sir2, silent information regulator 2 protein) is involved in the regulation of glucose and lipid metabolism. SIRT1 has been proposed to be an important regulator of insulin sensitivity. SIRT1 stimulates a glucose-dependent insulin secretion from pancreatic β cells and may influence directly or indirectly on insulin signaling pathway in insulin sensitive cells. Furthermore, SIRT1 in interaction with PGC1α could increase fatty acid oxidation and glucose uptake in muscle, which also can prevent insulin resistance. The aim of our study was to estimate the relationship between muscle SIRT1 and insulin signaling and the effects of hyperinsulinemia and NEFA elevation on skeletal muscle SIRT1 expression in humans.
Materials and methods: We examined 108 apparently healthy men with normal glucose tolerance (age: 23.30±2.7 years; BMI: 25.7± 4.0 kg/m2). In all subjects insulin sensitivity was evaluated by the 2 h euglycemic-hyperinsulinemic clamp. Additionally, in 20 subjects, clamp was prolonged to 6 hours. After one week, another 6 h clamp combined with Intralipid/heparin infusion was performed in 20 volunteers. In all subjects before the clamp muscle biopsy was performed. In 20 individuals with prolonged clamps, muscle biopsy were taken before and after 6 h clamp with and without Intralipid/heparin infusion. Tissue mRNA expression of SIRT1 and IRS1, IRS2, PIK3R1, PIK3CA, Akt2, SLC2A4, PGC1α was analyzed with Real Time PCR.
Results: Intralipid/heparin infusion caused 4-fold concentration NEFA in serum (p<0.001). Insulin sensitivity was decreased by about 40% after 6 h Intralipid/heparin infusion(p<0.001). Hyperinsulinemia increased muscle SIRT1 expression (p=0.016), whereas in the presence of Intralipid/heparin infusion this stimulatory effect of hyperinsulinemia was abolished. Hyperinsulinemia resulted in a significant increase in the expression of PIK3R1 (p<0.001), Akt2 (p=0.007), SLC2A4 (p=0.02) and PGC1α (p<0.001). Intralipid/heparin infusion abolished most of these changes. We observed a positive correlation between the expression of SIRT1 and expression of IRS2 (r=0.31, p<0.001), PIK3R1 (r=0.26, p=0.007), PIK3CA (r=0.26, p=0.008), Akt2 (r=0.45, p<0.001), SLC2A4 (r=0.24; p=0.011) and PGC1α (r=0.21; p=0.03) in the baseline state. Increase in muscle SIRT1 expression in response to hyperinsulinemia was related to the concurrent change in PGC1α (r= 0.46, p=0.049) and SLC2A4 (r=0.52, p=0.02).
Conclusion: Our results indicate that insulin increases muscle SIRT1 expression and this effect is abolished by circulating NFEA elevation. Thus, muscle SIRT1 may be involved in lipid- induced insulin resistance.
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OP 07 A glimpse at future diabetes therapy

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Pharmacokinetics, pharmacodynamics and tolerability of a novel glucokinase activator TMG-123, after single oral ascending doses in Japanse healthy subjects
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Background and aims: TMG-123 is a novel glucokinase activator with a postulated liver dominant mechanism of action and with the potential to deliver effective glucose-lowing in Type 2 diabetes mellitus. Two Phase I studies were conducted to evaluate the pharmacokinetics (PK), pharmacodynamics (PD) and tolerability of TMG-123 in Japanese healthy male subjects after single oral ascending doses.
Materials and methods: Two, double-blinded, randomized, placebo-controlled studies were conducted. Gradually increasing single oral doses of 1 mg up to160 mg TMG-123 or placebo were administered during fasting conditions. Each subject participated once, either on TMG-123 or on placebo, with the exception of one dose group in the second study. Subjects in this group participated on 2 occasions, once on TMG-123 / placebo given in fasting conditions and once on TMG-123 / placebo given in combination with a standardized breakfast. Eight dose levels were evaluated in the first study and four in the second study. The PK and PD variables were calculated by non-compartmental analyses.
Results: In total, 96 Japanese healthy male volunteers aged from 20 to 34 years were participated, and from 1 mg to 160 mg of TMG-123 or placebo were administered. TMG-123 was well tolerated up to 160 mg dose without serious adverse event or hypoglycemia, and most adverse events were mild. No clinically significant changes in ECG, vital signs, or safety laboratory assessments were observed. Following TMG-123 dosing in fasting conditions, TMG-123 was rapidly absorbed at all dose levels studied. The maximum concentration of TMG-123 in plasma (C_{max}) was generally reached within 1 hour after dosing. In the first study, C_{max} and AUC were increasing in dose up to 40 mg, whereas C_{max} / AUC were increasing in dose up to 80 / 160 mg in the second study. TMG-123 dosing in 30 minutes after the standardized breakfast had no effect on C_{max}, while AUC was slightly increased and the time reached C_{max} was delayed compared to fasting conditions. The plasma TMG-123 concentration dependent serum glucose decrease was observed up to around 200 ng/mL of plasma TMG-123 level, whereas plasma TMG-123 level was reached around 500 ng/mL in the studies. A serum glucose lowering effect was maintained during a fasting period. A serum insulin level was transiently increased at 0.5 hours post-dose to around 15 μU/mL over 80 mg dosing. No dose dependent serum glucagon changes were observed.
Conclusion: TMG-123 was well tolerated in single doses up to 160 mg, and no safety concerns were raised. TMG-123 was rapidly absorbed in fasting conditions and no significant food effect on C_{max} and AUC were observed. The PK / PD result suggests a liver dominant glucokinase activation of TMG-123 in humans.
Supported by: Teijin Pharma Limited

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ISIS PTP-1BRx, a novel PTP-1B antisense inhibitor, improves HbA_{1c} and body weight in patients with type 2 diabetes on metformin± sulphonylurea
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Background and aims: Protein-tyrosine phosphatase 1B (PTP-1B) is a negative modulator of insulin and leptin signaling. Inhibition of PTP-1B

action could address both diabetes and obesity, making it an attractive target for drug development. This study evaluated the safety, tolerability and efficacy of 6-month treatment with a PTP-1B antisense inhibitor (ISIS PTP-1B$_{Rx}$) in a double-blind placebo-controlled, Phase 2 study in overweight patients with Type 2 diabetes inadequately controlled on metformin with or without sulphonylurea (SU).

Materials and methods: Patients, mean age 57, 63% female, 53% Black, with a hemoglobin A1c ≥7.5% and a mean BMI of 34 kg/m^2 were randomized to ISIS PTP-1B$_{Rx}$ 200 mg or placebo administered as once weekly subcutaneous injections for 26 weeks. Patients had to be on a stable dose of metformin (≥1000 mg/day) alone or in combination with SU (≥10 mg/day). Seventy- five patients completed treatment and 69 were evaluated for the primary endpoint based on protocol specified criteria.

Results: Data are Mean ± SD; #Mean Change (Δ) from Baseline to Week 36 ISIS-PTP-1B$_{Rx}$ was well-tolerated and did not cause severe hypoglycemia, gastrointestinal side effects or clinically significant changes in hepatic or renal function.

Conclusion: In this study, ISIS PTP-1B$_{Rx}$ treatment was generally safe and well tolerated and resulted in significant reductions in HbA1c and body weight that continued after cessation of treatment. ISIS PTP-1B$_{Rx}$ showed an attractive profile that includes improved glycemic control without severe hypoglycemia and with the additive benefit of weight loss, supporting future development in patients with Type 2 diabetes.

Treatment Group	N	HbA1c (%)	Fructosamine (µmol/L)	BW (kg)	Leptin (ng/mL)	Total Adiponectin (µg/mL)
Placebo (Baseline) #Mean Δ	23	8.4±0.9 -0.18	315±55.7 -3.7	96.0±17.5 -1.4	14.1±9.4 1.8	4.7±2.4 -0.09
ISIS PTP-1B$_{Rx}$ 200mg (Baseline) #Mean Δ	46	8.8±1.0 -0.69	320±50.0 -33.2	91.5±18.7 -2.7	16.5±10.8 -2.5	5.5±3.5 0.64
p-value (ISIS PTP-1B$_{Rx}$ vs placebo)		0.034	0.005	0.012	0.089	0.037

Clinical Trial Registration Number: NCT01918865

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Improved postprandial glycaemic control with faster-acting insulin aspart in individuals with type 1 diabetes using CSII

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Background and aims: This double-blind, randomised, crossover, active-controlled trial compared 14 days of continuous subcutaneous insulin infusion (CSII) of two formulations of faster-acting insulin aspart (faster aspart) with CSII of insulin aspart (IAsp) in 43 adults with type 1 diabetes. Only data for the faster aspart formulation undergoing further development are presented.

Materials and methods: The primary endpoint was the mean change in postprandial glucose response 2 h after a standardised meal test (individualised insulin dosing by bolus calculator), as evaluated by mean change in plasma glucose ($\Delta PG_{av,0-2h}$). Subjects had blinded continuous glucose monitoring (CGM) during the trial.

Results: Faster aspart provided statistically significant lower $\Delta PG_{av,0-2h}$, compared with IAsp (3.03 vs 4.02 mmol/L; mean difference [95% CI]: -0.99 [-1.95; -0.03]; Table). The greater glucose-lowering effect demonstrated at meals with faster aspart vs IAsp was confirmed by interstitial glucose (IG) profiles, with the largest differences at breakfast (Table). The duration of low IG (≤3.9 mmol/L per 24 h) was significantly longer for IAsp (2.45 h) than faster aspart (2.03 h; mean difference [95% CI]: -0.42 [-0.72; -0.11]. No new safety findings were observed with faster aspart compared with IAsp.

Conclusion: In summary, faster aspart had significantly greater glucose-lowering effect than IAsp after a standardised meal, with findings confirmed by CGM for all meals, and less time spent with low glucose levels, as measured by IG.

Table: Glucose-lowering effect of faster aspart vs IAsp

	Faster aspart: LSMean (mmol/L) n=43	IAsp: LSMean (mmol/L) n=42‡‡	Mean treatment difference (mmol/L) [95% CI]
ΔPG_{av}			
0–2 h†	3.03¶	4.02¶	–0.99* [–1.95; –0.03]
0–1 h‡	1.89¶	2.39¶	–0.50 [–1.07; 0.07]
0–4 h‡	3.59¶	4.32¶	–0.73 [–2.00; 0.54]
IG increment			
All meals, 60 min	0.63	1.29	–0.66*** [–0.95; –0.37]
after§	0.50	1.07	
All meals, 120 min	1.12	2.04	–0.58** [–0.97; –0.19]
after§	0.67	1.79	
Breakfast, 60 min			–0.92*** [–1.45; –0.38]
after§			
Breakfast, 120 min			–1.13* [–2.03; –0.23]
after§			

*P<0.05; **P<0.01;***P<0.001; †primary endpoint; ‡secondary endpoint; §CGM efficacy endpoint, IG values were recorded every 5 min; ¶Eight subjects received fewer than the planned 600 Kcal and one subject received the IAsp dose after the start of the meal in the meal test. These profiles are excluded from the meal test analysis; ‡‡One patient was exposed to faster aspart treatment and withdrawn after 3.9 days of treatment with IAsp due to non-compliance with the protocol

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Supported by: Novo Nordisk

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Basal insulin peglispro is superior to insulin glargine in reducing HbA$_{1c}$ in insulin-naïve patients with type 2 diabetes treated with oral antihyperglycaemic drugs: IMAGINE 2

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Background and aims: Basal insulin peglispro (BIL) is a novel basal insulin with a flat activity profile which has a hepato-preferential action resulting from reduced peripheral effects. The primary objective of this Phase 3, double-blind, treat-to-target study was to assess whether BIL was non-inferior (margin=.4%) to insulin glargine (GL) at 52 weeks in reducing HbA1c when added to prestudy oral antihyperglycaemic medications in patients with type 2 diabetes.

Materials and methods: Patients were randomised to evening dosing of BIL (N=1003) or GL (N=535); 168 patients underwent magnetic resonance imaging to assess liver fat content. Testing of the primary objective for superiority was pre-specified with adjustment for multiplicity.

Results: BIL-treated patients had statistically superior HbA1c change at week 52 compared to GL-treated patients (-1.6 vs -1.3%; Δ=-.3% [95% CI: -.40, -.19]), with lower HbA1c (6.9 vs 7.2%, p<.001) and more at goal (HbA1c ≤6.5%: 36 vs 24%, p<.001; HbA1c <7.0%: 58 vs 43%, p <.001). Within-day glycaemic variability using standard deviation (SD) of SMBG profiles was lower for BIL (1.7±.03 vs 1.9±.04 mmol/L [31 ±.5 vs 34±.7 mg/dL], p=.003), as was between-day fasting glycaemic variability (SD of the prior 7 days) (.9±.02 vs 1.0±.03 mmol/L [16±.4 vs 17±.5 mg/dL], p=.02). Total hypoglycaemia rates were similar (BIL vs GL: 1.16±.06 vs 1.21±.07 events/patient/30 days) as was incidence of severe hypoglycaemia (.4 vs .6%). Nocturnal hypoglycaemia rates were lower with BIL (.30±.02 vs .40±.03 events/patient/30 days, p<.001). More patients had HbA1c <7% without nocturnal hypoglycaemia

(LOCF) with BIL (26%) than with GL (15%), p<.001. At week 52, weight gain was less with BIL (2.1±.2 vs 2.6±.2 kg, p=.046), but insulin dose was higher (.45±.01 vs .42±.01 U/kg, p=.022). Thus BIL was titrated to a higher dose than GL with similar total hypoglycaemia, lower HbA1c, and less weight gain. At week 52, triglycerides increased with BIL and decreased with GL (.12±.03 mmol/L [11±2 mg/dL] vs -.08±.04 mmol/L [-7±3 mg/dL], p<.001). HDL- and LDL-cholesterol did not differ between groups. Adjudicated cardiovascular events were similar between BIL and GL. ALT increased 4.1 IU/L with BIL and decreased 2.0 IU/L with GL (p <.001). More BIL patients had ALT ≥3X ULN (2.3% vs .6%, p=.012) but none met Hy's law criteria for acute, severe drug-induced liver injury. Liver fat content was unchanged with BIL (-.6±.5% p=.232) but decreased 3.1 ±.7% with GL (p<.001; BIL vs GL, p=.002). Injection site reactions were more common with BIL (3.5% vs .6%, p<.001) and the majority of these were lipohypertrophy (2.1 vs .4%, p=.007).

Conclusion: At 52 weeks in this double-blind, treat-to-target study of insulin-naïve patients with type 2 diabetes, BIL provided greater reduction in HbA1c, less nocturnal hypoglycaemia, less weight gain, and higher triglycerides compared to GL, consistent with reduced peripheral insulin action and a hepato-preferential effect of BIL. Liver fat content was unchanged from baseline with BIL but decreased with GL.

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Unique profile of the weekly insulin HM12470: very slow onset of action, rapid off-rate similar to insulin, and absence of insulin receptor downregulation

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Background and aims: The novel long-acting basal insulin HM12470 consists of an insulin analog (Insulin 115) conjugated to the non-glycosylated FC region of a human immunoglobulin fragment via non-peptidyl linker and is developed for once-weekly administration. Previously, extended pharmacokinetics and prolonged glucose lowering efficiency of HM12470 have been shown in rodents. The aim of the present study was to characterize the early steps of HM12470 signaling at the receptor and post-receptor level and to assess the metabolic and mitogenic potency of this analog in vitro.

Materials and methods: The off-rate of HM12470 was measured in H9C2-E2 cells by a competition binding assay with 125I-labelled regular insulin. The impact of HM12470, Insulin 115, AspB10 and regular insulin on the insulin signaling cascade was assessed in H9C2-E2, primary human vascular smooth muscle cells (hVSMC) and primary human skeletal muscle cells (hSkMC) by Western Blot analysis. To address functional endpoints, we analysed proliferation in hVSMC by BrdU incorporation and glucose uptake in hSkMC using 14C-labelled 2-deoxy-glucose.

Results: In H9C2-E2 cells overexpressing the human insulin receptor, we observed an off-rate for HM12470 very similar to regular insulin. At the receptor level, HM12470 and Insulin 115 showed no differences in the phosphorylation pattern of tyrosine 1146, 1150/1151 and 972 in comparison to insulin. HM12470 did not acutely activate Akt or MAPK signaling pathways in human skeletal (hSkMC) and human vascular smooth muscle cells (hVSMC). However, upon long-term stimulation we could detect a significant Akt phosphorylation after 24 or 48 h exposure to HM12470 in hSkMC and hVSMC, respectively. Remarkably, in the chronic treatment HM12470 did not induce downregulation of the insulin receptor in both hVSMC and hSkMC. Under these conditions, both regular insulin and AspB10 insulin completely abrogated the insulin receptor. At 100 nM, HM12470 did not induce proliferation of hVSMC in sharp contrast to AspB10 insulin (2-3 fold stimulation). Acute stimulation of glucose uptake in hSkMC was obtained at EC50 values of 36.1 nM and 154.9 nM for regular insulin and HM12470, respectively. However, after 24 h exposure the HM12470-induced glucose uptake was similar to insulin.

Conclusion: In conclusion, the long-acting basal insulin HM12470 shows a flat profile of action without increased mitogenic potency and downregulation of the insulin receptor in vitro. Thus, HM12470 appears safe and it does not desensitize target cells under conditions of chronic exposure and therefore represents an excellent candidate for a weekly basal insulin.

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Glucagon nasal powder: an effective alternative to intramuscular glucagon in youth with type 1 diabetes

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Background and aims: Intramuscular glucagon (IM), in weight adjusted 0.5 and 1.0 mg doses, is the only currently available pharmacologic treatment for severe hypoglycemia in children and adolescents outside of the hospital setting. Unlike current IM emergency kits, needle-free dry-powder intranasal glucagon (IN) does not require reconstitution prior to administration. Having demonstrated that a 3 mg IN dose of glucagon effectively reverses hypoglycemia in adults with T1D, this study examined the dose response relationships of 2 mg and 3 mg IN glucagon in youth 4-<17 years of age.

Materials and methods: 45 youth with T1D were studied at 7 centers participating in the T1D Exchange. The two youngest cohorts (4-<8; 8-< 12 yrs) were randomized to receive either IN administrations, of 2 mg and 3 mg IN on two separate days in double blind, random order or a single weight based-dose of IM glucagon. Subjects 12-<17 yrs received in random order, 1 mg IM at one session and 3 mg IN at another session. Glucagon was given after blood glucose was lowered to <80 mg/dL and the primary outcome was a ≥25 mg/dL rise in plasma glucose from nadir.

Results: The primary efficacy outcome was met after all IM and IN doses of glucagon within 10-20 minutes (Table) with one exception- a 6 yo who immediately blew his nose following a 2-mg dosage. Lack of absorption of the intranasal dose is supported by his low peak glucagon (324 pg/mL). Time to peak and peak glucagon levels were similar in both IM and IN conditions. Transient nausea (with or without vomiting) occurred with 67% of IM sessions vs. 42% of IN sessions (P=0.06).

Conclusion: These data support the efficacy of a novel glucagon nasal powder delivery system for treatment of hypoglycemia in youth with T1D. Given the transient nature of adverse effects when they occur, we conclude that a single 3 mg IN dose can be safely used across the 4-<17-year range of pediatric patients.

	4 to <8 years old			8 to <12 y/o			12 to <17 y/o	
	IM** N=6	2mg IN N=10	3mg IN N=10	IM** N=6	2mg IN N=11	3mg IN N=11	1mg IM N=12	3mg IN N=12
Achieved primary outcome[0] n (%)	6 (100)	9 (90)	10 (100)	6 (100)	11 (100)	11 (100)	12 (100)	12 (100)
	IM N=6	2mg IN+ N=9	3mg IN N=10	IM N=6	2mg IN N=11	3mg IN N=11	1mg IM N=12	3mg IN N=12
Time (minutes) post treatment for 100% treatment success	10	20	15	20	20	15	20	20
Mean Nadir Blood Glucose (mg/dL)	71	67	66	72	76	72	69	73
Mean Peak Blood Glucose (mg/dL)	211	187	198	205	201	206	194	178
C_{max} glucagon (pg/mL)*	6591.2	3422.7	3551.5	4743.0	2776.3	5664.3	4277.2	2997.4
T_{max} glucagon (hr)	0.33 (0.08-0.50)	0.25 (0.17-0.25)	0.29 (0.17-0.50)	0.29 (0.08-0.50)	0.25 (0.17-0.33)	0.25 (0.17-0.50)	0.29 (0.08-0.50)	0.33 (0.25-1.50)

**doses: 0.5 mg if weight <25 kg and 1.0 mg if weight ≥25 kg

[0]≥25 mg/dL rise in blood glucose above nadir

+Excludes the subject who blew his nose immediately after dosing and did not meet the primary outcome.

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OP 08 The calm before the storm: predicting metabolic disease

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Longitudinal trajectories of insulin sensitivity and beta cell function before the development of type 2 diabetes in Japanese individuals

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Background and aims: Evidence is limited about long-term trajectories revealed through detailed assessments of insulin and glucose concentrations before the development of type 2 diabetes (T2D). We aimed to clarify the time point of deteriorating insulin sensitivity and beta-cell function that could be observed during the development of T2D among Japanese individuals.

Materials and methods: We investigated data on 474 Japanese individuals without T2D (T2D indicated by a self-report of clinician-diagnosed diabetes, fasting glucose concentrations of ≥7.0 mmol/L, HbA1c ≥6.5% or 2-h glucose concentrations of ≥11.1 mmol/L) who had available data on glucose and insulin concentrations. We assessed glucose and insulin concentrations during a 75-g oral glucose tolerance test over a 10-y follow-up. The homeostasis model assessment of insulin resistance (HOMA-IR), insulinogenic index (IGI) and the Matsuda index of whole body insulin sensitivity were calculated. The insulin secretion/insulin resistance index (disposition index, DI) was calculated as the product of the Matsuda index and IGI. Stumvoll first-phase and second-phase insulin release during OGTT were also evaluated.

Results: Individuals who did not develop T2D (n=420) had consistently low mean fasting values (<5.3 mmol/L) and postprandial glucose concentrations at 30 min (<8.6 mmol/L), 60 min (<8.4 mmol/L), 90 min (<7.3 mmol/L) and 120 min (<6.7 mmol/L) over 10 y. Those who later developed T2D (n=54) had higher glucose concentrations at 0 to 120 min even 10 y before diagnosis than those who did not develop T2D; incident cases of T2D also experienced a sharp increase in these glucose measures 3 y before diagnosis. A large difference between cases and non-cases was observed for glucose measurements at 60 min over the total observational period while glucose concentrations at 180 min specifically deteriorated only 1-2 y before diagnosis. Glucose concentrations at 60 min was significantly predictive of future T2D independently of glucose concentrations at 0 min and at 120 min. Interestingly, those who later developed T2D had constantly reduced IGI values, reduced Stumvoll insulin release levels and reduced DI values. These values remained low and did not markedly increase in the late stage before diagnosis. A sharp increase in the HOMA-IR in the late stage before diagnosis predicted T2D. Stumvoll insulin sensitivity index levels were also deteriorated in the late stage of the disease course.

Conclusion: Japanese individuals who later developed T2D had clearly elevated glucose concentrations at 60 min rather than other glucose measurements even 10 y before diagnosis. Those who developed T2D also had consistently reduced beta-cell function over 10 y before diagnosis, values similar to those observed at diagnosis.

Supported by: JSPS

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Factors affecting the risk for the first appearance of an islet autoantibody in The Environmental Determinants of Diabetes in the Young (TEDDY) study

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Background and aims: Autoantibodies against Insulin (IAA), GAD65 (GADA) and IA-2 (IA-2A) are detectable for a variable period of time prior to diabetes onset. In the TEDDY study the incidence of IAA only peaked within the first year of life and declined over the following 5 years. GADA only increased till the second year and remained relatively constant. IA-2A only was rare. GADA only were more common than IAA only among HLA-DQ2/2 but less common among HLA-DQ4/8 children. The aim was to study prenatal factors that may affect the risk for HLA-dependent autoantibody appearance.
Materials and methods: Children (n=8676) with HLA-DQ high risk genotypes (DQ2/8, DQ8/8, DQ4/8 and DQ2/2) were enrolled at 3-4.5 months of age and followed with standardized autoantibody assessments quarterly through the first 4 years of life and then semiannually thereafter. Non-diabetic mothers of singleton infants (n=6,947) filled out a questionnaire 3-4.5 months after pregnancy. Lower birth weight was defined as the lower 25% of the TEDDY children's birth weights.
Results: The risk for IAA only (n=197) was increased in Finland and Sweden but not in Germany compared to the US but not for GADA only (n=192). The risk for both autoantibodies was increased among first degree relatives and IAA only among boys. Maternal smoking was associated with country (Europe), maternal BMI, drinking during the 3rd trimester (>2 drinks/month), and maternal infections (lower respiratory tract infection, skin infection or rash, genital infection). After adjusting for country, type 1 diabetes in a first degree relative, HLA genotypes and gender, none of these maternal factors were associated with an islet autoantibody. However, lower birth weight, but not maternal smoking, was independently associated with a decreased risk for GADA only but not for IAA only.
Conclusion: Prenatal factors may affect the risk for HLA-associated first appearance of either IAA or GADA.
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Using genomic information to differentiate diabetes aetiology in young-adult onset diabetes

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Background and aims: Establishing the aetiology in young adult-onset diabetes can be challenging, in view of the overlapping features between common diabetes types. Current tools include clinical parameters and laboratory tests but are often inadequate to allow firm clinical decisions to be made. The recent discovery of a large number of variants associated with increased risk both for type 1 (T1D) and type 2 diabetes (T2D), offers a potential adjunct in the differential diagnosis of common diabetes types. We assessed whether individual genetic risks using known T1D and T2D risk variants offer useful diagnostic discrimination between T1D and T2D diagnosed in early adulthood.
Materials and methods: North European subjects (n=715) with diabetes onset <45 years were provisionally classified as T1D (n=293), T2D (n=370) and LADA (n=52), based on the presence of pancreatic antibodies, time to insulin use from diagnosis, and c-peptide level. We used common risk variants for T1D (47 variants) and T2D (65 variants), plus high risk and protective variants for T1D in the Major Histocompatibility Complex (23 variants) to calculate weighted Genetic Risk Scores (GRS) for T1D (T1DGRS) and T2D (T2DGRS). Differences in GRS between clinical parameters, diabetes subtypes and progression to insulin were estimated with parametric and non-parametric tests. The area under the receiver operator curve (AUC) was calculated for discriminating T1D and T2D using the T1DGRS and T2DGRS.
Results: Higher T1DGRS was associated with diagnosis <18 years ($p=2\times10^{-13}$), undetectable c-peptide ($p=2\times10^{-9}$) and insulin vs. tablets as initial or current treatment (both $p<10^{-22}$), features consistent with T1D. Higher T2DGRS was observed with diet (p=0.002) or tablet use (p=0.04) vs. insulin therapy at diagnosis and with current oral therapy (p=0.0002), characteristics suggestive of T2D. The difference in T1DGRS and T2DGRS between clinically defined T1D vs. T2D cases was highly significant (both $p<10^{-22}$), as was the difference between T2D vs. LADA labelled subjects ($p<10^{-22}$ and p=0.0001 respectively). Non-T1D cases starting insulin within 1 year from diabetes diagnosis had higher T1DGRS compared to the remainder (p=0.0004), therefore estimating T1DGRS at diagnosis could aid in tailoring management. The AUC for clinically defined T1D and T2D patients using the T1DGRS and T2DGRS were 80% and 64%, respectively, indicating that T1DGRS on its own can contribute to the discrimination between clinically labelled T1D and T2D.
Conclusion: Genetic Risk Scores correlate well with traditional diabetes classification parameters and the clinically defined diabetes diagnosis. T1DGRS may also predict early progression to insulin in non-T1D individuals. In summary, genomic information could be a useful adjunct in the differential diagnosis and management of young adult onset diabetes from diagnosis.
Supported by: NIHR, BRC

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A signal near TMEM170A is associated with coronary artery disease and SNPs near IL15RA/IL2RA and THY1 may interact with diabetes status to modify the risk of CAD

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Background and aims: Subjects with type 2 diabetes (T2D) are three or four times more likely to suffer from coronary artery disease (CAD) than non-diabetic individuals. Subjects with T2D have accelerated and a larger burden of atherosclerosis compared to non-diabetic individuals. We hypothesise that there may be different genetic factors that contribute towards these differences.

Materials and methods: We combined summary statistics from 1000G imputed, HapMap 2 imputed and CardioMetabochip genotypes from 24,257 subjects with T2D (10.012 CAD cases and 14,245 CAD free controls) and 42,384 non diabetic individuals (17,694 CAD cases and 24,690 CAD free controls) in a fixed effects variant weighted meta-analysis and performed a test for interaction with diabetes status. Six hundred and eighty six independent SNPs (distance>100Kb/R^2<0.3) analyses based on p value of association <1E-4 for replication from the diabetes stratified and interaction analysis. These associations were replicated in 11,537 patients with T2D (3,706 CAD cases and 7,831 CAD free controls) and 106,250 non-diabetic individuals (12,988 CAD cases and 93,262 CAD free controls).
Results: A known CAD SNP, rs4977574 in the 9p21.3 region, was the top signal for CAD in non-diabetic individuals (P=7.7E-41) and was also genome-wide significant for CAD in subjects with T2D (P=3.9E-9). Rs11072811, a known CAD SNP near *ADAMTS7*, was the top signal for CAD in subjects with T2D (OR (95%CI)=1.05 (1.02-1.09), EAF=0.52, P=9.7E-11) but was not genome wide significant for CAD in non-diabetic individuals (OR (95%CI)=1.02 (0.99-1.05), EAF=0.52, P=2.8E-3). There was some evidence for interaction (P=4.5E-3) that was not significant after correction for multiple testing. We identified a genome wide significant hit near *TMEM170A* that had been previously reported (not at genome wide significance) for CAD and carotid intima thickness (cIMT). Rs6564261 (R^2 with rs4888378>0.8) was associated with CAD in non-diabetic individuals (OR (95%CI)=1.04 (1.01-1.07), EAF=0.62, P=2E-10) with no evidence of interaction with diabetes status (P=8E-2). Two SNPs did show evidence for interaction with diabetes status, with the same direction of effect in the discovery and replication analyses and an interaction p<0.05 in the replication. Rs79705396, near *IL15RA* and *IL2RA* (P=5.2E-6), and rs7952366, near *THY1* (P=1.6E-5).
Conclusion: We reported a locus for CAD near *TMEM170A* at genome wide significance that has been associated with cIMT, and two SNPs that may interact with diabetes status to modify the risk of CAD near genes that may have a biological role in the atherosclerosis.
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Serum lipidome as an independent predictor of progression to type 2 diabetes: the METSIM study

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Background and aims: There is a need for early markers to track progression from a state of normal glucose tolerance through pre-diabetes (impaired fasting glucose and/or impaired glucose tolerance) to type 2 diabetes (T2D). Several diabetes risk models and scores have been developed as prognostic tools. However, these are mainly based on established risk factors of T2D and they lack the specificity required for clinical practice.
Materials and methods: We applied global lipid profiling based on ultra-high performance liquid chromatography (UHPLC) coupled to quadrupole time-of-flight (QTOF) mass spectrometry (MS) at baseline and at 5-year follow-up plasma samples of well phenotyped male participants (107 T2D progressors, 216 matched normoglycemic controls; dataset 1) from the longitudinal study METSIM (METabolic Syndrome In Men) to identify lipidomic profiles of progression to T2D, and to develop a lipid-based predictive model for T2D. The selection of progressors was based on fastest progression to T2D and greater glucose AUC at follow-up. Age-matched controls were selected as non-progressors. In addition, we performed global lipidomic profiling at baseline of a larger subset of the previous cohort (dataset 2), which was characterized by a wide distribution in terms of glucose tolerance (n=640), including non-progressors with a high percentage of subjects with impaired fasting glucose (IFG; 60%), subjects with HbA1C between 5.7-6.4% (11%), subjects with impaired glucose tolerance (IGT; 2%), both IFG+IGT (6%), and subjects with normal glucose tolerance (21%). 20 subjects in dataset 2 progressed to T2D during the follow-up.
Results: A total of 280 lipids were analyzed. The global lipidome was first surveyed by clustering the baseline data from dataset 1 using Bayesian model-based clustering. By applying linear mixed models to the resulting 13 clusters, eight of them showed significant differences in their lipid mean levels between progressors and non-progressors at baseline, after adjusting for BMI. Four clusters consisting only of triglyceride species (grouped by different carbon chain lengths and number of double bounds in their structures) and a cluster consisting mainly of phospholipids were upregulated in T2D progressors (adjusted p-value <0.05). Two clusters containing sphingomyelins and ether phospholipids were downregulated. A similar pattern was observed when dataset 2 was surveyed. Those non-progressors classified as subjects with IFG at baseline showed similar yet less pronounced lipid changes at the cluster level as progressors to T2D. Selected lipids were then considered to build a lipid-based predictive model of T2D with the dataset 1, by using stepwise logistic regression. We compared the candidate models with the Finnish Diabetes Risk Score (FINDRISC) based-model applied to our data. The candidate lipid marker was an independent predictor of T2D and improved the predictive model based on FINDRISC.
Conclusion: Our study indicates that a lipid molecular signature predictive of diabetes is present already years before the diagnosis of diabetes, independent of other risk factors
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Non-coding RNA biomarkers of diabetes complications

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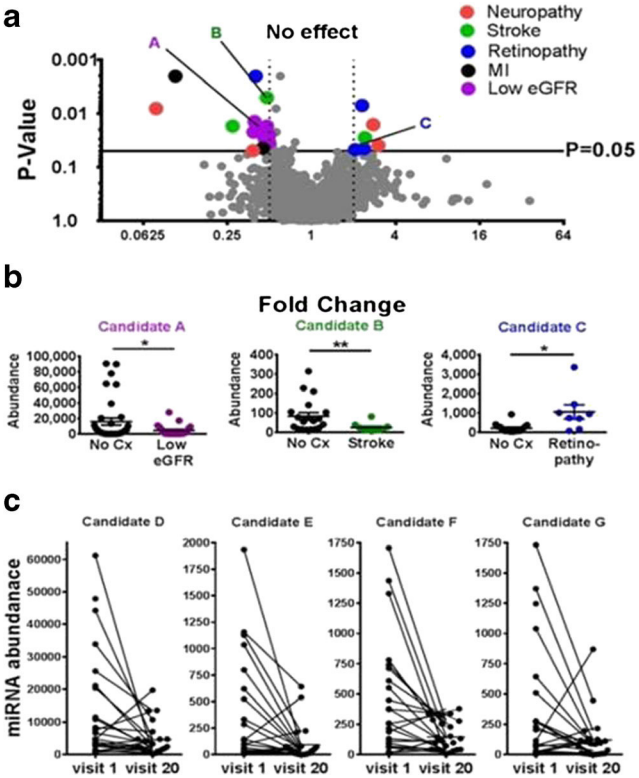
Background and aims: Type 2 Diabetes (T2D) increases the risk of cardiovascular disease (CVD), and other vascular complications including retinopathy and neuropathy and is now well-accepted to be an outcome of intra-uterine programming and exposure to environmental (lifestyle) factors during post-natal life. It is essential and timely to identify early biomarkers of T2D and its complications. In recent years microRNAs (miRNAs), a subset of small non-coding (nc) RNAs, have been detected in circulation and demonstrated as promising biomarkers, mostly in cancer research. We aim to validate circulating ncRNA and DNA methylation signatures for T2D and its vascular complications using two unique longitudinal cohorts i) the Pune Maternal Nutrition study (PMNS) comprising of 18 year follow up study of undernourished children and their parents from preconception period, 20% of whom have now developed insulin resistance at 18 years of age ii) the FIELD study, which demonstrated that, relative to placebo, long-term fenofibrate improved some CVD and all microvascular complications with 9,795 subjects enrolled in study and analysed at baseline, 1 and 5 years and in 1,744 of these individuals at 12 years. We present epigenetic (DNA methylation) and ncRNA signatures for T2D and its complications over lifetime and response to therapy, enabling early management of at-risk individuals and facilitating development of novel therapeutics.
Materials and methods: Circulating miRNAs in plasma were assessed from i) PMNS study subjects at 18, 12 and 6 years as well as at their birth and from their parents (at conception) and ii) T2D subjects in the FIELD cohort (Fenofibrate Intervention and Event Lowering in Diabetes) with retinopathy, neuropathy, low eGFR,

ACR, non-fatal stroke or non-fatal myocardial infarction and were compared to individuals without complications using ultra-high throughput qPCR profiling. DNA methylation in both study cohorts was assessed on Illumina 450 K DNA methylation arrays.
Results: We observed that circulating miRNAs in five T2D complication groups Vs no complications were differentially expressed as seen in volcano plot (Figure 1A). Some of the miRNAs identified in the initial high throughput analysis (+/- 2-fold change and $P<0.05$) were also found to be significantly different in multiple samples when validated using low-throughput high sensitivity quantitative (q)PCR. We also found serum levels of four candidate microRNAs related to vascular health to be lower in FIELD T2D subjects at visit 20 (5 years) Vs visit 1 (baseline).
Conclusion: miRNAs are found to be differentially expressed in individuals with diabetic complications, which could allow early identification of at risk individuals, thereby providing a vital opportunity to improve heath prior to clinical onset.



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OP 09 The price of diabetes care

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The economic consequences of clinical inertia in treatment intensification for type 2 diabetes mellitus in the UK

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Background and aims: Chronic hyperglycemia may lead to severe and debilitating micro- and macrovascular complications and an elevated risk of premature mortality. While there is no cure for T2DM, current treatment recommendations suggest treating patients with agents to maintain near normal HbA1c levels. Despite many available treatments, T2DM gradually worsens over time and treatment intensification is required to meet HbA1c goals. Recent evidence confirms clinical inertia in treatment intensification in everyday UK practice; patients taking 1 or 2 oral antihyperglycemic drugs (OADs) waited a median of 1.5 and 7.2 years, respectively, after exceeding a treatment goal of HbA1c <7.5% before being prescribed rescue therapy. Reducing clinical inertia is expected to lead to better health outcomes, but these benefits occur over long time horizons. This analysis used modeling techniques to estimate the potential magnitude of the health benefits associated with reducing clinical inertia in terms of treatment intensification for patients with T2DM receiving mono- or dual therapy in the UK.
Materials and methods: The Economic and Health Outcomes Model of T2DM (ECHO-T2DM), a validated, long-term stochastic microsimulation model, was used to estimate the potential health benefits over 30 years of reducing clinical inertia in the treatment of T2DM for patients taking 1 OAD (monotherapy) and for those taking 2 OADs (dual therapy) in the UK. For the monotherapy simulation, patients were assigned metformin (MET) at baseline and sulphonylurea (SU) was added as the 2nd line treatment. For 3rd line, SU was discontinued and basal insulin was added. Prandial insulin was specified as the add-on in 4th line. For the dual therapy simulation, patients in both arms were assigned MET plus SU at baseline; the same insulin rescue sequence with basal and then prandial was used. In each of these simulations, patients were separated into 2 arms based on assumptions about the delay in treatment intensification. In the "No Delay" arm, treatment intensification was triggered immediately when HbA1c >7.5% (in line with UK norms). In the "Delay" arm, intensification at each step was postponed based on mean values derived from the recent UK real world evidence study; add-on with SU was delayed by 2.16 years and add-on with basal insulin by 10.63 years. Baseline patient characteristics were sourced from The Health Improvement Network (THIN) database in the UK, separately for patients starting on mono- and dual therapy. Treatment effects for the OADs were obtained from published clinical trials.
Results: With mono- and dual therapy, discounted life-years (LYs) were 0.123 and 0.180 larger over 30 years, respectively, in the No Delay arm compared with the Delay arm, in line with modest relative risk reductions (RRRs) in the occurrence of myocardial infarctions and stroke (4.7% and 4.6% for monotherapy and 6.3% and 5.7% for dual therapy, respectively). There were sizable RRRs in the occurrence of retinopathy and neuropathy outcomes. For example, RRRs for proliferative diabetic retinopathy and severe vision loss were ~50% for monotherapy and more than 55% for dual therapy; the RRRs for lower extremity amputation were 10.6% for monotherapy and 14.0% for dual therapy.
Conclusion: These simulation results suggest that reducing clinical inertia in the UK, and likely elsewhere, has the potential to improve long-run patient outcomes.
Supported by: Janssen Global Services, LLC

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A regimen-based measure of adherence and its association with healthcare costs in patients with type 2 diabetes and patients with both cardiovascular disease and type 2 diabetes mellitus

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Background and aims: In order to achieve recommended HbA1c targets, many patients require multidrug regimens, but these regimens present increased hurdles to patient adherence. Adherence may be particularly important for T2DM patients with cardiovascular disease (CVD) due to increased risks associated with poor HbA1c control for these patients. A common measure of adherence, proportion of days covered (PDC) ≥80% by any diabetes medication, may overestimate adherence to multidrug regimens if patients fill each drug inconsistently but have some drugs on hand most of the time. This study develops a refined regimen-based measure of adherence that accounts for drug regimen changes for patients with T2DM and a subset of patients with both T2DM and cardiovascular disease.

Materials and methods: A retrospective cross-sectional study was conducted using data from Optum-Humedica for adult patients with T2DM that included person-linked claims, electronic medical records (EMR), and vital records for the period 1/2007-3/2014. PDC and health care costs (medical, pharmacy, and total) were assessed over the most recent one year period with continuous enrollment and at least 1 diabetes drug fill. Patients were also required to be continuously enrolled for at least 3 months prior so that drugs on hand at the start of the year could be accurately assessed. Patients with both T2DM and CVD were identified based on diagnosis codes. Adherence was measured based on regimen-based PDC≥80% accounting for initiation, switching, discontinuation, and stockpiling at the class level and compared with the standard adherence measure based on the percent of days with any diabetes drugs on hand. Healthcare costs were estimated using data on estimated allowed amount for each claim. Generalized linear models (log link and Poisson distribution) were used to estimate excess costs related to non-adherence after adjusting for demographic and health plan characteristics.

Results: 26,063 patients met study criteria and had a mean age of 61 years, 52% male, mean HbA1c of 7.3% and mean healthcare cost of $18,047. 26% of patients (N=6,652) had CVD. Non-adherent patients were younger (mean age 59 vs. 64, $p<0.001$) and had higher mean HbA1c (7.5% vs. 7.1%, $p<0.001$). Non-adherent patients (N=13,666, 52%) cost 29% more than adherent patients ($20,297 vs. $15,761, $p<0.001$) using the regimen-based measure. Among patients with CVD and T2DM, non-adherent patients cost 35% more than adherent patients ($40,413 vs. $29,859 $p<0.001$) and nearly twice the cost vs. all patients with T2DM. 52% of all patients filled multiple drug classes. The fraction of multidrug patients classified as non-adherent increased to 57% (regimen-based) from 36% (standard). The excess healthcare cost of non-adherent multidrug patients was 20% higher compared to adherent patients ($4,508 [regimen-based] vs. $3,750 [standard] $p<0.001$). A parsimonious model with only age categories as control variables yielded similar results.

Conclusion: A measure of PDC which more closely approximates T2DM medication regimen shows that standard measures have misclassified non-adherent multidrug patients and consequently underestimated the burden of non-adherence. Estimates of medication adherence in T2DM should be based on the patient's regimen and not simply any diabetes medication on hand. Among T2DM patients with CVD, the economic burden of non-adherence is higher than in other T2DM patients.

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Post-prandial hyperglycaemia (PPH): missed work time and reduced productivity among people with type 1 and type 2 diabetes in the US, UK and Germany

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Background and aims: Post-prandial hyperglycaemia (PPH) is a persistent challenge for diabetes management. Unfortunately, the impact of PPH episodes on work absenteeism and functioning while at work among people with diabetes is not well understood. The purpose of this study was to investigate the impact of PPH episodes on missed work time and work productivity among people with type 1 (T1DM) and type 2 (T2DM) diabetes in the US, UK, and Germany.

Materials and methods: Data were collected in a web survey of 906 adults diagnosed with T1DM (39%) or T2DM (61%) and treated with self-administered bolus insulin in the US (40%), UK (26%), and Germany (34%). Experience of PPH and missed work time/work productivity issues due to PPH were respondent-reported.

Results: A total of 519 respondents (57%) reported working for pay, working an average of 35.2 hours per week. Inadequate post-prandial glucose (PPG) control among both working and non-working respondents was common. Among respondents who worked for pay, 68% experienced difficulty getting their blood glucose (BG) stable after eating during the past week, and 27% experienced PPH episodes 3 or more times in the past week. Among working respondents, 27% reported missing at least some work the last time they experienced a PPH episode. A total of 10% of working respondents indicated that they missed a full day of work the last time they experienced a PPH episode, while 14% reported being late to work and/or 19% left work early. On average, respondents reported missing 168 minutes of work time in the previous week due to their BG being out of range after eating. Furthermore, 71% of working respondents indicated that they had work productivity issues the last time they experienced a PPH episode; 54% of respondents reported that they experienced difficulty focusing at work, 45% indicated that they were less productive at work, 44% reported needing to take a break at work, 28% indicated making more mistakes at work, 11% had to cancel or reschedule a work meeting/appointment, and 10% missed meetings at work. There were no significant differences in missed work time due to PPH by diabetes type or by country. Compared to those with T1DM, respondents with T2DM were significantly more likely to report experiencing any work productivity issues due to PPH (77.0% vs. 62.6%, $p<.05$), finding it difficult to focus at work due to PPH (62.8% vs. 43.5%, $p<.01$), and being less productive at work due to PPH (50.0% vs. 37.4%, $p<.05$). The association between PPH and work productivity also differed somewhat by country. German respondents were significantly more likely than those in the US and UK to report any work productivity issues due to PPH (Germany, 83.5%; US, 64.5%; UK, 61.6%, $p<.01$), making more mistakes at work due to PPH (Germany, 37.1%; US, 24.7%; UK, 19.2%, $p<.05$), and finding it difficult to focus due to PPH (Germany, 64.9%; US, 46.2%; UK, 50.7%, $p<.05$).

Conclusion: The results indicate that the experience of PPH episodes among people with T1DM and T2DM treated with bolus insulin is common and significantly associated with missed work time and reduced work productivity. The findings highlight the need for better clinical management of PPH, particularly for people with T1DM or T2DM treated with self-administered bolus insulin and engaged in the workforce. The results also suggest that PPH may be associated with economic costs related to missed work time and reduced work productivity.

Supported by: Novo Nordisk

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The importance of appropriately incorporating the effects of hypoglycaemia within a health economic model when hypoglycaemia rates are high

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Background and aims: Hypoglycaemia is an important determinant of health utility and thus the cost-effectiveness of blood glucose lowering therapies in people with diabetes. Standard health economic evaluations typically attribute a per-event disutility (PED) to clinical outcomes including hypoglycaemia. Increasingly in diabetes sources of real-world data are being used to conduct health economic evaluations in which patients may have very high rates of hypoglycaemia. Importantly, there is growing evidence of the diminishing marginal effects (DME) of hypoglycaemia on health utility as frequency increases. The objective of this study was to compare PED versus DME on predicted quality adjusted life expectancy (QALE) using an established diabetes model.
Materials and methods: This study used the CORE Diabetes Model (CDM) and published real-world audit data for patients with type 1 diabetes switching to insulin degludec (ID) from either insulin glargine or detemir (IGD). Mean (± SD) baseline profiles were age 35.0 years (±11.4); diabetes duration 18.2 years (±7.5); HbA1c 9.4% (±0.8); weight 77.0 kg (±10.7) and 3.9 hypoglycaemia (severe and non-severe) events per week (±0.9). Mean change in clinical variables was HbA1c -0.5% (±0.3); weight +0.8 g (±1.6) and hypoglycaemia events/week -3.6 (±0.9). A baseline cohort was projected over a lifetime using the CDM using published PED disutility (-0.0052) and published DME disutility (0.0141*[number of events]^0.3393) with and without treatment effects applied to calculate life expectancy (LE) and QALE associated with the pre and post-switch profiles. Results were discounted at 3.5%.
Results: Overall discounted LE was 18.8 years and 19.06 years for IGD and ID profiles respectively and discounted QALE (ignoring the impact of hypoglycaemia) was 12.13 and 12.4 for IGD and ID respectively. Discounted QALE was reduced by 1.85 (IGD) and 0.849 (ID) using the DME approach applied to hypoglycaemia rates of 3.9 and 0.4 events/week respectively. Using the PED approach discounted QALE was reduced by 22.96 (IGD) and 2.355 (ID). The PED approach resulted in a QALE prediction less than zero for IGD.
Conclusion: Failure to adequately accommodate the relationship between hypoglycaemia frequency and quality of life within health economic evaluations may lead to misleading predictions and estimates of QALE that lack face validity; this is particularly so when hypoglycaemia event rates are high.

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A first evaluation of the cost-effectiveness of CSII versus MDI in type 2 patients: projection of clinical and economic benefits in the Netherlands

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Background and aims: Literature in type 1 patients demonstrated that CSII was often cost-effective over MDI in uncontrolled patients and in various countries. However, this question remained unaddressed so far for type 2 patients. The present study is the first investigating and reporting health economic outcomes for CSII versus MDI in type 2 patients based on improvements in glycemic control since new clinical data were published in type 2 patients in 2014.
Materials and methods: We published the results of the OpT2mise randomized controlled trial, during which they followed 331 people with type 2 diabetes who were on MDI with at least 3 injections per day and uncontrolled HbA1c. Mean HbA1c at baseline was 9% with average age of 56 years. At 6 months, mean HbA1c had decreased by 1.1% in the CSII group and by 0.4% in the MDI group. The CORE Diabetes Model (CDM) was used in order to project the long-term clinical and economic benefits of these interventions in type 2 patients. The CDM has been widely published and validated. For type 2 patients, it is based on the published UKPDS risk equations amongst others published sources. The CDM uses the baseline risk factors combined with the impact of CSII and MDI to assess annually the risk of developing complications and mortality. Unit costs are attached to each individual diabetes-related complication. Dutch costs (complications and interventions) come from various published and official sources. To reflect the decrement in quality of life due to diabetes, a specific utility value was also associated to each complication. These utilities are taken from a published systematic literature review. Costs and clinical outcomes were discounted at 4% and 1.5% p.a. respectively. The health economic analysis has been conducted from a Dutch third party payer perspective. Sensitivity analyses were also conducted to test the robustness of the results.
Results: Undiscounted life expectancies were improved for CSII versus MDI by 0.785 years. Total QALYs were 9.38 and 8.95 respectively for CSII and MDI.. The breakdown of costs indicated that around 50% of these costs were attributable to diabetes related complications. Extra acquisition costs of CSII versus MDI were partially offset by the reduction in complications. The ICER has been estimated (in EUR/QALY) at 62,895 and at 60,474 when indirect costs were included.
Conclusion: These results indicate that accordingly to commonly accepted threshold CSII could be considered a cost-effective intervention compared to MDI in uncontrolled type 2 patients in the Netherlands. CSII also increased the life expectancy of uncontrolled patients as well as reduced morbidity and improved their quality of life.

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Weight change over time and its link to healthcare costs among newly-diagnosed type 2 diabetes patients in Sweden

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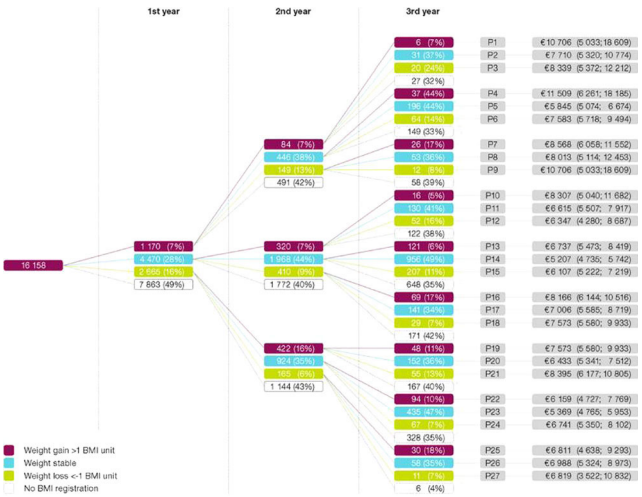
Background and aims: Weight control is an important parameter in management of patients with type 2 diabetes (T2DM), however actual knowledge on the impact of weight changes in relation to health care resource use is scarce. The aim was to comprehensively describe relative weight (body mass index, BMI) change pathways by using repeated BMI measurements in newly diagnosed type T2DM patients and link that to healthcare resource utilization costs from primary and hospital care.
Materials and methods: Between 1999-2009, all newly diagnosed T2DM patients in 84 primary care centers (almost 10% of all primary care centers in Sweden) were extracted. Patients eligible for the study had BMI measurement at diagnosis and subsequent BMI measurements at 12, 24, and 36 months were identified. Patients with BMI<18, cancer and heart failure at T2DM diagnosis were excluded from the analysis due to the risk of unintentional weight loss. Three BMI change categories were defined based on BMI change over a period of one year: weight gain (≥1 BMI unit increase), weight stable (<1 BMI unit change), and weight loss (≥1 BMI unit decrease). Based on the observed individual BMI change sequence over three consecutive years, patients were assigned to one of totally 27 possible BMI change pathways (P1-P27, Figure). The most common pathways were identified by estimating a conditional probability of a patient moving between BMI categories over a period of one year.

Mean three year healthcare costs for each pathway were estimated by applying Swedish unit costs to the healthcare resource data extracted from electronic patient journals and the Swedish National Patient register.

Results: Of the 16,158 newly diagnosed T2DM patients, 8,295 had a BMI measurement at baseline and year 1; 4,888 at year 1 and 2; and 3,116 at all three years. Patients eligible for BMI change calculations in the 1st year were 44% women, mean age 61 years, had mean HbA1c 6.8% and BMI 30.8 kg/m2. The most frequently observed BMI change pathway was associated with weight stability over 3 years (P14, €5,207). The second and third most common pathways were associated with weight loss or gain in year 1 and weight stability in subsequent years (P23, €5,369 and P5, €5,845, respectively). Over 3 years, the highest healthcare costs were observed among individuals with weight gain in year 1 (€8,335, average costs of P1-P9) whereas patients whose remained weight stable or had weight loss in year 1 on average incurred €6,907 (average costs of P7-18) and € 6,810 (average costs of P19-P27), respectively.

Conclusion: In newly diagnosed diabetes, an initial moderate weight gain leads to increased healthcare costs. Costs of interventions that maintain weight in patients with diabetes should be considered in the context of costs associated with weight gain.



Supported by: AstraZeneca

OP 10 Incretins: that gut feeling

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Impaired sensitivity to incretins and adaptation of the incretin response in type 2 diabetes

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Background and aims: In type 2 diabetes (T2D), the sensitivity to incretin hormones, determined by exogenous hormone infusion, is reduced. Whether this holds true under more physiological conditions, e.g. during an OGTT, has not been determined. In addition, the relationship between sensitivity to incretins and the incretin response has not been explored.

Materials and methods: We used 25, 75, and 125 g OGTT and isoglycaemic glucose infusions in patients with T2D (n=8) and matched (n=8) normal glucose tolerant (NGT) subjects (3 males; age: 57±1 (mean ± SE) years; BMI: 29±0.3 kg/m^2), and a beta cell mathematical model to assess the time course of incretin potentiation (Pincr) of insulin secretion. Total glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP) were also measured, thus allowing evaluation of the relationships between the time course of incretin hormones and Pincr.

Results: Pincr increased dose-dependently during the OGTTs and then returned to baseline. As expected, the Pincr increase was markedly blunted in T2D. Across all glucose doses Pincr was linearly correlated in every subject with GLP-1 and GIP concentrations; the correlation was stronger in NGT (average r=0.64 and 0.61, for GLP-1 and GIP) than in T2D (r=0.46 and 0.47), due to the flatter Pincr response in T2D. The slope of the individual relationships was used as an index of beta cell sensitivity to GLP-1 and GIP. Of note, this assessment of incretin sensitivity is not intrinsically related to the magnitude of the incretin response. The sensitivities to GLP-1 and GIP were correlated (r=0.66, p<0.006). GLP-1 sensitivity was impaired in T2D (0.008±0.002 l/pmol) vs. NGT (0.020±0.006 l/pmol, p<0.027). However, there was a wide overlapping between the two groups (range: 0.007-0.045 l/pmol in NGT vs. 0.002-0.015 l/pmol in T2D). Using all OGTT data, the GLP-1 AUC was inversely related to GLP-1 sensitivity, with the glucose dose as a factor increasing AUC, and a steeper slope in NGT vs. T2D (r=0.81, p< 0.0001). Similar results were obtained for GIP (GIP sensitivity: 0.003± 0.001 and 0.013±0.006 l/pmol in T2D and NGT, respectively, p<0.030). This relationship suggests the existence of a compensation mechanism that upregulates incretin secretion in presence of decreased sensitivity to incretins; this compensation is impaired in T2D.

Conclusion: This analysis indicates that relative incretin insensitivity is partly compensated for by higher incretin secretion, both in NGT and T2D. In T2D we found both a moderate impairment in incretin sensitivity and a defective compensation of the incretin secretion.

Clinical Trial Registration Number: NCT00529048

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HNF-4 gamma and HNF-4 alpha have opposite effects on diet-induced diabetes

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Background and aims: Transcription factors, HNF-4α and HNF-4γ belong to nuclear receptor superfamily whose ligands are fatty acids. HNF-4α plays pleiotropic roles in liver functions. In Human, heterozygous mutations in HNF4A gene lead to Maturity Onset Diabetes of the Young Type I (MODY I). In intestine, our team showed that HNF-4α controls epithelium homeostasis and HNF-4γ impacts the glucose homeostasis through a decreased incretin effect. Furthermore, HNF-4α and HNF-4γ have opposite effect on abundance and differentiation of enteroendocrine cells producing incretin hormones. We test thus the hypothesis that HNF-4α and HNF-4γ have opposite effects in susceptibility to diet induced type 2 diabetes (T2D).
Materials and methods: We used 2 mouse models: 1- a constitutive and total Hnf4g gene invalidation and 2-an inducible and intestine specific Hnf4a gene invalidation, fed high-fat/high-fructose diet for 4 weeks. We performed oral and intraperitoneal glucose tolerance tests and measured insulin and enterohormone plasma concentrations to evaluate the T2D susceptibility. GLP-1-positive cell number was measured by immunohistology. The expression of transcription factors involved in the differentiation of enteroendocrine cells (Ngn3, NeuroD1, PAX6 and PAX4, ISL1, FOXA1 and FOXA2, NKX2.2, ARX, PDX1) and of some enterohormones (CCK, PYY, GIP and GCG) was studied by RT-qPCR.
Results: We show that the invalidation of Hnf4g gene protects mice from weight gain and glucose intolerance, induced by 4 weeks of diet rich in lipids and fructose, that is observed in control mice. Contrary to intestinal HNF-4a invalidation, Hnf4g invalidation improves oral glucose tolerance but not intraperitoneal glucose tolerance. The high-fat/high-fructose diet does not further modify the impact of Hnf4g invalidation on the abundance of GLP-1 cell number and the transcription factor expression involved in enteroendocrine lineage.
Conclusion: We demonstrate for the first time that HNF-4γ increases the susceptibility to diet induced T2D. This occurred in part through intestine action: a decrease of incretin hormone secretion mediated by lower enteroendocrine cell differentiation. Targeting the enteroendocrine cell differentiation through nuclear receptors HNF-4 might be a new therapeutic approach in DT2.
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Differentiation and abundance of enteroendocrine cells are impaired in obese and diabetic obese subjects

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Background and aims: A worldwide obesity and diabetes epidemic is spreading in humans since two decades. Gastric bypass, the most effective treatment for morbid obesity, is leading to diabetes improvement or resolution. This occurs partly through changes in gut hormone secretions, which can rely on differentiation, number and function of enteroendocrine (EE) cells**.** However, it is not known if an alteration of EE cell differentiation could explain the lower level of enterohormone in obese subjects. This study evaluates the impact of obesity and type 2 diabetes (T2D) on the expression of genes coding for enterohormones and for transcription factors responsible for the commitment of EE cell subtypes in obese and obese-diabetic subjects.
Materials and methods: Jejunum samples were obtained from gastric bypass of obese (n=37) and obese-diabetic subjects (n=21) or from a set of non-diabetic normal weight individuals (lean) obtained through collaboration with surgeons (n=22). We used an integrated strategy combining immunohistological phenotyping of EE cell types and gene expression studies on isolated jejunal epithelial cells by RT-qPCR using Taqman® Arrays Cards. Expression of transcription factors involved in the differentiation of EE cells (*Ngn3*, *NeuroD1*, *PAX6* and *PAX4*, *ISL1*, *FOXA1* and *FOXA2*, *NKX2.2*, *ARX*, *PDX1*) and of some enterohormones (*CCK*, *PYY*, *GIP* and *GCG*) were studied. The statistic tests were a non-parametric Kruskal-Wallis test (lean/obese status) or an ANOVA test (lean/obese/obese-diabetic status).
Results: We have shown that the number of EE L-cells (GLP-1), K-cells (GIP) and I-cells (CCK) in the jejunum of obese subjects is lower than in lean control subjects (50%, 30%, and 30% respectively, $p<0.05$), in accordance with reported low levels of plasma hormones. We then evaluated EE cell differentiation by low density arrays according to the obese/lean status. We showed that *PAX6* gene expression was significantly decreased ($p<0.001$) between obese and lean subjects. Moreover, *PAX6, NKX2, ARX, CCK, GCG* genes were found to be differentially expressed ($p<0.001$) between the three groups of subjects, obese, obese-diabetic and lean subjects. We showed that obesity impacted significantly the EE GLP-1 cell lineage whereas T2D in obesity impacted preferentially on CCK cell lineage.
Conclusion: Our study highlights differences on gene expression profile according to the metabolic status of individuals. Obesity and T2D modulate the EE lineage in humans by limiting the abundance of EE cell type in the absorptive segment of the intestine. Deregulation of the EE differentiation could cause alterations in enterohormone secretions observed in obese and obese diabetic subjects.
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Neuromedin U is a mammalian decretin hormone suppressing insulin secretion through NmuR1

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Background and aims: Decretins are enteroendocrine hormones postulated from classical studies of starvation in humans and other mammals to suppress insulin secretion and provoke 'starvation diabetes'. We recently identified a decretin in Drosophila called Limostatin (Lst) that is induced by carbohydrate restriction and suppresses insulin output by signaling through the G-protein coupled Limostatin Receptor (LstR). Our work on isolated human islets suggested that the hormone Neuromedin U (Nmu) and its islet β-cell receptor NmuR1 might be mammalian orthologues of Lst/LstR. To test this hypothesis, we investigated in vivo Nmu signaling in mice.
Materials and methods: We used mice homozygous for a null NmuR1 allele and Nmu-eGFP transgenic mice to investigate Nmu regulation and signaling in vivo. We also generated novel sensitive enzyme linked immunosorption assays (ELISA) to measure Nmu in mouse and human serum.
Results: Nmu mRNA and protein were produced abundantly in mouse stomach and intestinal enteroendocrine cells. We detected a two-fold increase of serum Nmu levels in mice after 72 hours fasting, accompanied by increased Nmu mRNA expression in Nmu-EGFP+ cells. In this setting, oral glucose challenge evoked hyperglycemia accompanied by

relative hypoinsulinemia, effects not observed after overnight fasting or in mice lacking NmuR1. Glucose-stimulated insulin secretion by isolated mouse islets revealed that Nmu inhibits insulin release, an effect abolished in NmuR1 mutant islets. Unexpectedly, we discovered that during the latter phases of standard oral glucose tolerance testing after overnight fasting, serum Nmu concentration increased as insulin levels declined in control mice. By contrast, insulin levels at this stage remained elevated in NmuR1 mutant mice, revealing that Nmu may suppress insulin secretion in physiological states distinct from starvation. Consistent with this view, in mice fasted 24 hours we found Nmu injection that increased serum Nmu levels two-fold led to reduced insulin levels, increased glucagon levels, and hyperglycemia. Nmu administered in this way during oral glucose challenge was also sufficient to impair glucose-stimulated insulin secretion and glucose tolerance. Thus, a small increase of serum Nmu levels in physiological settings had a marked impact on the output of insulin and glucagon, and metabolic homeostasis.

Conclusion: Here we show that, in pathological settings like prolonged fasting, Nmu functions as a decretin to suppress insulin output through NmuR1 and provokes starvation diabetes. Our studies also unexpectedly revealed how inter-organ Nmu signaling between the gastrointestinal tract and endocrine pancreas may be regulated to coordinate changes in secretion of insulin and other hormones during physiological feeding. This previously unidentified function of Nmu suggests that drugs modulating this entero-insular axis may be useful in physiological and disease states, including diabetes mellitus.

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Incretin effects of branched-chain amino acids

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Background and aims: Incretin effect described first in 1986 by Nauck describes different insulin response to glucose load after oral vs. intravenous administration. Intestinal hormones incretins, namely GLP-1 and GIP, are responsible for this augmented insulin secretion after oral administration. Other nutrients than glucose are capable of stimulating insulin secretion in the same glucose-independent incretin way. Branched chain amino acids (BCAA) are known to exert insulinogenic effect. Whether this effect is also mediated by incretins has not been well addressed in literature. The aim of this study is to show that oral administration of BCAA elicits higher insulin and incretin response when compared to intravenous route of the same dose of BCAA.

Materials and methods: Twenty four healthy, male Caucasian subjects were recruited, mean age was 25.5 years, and mean weight was 76.2 kg. Two tests were performed on a different day after an over-night fast. First test (IV test) comprised of intravenous application of BCAA solution (Nutramin VLI, Fresenius Kabi) in total dose of 0,4 g/kg in a 2 hours infusion (mean total dose of BCAA 30.5 g). Second test (PO test) comprised of oral ingestion of BCAA capsules (BCAA Nutrend) in a single dose of 0.4 g/kg. Plasma levels of glucose, insulin, GLP-1 and GIP were measured at time intervals for up to 240 minutes. Insulin secretion response was assessed as incremental area under the curve (iAUC) and was calculated using the trapezoid model.

Results: Oral administration of BCAA induced higher insulin response than intravenous (iAUC 0-240 min PO 22.48±29.3 vs. IV 7.91±21.8 mIU/L, p<0, 05). The peak plasma insulin concentration were observed at 60 minute in PO test (PO 4.85±3.88 vs. IV 1.92±2.92 mIU/L, p<0, 05) and at 90 minutes in IV test respectively (PO 4.49±4.37 vs. IV 2.65±3.48 mIU/L, p=0, 14). PO test induced considerably higher GLP-1 (iAUC 0-90 min PO 9.03±5.7 vs. IV 0.97±3.45 pmol/l, p<0,001) and GIP response (iAUC 0-90 min PO 36.72±52.59 vs. IV -16.27±56.18 pg/ml, p< 0,005). The plasma GLP-1 peak was at 60 minute (GLP-1 max 2.94± 2.25 pmol/l) and GIP peak at 30 minute (GIP max 13.79±18.03 pg/ml) in PO test. Plasma glucose levels declined in the same pattern during both tests (iAUC 0-240 min PO -8.94 mmol/L, IV -8.49 mmol/L) reaching its minimum at 210 minute in PO test (3.86±0.24 mmol/L) and 120 minute in IV test respectively (3.78±0.24 mmol/L).

Conclusion: We found that oral administration of BCAA elicits significantly higher insulin and incretin response when compared to intravenous application. We conclude therefore that BCAA effect on insulin secretion is at least partially incretin dependent.

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NHE3 blockade ameliorates glucose intolerance via its inhibitory action on SGLT1-mediated intestinal glucose absorption in diabetes

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Background and aims: Na^+-glucose cotransporter 1 (SGLT1) plays a pivotal role in regulating intestinal glucose uptake and has a profound impact on glucose homeostasis and type 1 and 2 diabetic states. Interestingly, we have previously shown that angiotensin II (AngII), via its receptor AT1R, has an inhibitory action on SGLT1 function. In light of these findings, investigation is warranted to identify additional modulators of SGLT1. Therefore, the present study aimed to unravel a previously unknown role of sodium-hydrogen exchanger 3 (NHE3), a critical transporter for sodium homeostasis, in the regulation of SGLT1-mediated intestinal glucose absorption, and to elucidate its downstream signaling cascades, as well as further exploring a potential interaction of the "AT1R-NHE3-SGLT1" signaling axis in the control of intestinal glucose uptake and glucose homeostasis, and thus its consequence on diabetes.

Materials and methods: ^{14}C-glucose absorption in human and mouse (*m+/db & db/db*) jejunums, and human Caco2 cells after incubation with EIPA (NHE3 blocker) was measured by liquid scintillation counting. Changes in plasma levels of fasting blood glucose of mice after gastric gavage with EIPA were studied by OGTT. NHE3 was also downregulated by siRNA-mediated transient knockdown and overexpressed in Caco2 cells. Intracellular pH (pH_i) and its recovery rate after incubation with AngII, L162313 (non-peptide AT1R agonist), losartan (AT1R blocker) and KT5823 (PKG blocker) were measured using the fluorescent dye, BCECF-AM.

Results: NHE3 blockade inhibited SGLT1-mediated glucose uptake in a GLUT2-independent manner across the 3 study models consistently, i.e. human jejunum (1.0 mM EIPA: -41.6%, p<0.0001), human Caco2 cells (1.0 mM EIPA: -40.5%, p<0.0001), and mouse jejunum (1.0 mM EIPA: *m+/db*, -76.1%, p<0.0001; *db/db*, -44.1%, p<0.0001). In parallel, knockdown of NHE3 in Caco2 cells significantly reduced SGLT1-mediated glucose uptake (-29.1%, p<0.0001) and mRNA levels of SGK1 (-34.5%, p< 0.001) and SGLT1 (-63.0%, p<0.0001); whereas overexpression of NHE3 reversely upregulated SGLT-1 mediated glucose uptake (+52.1%, p<0.001) and mRNA levels of SGK1 (+161.3%, p< 0.001) and SGLT1 (+89.8%, p<0.001). For the effect on glucose metabolism, NHE3 blockade did not significantly alter the blood glucose levels in *m+/db* mice (0.5 mM EIPA: -9.2%, p>0.05) but remarkably reduced those of *db/db* mice (0.5 mM EIPA: -33.7%, p<0.0001), as shown by OGTT. For the study of AT1R, AngII inhibited NHE3-mediated pH_i recovery (1 μM: -34.6%, p<0.01), of which its effect was abolished by blockade of AT1R (1 μM losartan: -3.93%, p>0.05) and PKG (1 μM KT5823: -4.61%, p> 0.05). Activation of AT1R attenuated protein expressions of NHE3, as revealed by Western blot (10 μM L162313: -51%, p<0.0001).

Conclusion: Collectively, our data reveal for the first time that NHE3 blockade reduces glucose intolerance in diabetes by inhibiting SGLT1-mediated intestinal glucose absorption, probably via SGK1 mediation. Such an interaction may be modulated by an upstream inhibitory action of AT1R-PKG activation on NHE3. Our study provides a perspective of counteracting hyperglycemia by targeting intestinal glucose uptake, and opens an avenue for the potentially clinical use of NHE3 blockade in the regulation of post-prandial hyperglycemia for diabetic patients.
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OP 11 Healing the diabetic foot

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Incidence and predictors of ulceration and amputation in a Dutch type 2 diabetes mellitus population

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Background and aims: Ulceration and amputation are burdensome complications of type 2 diabetes (T2DM). A reliable method that predicts these outcomes would help in the identification of patients at risk of ulceration and amputation. We therefore aimed to identify predictors of ulceration and amputation.
Materials and methods: This study was conducted in 5,466 patients whocwere treated by the Diabetes Care System (DCS) in the West-Friesland region of the Netherlands between 2011 andc2013. In these patients, a general foot inspection was performed, in linecwith the SIMM's classification, to check for foot deformities or local pressure points. Patients were examined to obtain information on peripheral neuropathy, vibration sensation, peripheral arterial disease and limited joint mobility. Diabetic foot ulceration was defined as a skin lesion distal to the ankle. Information of foot ulceration and amputation in the last year, was obtained by self-report during the annual screening. According to this examination, patients were categorized according to the SIMM's risk classification. The primary outcome of this study was the incidence of ulceration or amputation (SIMM's category 3). Potential predictors of ulceration and amputation, other than SIMM's category 1 and 2 were also determined during the annual screening including age, sex, diabetes duration, smoking status, BMI, blood pressure, HbA1c, lipid levels. The prevalence of the different SIMM's risk categories and the 2-year incidence ulceration and amputation was determined. Predictors for ulceration and amputation were identified out of easily available clinical measurements performed at baseline, using backward logistic regression analyses using the Akaike information criterion, with a significance level of 15.7% ($p<0.157$).
Results: Two-year incidence of ulceration and amputation was 1.9%. SIMM's risk classification 1 (OR 4.03 (95% CI 2.34-6.92)) and 2 (OR 3.92 (95% CI 2.50-6.15)) Current smoking (OR 1.81 (95% CI 1.14-2.88)), BMI (OR 1.03 (95% CI 1.00-1.07)) and HbA1c (OR 1.02 (95% CI 1.00-1.03)) were identified as predictors for ulceration and amputation (Table).
Conclusion: This study identified risk factors for the prediction of ulceration and amputation. The predictors are factors routinely measured during usual diabetes care, which will allow for a translation to clinical care. Using this classification system, it provides a method to select patients at higher risk for ulceration and amputation and distribute medical care to those at most need.

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Magnetic resonance imaging shows wide-spread bone marrow oedema in the acute Charcot foot and this can be quantitated by a novel scoring system

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Background and aims: Magnetic resonance imaging (MRI) is an established modality in the diagnosis of acute Charcot foot. It shows the extent of bone and joint damage not only in areas presenting with radiological changes but also in areas which appear "normal" on standard foot

and ankle radiographs. We devised a novel semi-quantitative bone marrow oedema (BMO) score that can be used on foot and ankle MRI scans in patients with acute Charcot foot. The aim of this study was to investigate the extent of BMO on MRI scans in patients with acute Charcot foot and to determine the inter- and intra-observer reliability of the BMO score.

Materials and methods: We studied 45 patients presenting to a single centre with a recent onset of acute Charcot foot. All bones (proximal phalanges, medial and lateral sesamoids, metatarsals, tarsals, distal tibial plafond, medial and lateral malleoli) were scored individually by three observers for the extent of oedema (BMO score: 0- no oedema; 1-oedema50% on two planes) on a non-contrast MRI scan (Siemens Avanto 1.5 T, dedicated foot and ankle coil). Zones of involvement were assessed using Sanders and Frykberg's classification (pattern I - metatarsal-phalangeal joints; pattern II - metatarsal-tarsal joint; pattern III - tarsal joints; pattern IV - ankle joint and pattern V - calcaneum). The inter-observer and intra-observer reliability for assessing the total BMO score and identifying the zones of involvement were also determined.

Results: The MRIs showed extensive bone marrow oedema throughout the foot involving several combinations of patterns and only 2 cases presented with a classical single pattern of involvement - pattern II (1 case, 2.2%) and pattern IV (1 case, 2.2%). The two most common presentations were the combination of patterns I+ II+III, which was observed in 13 cases (28.9%) and the combination of patterns II +III, which was observed in 12 cases (26.7%). Five cases presented with a combination of patterns I+II (11.1%) and further 5 cases presented with a combination of patterns I+II+III+IV (11.1%). Less frequent presentations included a combination of patterns II+III+IV noted in 3 cases (6.7%) and patterns I+II+IV noted in 2 cases (4.4%). The remaining presentations were noted only in single cases. These included a combination of patterns I+III (1 case, 2.2%), patterns I+II+IV (1 case, 2.2%), patterns I+II+III+IV+V (1 case, 2.2%). The total BMO score showed no significant difference between the three observers (Interclass Correlation Coefficient 78% (95%CI 60% to 95%), p=0.47) and there was good intra-observer reliability (2 replications of 10 scans - mean difference -0.179 (95%CI -2.031 to 1.674), p=0.21). There was very good intra-observer reliability for the identification of patterns I, II and III (Kappa coefficients 75%, 100%, 79% respectively) and moderate for patterns IV and V (Kappa coefficients 43% and 47%, respectively).

Conclusion: We report an extensive distribution of BMO on foot and ankle MRI scans in diabetic patients with acute Charcot foot, which has not been previously noted. The total BMO score has very good intra- and inter-observer reliability. MRI imaging of the whole foot should be considered when Charcot foot is suspected to allow full appreciation of the extent of bone and joint damage. The proposed semi-quantitative BMO score can become a novel tool in the assessment of the acute Charcot foot.

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Improved 6-year survival in patients with chronic diabetic foot ulcers after hyperbaric oxygen therapy: outcome from a randomised double-blind study

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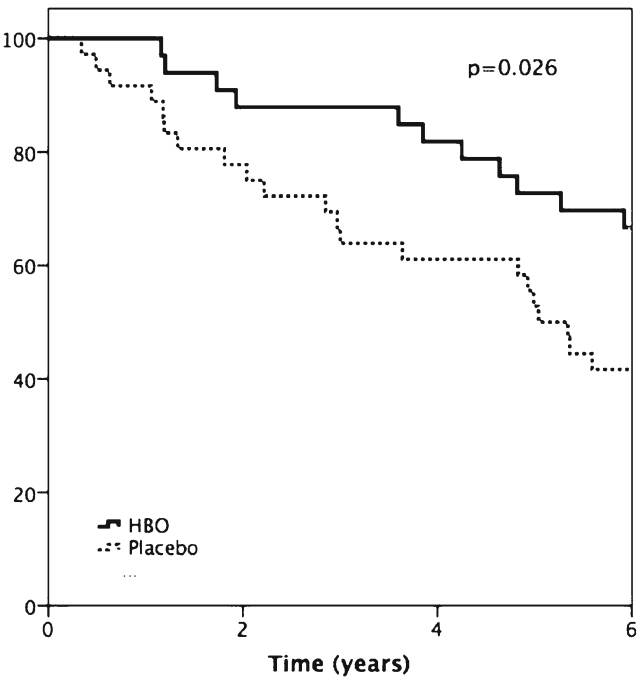
Background and aims: Presence of a chronic diabetic foot ulcer (DFU) is associated with increased mortality. Hyperbaric Oxygen Therapy (HBO), a medical treatment for chronic DFU in selected patients, increases tissue oxygenation, stimulates angiogenesis and restores stem cell mobilization from bone marrow. We have previously shown, beside improved healing rates, an increased 3-year survival in DFU patients receiving HBO compared to placebo (89.5 vs. 70.3%, p=0.04). The aim of this study was to evaluate whether this plausible effect persists in a 6-year perspective.

Materials and methods: Patients with chronic DFU were included in a prospective randomized double-blind placebo-controlled study evaluating the effect of 40 treatment sessions with HBO (2.5 ATA, 90 minutes) compared to hyperbaric air (placebo). Patients receiving >35 treatment sessions (per-protocol requirement) were included in the analysis. Mortality data were received from the Swedish National Death Registry. Non-parametric statistics were used to assess differences between groups. Survival was estimated with Kaplan-Meier curves and Log-rank test. Data are given as median and percentages. A two-tailed p-value <0.05 was taken as statistically significant.

Results: 75 patients (38 HBO and 37 placebo) with a median age of 67 and 71 years (HBO and placebo, respectively, n.s) and a diabetes duration of 22 and 21 years (n.s) were included in the analysis. Baseline co-morbidities were similar between groups, as were prescription patterns of antidiabetic and cardiovascular protective drugs. Baseline QTc time on ECG were similar between groups. The 6-year survival rate was higher in HBO compared to placebo treated patients (63.2 vs. 40.5%, p<0.05). The deceased patients were 68.2 years old at baseline in the HBO group compared to 73.3 years in placebo group (p=0.18). Since age could be a plausible confounding factor, a subgroup analysis excluding patients <50 years, was performed (Figure). In this analysis of 69 patients (33 HBO and 36 placebo) aged 71.2 and 71.0 years respectively (n.s.), the difference in 6-year survival persisted (63.2% in HBO vs. 40.5% in placebo, p<0.05) and no difference in baseline age of non-survivors was found (71.9 vs. 73.3 years). QTc time between survivors and non-survivors at time of inclusion, did not differ in the HBO-group (425 vs. 432 ms, p=0.85), whereas a difference was seen in the placebo group (419 vs. 434 ms, p=0.02), with a longer QTc time among non-survivors. Similar outcome was found in the subgroup analysis excluding patients <50 years of age.

Conclusion: Our study indicates that HBO may improve 6-year survival in patients with chronic diabetic foot ulcers. Whether this finding is due to improved healing rates and thus less co-morbidity or whether the effect might be mediated by an improved cardiac microcirculation and perhaps a protective effect against further QTc prolongation needs to be further evaluated.



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Capsaicin 8% patch in painful diabetic peripheral neuropathy: a randomised, double-blind, placebo-controlled study

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Background and aims: The capsaicin 8% patch has demonstrated analgesic efficacy over 12 weeks in patients with post-herpetic neuralgia and HIV-associated distal sensory polyneuropathy, as well as non-inferior efficacy to pregabalin in peripheral neuropathic pain, with a faster onset of action, fewer systemic side effects and greater treatment satisfaction. STEP evaluated the efficacy and safety of the capsaicin 8% patch versus placebo in patients with painful diabetic peripheral neuropathy (PDPN).
Materials and methods: STEP was a Phase III, multicentre, double-blind, placebo-controlled, 12-week study. Patients with painful, distal, symmetrical, sensorimotor diabetic polyneuropathy of at least 1 year's duration, glycosylated haemoglobin ≤11% and average daily pain score ≥4 (question 5 of Brief Pain Inventory-Diabetic Neuropathy [BPI-DN]), were randomised (1:1) to either capsaicin 8% patch or placebo patch to painful areas of the feet for 30 minutes. The primary endpoint was percentage change in average daily pain score from baseline to between Weeks 2-8. Secondary endpoints included weekly average daily pain, 30% responder rates, BPI-DN sleep interference score and weekly sleep interference score.
Results: Overall, 369 patients with mean [SD] duration of PDPN 5.8 [4.0] years, glycosylated haemoglobin 7.3% [1.3] and average daily pain 6.5 [1.4], were randomised to capsaicin 8% patch (n=186) or placebo patch (n=183). In the primary analysis, a statistically significant greater mean reduction in average daily pain score was observed for capsaicin 8% patch versus placebo from baseline to between Weeks 2-8 (-27.4% versus -20.9%; p=0.025). Greater reductions in weekly average daily pain score were observed from Week 3 onwards with capsaicin versus placebo (p<0.05 each week, except at Week 6). A greater proportion of the capsaicin versus placebo group achieved at least a 30% reduction in average daily pain score from baseline to between Weeks 2-8 (39.8% versus 32.8%; p=0.108), and also Weeks 2-12 (40.9% versus 31.7%; p=0.05). Greater improvements in sleep interference score were observed with capsaicin versus placebo from baseline to between Weeks 2-8 (p=0.030), and also Weeks 2-12 (p=0.020). In addition, greater improvements in weekly sleep interference score were observed with capsaicin versus placebo from Week 6 onwards (p<0.05 each week, except at Week 10). Apart from transient application site reactions, treatment-emergent adverse events (TEAEs) in the capsaicin and placebo arms were very similar, and no discontinuations due to drug-related TEAEs occurred in either arm.
Conclusion: The capsaicin 8% patch provided statistically significant improvements in pain relief and sleep quality compared with placebo in patients with PDPN, with no new safety issues. The STEP study extends the range of peripheral neuropathic pain aetiologies for which the capsaicin 8% patch has demonstrated efficacy and safety.
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Impact of chronic renal failure on the clinical outcome of stem cell therapy in diabetic patients with critical limb ischaemia

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Background and aims: Therapeutic vasculogenesis by autologous stem cell therapy is a new treatment method for diabetic patients with critical limb ischemia (CLI). Chronic renal failure (CRF) can be one of the main factors that influence the prognosis and amputation rates in patients with diabetic foot disease, especially with CLI. The aim of our study was to assess the impact of CRF on the outcomes of stem cell therapy in diabetic patients with CLI.
Materials and methods: Sixty-two patients with CLI persisting after unsuccessful standard revascularization, treated by autologous stem cells in our foot clinic from January 2008 to October 2014 were included in the study. Patients were divided in a CRF group (17 patients) and a non-CRF group (42 patients). Patients with the same inclusion criteria treated conservatively in the same period and diagnosed to have CRF formed a control group (21 patients). CRF was defined as chronic kidney disease stages 4-5 (glomerular filtration rate<29 ml/min/1.73 m2). Mean follow-up was 17.9±13.1 months. Death and major amputation were assessed during the follow-up; the changes in transcutaneous oxygen pressure (TcPO2) were evaluated at 6 months the after cell treatment.
Results: The increase of TcPO2 after 6 months was significantly higher in the non-CRF group (25.7±12.5 mm Hg) in comparison with the CRF group (16.1±11.3 mm Hg, p=0.032) and control group (9.2±5.4 mm Hg, p=0.002). TcPO2 was significantly increased in both cell therapy groups after 6 moths compared to baseline (p=0.0008, p=0.0002), we observed no change in control group. No significant difference in mortality rates during the follow-up between CRF and control groups was observed (5/17 vs. 6/21, NS), non-CRF group revealed significantly lower mortality (6/42, p=0.039); all deaths were without a casual link to cell treatment. The major amputation rate in surviving patients was significantly lower in the non-CRF group compared to the control group (10/36 vs 8/15, p=0.0034), comparison of CRF and control groups revealed no difference (5/12 vs 8/15, NS).
Conclusion: Our study showed that stem cell therapy of patients with CLI and chronic renal failure significantly improved ischemia in comparison with chronic renal patients treated conservatively, but did not influence the mortality and rates of major amputation. Patients with normal renal function treated by stem cells had significantly lower mortality, amputation rate and higher improvement of ischemia. The therapeutic vasculogenesis by autologous stem cells should always be considered in patients on hemodialysis.
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Local application of human stem cells accelerated wound healing in diabetic rat model

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Background and aims: Wounds associated with diabetes are recalcitrant to healing due to many cellular and molecular aberrations as a result of diabetic complications such as neuropathy, immune impairment and

ischemia. There is increasing evidence that bone marrow mononuclear cells (BM-MNC), their subpopulation - mesenchymal stem cells (BM-MSC) and adipose tissue derived mesenchymal stem cells (AT-MSC) can differentiate into haematopoietic and non-haematopoietic cells, that can be related to wound healing. The aim of our study was to compare the process of wound healing in diabetic rats treated with the various types of stem cell suspensions of human BM-MNC, BM-MSC and AT-MSC obtained from diabetic and nondiabetic donors.

Materials and methods: Human BM-MNC, BM-MSC and AT-MSC were isolated from diabetic and nondiabetic donors by standard methods. BM-MNC concentrate, BM-MSC and AT-MSC in the 2nd passage were divided in aliquots and cryopreserved till application to rats. Diabetes in 56 Wistar rats was induced by a single intraperitoneal injection of streptozotocin 64 mg/kg bw and confirmed 48 h later by a blood glucose level higher than 15 mmol/l. 14 days later, the animals were divided into 7 groups of 8 rats. In each animal, one full thickness skin wound 0.8 cm in diameter was created by a punch biopsy device in the area of the back. The cell suspension from diabetic or non-diabetic donors was injected into the base of the wound, and its close surroundings: 1) 0.5 ml of BM-MNC concentrate; 2) 1 million of BM-MSC in a volume of 0.5 ml or 3) 1 million of AT-MSC in volume of 0.5 ml. The seventh group (Controls) of 8 rats undergone all the procedures except the application of cell suspension. All animals had a wound covered with silicon dressing and also were treated daily with subcutaneous long acting insulin and immunosuppression. Photographs and measurements of the wound has been taken for the assessment of the effect of cell therapy on wound healing after 3, 7 and 14 days of the appropriate therapy.

Results: The sizes of the wounds before treatment were comparable in all groups. After 3, 7 and 14 days, there was a significant reduction of wound size in all groups, including controls ($p<0.05$). After 7 days, a significant acceleration of healing in all BM-MNC and BM-MSC groups in comparison with the controls (all $p<0.05$) was seen, but acceleration in both AT-MSC groups was not significant. After 14 days, there was a significant acceleration of wound healing in all cells groups in comparison with controls, the average area reduction was 99.2% and 94.4% in diabetic and nondiabetic BM-MNC groups; analogous 97.8% and 98.6% in BM-MSC; 96.6% and 95% in AT-MSC; but only 83.8% in controls (all $p<0.05$).

Conclusion: In our experiment, the local application of human stem cells derived from bone marrow and adipose tissue accelerated wound healing in diabetic rats compared with controls receiving only standard therapy; a significant difference between the diabetic and nondiabetic cell suspensions was not found. AT-MSC were slower to improve healing after 7 days, but after 14 days, there were not significant differences between the types of used stem cells. Topical treatment of wounds by stem cells could be a promising therapy for patients with diabetes.

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OP 12 Effects of novel therapies on the vasculature

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Dipeptidyl peptidase-4 inhibitor anagliptin alleviates lipotoxicity-induced hepatic insulin resistance and steatohepatitis in mice

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Background and aims: Nonalcoholic fatty liver disease (NAFLD) is a form of a lipotoxic liver injury that can impair systemic insulin resistance and progress to nonalcoholic steatohepatitis (NASH). We previously demonstrated that excessive hepatic lipid accumulation promotes the activation of macrophages/Kupffer cells, resulting in exacerbated insulin resistance and hepatic inflammation. However, there are few promising treatments targeting lipotoxicity-mediated hepatic activation/polarization of macrophages in NASH. Dipeptidyl peptidase-4 (DPP-4) is widely expressed, including in immune cells such as monocytes/macrophages. In this study, to test the hypothesis that DPP-4 plays a role in immune cell-mediated insulin resistance and NAFLD progression to NASH, we examined the effect of anagliptin, a DPP-4 inhibitor, on diet-induced NAFLD.

Materials and methods: Eight-week-old C57BL/6 mice were fed a high-cholesterol/high-fat (CL) diet with or without 0.3% anagliptin (CL + Ana) for 14 weeks. The liver histology, insulin sensitivity, and DPP-4 activity were examined. Next, we quantified intrahepatic immune cells using a fluorescence-activated cell sorter (FACS).

Results: After 14 weeks of feeding, histological examination revealed hepatic steatosis and inflammation in mice fed the CL diet. The mice exhibited hyperinsulinemia and glucose intolerance, indicating that mice fed the CL diet developed NASH associated with insulin resistance. Anagliptin administration improved the hepatic steatosis by decreasing TG and FFA levels by 23% and 50%, respectively. Anagliptin also inhibited the development of hepatic inflammation and fibrosis, lowering F4/80+ macrophage/Kupffer cell infiltration and the hydroxyproline content in the liver. Importantly, hepatic DPP-4 protein expression was increased in mice fed the CL diet compared with mice fed normal chow (NC), while anagliptin significantly suppressed DPP-4 expression. Moreover, in the CL group, the DPP-4 activity increased 2.2-fold and 1.4-fold in the plasma and liver, respectively, compared with the NC group, whereas anagliptin markedly reduced the DPP-4 activity in both plasma and liver by 88% and 65%, respectively ($p<0.01$). Anagliptin administration in mice fed the CL diet led to improved glucose intolerance and hyperinsulinemia and enhanced insulin signal assessed by IRβ and Akt phosphorylation in the liver. These findings were associated with endoplasmic reticulum stress reduction (GRP78/CHOP and phospho-eIF2α) and MAPK (JNK/p38MAPK) and NFkB attenuation in the liver. To assess the effect of anagliptin on hepatic inflammation further, intrahepatic leukocytes were quantified using FACS. Hepatic macrophages identified as CD45+CD11b+F4/80+ cells were decreased in the CL + Ana group by 26% compared with mice fed the CL diet. In addition to a slight reduction of the total macrophage content in the liver, mice fed CL + Ana had 38% fewer CD11c+CD206- (M1)-type macrophages, but 24% more CD11c-CD206+ (M2)-type macrophages than mice fed the CL diet, resulting in a predominance of M2 over the M1 macrophage population.

Conclusion: DPP-4 activity in both liver and plasma is increased significantly in the dietary model of NAFLD associated with insulin resistance. Anagliptin suppresses lipotoxicity-induced aberrant activation of DPP-4 and attenuates hepatic insulin resistance and steatohepatitis by regulating M1/M2 status in hepatic macrophages.

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Dipeptidyl peptidase-4 inhibitor suppresses inflammation in U937 monocytes by inhibiting phosphorylation of caveolin-1 through CD26

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Background and aims: We reported that dipeptidyl peptidase-4 inhibitors (DPP-4i) confer an anti-atherosclerotic effect in apolipoprotein-E null mice by incretin-dependent and -independent manner. To elucidate the molecular mechanisms, we focused on the effect of DPP-4i on expression of proinflammatory cytokines in monocytes.
Materials and methods: Cultured U937 human monocytes were treated with or without 10 ug/ml of Lipopolysaccharide (LPS) and teneligliptin (1-10 nM). Treated cells lysates, and RNA were collected for Western blotting and real-time reverse transcription polymerase chain reaction. Some cells were treated with teneligliptin (5 and 10 nM) or vehicle with LPS, and RNA was subjected to microarray analysis.
Results: Teneligliptin (1-10 nM) widely suppressed the expression of inflammatory molecules, such as IL-1β, IL-6, TNF-α, NF-κB, TLR-4, MCP-1, CCR2, IL-8, IP-10, and RANTES induced by LPS without affecting cell proliferation. Treatments with antibody (Ab) or small interfering (si) RNA against CD26 mimicked anti-inflammatory effect of teneligliptin. Microarray analysis failed to detect a specific molecule to explain anti-inflammatory action of teneligliptin, suggesting posttranslational mechanisms. We nominated caveolin (Cav)-1, a signaling protein associated with caveolae, as a candidate molecule because it has been reported that Cav-1 binds to CD26 via DPP-4 catalytic site, which mediates inflammatory signals. Anti-Cav-1 polyclonal antibody [H97] recognizing 82-178 amino acids of Cav-1 significantly suppressed IL-1β, IL-6, TNF-α, and NF-κB mRNAs, whereas anti-Cav-1 monoclonal antibody [7c8] reacts epitope between residue 32 and the C-terminus of Cav-1 did not. Cav-1 gene expression was not altered by LPS or teneligliptin, however, Cav-1 was phosphorylated by LPS and teneligliptin attenuated LPS-induced p-Cav-1 generation. Likewise, anti-CD26Ab suppressed p-Cav-1generation. Anti-Cav-1Ab [7c8] suppressed slightly but anti-Cav-1Ab [H97] strongly suppressed p-Cav-1 generation by LPS. Induction of siRNAs into CD26 and Cav-1 genes successfully suppressed gene expression of CD26 and Cav-1, which resulted in significantly suppression of LPS-induced expression of IL-1β, IL-6, TNF-α, and NF-κB mRNAs. CD26siRNA and Cav-1siRNA tended to suppressCD86 mRNA, a downstream of NF-κB signal. ERK1/2 was phosphorylated by LPS, and teneligliptin, Anti-CD26Ab and anti-Cav-1Ab [H97] all suppressed p-ERK1/2 generation. Likewise CD26siRNA and Cav-1siRNA suppressed p-ERK1/2 generation. IRAK-4, a master molecule of downstream of TLR-4 signaling, was phosphorylated by LPS. Teneligliptin significantly suppressed LPS-induced p-IRAK-4 generation. Anti-CD26Ab and anti-Cav-1Ab[H97] tended to suppress p-IRAL-4 expression.
Conclusion: The present study suggests that a DPP-4i suppresses LPS-induced inflammation in human monocytes by inhibiting interaction of CD26 and Cav-1, which resulted in suppressed TLR4/ IRAK-4 mediated NF-kB and MAPK pathways.

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Dipeptidyl peptidase 4 inhibition by gemigliptin prevent abnormal vascular remodelling mediated by Nrf2 activation

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Background and aims: Dipeptidyl peptidase-4 (DPP-4) inhibitors exert a potent anti-hyperglycemic effect and reduce cardiovascular risk in type 2 diabetic patients. Several experimental studies have shown that in addition to glucagon-like peptide-1 augmentation, DPP-4 inhibitors including sitagliptin and anagliptin have a beneficial effect in cardiovascular diseases such as atherosclerosis and cardiac infarction that involves reactive oxygen species. Here, we show that gemigliptin, a new DPP-4 inhibitor, can directly attenuate the abnormal proliferation and migration of vascular smooth muscle cells (VSMCs) via enhanced NF-E2-related factor 2 (Nrf2) activity.
Materials and methods: The carotid artery ligation model was performed for the assessment of neointimal hyperplasia in male C57BL/6 N mice treated with different concentrations of gemigliptin in a normal chow diet (0, 0.04, 0.09, and 0.27% in diet). VSMCs were isolated from the thoracic aortas of four-week-old SD rats and the cells from the third to fifth passages were used for in vitro experiments: WST-1 assay, FACS analysis, RT-PCR and Western blotting, and wound healing assay. The expression of DPP-4 in VSMCs was knockdown by small interfering RNA (siRNA) using Lipofectamine® RNAiMAX .
Results: Gemigliptin significantly prevented the neointimal hyperplasia induced by ligation injury in mouse carotid arteries, *in vivo*. Likewise, the proliferation of rat primary VSMCs stimulated by 20% FBS was significantly attenuated by gemigliptin in a dose dependent manner, confirmed by the decreased phospho-Rb leading to G1 cycle cell arrest, consequently. We found that gemiglitin enhanced Nrf2 activity by not only its mRNA expression but also increased Keap1 proteosomal degradation by p62, resulting in the induction of Nrf2 target genes such as NAD(P)H quinone oxidoreductase 1 and heme oxygenase 1. The anti-proliferative role of gemigliptin disappeared in VSMCs with DPP4 siRNA knockdown, indicating that the endogenous DPP4 in VSMCs mediated the effect of gemigliptin. Besides, gemigliptin diminished TNF-α-mediated cell adhesion molecules such as MCP-1, VCAM-1 and MMP2 activity in VSMCs.
Conclusion: Taken together, our data indicate that gemigliptin exerts a prohibitory effect on the proliferation and migration of VSMCs via Nrf2 activity for the prevention of cardiovascular diseases.

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Effects of DPP-4 inhibition on glomerular and tubular function in a rat model of ischaemia-reperfusion injury

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Background and aims: Dipeptidyl peptidase (DPP)-4 inhibitors have protective effects in ischaemia-reperfusion injury (IRI) of the heart and lung. DPP-4 shows strong renal expression and its substrates are present in the kidney. Therefore, we studied the effects of the DPP-4 inhibitors linagliptin, vildagliptin and sitagliptin on the outcome of IRI-induced acute kidney injury in uninephrectomised rats.

Materials and methods: Male Wistar rats obtained from Charles River (Germany) weighing 150–170 g were subjected to uninephrectomy. Two weeks later, the remaining kidney was exposed to IRI by clamping the renal artery for 30 min; sham surgery was performed without clamping. The rats (n=10–14/group) received DPP-4 inhibitor treatment once-daily with either linagliptin (1.5 mg/kg/day), vildagliptin (8 mg/kg/day), sitagliptin (30 mg/kg/day) or vehicle via gavage on the 3 consecutive days prior to IRI. An additional group was treated with sitagliptin until study end in order to inhibit DPP-4 activity during the entire experiment. This group was initially treated with sitagliptin 30 mg/kg/day; after induction of IRI, the dose was adjusted to 15 mg/kg/day because of renal failure.
Results: Levels of active glucagon-like peptide-1 increased 3–4 fold in all DPP-4 inhibitor treatment groups versus placebo up to 24 h after clamping (p<0.05 for all comparisons). Plasma cystatin C, a marker of glomerular filtration rate, peaked 48 h after clamping and increased from 1454±320 ng/ml in the sham-operated group to 1728±323 ng/ml in the placebo-treated group (p=0.059). However, this increase was unaffected by treatment with either linagliptin, vildagliptin, or sitagliptin. In contrast, DPP-4 inhibitor treatment elicited beneficial effects on tubular regeneration. The tubular dilatation score, assessed 1 week after induction of IRI, was significantly reduced in all treatment groups compared with placebo.
Conclusion: In rats with acute IRI of the kidney, DPP-4 inhibition does not alter glomerular function but may enhance tubular regeneration.
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The neuroprotective efficacy of the DPP-4 inhibitor linagliptin against stroke is not mediated by GLP-1 receptor activation

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Background and aims: Diabetes and obesity are strong risk factors for premature and severe stroke. Inhibition of dipeptidyl peptidase (DPP)-4 is a treatment for type 2 diabetes that prevents the inactivation of glucagon-like peptide (GLP)-1 and therefore increases levels of active GLP-1. In addition to their glycaemic actions, DPP-4 inhibitors have been shown to have neuroprotective effects in several experimental models. Specifically, our research group has shown that the DPP-4 inhibitor linagliptin is efficacious against stroke in normal and type 2 diabetic and obese mice. However, the mechanism of action of DPP-4 inhibitor-mediated neuroprotection remains largely unknown. The aim of this study was to determine whether the efficacy of linagliptin against stroke is mediated by GLP-1 receptor (GLP-1R) activation by performing stroke efficacy studies in normal versus knockout mice lacking the GLP-1R ($Glp1r^{-/-}$ mice).
Materials and methods: Adult male mice with or without functional disruption of GLP-1R were treated orally with linagliptin 10 mg/kg/day or vehicle for 7 weeks. There were 4 experimental groups: $Glp1r^{-/-}$ plus linagliptin, n=9; $Glp1r^{-/-}$ plus vehicle, n=8; wild type plus linagliptin, n=9; wild type plus vehicle, n=9. After 4 weeks of treatment, the mice were subjected to 30 min of transient middle cerebral artery occlusion to induce ischaemic stroke. Drug treatment was continued for 3 additional weeks until sacrifice. The severity of the ischaemic damage was measured by evaluating the stroke volume and by stereological counting of NeuN-positive surviving neurons in the striatum and cortex.
Results: Linagliptin treatment resulted in a strong trend, albeit not statistically significant, towards a reduction of ischaemic volume in wild type mice (mean linagliptin=6.5 mm^3 vs mean vehicle=11.8 mm^3). In the $Glp1r^{-/-}$ mice, the effect of linagliptin on the reduction of ischaemic volume reached significance (mean linagliptin=18.8 mm^3 vs mean vehicle=7.9 mm^3; p<0.001). The results also showed that linagliptin treatment resulted in a significant reduction of stroke damage in both wild type (mean linagliptin=2.27×10^6 vs mean vehicle=1.62×10^6; p<0.05) and $Glp1r^{-/-}$ mice (mean linagliptin=2.07×10^6 vs mean vehicle=1.18×10^6; p<0.0005) as measured by the number of surviving neurons in the striatum and cerebral cortex.
Conclusion: These results indicate that linagliptin-induced neuroprotection is mediated by a GLP-1R-independent mechanism. Because DPP-4 inhibitors can also increase the levels of other DPP-4 substrates that may have beneficial effects in the brain, our results should provide a strong rationale for identifying these factors.
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Amelioration of hyperglycaemia with SGLT2i prevents atherosclerosis through suppression of macrophage form cell formation in diabetic mice independent of insulin action

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Background and aims: Although diabetes is one of the leading causes of atherosclerosis, it is poorly understood the direct association between hyperglycaemia and atherosclerosis independent of insulin action. A SGLT2i is a recently developed oral anti-diabetic agent, which reduces plasma glucose levels by increasing urinary glucose excretion without affecting insulin secretion and action. We investigated how amelioration of glycaemia by SGLT2i is associated with macrophage-driven atherosclerosis in mouse models of type 1 and type2 diabetes.
Materials and methods: Male *Apoe-/-* mice receiving injection of streptozotocin (50 mg/kg i.p x 5 days,n=34) or saline (n=31) were randomly assigned to SGLT2i treatment (dapagliflozin, 1.0 mg/kg/day) or vehicle. Male *db/db* diabetic mice were maintained on the food containing SGLT2i (ipragliflozin, 1.0 mg/kg/day, n=14) or none (n=30). After 4 weeks, the peritoneal macrophages (Mφ) migrated by an i.p. injection of thioglycolate were collected to evaluate foam cell formation measured by incorporation of [^{3}H]-oleate into [^{3}H]-cholesteryloleate in the presence of ox-LDL. In the *Apoe-/-* mice, The atherosclerosis was assessed as en face atherosclerotic plaque area determined by oil red O staining. In the diabetic mice, gene expressions related to foam cell formation in Mφ were examined by RT-PCR.
Results: In the type1 diabetic *Apoe-/-* mice, the SGLT2i decreased the fasting blood glucose (FBG) , HbA1c, and glucose-AUC after oral glucose tolerance test (OGTT) compared to vehicle. The body weight, daily food intake, systolic blood pressure, or insulin and lipids were not changed by treatment with the SGLT2i in the diabetic and non-diabetic mice. In the diabetic *Apoe-/-* mice, the atherosclerotic lesions and foam cell formation were accelerated compared to the non-diabetic counterparts (2-3 folds, p<0.01), and significantly suppressed by the SGLT2i administration (33-34%, p<0.01). The atherosclerotic lesions was highly correlated to the degree of foam cell formation (r=0.95, p<0.01, n=15) and the HbA1c levels (r=0.86, p<0.01, n=15), but insignificantly to the cholesterol levels (r=0.31, p=0.12). In the *db/db* type 2 diabetic mice with hyperinsulinemia, the SGLT2i decreased the body weight, levels of plasma triglycerides, FBG, and HbA1c, and the glucose AUC with increased water intake by 3 times and doubled urine volume. Foam cell formation was suppressed by the SGLT2i (34%, p<0.01), which correlated to the HbA1c levels (r=0.88, p<0.01, n=36), but insignificantly to lipid levels (r=-0.46, p=0.06) or insulin (r=-0.15, p=0.56). Gene expressions of scavenger receptors, CD36 and Lox-1, and ACAT-1, a rate limiting enzyme for cholesterol esterification were up-regulated, while ABCA1, a key molecule for cholesterol efflux was down-regulated in

Mφ obtained from both diabetic mice. The SGLT2i decreased expressions of scavenger receptors and ACAT-1 by approximately 30 to 40% and increased ABCA1 by 42%.
Conclusion: The SGLT2is exert anti-atherogenic effects by normalization of blood glucose levels in both type1 and type2 diabetic mice through preventing foam cell formation of Mφ, which is independent of insulin action. Our results suggest that the foam cell formation regulated by scavenger receptors, ACAT-1, and cholesterol efflux is highly sensitive to glycaemia *ex.vivo*.

OP 13 Growing experience with GLP-1 receptor agonists

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Endogenous GLP-1 and treatment with liragluitde affect activation in reward and satiety related brain areas in response to palatable food

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Background and aims: Central nervous system (CNS) activation in response to food cues may play an important role in the regulation of food intake. Obesity is associated with a deficit in responsivity to palatable food in reward areas of the CNS, which may lead to an increase in food intake, as a means to compensate for understimulation of the CNS reward system. It is suggested that alterations in these CNS responses may contribute to the development of obesity and type 2 diabetes (T2DM). The gut-derived hormone glucagon-like peptide-1 (GLP-1) and GLP-1 receptor agonists (GLP-1RA) reduce appetite and body weight. It has been suggested that these effects are, at least to some extent, mediated by effects on the CNS. We hypothesized that, in humans, endogenous GLP-1 and treatment with a GLP-1RA influence activation in reward and satiety related areas in the CNS in response to intake of palatable food.
Materials and methods: Using functional magnetic resonance imaging (fMRI), we determined the effects of endogenous GLP-1 and treatment with the GLP-1 analogue liraglutide on acute CNS activation to the receipt of chocolate milk. In the first study, infusion of the GLP-1 receptor antagonist exendin 9-39, was used to block endogenous GLP-1 effects in healthy lean individuals (n=20, mean ± SD age 56.3±6.2 yrs, BMI 22.5± 1.7 kg/m2, 10 males) and obese patients with T2DM (n=20, mean ± SD age 59.3±4.1 yrs, BMI 32.0±4.7 kg/m2, 11 males). Infusion with exendin 9-39 was compared to placebo infusion, which were performed on two separate test visits. In the second study, in a cross-over design with only the T2DM patients, we compared CNS activation between treatment with liraglutide 1.8 mg and insulin glargine at two time points, i.e. after 10 days and 12 weeks of treatment.
Results: Reduced activation in response to the receipt of chocolate milk was observed in T2DM patients, compared to lean individuals, in right insula (p=0.04). Blockade of endogenous GLP-1 effects blunted the activation to chocolate milk receipt in healthy lean individuals in bilateral insula (right p=0.01, left p=0.03). Increased activations in response to chocolate milk were observed after 10 days of treatment with liraglutide, compared to insulin, in right insula (p=0.03) and right caudate nucleus (p=0.01), but these effect ceased to be significant after 12 weeks of treatment.
Conclusion: We found effects of GLP-1 and liraglutide in the insula and caudate nucleus, areas implicated in CNS reward and the central regulation of food intake. Our results indicate that endogenous GLP-1 contributes to the central regulation of feeding. Furthermore, compared to insulin, short-term treatment with the GLP-1 analogue liraglutide may improve the observed deficit in the responsivity of the CNS to palatable food in obese T2DM patients, which may contribute to the induction of weight loss observed during this treatment. However, there were no long-term effects of liraglutide on CNS activation. This may explain why weight loss does not proceed after the initial treatment period with liraglutide 1.8 mg.
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Supported by: Novo Nordisk

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A randomised double-blind trial of liraglutide to control HbA_{1c} in subjects with type 2 diabetes treated with multiple daily insulin injections (MDILiraglutide trial)

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Background and aims: Multiple daily insulin injections (MDI) are generally a final treatment option in subjects with type 2 diabetes (T2D) and poor glycemic control, but are often associated with weight gain and increased risk of hypoglycemia. Many individuals also have significantly reduced insulin production and do not reach glycemic targets. Randomized controlled trial data are lacking on whether adjunctive glucose lowering agents can improve glycemic control in these individuals.
Materials and methods: In total, 124 subjects with T2D treated with MDI at 14 hospitals in Sweden were randomized to liraglutide (L) or placebo (P) for 24 weeks. The primary endpoint was change in HbA1c from baseline to week 24 estimated by ANCOVA, adjusted for baseline HbA1c.
Results: Mean age at baseline was 63.8 and 63.6 years, HbA1c 74.6 mmol/mol (9.0%) and 74.4 mmol/mol (9.0%), BMI 33.7 and 33.5 kg/m^2 and total insulin dose 105.3 and 105.6 units in the L and P groups, respectively. In the Intention to Treat Analysis, HbA1c was reduced by 16.9 mmol/mol (1.5%) in the L group, compared to 4.6 mmol/mol (0.4%) in the P group, difference -12.3 mmol/mol (-1.1%, p< 0.0001). There were more in the L group (42.9%) than in the P group (5.1%), who reached HbA1c <53 mmol/mol=7.0% (p<0.0001). Body weight was reduced by 3.8 kg in L but not P subjects, +0.0 kg, difference -3.8 kg (p<0.0001). Total insulin dose was reduced by 18.1 U with L and 2.3 U with P, difference -15.8 U (p<0.0001). Fasting and postprandial glucose levels were lower in the L than P group, difference -1.5 and -2.0 mmol/l, respectively (p<0.01 for both). The mean glucose levels (p< 0.001) and standard deviation of glucose levels (p=0.001) estimated by masked continuous glucose monitoring were significantly lower in the L than P group, -1.9 and -0.5 mmol/l, respectively. There were no severe hypoglycaemia in either group. No significant difference existed in symptomatic or asymptomatic non-severe hypoglycaemias with values <4.0 or <3.0 mmol/l between the L and P groups. The most common type of hypoglycaemia was non-severe symptomatic hypoglycaemia <4.0 mmol/l, mean number during follow-up 1.29 and 1.24 in the L and P Groups respectively (p=0.96).
Conclusion: Adding liraglutide to individuals with T2D treated with MDI improves glycemic control without increased risks of hypoglycaemia, reduces weight and patients can lower their insulin doses.
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Supported by: Research initiated trial partly funded by Novo Nordisk

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Once-daily liraglutide vs lixisenatide as add-on to metformin in type 2 diabetes: a 26-week randomised controlled clinical trial

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Background and aims: Liraglutide (lira) and lixisenatide (lixi) are GLP-1 receptor agonists for the treatment of type 2 diabetes mellitus (T2DM), both to be injected once daily. The aim of the trial was to compare the efficacy and safety of lira vs lixi, as add-on to metformin (MET) in patients with T2DM not achieving adequate glycaemic control on MET alone.
Materials and methods: This was a 26-week, randomised, parallel group, open label trial. Patients (age ≥18 years, HbA1c 7.5−10.5% [58−91 mmol/mol], BMI≥20 kg/m^2) with T2DM were randomised 1:1 to lira 1.8 mg or lixi 20 μg as add-on to MET (maximum tolerated dose, 1000−3000 mg daily). Dose escalation and administration of lira and lixi were according to the approved label for both drugs at the time of the trial. Lira was administered once daily at any time of the day irrespective of meals. Lixi was administered once daily, within an hour prior to the morning or evening meal.
Results: 404 patients (mean age 56 years, male/female 60%:40%, BMI 35 kg/m^2, HbA1c 8.4% [68 mmol/mol], T2DM duration 6.4 years) were randomised. At Week 26, lira reduced mean HbA1c significantly more than lixi did (estimated treatment difference: −0.62%, 95% CI: −0.80 to −0.44; p <0.0001), with more patients reaching HbA1c goals of <7% and ≤6.5%, compared to lixi (Table). Lira was associated with greater improvement in the HOMA-B index. As HOMA-B was measured in the fasting state, the improvement was possibly determined by differences in drug exposure due to different half-lives of lira and lixi. Lira reduced fasting plasma glucose more than lixi. Greater reduction in mean 9-point self-measured plasma glucose was seen with lira. However, lixi had smaller post-prandial increments for the meal following injection compared to lira. Both drugs promoted similar body weight decrease (−4.3 kg for lira and −3.7 kg for lixi; p=ns). Systolic and diastolic blood pressure similarly decreased in both groups. The safety profile was overall similar between the two groups. The most common adverse events were gastrointestinal disorders (nausea: 21.8% for lira and lixi; diarrhoea: 12.4% for lira and 9.9% for lixi). Confirmed hypoglycaemic episodes (severe hypoglycaemia or PG <3.1 mmol/L) were rare (4 events in 3 patients for lira, and 8 events in 5 patients for lixi, p=ns), with no severe hypoglycaemic episodes.
Conclusion: Liraglutide was more efficacious than lixisenatide as add-on to MET in achieving glycaemic control, and may be associated with greater improvement in beta-cell function in patients with T2DM. Body weight and blood pressure reductions were similar, and both treatments were well-tolerated with low risk of hypoglycaemia and similar gastrointestinal adverse events profiles.

Table. Effect of liraglutide vs lixisenatide in patients with T2DM after 26 weeks of treatment

	Lira (n=202)	Lixi (n=202)	Lira–Lixi		
	Estimated change from baseline*	Estimated change from baseline*	Estimated difference*	95% CI	P-value*
HbA_{1c} (%)	-1.83	-1.21	-0.62	[-0.80; -0.44]	<0.0001
HbA_{1c} (mmol/mol)	-20.02	-13.28	-6.74	[-8.70; -4.78]	<0.0001
% of patients with HbA_{1c} < 7%	74.2	45.5	4.16§	[2.58; 6.73]	<0.0001
% of patients with HbA_{1c} ≤ 6.5%	54.6	26.2	3.66§	[2.31; 5.81]	<0.0001
FPG (mmol/L)	-2.85	-1.70	-1.15	[-1.51; -0.80]	<0.0001
Mean of 9-point SMPG (mmol/L)	-2.64	-1.89	-0.75	[-1.08; -0.42]	<0.0001
Post-breakfast increment for lixi morning injection (mmol/L) (n=134)	-0.88	-2.12	1.24	[0.69; 1.79]	<0.0001
Post-dinner increment for lixi evening injection (mmol/L) (n=43)	-0.53	-1.88	1.36	[0.44; 2.27]	0.0039
HOMA-B (%)	1.66#	1.29#	1.29§	[1.14; 1.45]	<0.0001
Body weight (kg)	-4.26	-3.67	-0.59	[-1.55; 0.38]	0.2347
Systolic blood pressure (mmHg)	-4.70	-3.49	-1.21	[-3.87; 1.45]	0.3722
Diastolic blood pressure (mmHg)	-2.62	-2.69	0.07	[-1.53; 1.67]	0.9318

95% CI: 95% confidence interval. FPG: fasting plasma glucose. HOMA-B: homeostatic model assessment-beta cell function. SMPG: self-measured plasma glucose. #: numbers are ratio to baseline. §: numbers are odds ratio for reaching HbA_{1c} targets, and estimated treatment ratio for HOMA-B (lira/lixi). *: all endpoints were analyzed using mixed model for repeated measurement with visit, treatment and country as fixed factors and baseline as a covariate, all nested within visit; except for the binary endpoints % of patients with HbA_{1c} < 7% and ≤6.5%, respectively, which were analysed using a logistic regression model with the same fixed factors and baseline HbA_{1c} as a covariate. Post-breakfast and post-dinner increments analysis included all liraglutide treated subjects regardless of injection time (n=202), and all lixisenatide subjects injecting in the morning (n=134) or in the evening (n=43) respectively.

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Continuous glucose monitoring in type 2 diabetes patients treated with once weekly dulaglutide or once daily glargine, both combined with insulin lispro (AWARD-4 substudy)

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Background and aims: Since higher glycaemic variability may predict potentially greater risk for both hypo- and hyperglycaemia, we compared continuous glucose monitoring (CGM) metrics during treatment with 1.5 mg and 0.75 mg doses of once weekly GLP-1 receptor agonist dulaglutide (DU) vs daily glargine GLA), both with prandial lispro (±metformin), in a subset of patients with type 2 diabetes (T2D) enrolled in the AWARD-4 trial.
Materials and methods: The study population comprised patients with T2D uncontrolled on conventional insulin regimens. Participants (n=144) had mean baseline age of 59.9 y, HbA1c of 8.57% [70.2 mmol/mol], and a BMI of 32.9 kg/m^2. A CGMS® iPro™ was used for 3 days, at baseline and 26 (primary endpoint) and 52 weeks after randomisation, to estimate between-group differences in 24 h glycaemic ranges, thresholds, and variability. Study participants also performed 8-point self-monitored blood glucose (SMBG).
Results: The mean decreases from baseline to 26 weeks in CGM glucose (see Table) were similar to those from 8-point SMBG (LSM±SE; DU 1.5 mg -3.3±0.4 mmol/L; DU 0.75 mg -3.1±0.3 mmol/L; GLA -2.8±0.3 mmol/L), with no significant differences between treatments. These results were similar at 52 weeks. Increases in % time within the 3.9-7.8 mmol/L range were not different between treatments; however, DU 1.5 mg increased % time within the 3.9-10.0 mmol/L range significantly more than GLA at 26 weeks. Treatment effect on % time <3.9 mmol/L at 26 weeks were not different, but at 52 weeks treatment with GLA significantly increased % time <3.9 mmol/L versus both DU doses. DU 1.5 mg had a greater reduction in glycaemic variability as measured by within-patient standard deviation at 26 weeks versus GLA with no significant differences between treatments at 52 weeks. Total mean amplitude of glucose excursions (MAGE) was not different between treatments.
Conclusion: At primary endpoint, after 26 weeks, DU 1.5 mg compared to GLA resulted in greater increase in % time with CGM glucose in the 3.9-10.0 mmol/L range and with lower glycaemic variability. Both dulaglutide doses were associated with smaller increase in % time <3.9 mmol/L at final endpoint (52 weeks). These results are consistent with reported lower risk of clinical hypoglycaemia with DU 1.5 mg versus GLA in the overall AWARD-4 but further investigations are need to evaluate potential causal relationship between the observed changes in glucose variability and episodes of hypoglycaemia.

CGM Glycaemic Measures Mean (SD) for BL values LS mean (SE) for 26 and 52 weeks	DU 1.5 mg (n=42)	DU 0.75 mg (n=50)	Glargine (n=52)
Mean CGM glucose (mmol/L)			
• Baseline	10.7 (2.6)	9.9 (2.4)	10.2 (2.7)
• Δ at 26 weeks	-2.8 (0.3)	-2.4 (0.3)	-2.5 (0.3)
• Δ at 52 weeks	-2.2 (0.3)	-2.3 (0.3)	-2.5 (0.3)
% Time 3.9–7.8 mmol/L glucose range			
• Baseline	22.0 (22.0)	26.0 (21.4)	29.9 (23.5)
• Δ at 26 weeks	33.9 (3.9)	22.8 (3.6)	25.2 (3.5)
• Δ at 52 weeks	22.4 (4.1)	22.0 (3.8)	26.1 (3.7)
% Time 3.9–10.0 mmol/L glucose range			
• Baseline	40.7 (25.0)	52.9 (26.4)	52.2 (27.4)
• Δ at 26 weeks	34.9 (3.1)#	24.3 (2.9)	24.7 (2.8)
• Δ at 52 weeks	24.1 (3.3)	25.6 (3.0)	21.6 (3.0)
% Time <3.9 mmol/L			
• Baseline	2.7 (10.8)	2.8 (6.5)	1.9 (3.2)
• Δ at 26 weeks	0.4 (1.2)	2.9 (1.1)	2.7 (1.1)
• Δ at 52 weeks	1.3 (1.3)#	1.1 (1.2)#	4.5 (1.2)
% Time >10.0 mmol/L			
• Baseline	55.7 (27.2)	43.0 (27.8)	44.8 (28.5)
• Δ at 26 weeks	-34.6 (3.2)	-27.2 (2.9)	-27.7 (2.9)
• Δ at 52 weeks	-25.8 (3.4)	-26.7 (3.1)	-26.6 (3.1)
% Time >13.9 mmol/L			
• Baseline	19.5 (20.1)	14.7 (18.4)	17.4 (21.7)
• Δ at 26 weeks	-15.0 (1.4)	-13.8 (1.3)	-12.7 (1.3)
• Δ at 52 weeks	-13.2 (1.5)	-14.3 (1.4)#	-10.5 (1.4)
Area below 3.9 mmol/L glucose (min·mmol/L)			
• Baseline	44.5 (228.1)	37.4 (101.7)	14.5 (26.6)
• Δ at 26 weeks	-10.9 (16.5)	27.9 (15.3)	6.5 (15.1)
• Δ at 52 weeks	-0.3 (13.8)	-3.4 (12.9)	24.9 (12.7)

2-sided p<.05 vs glargine
Abbreviations: Δ = change from baseline; % = percentage; CGM = continuous glucose monitoring; DU = dulaglutide; GLA = insulin glargine; LS = least squares; SD = standard deviation; SE = standard error.

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Once weekly dulaglutide does not increase the risk for cardiovascular (CV) events in type 2 diabetes: a prespecified meta-analysis of prospectively adjudicated CV events

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Background and aims: Patients with type 2 diabetes have a 2- to 4-fold greater risk for cardiovascular disease than those without diabetes. Dulaglutide is a once weekly glucagon-like peptide-1 receptor agonist recently approved for treatment of type 2 diabetes. This meta-analysis evaluates the cardiovascular risk in type 2 diabetes patients treated with dulaglutide in 9 randomised safety and efficacy trials (mean [median] treatment duration was 333 [358] days).
Materials and methods: Reported cardiovascular events were independently adjudicated by a blinded clinical endpoint committee. The primary endpoint was a composite of death due to cardiovascular causes, nonfatal MI, nonfatal stroke, or hospitalisation for unstable angina (4-component major adverse cardiovascular event or MACE). Additional prespecified endpoints, composite and individual, included adjudicated coronary revascularisations, hospitalisation for heart failure, or all-cause mortality. Cox proportional hazards regression model stratified by study was used to estimate the hazard ratio (HR) and confidence interval (CI); the model included treatment as a fixed effect with 2 levels for the factor (dulaglutide or control).
Results: The analysis included 6010 randomised patients (dulaglutide: 3885; all comparators [active or placebo]: 2125); cumulative exposure to dulaglutide or control was 3941 and 2223 patient-years, respectively. The demographic and baseline cardiovascular disease characteristics were well-balanced across groups. Twenty-six (0.67%) patients in the dulaglutide group vs 25 (1.18%) in the all comparators group experienced a primary MACE-4 event. Treatment with dulaglutide was not associated with an increase in the risk of experiencing a MACE-4 endpoint compared with control therapies (HR: 0.57; multiplicity-adjusted (98.02%) CI: 0.30, 1.10) (Table). Results for the 3- and 6-component MACE, and all-cause mortality were consistent with the primary analysis (HR <1.0 for

all). In the dulaglutide group, 23 (0.59%) patients experienced a 3-component MACE event (composite of death from CV causes, nonfatal MI, or nonfatal stroke) compared to 21 (0.99%) in the all comparators group (HR: 0.60; 95% CI: 0.33, 1.08).

Conclusion: These results suggest that dulaglutide does not increase the risk of MACE events in type 2 diabetes patients. The ongoing cardiovascular outcomes study, REWIND, will further assess cardiovascular safety of dulaglutide.

Table. Summary of 4-component MACE analysis

Endpoint Component	All Dulaglutide (N=3885) n (%)	All Comparators (N=2125) n (%)	Hazard Ratio[a] Estimate (adj. 98.02% CI)	Treatment Comparison p value[a]
Primary 4-Component MACE Endpoint	26 (0.67)	25 (1.18)	0.57 (0.30, 1.10)	.046
- Death from CV Causes[b]	3 (0.08)	5 (0.24)	0.35 (0.07, 1.87)	.119
- Nonfatal MI	9 (0.23)	14 (0.66)	0.35 (0.13, 0.95)	.014
- Nonfatal Stroke	12 (0.31)	4 (0.19)	1.61 (0.42, 6.20)	.411
- Hospitalization for Unstable Angina	3 (0.08)	6 (0.28)	0.28 (0.05, 1.46)	.054

a Calculated from a stratified Cox Proportional Hazards regression model; the p-values should be compared to the significance level of 0.0198 that resulted from adjusting for a second possible meta-analysis

b Death from CV causes was defined as a death resulting from an acute MI, sudden cardiac death, death due to heart failure, death due to stroke, and death due to other CV causes.

Source: Table 5.1, Dulaglutide CV Meta-analysis report.

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Advancing basal insulin glargine with prandial lixisenatide QD vs insulin glulisine QD or TID in type 2 diabetes: the GetGoal-Duo2 evidence-based trial

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Background and aims: To provide evidence on how to advance basal insulin (BI).

Materials and methods: We explored treatment options in poorly controlled, BI-treated (≥6 mo±1-3 OADs), predominantly obese adults with type 2 diabetes mellitus (T2DM) randomized to lixisenatide 20 μg QD (LIXI), insulin glulisine QD (GLU-1) or GLU TID (GLU-3), all added to insulin glargine (IG)±metformin, if HbA_{1c} remained ≥7-≤9% after a 12-week IG optimization run-in period after stopping other OADs. Co-primary endpoints at 26 weeks were (1) non-inferiority (95% CI upper bound <0.4%) in HbA_{1c} reduction with LIXI vs GLU-1 and (2) for LIXI vs GLU-3, either non-inferiority in HbA_{1c} reduction (2a) OR superiority (one-sided α=0.025) in body weight change (2b). FPG, PPG, IG dose, AEs and hypoglycaemia were assessed. Each arm randomized 298 pts (T2DM duration 12 yrs, BI duration 3 yrs, weight 89 kg).

Results: All co-primary endpoints were met as LIXI was non-inferior to GLU-1 and GLU-3 for HbA_{1c} reductions and statistically superior to both for body weight loss (Table). Documented hypoglycaemia was numerically and significantly lower with LIXI than with GLU-1 and GLU-3, respectively.

Conclusion: BI plus LIXI may become a preferred option to advance BI, attaining meaningful glycaemic targets with less hypoglycaemia and without negative impact on weight vs prandial insulin as Basal Plus or Basal Bolus for uncontrolled, BI-treated T2DM.

Table

	Lixisenatide 20 μg QD + insulin glargine (n=297)	Insulin glulisine QD + insulin glargine (n=298)	Insulin glulisine TID + insulin glargine (n=295)
FPG, mmol/L			
Screening (start run-in) mean ± SD	9.16 ± 2.94	9.28 ± 2.88	9.51 ± 2.96
BL (end run-in) mean ± SD	6.58 ± 1.83	6.85 ± 1.99	6.65 ± 1.89
Week 26 (LOCF) mean ± SD	6.59 ± 1.96	6.66 ± 1.94	6.71 ± 2.02
LS mean [95% CI] tx difference	–	−0.01 [−0.319, 0.298]	−0.17 [−0.475, 0.143]
2-h PPG post test meal, mmol/L*			
BL (end run-in) mean ± SD	14.12 ± 3.62	13.82 ± 3.52	14.56 ± 3.48
Week 26 (LOCF) mean ± SD	10.19 ± 3.91	12.20 ± 3.36	12.69 ± 3.85
LS mean [95% CI] tx difference	–	−2.07 [−3.288, −0.848]	−2.23 [−3.392, −1.065]
HbA_{1c},%			
Screening (start run-in) mean ± SD	8.51 ± 0.72	8.49 ± 0.72	8.51 ± 0.78
BL (end run-in) mean ± SD	7.76 ± 0.56	7.72 ± 0.58	7.79 ± 0.60
Week 26 (LOCF) mean ± SD	7.17 ± 0.77	7.21 ± 0.79	6.96 ± 0.73
LS mean [95% CI] tx difference	–	**−0.05 [−0.17, 0.06]†**	**0.21 [0.10, 0.33]†**
Insulin glargine dose, U/day			
Screening (start run-in) mean ± SD	41 ± 22	40 ± 22	39 ± 21
BL (end run-in) mean ± SD	67 ± 32	65 ± 32	65 ± 27
Week 26 (LOCF) mean ± SD	67 ± 36	64 ± 36	61 ± 29
LS mean [95% CI] tx difference	–	0.8 [−1.4, 2.9]	3.8 [1.7, 6.0]
Insulin glulisine dose, U/day			
Week 26 (LOCF) mean	–	10	20
Body weight, kg			
BL mean ± SD	90.1 ± 17.4	88.4 ± 15.9	90.0 ± 17.2
Week 26 (LOCF) mean ± SD	89.4 ± 18.1	89.3 ± 16.3	91.3 ± 17.3
LS mean [95% CI] tx difference	–	−1.7 [−2.3, −1.1]	**−2.0 [−2.6, −1.4]**
(p-value vs lixisenatide)	–	(p<0.0001)	**(p<0.0001)†**
Documented symptomatic hypoglycaemia (plasma glucose <60 mg/dL)‡			
% pts	31.5	37.5§	44.6¶
Number of events	325	384	595
Number of events/pt yrs	2.2	2.6	4.1
Estimated rate ratio	–	0.8	0.5
Lixisenatide:insulin glulisine [95% CI]	–	[0.5, 1.1]**	[0.3, 0.7]††
Severe hypoglycaemia, number of pts with events	0	2	0
Gastrointestinal AEs (number of pts with event)‡			
Nausea	25% (75)	2% (5)	1% (3)
Vomiting	9% (26)	2% (5)	2% (6)
Diarrhoea	7% (20)	3% (10)	1% (4)

*Subset of the mITT population treated with lixisenatide or insulin glulisine before breakfast; †**co-primary endpoints**; ‡safety population; §p=0.144; ¶p=0.001; **p=0.123; ††p<0.0001 vs lixisenatide.
AEs, adverse events; BL, baseline; CI, confidence interval; FPG, fasting plasma glucose; HbA_{1c}, glycated haemoglobin; LOCF, last observation carried forward; LS, least squares; mITT, modified intent to treat; PPG, postprandial plamsa glucose; QD, once daily; SD, standard deviation; TID, thrice daily; tx, treatment.
n numbers are for the mITT population (all pts who received ≥1 dose of study medication, with both a baseline assessment and ≥1 post-baseline assessment).

Clinical Trial Registration Number: NCT01768559

OP 14 The interplay between liver fat and insulin action

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Adverse impact of visceral fat on lipid and metabolic profile in fatty liver disease

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Background and aims: Abdominal/visceral (VF) adiposity is a major risk factor for metabolic syndrome and type 2 diabetes and could be implicated in onset and development of non alcoholic fatty liver disease (NAFLD). However, the relative role in the progression of liver damage and dysfunction is still unknown. Insulin resistance, lipotoxicity and inflammation are the main events that occur in FLD. The aim of this work was to evaluate the impact of visceral fat on histological liver damage, lipid profile and metabolic alterations in patients with proven NAFLD.
Materials and methods: In 34 non diabetic subjects with biopsy proven non alcoholic FLD and 8 controls (CT) we measured visceral, subcutaneous and hepatic fat by MRI, plasma concentrations of triglycerides (TG), total cholesterol, free fatty acids (FFA), and GGT. Gas chromatography mass spectrometry was used to assess FFAs composition. From plasma FFA we calculated the de novo lipogenesis index (DNL= palmitic/linoleic acid) and unsaturated to saturated fat ratio (PUFA/SFA). By use of tracers we evaluated lipolysis and endogenous glucose production (EGP) and calculated adipose tissue insulin resistance (Adipo-IR= Lipolysis x Insulin) and hepatic insulin resistance (Hep-IR=EGP x Insulin). Histology was scored according to Kleiner.
Results: Of the 34 recruited patients 10 were without fibrosis (F0), 24 with fibrosis score 1 to 4 (F1-4). Fibrosis was associated with a worse metabolic profile, with increased both adipo-IR (8.3±4.3 vs 7.4±6.0 vs 3.6±1.4) and HOMA index (3.2±1.9 vs 2.6±1.2 vs 1.3±0.4) in F1-4 vs F0 vs CT (all $p<0.05$ for F1-4 vs CT). In addition patients with F1-4, compared to F0 and CT, had higher VF (2.9±1.1 vs 2.1±0.6 vs 0.7±0.4 kg, $p<0.03$). VF and hepatic fat correlated with Adipo-IR ($r=0.42$, $r=0.64$) and HOMA index ($r=0.42$, $r=0.52$) and with increased plasma levels of TG ($r=0.53$, $r=0.52$) and DNL ($r=0.46$, $r=0.52$) all $p<0.05$. Also alterations in FFA composition, in particular reduced PUFA/SFA ratio, were associated with higher VF ($r=-0.41$) and hepatic fat ($r=-0.57$ $p<0.005$). Moreover, VF and hepatic fat correlated positively with MCP-1 ($r=0.52$, $r=0.55$) and oxLDL ($r=0.37$, $r=0.25$) and negatively with adiponectin ($r=-0.49$, $r=-0.53$), all $p<0.05$, documenting a pro-inflammatory profile.
Conclusion: In patients with NAFLD, hepatic fat is associated with metabolic derangements and HOMA, but Adipo-IR and visceral fat accumulation appear to provide a major contribution to liver damage and is associated with an adverse lipid and inflammatory profile.
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In massively obese patients with a metabolically healthy phenotype the prevalence of non alcoholic fatty liver disease is high and associated with sympathetic overactivity

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Background and aims: Metabolically healthy obese (MHO) individuals are relatively insulin sensitive, normotensive and have favourable glucose and lipid profiles. Obesity and metabolic syndrome (MS) were reported to be associated with sympathetic nervous system overactivity. Non alcoholic fatty liver disease (NAFLD) is considered as the hepatic component of MS. The role of inflammation in the development of steatohepatitis (NASH) and the relation with sympathetic activity and metabolic disorders has been suggested. This study aimed to determine whether in massively obese patients with MHO phenotype inflammatory markers, insulin resistance, cortisol and sympathetic activity are associated with NAFLD.
Materials and methods: In a prospective study of patients undergoing bariatric surgery, liver biopsies were obtained peroperatively. We included 66 patients (54 women) aged 36.4±9.6 years, body mass index 44.7± 0.9 kg/m^2. All of them were free of other liver disease. Liver changes were classified by histological analysis into 3 groups: normal liver, simple steatosis and steatohepatitis (NASH). MHO phenotype was defined as no or only one feature of the metabolic syndrome (IDF criteria). Biological measurements included plasma liver enzymes, lipids, glucose, cortisol, inflammatory markers (C-reactive protein (CRP), fibrinogen) and HOMA-IR. Sympathetic activity was assessed by plasma epinephrine and norepinephrine at fasting and 2 hours after an oral glucose challenge.
Results: Among the 66 patients 34 had steatosis and 16 NASH. Twenty-two patients had definite MHO status, including 8 with histologically normal liver, 11 with steatosis and 3 NASH. Among MHO patients, those with steatosis or NASH had higher post-glucose norepinephrine, afternoon cortisol levels and CRP levels ($p=0.04$, 0.01, 0.05 respectively). No other significant difference was detected between these patients and those with normal liver.
Conclusion: The prevalence of liver disease is high in massively obese patients with MHO phenotype. Those with NAFLD exhibit a more marked cluster of inflammation, hypercortisolism and sympathetic activity.

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The link between obesity and insulin resistance by hepassocin

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Background and aims: The prevalence of obesity has increased in most of the world over the past few decades, and the excess of body fat is associated with adverse health consequences. Numerous studies indicated that inflammation-mediated adipokines or hepatokines release led to the development of insulin resistance, the relationship among these factors was still obscure. Hepassocin was previously discovered as a hepatokine that exerted a lipogenic activity and led to insulin resistance. A recent study indicated that hepassocin also expressed in the adipose tissue. However, the role of hepassocin between obesity and insulin resistance is still obscure
Materials and methods: A total of 150 subjects with normal weight, overweight, and obesity (n=50 for each group) were enrolled. Serum hepassocin concentrations were measured by enzyme-linked immunosorbent assay. Overexpression or knockdown of hepassocin in the adipose tissue of high fat diet-fed mice was achieved by lenti-viral vector transfection to evaluate the effects of hepassocin in fat pad. In addition, 3 T3-L1 adipocytes were used to investigate the possible mechanisms in hepassocin-induced lipogenesis, and to clarify the role of hepassocin in obesity. Moreover, HepG2 cells were treated with hepassocin recombinant protein to evaluate the effect of hepassocin on hepatic insulin sensitivity.
Results: The serum concentrations of hepassocin were significantly increased in subjects with overweight, or obesity as compared with normal

weight group (P for trend <0.01). The expression of hepassocin was detected in the epididymal fat, and the epididymal hepassocin expression was increased in high fat fed mice, as compared with chow group. Interleukin-6 treatment induced the expression of hepassocin in 3 T3-L1 adipocytes. In addition, treatment with hepassocin in 3 T3-L1 adipocytes significantly increased the lipogenesis through an ERK1/2-dependent pathway. Overexpression of hepassocin in epididymal adipose tissue significantly increased the size of the fat pad in mice. Furthermore, hepassocin disrupted insulin signaling to increase glucose production in HepG2 hepatocytes.
Conclusion: Inflammation in adipose tissue increased the expression of hepassocin and the elevated hepassocin in circulation further led to the insulin resistance in liver, and affected hepatic glucose production. In addition, hepassocin increased lipogenesis in adipocytes to induce obesity. Thus, we provided a novel mechanism of obesity-induced insulin resistance by hepassocin.

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Glucagon-Like Peptide-1 (GLP-1) and its receptor agonist, exendin-4, affect cholesterol metabolism by regulating SREBP2 in hepatocyte
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Background and aims: Type2 diabetes mellitus (T2DM) causes dyslipidemia characterized by enhance of production of very low-density lipoprotein (VLDL) and LDL, and decrease of high-density lipoprotein (HDL). Glucagon-like peptide-1 (GLP-1) has been implicated in decreasing postprandial Apo-B48, chylomicron, triacylglycerol in animal models and patients with T2DM, by reducing intestinal lipoprotein production. To date, GLP-1 has some effects on cholesterol metabolism, but it remains to be completely elucidated. In this study, we investigated the effect of GLP-1 on cholesterol metabolism in liver of mice and Huh7 hepatocytes.
Materials and methods: (1) in vivo study, ten-weeks-old male C57BL/6J mice (n=16) were treated with the agonist of GLP-1 receptor, exendin-4 (ex-4, 10 nmol/kg body weight) or saline (control) by intraperitoneal injection for 5 days. Serum biological analysis was performed. Hepatic cholesterol contents were determined by Folch method. Protein levels and mRNA expressions involved with cholesterol metabolism in mice liver and small intestine were analyzed by western blotting and quantitative real-time PCR, respectively. (2) In vitro study, cultured Huh7 hepatocytes were treated with 0, 100, or 500 nM GLP-1(7-36) amide and LDL (100 μg/mL) in medium containing 10% lipoprotein derived serum (LPDS). Cellular protein and mRNA levels were analyzed described above.
Results: (1) The change in body weights did not differ between ex-4 treated mice and control mice. Serum VLDL- and LDL-cholesterol levels were significantly decreased in ex-4 treated mice than control mice (9.2± 1.4 vs. 6.3±1.8 mg/dL, 11.6±2.3 vs. 8.8±2.0 mg/dL, p<0.05). Hepatic protein levels of sterol regulatory element-binding protein (SREBP2) and LDL receptor (LDLR) were significantly increased by ex-4 treatment. Ex-4 increased hepatic mRNA expression levels of hydroxymethylglutaryl-CoA synthase and reductase, which are related to cholesterol synthesis, by 2.0-(p=0.03) and 1.5-folds (p=0.005), respectively. It decreased those of ATP-binding cassette transporters-G5/8 (ABCG5/8), which are related to cholesterol excretion, by 0.6- (p=0.02) and 0.7-folds (p=0.01), respectively. Ex-4 did not change hepatic cholesterol contents and the mRNA expression levels of cholesterol metabolism-related genes in small intestine. (2) In Huh7 cells, 500 nM GLP-1 increased the protein levels of SREBP2 and LDLR. 100 nM GLP-1 significantly increased mRNA expression of SREBP2 and LDLR by 1.5- (p=0.01) and 2.5- (p=0.01) folds while it significantly decreased those of ABCG5/8 by 0.3- (p=0.02) and 0.5- folds (p=0.04), respectively. 100 or 500 nM GLP-1 enhanced phosphorylated Erk1/2 associated with activation of insulin signal pathway.
Conclusion: Ex-4 decreased serum levels of VLDL- and LDL-cholesterol by increasing the hepatic expression of SREBP2 and LDLR in C57BL/6J mice. In culture hepatocytes, GLP-1 also increased the SREBP and LDLR expression, suggesting that ex-4/GLP-1 increases the uptake of VLDL and LDL in liver and reduce serum cholesterol independent of insulin.

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Hepatic FGF21 protects mice against diet-induced lipid dysregulation and insulin resistance
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Background and aims: Fibroblast growth factor 21 (FGF21) is a potent metabolic hormone produced by a number of organs. It has been shown to confer multiple metabolic benefits on obesity and diabetes. Therapeutic administration of FGF21 or its analog protects against diet-induced obesity and hyperglycemia in both rodents and humans. However, the physiological roles of FGF21 remain ambiguous. Since the liver is the major site of production for circulating FGF21, we propose that the endocrine actions of FGF21 derived from the liver may account for its metabolic effects on lipid metabolism.
Materials and methods: We therefore generated the liver-specific FGF21 KO (LiverKO) mouse strain by crossbreeding transgenic mice bearing a conditional FGF21 allele with the exons 2 and 3 flanked by LoxP sites with mice expressing Cre under the control of the albumin promoter. LiverKO mice and their age-matched wild-type (WT) littermates were fed a high fat diet (HFD) for 20 weeks and assessed for the metabolic phenotypes.
Results: Circulating FGF21 was significantly elevated in WT mice during fasting or HFD feeding. However, this elevation of circulating FGF21 level was completely abolished in LiverKO mice, suggesting the liver is the major source of circulating FGF21. LiverKO mice treated with HFD showed a significant reduction in body fat mass when compared to WT mice, as revealed by NMR body composition analyzer suggesting resistance to obesity was mainly attributed to the loss of hepatic FGF21. Interestingly, although LiverKO mice had lean phenotype, they developed more severe glucose intolerance and insulin resistance after HFD feeding as shown by GTT and ITT, respectively. In addition, LiverKO mice had higher serum triglyceride and free fatty acid (FFA) levels suggesting that lipid homeostasis is impaired. It is possible that hepatic FGF21 inhibits lipolysis while promotes lipid uptake in adipocytes.
Conclusion: These data collectively suggest that circulating FGF21 is mainly derived from the liver. Hepatic FGF21 regulates lipid metabolism in adipocytes, and therefore protects mice against diet-induced hyperglycemia and insulin resistance possibly via reducing ectopic lipid accumulation in non-adipose tissues such as the liver and skeletal muscles.
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Monounsaturated fatty acid enriched high fat diets attenuate obesity induced hyperinsulinaemia concurrent with an altered hepatic lipidome
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Background and aims: Altered lipid metabolism underlies many metabolic disorders such as obesity and Type 2 Diabetes Mellitus (T2DM).

Excess lipids and their metabolites act as metabolic stressors to induce inflammation and promote insulin resistance (IR). Lipidomics represents a novel tool to identify lipid biomarkers of disease. A lipidomic approach sought to investigate the differential effect of saturated fatty acid (SFA) versus monounsaturated fatty acid (MUFA) high-fat diet (HFD) challenges on the hepatic lipidome and an IR profile.

Materials and methods: C57BL/6 mice were fed SFA-HFD (45% palm oil), MUFA-HFD (45% olive oil) or chow diet for 24 weeks. GTT, ITT and an insulin secretory response determined insulin sensitivity. The hepatic lipidome of 36 mice was quantified by Biocrates Austria. Electrospray ionization-MS/MS screened glycerophospholipids and sphingolipids. The lipidomic database was combined with phenotypic data. Multivariate principle component analysis (PCA) and regression analysis were performed using SPSS. A novel visualization approach, manually superimposed liver lipidomic data onto the online LipidMaps sphingolipid biosynthesis pathway using a BioPAX level 3 format to differentiate the synthesis of these lipids between diets.

Results: MUFA-fed mice had reduced hyperinsulineamia compared to SFA-fed mice despite equivalent obesity. PCA combined with regression analysis highlighted that a high-SFA/high-ceramide/low-PE cluster was predictive of an IR phenotype. Hepatic sphingolipid synthesis was downregulated following a MUFA-HFD compared to chow and SFA-HFD.

Conclusion: Dietary intake of MUFA, can attenuate obesity-induced hyperinsulinaemia concurrent with an altered hepatic lipidome. A novel visualization of LipidMaps illustrated that MUFA-HFD prevented sphingomyelin or ceramide synthesis, which were associated with IR. Further work is required to determine the mechanistic basis of the altered hepatic lipidome, wherein to what extent can replacement of dietary SFA with MUFA, prevent IR by modifying hepatic lipid biology.

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OP 15 In vivo veritas

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Pancreatic beta cell function, insulin sensitivity and metabolic phenotypes in type 2 diabetes at the time of diagnosis. The Verona newly diagnosed type 2 diabetes study

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Background and aims: Presence and frequency of beta cell (BC) dysfunction (BCD) and insulin resistance (IR) in patients with newly diagnosed type 2 diabetes mellitus (NDT2D) are imperfectly known, because previous studies used small cohorts and/or only surrogate indexes of BC function and IR. We sought to assess BC function and IR with state-of-art methods in the VNDS.

Materials and methods: In 712 GADA-negative, drug naïve, consecutive Italian NDT2D patients we assessed: 1. standard parameters; 2. insulin sensitivity (IS) by the euglycaemic insulin clamp); 3. BC function by state-of-art modeling of prolonged (5 hours) OGTT-derived glucose/C-peptide curves. Thresholds for BCD and IR were the 25th percentiles of BC function and IS assessed with the same methods of the VNDS in Italian subjects with normal glucose regulation of the GENFIEV (n=340) and GISIR (n=386) studies, respectively.

Results: In the VNDS, 89.8% [95% C.I.: 87.6 - 92.0%] and 87.8% [85.4 - 90.2] patients had BCD and IR, respectively. Patients with only one defect were 19.7% [16.8 - 22.6]. Isolated BCD and isolated IR were present in 10.9% [8.6 - 13.2] and 8.9% [6.8 - 11.0] patients, respectively. Coexistence of BCD and IR was observed in 78.9% [75.9 - 81.9] of the patients. 1.4% [0.5 - 2.3] of the patients had no detectable alterations in BC function and IS. Patients (19.7%) with only one metabolic defect had lower BMI, fasting glucose, HbA1c, triglycerides and BC function, and higher HDL-cholesterol and IS than patients with both BCD and IR ($p<0.01$ or less after Bonferroni's correction).

Conclusion: In conclusion, in NDT2DM patients: 1. at least 75.9% have both BCD and IR; 2. At least 87.6% and 85.4% have BCD and IR, respectively; 3. At least 16.8% have only one defect and a significantly different (milder) metabolic phenotype compared to patients with both defects. These findings may be relevant to therapeutic strategies centered on the metabolic phenotype of the patient.

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Cardio-vascular changes associated with impaired as compared with normal glucose tolerance in obese patients. Role of sympathetic activity

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Background and aims: An increase in cardiac output and alterations in cardio-vascular autonomic activity are clearly demonstrated in obese patients. Glucose intolerance is associated with more marked alterations of

cardiac autonomic function. This study aimed to compare cardio-vascular autonomic activity and hemodynamic parameters in obese patients with normal (NGT) or impaired glucose tolerance (IGT).
Materials and methods: Sixty-six obese patients were included and classified according to OGTT as NGT (n=38) or IGT (n=28). NGT and IGT patients were similar for sex ratio, age and BMI (38.4±4.1 and 37.4± 4.3 kg/m^2, respectively). All were normotensive or had well-controlled hypertension and were free of cardio-vascular history. Arterial stiffness (carotid-to-femoral pulse wave velocity), central and peripheral blood pressure and left ventricular ejection time (LVET) were assessed by applanation tonometry (SphygmoCor®). Cardiac sympathetic activity (LF-HR) and sympatho-vagal balance (LF/HF-HR) were determined by spectral analysis of heart rate variations (Task Force Monitor® digital plethysmography). Cardiac output, stroke volume (SV), cardiac index (CI) and thoracic fluid content (TFC) were measured by thoracic impedance. The stroke volume to pulse pressure ratio (SV/PP, an index of total arterial compliance) was calculated from SphygmoCor® aortic and peripheral blood pressure measurements.
Results: Blood pressures and pulse wave velocity were similar in both groups. Compared with NGT, IGT patients had higher CI (2.3±0.5 vs 1.8 ±0.7 l/min*m2), SV (68±19 vs 55±17 ml) and TFC and longer LVET (317±25 vs 304±25 ms), and lower total peripheral resistance index ($p<0.04$ to $p<0.002$). The SV/PP ratio derived from aortic (1.1±0.4 vs 2.2± 0.8) and peripheral (0.8±0.2 vs 1.7±0.5) pulse pressure were significantly higher in IGT patients ($p=0.002$, $p<0.0001$). In the overall population, plasma glucose at fasting and 2 hours after the oral glucose challenge correlated with LF/HF-HR ($r=0.27$, $p=0.02$; $r=0.26$, $p=0.03$), even after adjusting for age and BMI.
Conclusion: In obese patients, IGT is accompanied by an increase in left ventricular output and ejection duration and hypervolemia, all probably favoured by higher sympathetic activity. IGT patients also have a better total arterial compliance, in line with lower peripheral resistance.

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Influence of replacement therapy on metabolic profile in male patients with type 2 diabetes mellitus and androgen deficiency

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Background and aims: Low testosterone levels are observed in about 40% of men with diabetes mellitus type 2 and are associated with metabolic disorders, abnormalities of body composition and glycemic control. The aim of our study was to investigate the influence of testosterone replacement therapy on lipids, anthropometric parameters and HbA1c level in patients with diabetes mellitus type 2 and testosterone deficiency
Materials and methods: We investigated 102 male patients with diabetes mellitus type 2, aged 53 to 68 years, with BMI from 27.5 to 38.5 kg/m2.We assessed total (TT) and free testosterone (fT) levels, lipids, HbA1c, body mass index (BMI), waist-to-hip ratio (WHR) and waist circumstances (WC). Patients were divided into two groups: 1) treatment group, where we used diet, antidiabetic therapy and testosterone replacement therapy during 12 months; 2) control group where we used diet and antidiabetic therapy. IN treatment group PSA and prostate volume were controled.
Results: In treatment group baseline mean TT and fT levels were 8.24 nmol/l and 0,256 nmol/l, respectively and in control group 8.45 nmol/l and 0.265 nmol/l respectively, and did not differ significantly between groups. During testosterone replacement therapy mean TT and fT levels increased to 16.45 nmol/l and 0.567 nmol/l, respectively. After 12 months of treatment we observed significant improvement of lipids profile in both groups (decreasing of total cholesterol from mean 256.4 mg/dl to 221.8 mg/dl in treatment group and from 249.4 mg/dl to 238.2 mg/dl in control group and decreasing of LDL-cholesterol from 179.3 mg/dl to 147.4 mg/dl in treatment group and from 175.5 mg/dl fo 163.3 mg/dl in control group) but changes in testosterone group were higher ($p<0.05$ and $p<0.02$, respectively). We observed also decreasing of HbA1c levels in testosterone group (8,3 vs. 7.2%; $p<0,001$) and in control group (8,4 vs. 7,6%; $p<0.001$) but there were also significant differences between both groups ($p<0,02$). BMI values decreased but changes between groups did not differ significantly. WHR and WC values also decreased in both groups ($p<0.002$ and $p<0,01$; respectively), but changes in testosterone group were higher ($p<0,02$ and $p<0,05$; respectively). The method of antidiabetic therapy (oral or insulin) had not significant impact on changes of lipids, HbA1c and anthropometric indexes during study. PSA and prostate volume did not changes significantly during treatment.
Conclusion: Testosterone replacement therapy in men with diabetes mellitus type 2 and androgen deficiency testosterone influenced positively on lipids, body composition and HbA1c. This treatment is safe and routine testosterone measurement should be taken under consideration in diabetic men with testosterone deficiency.

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The impact of TSH increment on incident type 2 diabetes mellitus in euthyroid subjects

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Background and aims: It is widely known that thyroid function influences glucose metabolism. However, there have been no longitudinal studies to determine the association between thyroid function and diabetes. Therefore, the objective of this study was to investigate the incidence of type 2 diabetes mellitus (T2DM) according to the change of TSH level.
Materials and methods: A total of 23,063 participants had taken thyroid function tests (thyroid-stimulating hormone [TSH], total or free thyroxine [T4] and triiodothyronine [T3]) between 2006 and 2012 through a yearly health check-up program. Among them, 16,921 euthyroid subjects without diagnosed T2DM were enrolled in the study. Euthyroid was defined as having normal total T4 (4.7 to 12.5 ng/dL) or free T4 (0.78 to 1.85 ng/dL) and normal total T3 (76 to 190 ng/dL) with normal TSH level (0.4 to 4.2 μIU/L). TSH changes were calculated from TSH ratio as dividing TSH level at the end of follow-up or until a year before the last date of diabetes diagnosis into baseline TSH level.
Results: During 86,010 person-years of follow-up, there were 726 incident cases of T2DM. In cox proportional hazards models, the higher TSH ratio was significantly associated with the increased risk of incident T2DM (HR=1.10, 95% CI : 1.02-1.18, p=0.011) after adjusting baseline HbA1c, fasting glucose, age, sex, BMI, TG , HDL and LDL. In comparison with subjects in the lowest tertile (0.6±0.2), the subjects in the highest tertile of TSH ratio (1.9±1.0) also had a greater risk of incident T2DM (HR=1.39, 95% CI : 1.16-1.66, p for trend<0.001). Most of the subjects (4814, 83.0%) in the hightest tertile of TSH ratio maintained an euthyroid status. However, either baseline TSH level or tertiles were not associated with the risk of incident T2DM.
Conclusion: This study demonstrated that TSH increment, rather than baseline TSH level itself, can be an additional risk factor of incident T2DM even in the subjects with preserving euthyroidism.

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Apelin administration improves insulin sensitivity: proof of concept study in overweight healthy men

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Background and aims: Apelin is a recently identified adipokine that influences various biological systems through the activation of its G protein-coupled receptor (APJ). This adipokine is thought to exert beneficial actions on glucose metabolism since apelin administration has been demonstrated to improve glucose tolerance in various murine models by enhancing insulin sensitivity in peripheral tissues such as skeletal muscles and white adipose tissues. The aim of the present "APELINS" proof of concept study was to determine the influence of apelin administration on insulin sensitivity in humans.
Materials and methods: We designed a Phase I, double-blind, placebo-controlled, cross-over study which included non diabetic overweight male volunteers to successively assess the efficacy and the tolerance of two doses of (pyr1)-Apelin-13 versus placebo. A first group of subjects received 9 nmol/kg (n=8), and, after safety data review by an independent expert committee, a second group received 30 nmol/kg (n=8). Each volunteer participated in two hyperinsulinemic (1 $mU.Kg^{-1}.min^{-1}$) euglycemic clamps spaced from 7 to 21 days. The primary endpoint (ΔGIR) was the difference between the glucose infusion rate (GIR) measured during the steady state ending the "investigational product" perfusion (GIRperfusion 210th-240th min) and the GIR measured during the first steady state ending the "basal" phase (GIRbasal 90th -120th min).
Results: Main clinical characteristics of male volunteers were as follows: 32.8±6.8 yr, BMI: 27.6±1.4 kg/m^2, waist circumference: 99.3±4.7, fasting plasma glucose: 94±8 mg/dl, A1c: 5.4±0.3%. Mean concentrations of total plasma apelin were similar and stable in the different groups during the "basal" phase and placebo administration (from 277±106 to 305±59 pg/ml), but raised to 1332±1525 pg/ml and 4815±1874 respectively during the steady states ending the perfusion of low dose and high dose apelin. At low dose (9 nmol/kg), apelin administration induced a non significant increase in ΔGIR versus placebo (+2.21±0.54 vs+1.57± 0.53 mg/kg/min, p=0.06). A significant improvement in insulin sensitivity was observed with the dose of 30 nmol/kg (ΔGIR: + 1.72± 0.47 mg/kg/min with apelin administration versus + 0.89±0.62 mg/kg/min with placebo, p=0.03). Cardiovascular monitoring and safety reports did not reveal any specific side effect of apelin administration.
Conclusion: The present data provide the first demonstration of the insulin sensitizing action of apelin in humans, as recently suggested by animal studies, and thus open new therapeutic perspectives. The apelin/APJ pathway shoud now be considered for the development of alternative strategies aiming to fight against insulin resistance in type 2 diabetic subjects.
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Insulin sensitivity to glucose uptake is similarly changed across insulin sensitive tissues: evidence from a positron emission tomography study

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Background and aims: Insulin resistance is the major pathophysiological feature of type 2 diabetes. Skeletal muscle, liver, and adipose tissue are the key insulin sensitive tissues in humans. Impaired effect of insulin is reflected by reduced glucose uptake (GU) into these tissues. No previous study has investigated whether insulin sensitivity to GU differs across the insulin sensitive tissues in the same individual. To investigate this question, we measured GU in skeletal muscle, liver, and adipose tissue in a single session using positron emission tomography (PET) and euglycemic-hyperinsulinemic clamp.
Materials and methods: The study population consisted of 141 subjects without type 2 diabetes, who had previously participated in PET studies. PET-studies included the measurement of GU in the liver, quadriceps femoris muscle, and intra-abdominal and abdominal subcutaneous adipose tissue during the euglycemic-hyperinsulinemic clamp using fluorine-18 labelled deoxyglucose and PET. Principal component analysis (PCA) was used to analyze the rates of GU across the different tissues. Eigenvalue 1 was used as a threshold for component extraction.
Results: In PCA analysis, skeletal muscle, intraperitoneal adipose tissue and liver GU loaded on one single principal component indicating that the rates of GU were similarly changed across the tissues. The model explained 53% of the variance of GU. When intraperitoneal adipose tissue was substituted by abdominal subcutaneous adipose tissue the results remained almost unchanged, and the model explained 55% of the variance of GU. PCA resulted in one principal component even after the inclusion of BMI and age into the model.
Conclusion: PCA analysis showed that over half of the variance in the insulin stimulated GU in skeletal muscle, liver, and adipose tissue was explained by a single principal component. Our results indicate that the insulin sensitivity to GU is similarly changed across the insulin-sensitive tissues, independently of obesity and age.
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OP 16 The stressed beta cell

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TYK2, a candidate gene for type 1 diabetes, modulates apoptosis and the innate immune response in human pancreatic beta cells

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Background and aims: Type 1 diabetes (T1D) is a chronic autoimmune disease characterized by the specific destruction of pancreatic β-cells. Linkage and genome-wide association studies have identified >50 loci across the human genome associated with risk of T1D, including two SNPs in the TYK2 gene. These SNPs are predicted to decrease TYK2 function and are associated with a decreased risk to develop T1D. TYK2 is a member of the JAK family of tyrosine kinases that play a critical role in the interferons (IFN) signaling via phosphorylation and activation of STAT proteins. We presently evaluated whether TYK2 plays a role in human pancreatic β-cell apoptosis and production of pro-inflammatory mediators.
Materials and methods: Human β-cell line EndoC-βH1 cells and dispersed human islets were transfected with small interfering (si)RNA targeting TYK2 (inhibition of >50%) and subsequently exposed to intracellular polyinosinic-polycitidilic acid (PIC; a synthetic viral double-stranded RNA) or IFNα. Activation of the IFN signaling pathway was evaluated by Western blot of P-STAT1/STAT1 and P-STAT2/STAT2. Expression and release of IFNα, IFNβ and the chemokine CXCL10 were determined by RT-PCR and ELISA, respectively. Viability assays were performed by Hoechst/Propidium Iodide staining. Expression of MHC class I proteins was evaluated by RT-PCR, immunofluorescence and flow cytometry.
Results: Pathway analysis of candidate genes expressed in human islets identified a central role for interferon-regulated pathways. TYK2 knockdown (KD) decreased by 50-70% (p 0.05) PIC-induced STAT1/2 activation in human β-cells. Both mRNA expression and release of IFNα, IFNβ and CXCL10 were increased by 2-400 fold in human β-cells after PIC transfection, which was partially prevented by TYK2 KD in the case of IFNα and CXCL10, but not IFNβ ($p<0.001$; n=3-4). Expression of MHC class I proteins, a hallmark of early β-cell inflammation, was prevented by TYK2 silencing after both PIC or IFNα treatments. This decreased type I IFN response was paralleled by prevention of PIC-induced apoptosis in TYK2-silenced β-cells (36% and 26% of protection in EndoC-βH1 and dispersed human islets, respectively; p 0.05; n=4-5). Protection against apoptosis was confirmed by lower expression of cleaved caspase 3 in TYK2-inhibited cells.
Conclusion: The present findings suggest that the T1D candidate gene TYK2 regulates apoptotic and pro-inflammatory pathways in human pancreatic β-cells via modulation of IFN signaling and expression of MHC class I proteins. TYK2 may play a key role in the "dialog" between β-cells and the immune system in early T1D.
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The hepatokine fetuin-A has TLR4 dependent and independent effects in islets

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Background and aims: An increased level of plasma fetuin-A in humans strongly predicts the incidence of T2DM. In adipocytes, fetuin-A augments fatty acid-induced inflammation in a TLR4-dependent manner. We previously showed that in mouse islets fetuin-A increased the mRNA levels of pro-inflammatory cytokines (IL-1β, MCP-1) in a TLR2/4-dependent manner. In spite of increased cytokine production and JNK phosphorylation, chronic exposure to fetuin-A did not augment apoptosis. In contrast, palmitic acid induced JNK phosphorylation and apoptosis without stimulation of cytokine production. The present study aims to decipher the signalling pathways activated by fetuin-A and its contribution to the lipotoxic effect of palmitic acid.
Materials and methods: Isolated islets from WT and TLR4 KO mice were cultured in medium supplemented with palmitic acid (60 μmol/l) in the presence of either serum albumin (0.6 mg/ml) or fetuin-A (0.6 mg/ml) for 1 h to 48 h. Protein phosphorylations were analyzed on western blots. Changes in gene expression were quantified by RT-PCR. Apoptosis was estimated by TUNEL staining of isolated islet cells and subcellular localisation of proteins by confocal microscopy.
Results: In agreement with observations made in other tissues, fetuin-A induced stimulation of cytokine production was mediated through TLR4, since the effect was absent in islets of TLR4 KO mice. When the islets of WT mice were treated with clodronate (0.5 mg/ml) in order to deplete them from immune cells, the effect of fetuin-A on cytokine production was abrogated. Furthermore, fetuin-A did not induce nuclear accumulation (i.e. activation) of the transcription factor NFkB in isolated islet cells, suggesting that the endocrine cells were not the source of cytokines. Surprisingly, fetuin-A stimulated phosphorylation of the pro-apoptotic JNK in isolated islets of WT but also of TLR4 deficient islets. In contrast, palmitic acid stimulated JNK in a TLR4 dependent manner. Consequently, in TLR4 KO islet cells palmitic acid-induced apoptosis was inhibited, while addition of fetuin-A restored the cytotoxic effect of palmitic acid.
Conclusion: These results suggest that in islets fetuin-A may worsen lipotoxicity through TLR4-dependent stimulation of cytokine production in immune cells and TLR4-independent activation of JNK in endocrine cells.

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Loss of the pro-apoptotic BH3-only protein BIM results in increased beta cell mass in a mouse model of type 2 diabetes

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Background and aims: Loss of beta cell mass is a feature of type 2 diabetes, and evidence suggests that this is the result of apoptosis. We identified that the high concentrations of glucose induce ER and oxidative stress, which results in islet cell apoptosis mediated by pro-apoptotic BH3-only molecules BIM and PUMA. Islets isolated from organ donors with type 2 diabetes had increased expression of BIM and PUMA compared with non-diabetic donors, suggesting these factors are also important in loss of beta cells in humans. We hypothesized that blocking beta cell apoptosis in type 2 diabetes would improve glucose homeostasis, and tested this by examining the effects of BIM deficiency in the leptin receptor mutant $Lepr^{db/db}$ mouse model of type 2 diabetes.

Materials and methods: BIM-deficient mice were crossed with $Lepr^{db/db}$ mice to generate three groups of male and female mice: $Lepr^{db/db}BIM^{+/+}$, $Lepr^{db/db}BIM^{+/-}$ and $Lepr^{db/db}BIM^{-/-}$ mice. Corresponding non-diabetic $Lepr^{db/+}$ mouse groups were used as controls. Fasting blood glucose (FBG) was measured at 6, 10, 14 and 18 weeks of age. Intraperitoneal glucose tolerance test was performed in 16-week-old mice and serum insulin was quantified by ELISA. Islet morphology was studied in H&E, insulin and TUNEL stained pancreatic sections. To quantify islet volume, we performed optical projection tomography (OPT), 3-dimensional imaging of whole pancreas stained with anti-insulin antibodies. Abdominal fat was quantified after micro-CT scanning.
Results: Deficiency of BIM significantly protected $Lepr^{db/db}$ mice from development of hyperglycaemia. FBG in female $Lepr^{db/db}BIM^{-/-}$ mice was in the non-diabetic range (FBG in 18 week old mice: $Lepr^{db/db}BIM^{-/-}$=10.1±1.5 vs $Lepr^{db/db}BIM^{+/+}$=26.2±0.7 mmol/l). Compared to $Lepr^{db/db}BIM^{+/+}$ mice, $Lepr^{db/db}BIM^{-/-}$ mice also had improved glucose tolerance. $Lepr^{db/db}$ mice heterozygous for BIM ($Lepr^{db/db}BIM^{+/-}$) had an intermediate phenotype. We observed a striking increase in islet size in BIM-deficient $Lepr^{db/db}$ mice by histology, while the beta cell size was similar between $Lepr^{db/db}BIM^{+/+}$ and $Lepr^{db/db}BIM^{-/-}$ mice. This suggests that increase in islet size was due to an increase in number of beta cells. Quantification of islet volume with OPT revealed a significant increase in islet volume in BIM-deficient compared with wild-type $Lepr^{db/db}$ mice ($4.2±0.7×10^6$ vs $2.1±0.4×10^6$ μm3). The increased islet volume was accompanied by a two-fold increase in serum insulin concentration (19.3±2.1 vs 9.7±2.3 μg/L). We detected reduced number of TUNEL positive cells in BIM-deficient compared with wild-type $Lepr^{db/db}$ islets indicating that the islet phenotype is secondary to apoptosis inhibition. Further, compared to $Lepr^{db/db}$ mice wild-type for BIM, $Lepr^{db/db}$ mice deficient in BIM gained more weight (20-week old female body weights: 66.2±1.6 vs 60.8±0.8 grams) and had increased abdominal fat (% abdominal fat volume: 73.9±4.1 vs 42.2±3.5). These changes in body weight could be due to increased circulating insulin concentration in $Lepr^{db/db}BIM^{-/-}$ mice.
Conclusion: Our data suggest that BIM has a direct role in beta cell apoptosis in vivo in type 2 diabetes, and indicate that inhibition of beta cell apoptosis can prevent the loss of islet cell mass, allowing the islet to compensate for the increasing insulin demand caused by insulin resistance and obesity.
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Thrombospondin 1: a master regulator of the anti-oxidant defence in pancreatic beta cells

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Background and aims: Western diets rich in saturated fats contribute to the increasing prevalence of type 2 diabetes. Saturated fatty acids, such as palmitate, induce endoplasmic reticulum (ER) stress and cause lipotoxic beta-cell death. Thrombospondin 1 (THBS1) is a glycoprotein expressed in the ER and secreted to the intercellular space. THBS1 has been shown in other tissues to promote adaptive ER stress signaling. THBS1-/- mice have impaired glucose tolerance and glucose-stimulated insulin release. In this study we interrogated the putative role of THBS1 in lipotoxic beta-cell death.
Materials and methods: Apoptosis was evaluated using nuclear dyes, mRNA expression by qPCR, and protein expression/phosphorylation by Western blot. Gene silencing and overexpression were achieved by RNA interference and adenoviral vectors, respectively. The data are based on 3-5 independent experiments.
Results: Palmitate reduced THBS1 protein expression in human islets by 73±3% ($p<0.05$) and in rat beta-cells by 71±3% ($p<0.05$). THBS1 knockdown (KD) sensitized beta-cells to palmitate-induced apoptosis (from 24±2 to 48±2% apoptosis, $p<0.05$), while its overexpression was protective (from 24±2 to 18±2% apoptosis, $p<0.05$). Different from previous findings in cardiomyocytes, THBS1 KD or overexpression did not modulate ATF6 signaling in beta-cells. Reactive oxygen species were induced by THBS1 KD (by 43±9%, $p<0.05$) and reduced by THBS1 overexpression (by 38±4%, $p<0.05$) in palmitate-treated cells. The anti-oxidant enzymes glutathione s-transferase M1 (GSTM1) and catalase were induced by palmitate (by 5.3±0.7 and 3.3±0.3-fold, respectively, $p<0.05$) and further increased by THBS1 overexpression (by 44±9% and 48±2% compared to palmitate, $p<0.05$). GSTM1 expression was reduced in islets from THBS1-/- mice (by 55±7% compared to wild-type mice, $p<0.05$), showing that THBS1 also regulates the anti-oxidant response in vivo. THBS1 overexpression increased by 75±9% ($p<0.05$) the activity of an anti-oxidant response element (ARE) reporter. The transcription factor NRF2 is known to bind to ARE. NRF2 KD in THBS1 overexpressing cells resulted in decreased GSTM1 and catalase mRNA expression (by 5.6±1.3 and 3.5±0.5-fold, $p<0.05$), showing that NRF2 mediates the THBS1-dependent induction of anti-oxidant stress enzymes. The PERK inhibitor GSK2606414 reduced NRF2 activation in THBS1 overexpressing cells by 2.5±0.5-fold and down-regulated GSTM1 and catalase mRNA by 60±9% and 57±3% ($p<0.05$), indicating that THBS1 activates NRF2 via the ER stress transducer PERK. Importantly, THBS1 KD induced JNK phosphorylation (by 75±16%, $p<0.05$) and expression of the proapoptotic BCL-2 family member PUMA (51±8%, $p<0.05$), providing a mechanistic explanation for the increase in beta-cell apoptosis following THBS1 inhibition.
Conclusion: THBS1 activates NRF2 via PERK, thus orchestrating a protective anti-oxidant defense mechanism against lipotoxicity in beta-cells. These data contribute to a better understanding of the mechanisms leading to beta-cell failure during metabolic stress and point to THBS1 as an interesting target to prevent oxidative stress in type 2 diabetes.
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The hippo kinase LATS2 impairs pancreatic beta cell survival and function

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Background and aims: Both type 1 and type 2 diabetes mellitus result from an absolute or relative decline in pancreatic β-cell function and/or mass. Apoptosis and loss of function are hallmarks of β-cell failure and the fundamental cause of diabetes. Large-tumor suppressors 2 (LATS2), a core component of the Hippo signaling pathway, is a ubiquitously expressed serine/threonine kinase and involved in multiple cellular processes such as proliferation, stress responses, and apoptosis. So far, the role of LATS2 in the β-cell is not known. Our aim was to investigate whether LATS2 is activated in the β-cell under diabetic conditions and whether its hyper-activation or inhibition would regulate β-cell death and insulin secretion.
Materials and methods: Rat β-cell line INS-1E and isolated human islets were exposed to a diabetic milieu (IL-1β/IFNγ or increased glucose concentration (22.2 mM)). Phospho-LATS1/2, LATS2, MOB1 and β-cell apoptosis (Caspase 3 & PARP cleavage) were analyzed by Western blotting. β-cell apoptosis was also analyzed by TUNEL staining and insulin, Pdx1, Nkx2.2, Nkx6.1, Neurod1, MafA, GCK, Slc2a2, KCNG11 and Abcc8 mRNA expression by real-time PCR (RT-PCR). LATS2 was overexpressed by plasmid transfection or adenovirus infection, LATS2

inactivation was performed by overexpressing dominant-negative LATS2 (dn-LATS2) or specific siRNA to LATS2 in both INS-1E cells and human islets. Insulin secretion in response to glucose (GSIS at 2.8 mM and 16.7 mM glucose), Glibenclamide (1 uM) and KCl (35 mM) in human islets in vitro were examined by ELISA.

Results: LATS1/2 phosphorylation (at S909/872 for LATS1 and LATS2) was increased in INS-1E cells exposed to pro-inflammatory cytokines: IL-1β/IFNγ or increased glucose concentration, indicating higher activity of LATS kinases under diabetic conditions. This correlated with MOB1 (LATS2-associated protein) up-regulation and increased β-cell apoptosis. LATS2 overexpression increased the number of TUNEL-positive β-cells in human islets and induced caspase-3 and PARP cleavage in human and rodent β-cells. Inhibition of endogenous LATS2 activity by siRNA knockdown or overexpression of dominant negative LATS2 protected INS-1E cells and human islets from chronically elevated glucose- and cytokines-induced apoptosis. LATS2 silencing abolished diabetic milieu-induced MOB1 up-regulation suggesting LATS2-dependent regulation of MOB1 in β-cells. Consistently, MOB1 knockdown blocked LATS2- and glucotoxicity-induced caspase-3 and PARP cleavage in INS-1E cells indicating a major role of MOB1 in LATS2-induced β-cell apoptosis. Also, LATS2 overexpression impaired GSIS in human islets without significant changes neither on insulin content and gene expression nor on genes involved in glucose sensing (Slc2a2 and GCK), insulin transcription (Pdx1, NeuroD, MafA) and ATP-dependent K+ channel subunits (Kcnj11 and abcc8). LATS2 overexpression also abolished insulin secretion stimulated by the insulin secretagogues KCl and glibenclamide, suggesting that the insulin secretory defect may occur at a step down-stream of calcium influx.

Conclusion: Our results show that LATS2 hyper-activation leads to β-cell death and loss of function and its inhibition protects from apoptosis. Blocking of LATS2 may be a successful strategy to improve β-cell survival and function in diabetes.

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Metabolic stress induces aberrant G-protein prenylation in pancreatic beta cells

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Background and aims: Activation of specific small G-proteins [Arf6, Cdc42 and Rac1] is implicated in glucose-stimulated insulin secretion [GSIS] in clonal β-cells, normal rodent and human islets. We recently demonstrated requisite roles for G-protein prenylation in GSIS. We identified three classes of prenylating enzymes in pancreatic β-cells. Farnesyltransferase [FTase] and geranylgeranyltransferases [GGTase-I] prenylate Ras and Rho G-proteins, respectively. GGTase-II mediates prenylation of Rab G-proteins. The aim of the current study is to assess alterations, if any, in FTase/GGTase-I signaling in pancreatic β-cells in in vitro and in vivo models of metabolic stress, impaired insulin secretion and T2DM.

Materials and methods: Normal rat islets or INS-1 832/13 β-cells were exposed to glucotoxic [20 mM; 24 hr], lipotoxic [0.3 mM palmitate; 24 hr] or thapsigargin [0.25 μM; 24 hr] conditions. FTase and GGTase-I activities were assayed by radiometric methods. Caspase-3 activation and FTase/GGTase-α degradation were quantified by Western blotting.

Results: Exposure of INS-1 832/13 β-cells to gluco- or lipotoxic conditions markedly attenuated [~50%] FTase and GGTase activities. A significant increase in caspase-3 activity and degradation of the common α-subunit of FTase/GGTase [FTase/GGTase-α] were also seen under these conditions. Thapsigargin, a known inducer of endoplasmic reticulum [ER] stress, promoted caspase-3 activation, FTase/GGTase-α degradation and reduction [~70%] of FTase and GGTase activities. Compatible with our in vitro findings, caspase-3 activity and FTase/GGTase-α degradation were markedly increased in islets derived from the Zucker diabetic fatty [13 weeks] rats compared to those from the age-matched Zucker lean controls. Consequentially, a significant increase in the abundance of unprenylated Rap1, a geranylgeranylated protein, was noted in pancreatic β-cells exposed to gluco or lipotoxic conditions. In addition, forced inactivation of G-protein prenylation [via depletion of endogenous pools of mevalonic acid] using simvastatin also resulted in accumulation of unprenylated Rap1 in these cells. Lastly, glucotoxic conditions promoted sustained activation of Rac1, a geranylgeranylated protein, activation of stress kinase [p38MAPK], nuclear lamin-B degradation and inhibition of GSIS in rat islets and INS-1 832/13 cells.

Conclusion: Based on our findings we propose that metabolic stress induces aberrant G-protein prenylation signaling events leading to stress kinase activation and loss in β-cell function including GSIS.

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OP 17 Knives, not forks

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Unravelling the effects of endogenous incretin hormones on glucose metabolism after Roux-en-Y gastric bypass surgery

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Background and aims: Roux-en-Y gastric bypass (RYGB) induces weight loss and diabetes remission. Exaggerated meal-induced secretion of glucagon-like peptide-1 (GLP-1) is important for enhanced postprandial insulin secretion and improved glucose metabolism after surgery, but the role of glucose-dependent insulinotropic polypeptide (GIP) is debated. Administration of a DPP-4 inhibitor as sitagliptin (sita) prevents the degradation of the active forms of GLP-1 and GIP, while it is possible to block the effect of GLP-1 with the GLP-1 receptor antagonist exendin 9-39 (Ex9). We investigated the individual and combined effects of DPP-4 inhibition and GLP-1 receptor blockade to understand the relative importance of the endogenous incretin hormones on glucose metabolism after RYGB.
Materials and methods: In a placebo-controlled, single-blinded, cross-over design, twelve glucose tolerant patients (age 35.4±7 (mean±SEM) years, gender (f/m) 8/4, BMI 33.5±6 kg/m2) were studied 5.3±1 month after RYGB. On four separate experimental days patients underwent a standard 4 h mixed meal test (356 kcal, 53E% carbohydrate, 33E% fat, 14E% protein) with either 1) placebo (plac); 2) sita (100 mg 12 h and 2 h before study start); 3) Ex9 infusion (900 pmol×kg-1×min-1); or both 4) sita and Ex9 (Ex9/sita) in a randomized order. Pre-hepatic insulin secretion rates (ISR) were calculated from deconvolution of C-peptide and beta-cell glucose sensitivity (beta-GS) was used to characterize the dose-response relationship between glucose concentration and ISR. Results were analysed by linear mixed effects models.
Results: GLP-1 receptor antagonism by Ex9 infusion increased glucose excursions compared with placebo while no effect of DPP-4 inhibition was seen (incremental (i)-AUC glucose: plac 92±44 mmol/L×min, Ex9 192±44 $p<0.01$, sita 112±43 $p=0.47$). Ex9 and Ex9/sita had comparable effects on glucose concentrations (iAUC glucose: Ex9 192±44 vs. Ex9/sita 163±39, $p=0.27$). Insulin secretion evaluated by iAUC ISR and beta-GS decreased during Ex9 infusion, whereas no significant effect of sita was seen compared with placebo (iAUC ISR: plac 933±96 pmol×kg-1, Ex9 713±83 $p<0.01$, sita 963±123 $p=0.95$. Beta-GS: plac 4.8±0.8 (pmol×kg-1×min-1)/mmol/L, Ex9 3.8±0.7 $p=0.01$, sita 5.7±1.2 $p=0.26$). Both measures of beta-cell function deteriorated equally during Ex9 infusions with and without sita (iAUC ISR: Ex9 713±83 vs. Ex9/sita 660±53, $p=0.65$ and beta-GS: Ex9 3.8±0.7 vs. Ex9/sita 3.1±0.5 $p=0.09$). Total AUC of intact GLP-1 increased 3-fold ($p<0.01$) and intact GIP 1.6-fold ($p<0.01$) on sita days, thereby indicating an effective DPP-4 inhibition.
Conclusion: After RYGB, infusion of the GLP-1 receptor antagonist, Ex9, induced deteriorated glucose tolerance and impaired postprandial insulin secretion, whereas DPP-4 inhibition had no effect on glucose tolerance or insulin secretion. DPP-4 inhibition also failed to improve beta-cell function when the GLP-1 receptor was blocked. This finding underscores the importance of the exaggerated GLP-1 secretion for improved glycaemic control after RYGB, but does not support a major role of GIP for improved glucose tolerance and beta-cell function after RYGB.
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The expression of genes involved in intestinal gluconeogenesis is altered in morbidly obese subjects with higher insulin resistance

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Background and aims: It has been suggested that small intestine might possess the capacity to produce glucose, being induced in insulin resistance states. It has been shown that genes involved in gluconeogenesis are expressed in human small intestine. However, there is little information about intestinal gluconeogenesis in humans and the alterations that high insulin resistance may produce. The aim of this study is to evaluate the expression of genes involved in intestinal gluconeogenesis in morbidly obese subjects in states of insulin resistance and also to evaluate the effect of metformin in their expression.
Materials and methods: The study was undertaken in 45 morbidly obese subjects who underwent to Roux-en-Y gastric bypass. The morbidly obese subjects were classified in subjects with homeostasis model assessment of insulin resistance (HOMA-IR) value lower than 4.7 (MO-low-IR), subjects with HOMA-IR value higher than 4.7 (MO-high-IR) (both groups without treatment for T2D) and subjects with T2D who were only receiving metformin treatment (MO-metf-T2D). The jejunal biopsy samples used to evaluate the mRNA expression levels were obtained during the bariatric surgery, 40 cm from the ligament of Treitz.
Results: In MO-high-IR group, glutaminase (GLS) ($p=0.034$), phosphoenolpyruvate carboxykinase (PEPCK) ($p=0.027$) and glucose 6-phosphatase (G6Pase) ($p=0.024$) mRNA expression levels were significantly higher than in MO-low-IR group. In MO-metf-T2D group, G6Pase ($p=0.014$) and GLS ($p=0.049$) mRNA expression levels were significantly higher than in MO-low-IR group, whilst PEPCK mRNA expression levels were significantly lower ($p=0.001$). In MO-metf-T2D group, PEPCK ($p=0.001$) mRNA expression levels were significantly lower than in MO-high-IR group. Weight, BMI and waist circumference correlated positively with PEPCK mRNA expression levels ($r=0.416$; $p=0.025$; $r=0.398$; $p=0.033$; $r=0.446$; $p=0.043$, respectively). Insulin levels correlated positively with PEPCK ($r=0.347$; $p=0.045$), fructose 1,6-bisphosphatase (FBPase) ($r=0.319$; $p=0.046$) and G6Pase ($r=0.461$; $p=0.005$) mRNA expression levels. HOMA-IR value correlated positively with G6Pase ($r=0.424$; $p=0.010$) mRNA expression levels. GLS mRNA expression levels correlated positively with FBPase mRNA expression levels ($r=0.683$; $p<0.001$). G6Pase mRNA expression correlated positively with GLS ($r=0.703$; $p<0.001$) and FBPase ($r=0.654$; $p<0.001$) mRNA expression levels.
Conclusion: The mRNA expression of most of the genes involved in gluconeogenesis is increased in the jejunum of morbidly obese subjects with higher insulin resistance. This altered jejunal mRNA expression is attenuated in those morbidly obese subjects treated with metformin.

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CD163+ and CD206+ adipose tissue immune cells in obese subjects with type 2 diabetes mellitus: the effect of gastric plication and duodenal-jejunal bypass liner implantation

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Background and aims: CD163 and CD206 are considered the main markers of alternatively activated (M2) macrophages which are suggested as one of the main players in the resolution of adipose tissue low-grade inflammation. The aim of our study was to examine changes in CD163+ and CD206+ cells in subcutaneous adipose tissue (SCAT) of obese subjects with type 2 diabetes mellitus (T2DM) in the context of global metabolic improvements after selected bariatric procedures.
Materials and methods: Twenty-two obese subjects with T2DM undergoing either GP or DJBL were included into the study. Anthropometric and biochemical parameters were measured and SCAT samples from the abdominal region were taken at baseline, 1 and 6 (GP) or 10 months (DJBL) after intervention. M2 macrophage subpopulations were identified using flow cytometry and a combination of antigens including CD14, HLA-DR, CD163 and CD206.
Results: At baseline 2 different subsets of CD163+ (CD163+HLA-DR+CD14+ and CD163+HLA-DR+CD14-) and CD206+ cells (CD206+HLA-DR+CD14+ and CD206+HLA-DR+CD14-) were identified in SCAT according to the presence of CD14. Both interventions resulted in decreased body weight (BMI 43.2±1.8 vs. 36.0±2.1 kg/m2, p<0.001 for GP and 42.6±1.2 vs. 39.0±1.5 kg/m2, p<0.001 for DJBL) and improved glucose control (HbA1C 64.8±6.3 vs. 45.0±2.9 mmol/mol, p=0.004 for GP and 74.2±5.6 vs. 53.8±5.0 mmol/mol, p<0.001 for DJBL). In SCAT, both GP and DJBL significantly decreased CD163+HLA-DR+CD14+ (20.4±2.7 vs. 11.3±1.0%, p=0.010 for GP and 19.9±1.9 vs. 12.6±0.6%, p=0.025 for DJBL) and increased CD163+HLA-DR+CD14- cells (3.5±0.3 vs. 7.3±1.1%, p=0.001 for GB and 3.4±0.4 vs. 5.3±0.5%, p=0.029 for DJBL). CD206+HLA-DR+CD14+ cells also decreased (21.0±3.3 vs. 11.6±0.8%, p=0.013 for GB and 18.1±1.4 vs. 11.9±1.3%, p=0.039 for DJBL), while the CD206+HLA-DR+CD14- subset remained unchanged in both groups (2.0±0.2 vs. 2.7±0.3, p=0.43 for GP and 2.3±0.3 vs. 2.0±0.2, p=0.62 for DJBL).
Conclusion: Human adipose tissue immune cells with M2 macrophage phenotype seem to consist of several subpopulations which react differently to weight reduction. These changes might contribute to positive effects of GP and DJBL on metabolic control in obese T2DM subjects.
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Effects of ursodeoxycholic acid and chenodeoxycholic acid on insulin secretion after Roux-en-Y gastric bypass

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Background and aims: Roux-en-Y gastric bypass (RYGB) induces weight loss and improved glycaemic control. Bile acids have been proposed as an important contributor to the improved glycaemic control postoperatively since the plasma bile acid concentration increases substantially after RYGB. However, causality between increased plasma bile acids and effects on the glucose-regulatory pathways remain to be demonstrated. In healthy subjects and type 2 diabetic patients intragastric, intraduodenal and intrarectal infusions with bile acids increase secretion of glucagon-like peptide-1 (GLP-1), an important insulinotropic hormone. After RYGB, intraluminal bile acids may thus also contribute to increased postprandial GLP-1 secretion and thereby to increased postprandial insulin secretion. RYGB changes the gastrointestinal anatomy and makes it possible to reach the GLP-1 producing L-cells in the distal part of the intestine by an oral route. Therefore, we evaluated the effects of oral administration of ursodeoxycholic acid (UDCA) and chenodeoxycholic acid (CDCA) on insulin secretion in RYGB-operated patients.
Materials and methods: In a placebo-controlled, crossover study, we examined insulin secretion and glucose concentration in 10 RYGB-operated patients with normal glucose tolerance (age (mean±SEM) 37.9±3.4 years, BMI 29.2±1.3 kg/m^2, fasting plasma glucose 4.96±0.13 mmol/litre) at 32.6±1.1 months postoperatively. On three different days the patients ingested either 1) UDCA (750 mg); 2) CDCA (1250 mg) suspended in 150 mL water; or 3) 150 mL water alone (placebo). Blood samples were drawn for the following 180 minutes. Insulin secretion was evaluated using delta C-peptide$_{0\text{-peak}}$ and positive incremental AUC (pi-AUC, i.e. AUC above fasting concentration). Linear mixed-effects models were used for statistical analysis.
Results: Oral intake of CDCA increased insulin secretion slightly, but significantly, compared with placebo (delta C-peptide$_{0\text{-peak}}$: placebo: 45±13 pmol/L, CDCA: 140±27 pmol/L, p=0.0012; pi-AUC: placebo: 1900±643 pmol/L×min, CDCA: 7094±1447 pmol/L×min, p=0.02)). UDCA did not affect insulin secretion compared with placebo (UDCA: delta C-peptide$_{0\text{-peak}}$: 72±13 pmol/L, p=0.23; pi-AUC: 3119±1234 pmol/L×min, p=0.47). Plasma glucose was not affected by oral intake of neither UDCA nor CDCA.
Conclusion: In RYGB-operated patients with normal glucose tolerance, oral administration of CDCA increased insulin secretion significantly compared with placebo, whereas no effect was seen after oral administration of UDCA. However, the effect was small and no effect on glucose concentration was observed questioning a major role of bile acids for the improved glycaemic control after RYGB.
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Roux-en-Y gastric bypass improves gastrointestinal-mediated glucose disposal in type 2 diabetes whereas gastric banding and caloric restriction do not

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Background and aims: The mechanisms by which Roux-en-Y gastric bypass surgery (RYGB) mediates its glucose-lowering effect in type 2 diabetes are debated. The two predominant hypotheses include 1) improved glucose handling driven by gastrointestinal (GI) factors elicited by rerouting of nutrients through the GI tract, and 2) post-surgery caloric restriction promoting beneficial effects on glucose metabolism (independent of the altered GI anatomy/physiology). We evaluated the impact of RYGB vs laparoscopic adjustable gastric banding (LAGB) and surgery-matched caloric restriction alone (CAL), respectively, on GI-mediated glucose disposal (GIGD) in patients with type 2 diabetes.
Materials and methods: GIGD was calculated by comparing glucose amounts utilised during 50 g-OGTT and isoglycaemic i.v. glucose infusion (IIGI) (using variable glucose infusion rates), respectively (100%×(glucoseOGTT - glucoseIIGI / glucoseOGTT)), in obese patients with type 2 diabetes before and 1 month after either RYGB (n=40), LAGB (n=16) or CAL (n=11).

Results: As previously reported, all groups showed a significant and similar degree of weight loss, and AUC for plasma glucose during OGTT improved in all groups with RYGB eliciting the most robust improvement. We observed a significantly increased GIGD following RYGB (Δ13.0±4.8%, p<0.01), whereas GIGD changes were insignificant after LAGB (Δ-1.7±9.5%, p=0.86 and CAL (Δ9.5±9.1%, p=0.32).
Conclusion: These data suggest that the improved glucose tolerance observed after RYGB is mainly driven by nutrient-rerouting through the GI tract rather than surgery-induced caloric restriction and ascribe improved GIGD a key role in remission of the diabetic state often observed in patients with type 2 diabetes undergoing RYGB.

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Bariatric surgery normalises splanchnic vascular responses to a mixed-meal and to GIP infusion

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Background and aims: Splanchnic vasculature regulates intermediary metabolism by facilitating nutrient delivery from intestine into systemic circulation and pancreas, and by affecting hepatic insulin clearance. Recent studies have suggested that disturbances in splanchnic vascular functions are associated with dysglycemia predisposing to development of type 2 diabetes. The present study was conducted to investigate the splanchnic vascular responses to a mixed-meal test, and to glucose-dependent insulinotrophic polypeptide (GIP) infusion before and after bariatric surgery.
Materials and methods: Ten morbidly obese (40.8±5.9 kg m^{-2}) diabetic subjects and 10 age-matched, lean (22.9±2.1 kg m^{-2}) subjects were recruited. Small intestinal, pancreatic and hepatic blood flow (BF) and volume (BV) before and after 300-kcal mixed-meal, and during GIP infusion (2 pmol kg^{-1} min^{-1}) were measured using radiotracers [^{15}O]H_2O and [^{15}O]CO, and PET. A standard 75-g oral glucose tolerance test was performed, and during imaging studies blood was continuously drawn to measure glucose, insulin, and incretin hormones (GIP and GLP-1). In obese subjects, these procedures were repeated six weeks after bariatric surgery.
Results: Compared to controls, obese diabetic subjects were slightly hyperglycemic (fasting plasma glucose 6.8±0.8 *vs.* 5.0±0.4 mM, P<0.0001) prior to surgery. In both groups, meal ingestion provoked significant increases in small intestinal BF (+61.8 and +92.2% *vs.* baseline, for lean and obese subjects, respectively, both P<0.05). While pancreatic BF was increased in lean subjects (1.9±0.6 *vs.* 1.6±0.4 ml ml^{-1} min^{-1}, P<0.05), this was not observed in obese subjects (NS). During GIP infusion, small intestinal BF was increased in both groups (+141 and +112% *vs.* baseline, for lean and obese subjects, respectively, both P<0.05). In contrast, pancreatic BF was decreased (1.2±0.3 *vs.* 1.6±0.4 ml ml^{-1} min^{-1}, P=0.01) only in lean subjects, whereas no response was observed in obese subjects (NS). Hepatic BV was decreased after meal ingestion and during GIP infusion in both groups (from -6.3 to -11.9%, P<0.05). Six weeks after bariatric surgery, 12% excess body weight loss was observed (-13.9 kg, P<0.01), and diabetes remission rate of 80% was reached. Postoperatively, small intestinal vascular responses to meal and to GIP infusion (+167 and +93.6% *vs.* baseline, respectively, both P<0.05) were unchanged when compared with the preoperative state. However, after surgery pancreatic vascular responses to meal (1.4±0.2 *vs.* 1.0±0.3 ml ml^{-1} min^{-1}, P<0.01) and to GIP infusion (0.9±0.3 *vs.* 1.1±0.3 ml ml^{-1} min^{-1}, P<0.05) were normalized and essentially similar to those of lean subjects.
Conclusion: Bariatric surgery in morbidly obese diabetic patients normalizes splanchnic vascular responses. Further studies are needed and should focus on investigating the many factors that affect splanchnic blood flow kinetics after a meal, and their association to insulin secretion, systemic delivery and hepatic clearance rate.
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OP 18 Pulling the cover off omics and metabolism

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Sequencing of 12,940 exomes identifies additional coding variants in *G6PC2* which influence fasting glucose levels through effects on protein stability or activity

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Background and aims: We recently identified coding variants in *G6PC2* (encoding the islet-specific glucose-6-phosphatase catalytic subunit) influencing fasting glucose (FG) levels in non-diabetic Europeans and demonstrated that these variants affected protein stability. In addition to single variant associations, gene-based analyses also revealed a robust association driven by multiple low-frequency/rare variants in the exome array analysis. To achieve a comprehensive assessment of the impact of coding variation in *G6PC2* on FG levels, especially that of rare variants not captured on the array, we combined exome sequence data with array data to identify additional coding variants. We coupled genetic analyses with functional characterisation to identify nonsynonymous *G6PC2* variants altering protein function, thus impacting on FG levels.
Materials and methods: Exome sequence data from 12,940 individuals consisting of type 2 diabetes cases and controls from five major ancestries was used to catalogue coding variants in *G6PC2*. For analysis of glycemic traits, 5,132 non-diabetic exome sequences were combined with previously published exome array data from 33,407 non-diabetic Europeans. Single variant analyses were performed within each ethnic group then combined in a trans-ethnic meta-analysis. *G6PC2* variants were characterised by assessing effects on protein expression (western blot) and/or activity (phosphatase assay).
Results: We identified 69 coding variants in *G6PC2* in our exome array and sequence data, including 3 previously reported functional variants that were shown to influence FG levels (H177Y, Y207S, V219L). We selected 10 further variants, all rare (minor allele frequency [MAF]<0.005), for functional follow-up based on either evidence of association with FG levels (S30F, I171T, S324P; single variant $P<0.05$), or biological relevance (variant maps to conserved active site). All the variants studied except for I171T-G6PC2 had significantly reduced protein levels (mean 12.1% of wild type [WT]; $P<0.001$) due to enhanced proteasome-mediated degradation. I171T-G6PC2 was expressed at levels similar to WT-G6PC2, however its position within the active site suggested an alternative mechanism. This was confirmed by activity assays which showed that the missense substitution affected protein activity. Using a glucose-6-phosphatase homologous model, phosphatase activity of the variant was decreased by 38.9% at 2.5 mM glucose-6-phosphate compared to WT ($P<0.05$). Both S324P and I171T are rare functional variants (MAF=0.0044 and 0.0012 respectively) that contributed to the gene-based signal at *G6PC2* in the exome array analysis.
Conclusion: We identified additional coding variants in *G6PC2*, mostly rare, providing a more complete inventory of genetic variation in this key regulator of beta cell function. Functional investigation demonstrated that many of these result in marked protein instability whilst one variant alters protein activity, consistent with a role in modulating FG levels.
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Role of the type 2 diabetes genome-wide association studies gene SLC30A8 in the pancreatic alpha cell

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Background and aims: Genome-wide association studies have revealed >90 loci in the human genome with a significant impact on type 2 diabetes risk. One such locus encodes a non-synomymous single nucleotide polymorphism in the SLC30A8 gene encoding the zinc transporter ZnT8, expressed almost exclusively in pancreatic islets. Changes observed in humans containing risk alleles and animals lacking ZnT8 have previously been attributed to alterations in insulin secretion from beta-cells. Though the role of the ZnT8 transporter in beta-cells has been studied extensively its role on glucagon secretion is largely unknown. Here, we investigate the role of ZnT8 in the alpha-cell by generating animals deleted for slc30a8 selectively in these cells.
Materials and methods: Slc30a8 was inactivated in alpha-cells by crossing animals bearing a floxed Slc30a8 gene with mice expressing Cre recombinase under a 1.6 kB fragment of the pre-proglucagon promoter, plus tdRFP expressed at the Rosa26 locus distal to a LoxP-STOP-LoxP cassette. Deletion was confirmed by qPCR on FACS-sorted alpha-cells. Glucose homeostasis was assessed by glucose tolerance and insulin tolerance test and by hypoglycaemic clamp studies. Intracellular free Ca2+ and Zn2+ were measured in tdRFP-labelled alpha-cells within the intact islet using the trappable fluorescent probes Fluo-2 and Fluozin-3, respectively.
Results: Glucose and insulin tolerance were normal in αSlc30a8KO mice. However, female KO mice required lower glucose infusion rates (KO 18.8±1.62 mg/min/kg vs control 28±1.27 g/min/kg, $P<0.001$, n=4-6) during hypoglycaemic clamps, and displayed enhanced glucagon release. Correspondingly, isolated islets from KO mice released more glucagon at 1 mM glucose than WT islets (0.18±0.009 vs 0.1±0.03 as a % of total, $P<0.01$, n=5) despite unchanged intracellular Ca2+ responses to glucose withdrawal (1 vs 17 mM). Additionally there was a tendency towards a higher α:β cell area in KO mice (0.2±0.015 vs 0.16±0.01, $P<0.5$, n=7). Cytoplasmic free Zn2+ concentrations were significantly lower in αSlc30a8KO alpha-cells (KO 1.14±0.007 vs control 1.33±0.02 F1/Fmin, $P<0.0001$, n=4).The expression of other ZnT family members was unchanged.
Conclusion: ZnT8 deletion from the mouse alpha-cell results in decreased intracellular zinc levels and increased glucagon secretion from isolated islets in vitro at low glucose conditions and in vivo during hypoglycaemia. These findings suggest that altered ZnT8 expression may affect glucagon levels in carriers of SLC30A8 variants in man, and may thus contribute to diabetes risk.
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A genome wide association study of IVGTT based measures of first phase insulin secretion reveals new physiology of known and novel variants

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Background and aims: Deterioration of insulin secretory capacity plays an important role in the development of type 2 diabetes yet the mechanisms underlying the process are not fully understood. As part of the GUINNESS consortium we meta-analysed 8 GWAS and 2 metabochip studies where first phase insulin secretion has been investigated through intravenous glucose tolerance tests (IVGTT) in an attempt to identify the genetic factors involved.
Materials and methods: The meta-analysis included 5 European (n=2090) and 3 non-European (n=1655) studies with GWAS data and 2 European studies with metabochip data (n=741). First phase insulin secretion was assessed with an IVGTT or hyperglycemic clamp and three different traits were defined in all cohorts: Acute Insulin Response to glucose (AIR), Peak Insulin (PeakIns) and Disposition Index (DI) (n=4486), all accounting for fasting glucose levels. GWAS studies were imputed using the 1000G reference panel. Variants were tested for association using linear regression adjusting for age and sex. In sub-analyses, BMI and Insulin Sensitivity Index (SI) were also used as additional covariates.
Results: Of known common type 2 diabetes variants, 7 were associated with both PeakIns and AIR at p<0.01, two *MTNR1B* and *CDKAL1* at genome wide significance for both traits (PeakIns $p=8\times10^{-16}$, $p=6\times10^{-13}$; AIR $p=1\times10^{-12}$, $p=1\times10^{-9}$). Comparing these associations to type 2 diabetes risks and measures based on oral glucose tolerance tests (OGTT) revealed some interesting patterns. The variant at *TCF7L2* is only modestly associated with first phase insulin despite having the strongest effect on diabetes risk. Whereas the diabetes variants at *HNF1A* ($p=4\times10^{-5}$ PeakIns) and *ZFAND6* ($p=8\times10^{-4}$ PeakIns) showed much stronger signals despite being unclassified in previous studies of diabetes intermediate traits using OGTT. Adjustments for BMI and SI did not substantially influence results. Putative novel associations for AIR were observed in population-specific analyses on chromosome 2 and 11 (both $P<5\times10^{-8}$) but these low frequency variants need further validation.
Conclusion: Our genome wide analysis was performed on the largest dataset for intravenously measured first phase insulin secretion. Common type 2 diabetes risk alleles at the *MTNR1B* and *CDKAL1* loci were the most strongly associated with our measures. For other variants, including those in *HNF1A* and *ZFAND6* our analyses provide new insight into physiological mechanisms of diabetes risk.

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The impact of known type 2 diabetes associated variants on circulating levels of GLP-1, GIP and glucagon during an oral glucose tolerance test

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Background and aims: Large-scale genome wide association studies (GWAS) have currently identified 68 genetic determinants of type 2 diabetes. For many of those variants, it is unclear through which mechanisms they exert their effect and little is known about whether these genes also affect incretin hormone levels and/or alpha-cell function. Our aim is to examine the impact of known type 2 diabetes associated variants on circulating levels of glucagon-like peptide-1 (GLP-1), glucose-dependent insulinotropic polypeptide (GIP) and glucagon during an oral glucose tolerance test (OGTT).
Materials and methods: Plasma levels of GLP-1, GIP and glucagon were examined in samples obtained during an OGTT in 1,547 individuals from the ADDITION-PRO cohort. Participants were given a standard 75 g OGTT after an overnight fast of ≥7 hours and blood samples were drawn at 0, 30 and 120 min for assessment of plasma GLP-1, GIP and glucagon levels. The incremental area under the curve of plasma GLP-1, GIP and glucagon were calculated from 0-30 minutes and from 0-120 minutes during the OGTT. Associations between 68 genetic variants and plasma levels of GLP-1, GIP and glucagon were studied using a linear mixed model (EMMAX) implemented in the EPACTS software package by the use of inverse-normalized residuals of the traits adjusted for age, sex and BMI.
Results: None of the associations of the 68 known type 2 diabetes risk variants with circulating plasma levels of GLP-1, GIP or glucagon during the OGTT were significant after correction for multiple testing. However, nominally associated (P<0.05) variants are reported in Table 1.
Conclusion: Known type 2 diabetes risk variants seem not to be strongly associated with altered secretion of GLP-1, GIP or glucagon. Large-scale genome-wide association studies of incretin hormone and glucagon release as well as studies of incretin hormone and glucagon action are needed in order to explain the genetic influence on these traits.

Table 1. Nominally associated (*P*<0.05) variants with plasma GLP-1, GIP and glucagon levels during an OGTT.

Time point	Plasma GLP-1	Plasma GIP	Plasma glucagon
Fasting	*TCF7L2* (rs7903146: -0.094 *P*=0.025)	*UBE2E2* (rs7612463: 0.139 *P*=0.021) *SPRY2* (rs1359790: 0.101 *P*=0.027) *PPARG* (rs1801282: -0.115 *P*=0.037) *ANK1* (rs516946: 0.091 *P*=0.047)	*HNF1A* (rs7957197: 0.120 *P*=0.014) *TMEM154* (rs6813195: 0.090 *P*=0.035) *MTNR1B* (rs10830963: -0.092 *P*=0.036) *HMGA2* (rs1531343: 0.141 *P*=0.045)
30 min	*ZMIZ1* (rs12571751: -0.103 *P*=0.010) *CAMK1D* (rs12779790: 0.148 *P*=0.021)	*ZBED3* (rs4457053: 0.090 *P*=0.039)	*PPARG* (rs1801282: 0.123 *P*=0.024) *HNF1A* (rs7957197: 0.098 *P*=0.045)
2 hours	*CDKN2A/2B* (rs10811661: -0.110 *P*=0.035) *TCF7L2* (rs7903146: -0.090 *P*=0.036) *CDKAL1* (rs10946398: 0.088 *P*=0.036) *HMG20A* (rs7177055: 0.086 *P*=0.045)	*TCF7L2* (rs7903146: -0.091 *P*=0.038)	*ANK1* (rs516946: 0.089 *P*=0.047)
incAUC 0-30 min	*CAMK1D* (rs12779790: 0.161 *P*=0.012) *ZMIZ1* (rs12571751: -0.088 *P*=0.028)	*ZBED3* (rs4457053: 0.093 *P*=0.033)	*PPARG* (rs1801282: 0.171 *P*=0.0015) *ADCY5* (rs11708067: 0.130 *P*=0.0025) *SLC30A8* (rs3802177: 0.105 *P*=0.011) *MTNR1B* (rs1387153: 0.102 *P*=0.020) *MTNR1B* (rs10830963: 0.099 *P*=0.023) *SPRY2* (rs1359790: -0.090 *P*=0.040)
incAUC 0-120 min		*TCF7L2* (rs7903146: -0.093 *P*=0.036) *IGF2BP2* (rs1470579: -0.089 *P*=0.042)	*PPARG* (rs1801282: 0.135 *P*=0.013) *MTNR1B* (rs10830963: 0.108 *P*=0.014) *ADCY5* (rs11708067: 0.102 *P*=0.019) *MTNR1B* (rs1387153: 0.104 *P*=0.019) *LPP* (rs6808574: -0.091 *P*=0.024) *SLC30A8* (rs3802177: 0.084 *P*=0.044)

Beta represents changes for each risk allele in the inverse-normalized residuals of the traits adjusted for age, sex and BMI.

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Effect of 36 hours fasting on genome-wide muscle gene expression in low and normal birth weight men

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Background and aims: An adverse fetal environment might cause low birth weight (LBW) that is associated with increased risk of metabolic diseases. We recently showed that healthy young LBW men cope in a metabolically preferential way with 36 hours of fasting compared to normal birth weight (NBW) men. We hypothesized that fasting might unmask differential metabolic and molecular changes in insulin sensitive tissues from LBW individuals. Therefore, we analyzed genome-wide gene expression in skeletal muscle from 20 young LBW and 17 matched NBW men during 36 h fasting, and after overnight fast in 8 LBW and 8 NBW men.
Materials and methods: Gene expression of more than 99% RefSeq genes was analyzed with Affymetrix HumanGene 1.0 ST arrays. The effect of fasting and LBW was analyzed with non-parametric tests and false discovery rate (FDR) was applied to correct for multiple testing.
Results: The fasting intervention caused increased lipolysis and FFA levels as well as whole-body insulin resistance in the young healthy men. Gene expression of 28,829 transcripts was examined in skeletal muscle. After FDR correction, we did not find any significant transcriptional differences in muscle from LBW versus NBW men. Therefore, we combined the groups when investigating the fasting induced mRNA response. Interestingly, 1,706 (6% of all) transcripts changed significantly in response to 36 h fasting with mTOR signaling, Wnt signaling and cancer related KEGG pathways being up-regulated whereas metabolic pathways including fatty acid synthesis and citrate cycle were down-regulated during 36 h fasting.
Conclusion: The genome-wide transcriptional profile in skeletal muscle in young healthy LBW men was not significantly different than in matched NBW men. In response to 36 h fasting, widespread mRNA changes were observed in skeletal muscle in the combined group of LBW and NBW men. Up-regulation of genes involved in mTOR signaling and Wnt signaling may contribute to beneficial metabolic effects of fasting.
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Selective metabolite biomarkers of isolated IFG (iIFG) and isolated IGT (iIGT)

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Background and aims: IFG and IGT are distinct prediabetic states which may be characterized by different metabolite profiles. This work focused on identifying metabolites selective for the non-overlapping conditions of iIFG and iIGT.
Materials and methods: A targeted metabolomic analysis of fasting plasma samples taken at time=0 of an OGTT was performed. Quantitative measurements for a panel of 23 metabolites previously associated with prediabetes and type 2 diabetes were made using the isotope dilution method. The data was rank normalized using the GenAbel package in R to create a normal distribution. The associations of metabolites for normal (normal glucose tolerance & normal fasting glucose) vs iIFT or iIGT were made using logistic regressions controlling for age, sex, and BMI. Odds ratios for a one SD change in the metabolite level, 95% confidence intervals and p values were calculated for each metabolite and reported as (OR (95% CI) p value). P values were adjusted with a false discovery rate of 0.1. Candidate biomarkers were identified using samples from the observational RISC study 3 year follow-up (normal n=623, iIFG n=220, iIGT n=56). The resulting candidate biomarkers were then validated in a second cohort, the DMVhi study (part of the DEXLIFE project), comprised of subjects at risk for type 2 diabetes (iIFG, iIGT, or FINDRISC score >12; normal n=485, iIFG n=104, iIGT n=31).
Results: In the RISC samples, 2-oxoleucine was associated with iIFG (1.36 (1.1-1.7) 3E-3) and had no significant association with iIGT. 2-Oxovaline was associated with iIFG (1.45 (1.2-1.7) 4E-5) and less so with iIGT (1.49 (1.1-2.0) 9E-3). alpha-Hydroxybutyric acid (AHB) was associated with iIGT (2.54 (1.9-3.5) 5E-9) and was not significantly associated with iIFG. Similar results were found in the DMVhi samples. Both 2-oxoleucine (1.65 (1.3-2.2) 0.001) and 2-oxovaline (1.58 (1.2-2.0) 0.001) were associated with iIFG and were not significantly associated with iIGT. AHB was associated with iIGT (2.75 (1.8-4.2) 4E-5) but less so with iIFG (1.31 (1.0-1.7) 0.05). In both cohorts, all three metabolites are associated with subjects having combined IFG and IGT.
Conclusion: Two branched-chain 2-oxoacids, 2-oxoleucine and 2-oxovaline, were found to be selective biomarkers for iIFG and AHB was found to be a selective biomarker for iIGT. The former are associated with elevated fasting glucose and the latter with impaired glucose disposal and may reflect different pathophysiological aspects of prediabetes. These biomarkers of different prediabetic states may have utility in identifying and stratifying subjects at risk for type 2 diabetes. Finally, these findings were seen in two different European cohorts having a total of 1519 subjects.
Supported by: DEXLIFE project (EU FP7 programme, Grant agreement no: 279228)

OP 19 Incretin-based therapy: novel agents, new indications

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Efficacy and safety of gemigliptin/metformin initial combination therapy versus either as monotherapy in drug-naïve patients with type 2 diabetes

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Background and aims: This study evaluated the efficacy and safety of initial combination therapy with gemigliptin and metformin vs. gemigliptin or metformin monotherapy in drug-naïve patients with type 2 diabetes.
Materials and methods: In this randomized, double-blind, active-controlled, Phase III trial, eligible patients with HbA1c greater than 7.5% were randomized to gemigliptin 50 mg q.d + metformin GR q.d (GEMI/MET, n=141), gemigliptin 50 mg q.d (GEMI, n=142), or metformin GR q.d (MET, n=150). From weeks 2-6, metformin GR was uptitrated in 500 mg/day increments to 2000 mg/day maximum in the gemigliptin/metformin and metformin groups. The primary endpoint was change from baseline in HbA1c after 24 weeks.
Results: Baseline demographics and clinical characteristics were generally well balanced across treatment groups (mean HbA1c 8.68%; age 53.9 years; BMI 25.9 kg/m^2, duration of T2DM 3.92 years). Mean daily metformin doses at week 24 were 1699 mg and 1868 mg for GEMI/MET and MET respectively. All treatment groups showed statistically significant reductions in HbA1c from baseline at week 24. Mean change in HbA1c from baseline was -2.06% for GEMI/MET versus -1.24% for GEMI and -1.47% for MET respectively ($p<0.0001$ for all comparisons of combination therapy vs. monotherapy). The differences in proportions achieving an HbA1c <7 or <6.5% were also statistically significant ($p<0.0001$) between the combination therapy and the respective monotherapy groups. After 24 weeks, FPG decreased in all treatment groups, to the greatest extent with GEMI/MET (Table). Adverse events (AEs) were reported in 61.0%, 53.5% and 58% of subjects on GEMI/MET, GEMI and MET, respectively. The GEMI group had the lowest incidence of drug-related adverse events relative to other groups. The incidence of hypoglycemia was very low (0.0~2.13%) and similar among treatment groups.
Conclusion: Initial combination therapy with gemigliptin and metformin was more effective in glycemic control than their monotherapy and was well tolerated in patients with type 2 diabetes.

	GEMI/MET (n=136)	GEMI (n=140)	MET (n=148)
HbA1c , %			
Baseline (SD)[a]	8.65 (0.88)	8.66 (0.90)	8.73 (0.91)
Change from baseline (SD)[a]	-2.06 (0.98)	-1.24 (0.96)	-1.47 (1.1)
Difference vs. GEMI (95% CI)	-0.82 (-1.02, -0.63)*	-	-
Difference vs. MET (95% CI)	-0.62 (-0.82, -0.41)*	-	-
At Week 24			
Subjects with HbA1c <7.0%, n (%)	112 (82.4)	57 (40.7)**	74 (50.0)**
Subjects with HbA1c <6.5%, n (%)	71 (52.2)	31 (22.1)**	33 (22.3)**
FPG (mg/dl)			
Baseline (SD)[a]	172.7 (47.7)	169.7 (42.5)	178.6 (49.7)
Change from baseline (SD)[a]	-57.0 (42.0)	-28.6 (37.8)	-47.6 (43.9)
Difference vs. GEMI (95% CI)	-26.61 (-33.72, -19.50)*	-	-
Difference vs. MET (95% CI)	-13.30 (-19.92, -6.68)*	-	-

[a] Mean (SD)
*P ≤0.001 for the between-group difference comparing combination therapy and both of its respective monotherapy and were analyzed using an ANCOVA model.
**P ≤0.001 for the between-group difference comparing combination therapy and both of its respective monotherapy and were analyzed using a Chi-square test.
Adjusted means based on ANCOVA in full analysis set with last observation carried forward imputation

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Omarigliptin, a once-weekly DPP-4 inhibitor, provides similar glycaemic control to sitagliptin in patients with type 2 diabetes mellitus inadequately controlled on metformin

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Background and aims: Omarigliptin (OMARI; MK-3102) is a potent, oral, once-weekly, dipeptidyl peptidase-4 (DPP-4) inhibitor in development for the treatment of patients with type 2 diabetes mellitus (T2DM). The convenience of an effective, well-tolerated, weekly oral drug may improve medication adherence. This global study compared the efficacy of OMARI 25 mg once weekly (q.w.) with that of sitagliptin (SITA) 100 mg daily (q.d.) in patients with T2DM and inadequate glycemic control on metformin monotherapy. The study also assessed the safety and tolerability of OMARI.
Materials and methods: This was a randomised, double-blind, non-inferiority study. Patients on metformin 1500 mg and an A1C ≥6.5% to ≤9.0% who met all other enrollment criteria entered a 2-week, single-blind, placebo run-in period, and were then randomised 1:1 to OMARI 25 mg q.w. or SITA 100 mg q.d. The primary efficacy analyses used the Full Analysis Set (FAS) population: patients who received at least one dose of study therapy and had a baseline or at least one post-randomization measurement. The primary hypothesis of non-inferiority (using an upper bound of 0.3%) of OMARI versus SITA in decreasing A1C at Week 24 was assessed using a constrained longitudinal data analysis model.
Results: Baseline characteristics were balanced across the two groups; baseline A1C was approximately 7.5%. At Week 24, OMARI and SITA both reduced A1C and FPG from baseline (Table). The upper bound of the two-sided 95% confidence interval for the between-group difference in reduction of A1C was 0.08% confirming non-inferiority. Changes from baseline in A1C were greater in subgroups with higher baseline A1C (Table). The percentage of patients with one or more adverse event was 36.3% and 40.6% in the OMARI and SITA groups, respectively, with no notable differences in specific adverse events. The incidences of serious adverse events, drug-related adverse events and discontinuations were similar between treatment groups. The incidences of hypoglycemia were 3.7% and 4.7% in the OMARI and SITA groups, respectively, with 1 severe hypoglycemia event reported in the OMARI group.
Conclusion: In patients with T2DM and inadequate glycemic control on metformin, once-weekly OMARI 25 mg provided similar improvement in glycemic control compared to SITA 100 mg q.d. Treatment with both agents was associated with a low incidence of hypoglycaemia and both agents were well-tolerated.

Change from Baseline in A1C (%)					
Treatment	N	Baseline Mean	Week 24 Mean	Change from Baseline LS Mean (95% CI)	Difference in LS Means (95% CI)
Omarigliptin 25 mg	322	7.52	6.99	-0.47 (-0.55, -0.38)	-0.03 (-0.15, 0.08)†
Sitagliptin 100 mg	320	7.49	7.01	-0.43 (-0.51, -0.35)	
Subgroup Analysis of Change from Baseline in A1C (%)					
Treatment	N	Baseline Mean	Week 24 Mean	Change from Baseline LS Mean (95% CI)	Difference in LS Means (95% CI)
Baseline A1C ≥ 7.0% and <8.0%					
Omarigliptin 25 mg	149	7.43	7.01	-0.39 (-0.51, -0.27)	0.01 (-0.16, 0.19)
Sitagliptin 100 mg	145	7.40	6.92	-0.40 (-0.53, -0.28)	
Baseline A1C ≥ 8.0%					
Omarigliptin 25 mg	86	8.55	7.68	-0.79 (-0.99, -0.58)	-0.08 (-0.37, 0.21)
Sitagliptin 100 mg	88	8.47	7.74	-0.71 (-0.91, -0.51)	
Change from Baseline in FPG (mmol/L)					
Treatment	N	Baseline Mean	Week 24 Mean	Change from Baseline LS Mean (95% CI)	Difference in LS Means (95% CI)
Omarigliptin 25 mg	322	8.9	7.9	-0.8 (-1.0, -0.6)	-0.2 (-0.5, 0.0)‡
Sitagliptin 100 mg	320	8.5	8.0	-0.5 (-0.7, -0.4)	

Based on a cLDA model including terms for treatment, time, and the interaction of time by treatment, with the constraint that the mean baseline is the same for all treatment groups.
N = Number of patients in the population. CI = Confidence Interval, LS = Least Square.
†p=0.561; ‡p=0.089

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Improved glucose control without increased hypoglycaemia risk with insulin glargine/lixisenatide fixed-ratio combination (LixiLan) vs insulin glargine alone

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Background and aims: In a 24-week, proof-of-concept study in insulin-naïve patients with type 2 diabetes on metformin (N=323; mean baseline HbA_{1c} 8.0%, age 56.7 yrs, diabetes duration 6.7 yrs), once-daily LixiLan demonstrated greater HbA_{1c} reductions than insulin glargine alone, with a change from baseline of -1.8% vs -1.6%, respectively (p=0.01); endpoint HbA_{1c} of 6.3% vs 6.5%, respectively, and 84% vs 78% of patients achieved HbA_{1c} <7%. The rate of symptomatic hypoglycaemia was similar (~25%) in both groups. This post hoc exploratory analysis was conducted to determine whether the risk of symptomatic hypoglycaemia was impacted by the magnitude of HbA_{1c} reductions.
Materials and methods: Hypoglycaemia rates were assessed in subgroups based on achieving HbA_{1c} categories (<6%; ≥6 - <6.5%; ≥6.5 - <7%; ≥7%) and reduction in HbA_{1c} from baseline (≤1%; >1 - ≤1.5%; >1.5 - ≤2%; >2%) at Week 24, last observation carried forward. Cochran-Armitage Trend Tests (2-sided) and a regression analysis assessing non-homogeneous rates of hypoglycaemia across groups were performed.
Results: Neither test showed a relationship between hypoglycaemia rates and HbA_{1c} (p>0.1; Table).
Conclusion: Reaching lower near-normal HbA_{1c} levels and achieving greater HbA_{1c} reductions with LixiLan vs insulin glargine alone did not increase rates of hypoglycaemia at any level of glucose control.

Table. Number* of patients reporting symptomatic hypoglycaemia† in each HbA_{1c} category or mean change subgroup at Week 24

Parameter	HbA_{1c}	Incidence of symptomatic hypoglycaemia, n/N‡	
		LixiLan N=160 (%)	Insulin glargine alone N=161 (%)
HbA_{1c} level reached at Week 24§	<6%	12/52 (23.1)	9/29 (31.0)
	≥6 – <6.5%	15/60 (25.0)	14/62 (22.6)
	≥6.5 – <7%	5/23 (21.7)	11/35 (31.4)
	≥7%	8/25 (32.0)	6/35 (17.1)
Absolute mean reduction in HbA_{1c} from baseline§	≤1%	10/29 (34.5)	9/48 (18.8)
	>1 – ≤1.5%	10/44 (22.7)	15/42 (35.7)
	>1.5 – ≤2%	4/30 (13.3)	6/25 (24.0)
	>2%	16/57 (28.1)	10/46 (21.7)

*Data are for patients from the modified intent-to-treat population with relevant baseline and Week 24 last observation carried forward measurements.
†Symptomatic hypoglycaemia was defined as an event with typical symptoms of hypoglycaemia and 'documented' when accompanied by a measured plasma glucose concentration of ≤3.9 mmol/L (70 mg/dL).
‡While n indicates the number of patients with symptomatic hypoglycaemia, N shows the number of patients achieving a specific HbA_{1c} level or reduction. A total of 40 patients in each treatment group had symptomatic hypoglycaemia during the 24-week treatment period.
§There was no statistically significant relationship between hypoglycaemia rates and HbA_{1c} levels reached at Week 24 and absolute mean reduction in HbA_{1c} from baseline (p>0.1).

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A randomised, double-blind, placebo-controlled, 39 week trial of ITCA 650 as add-on therapy in type 2 diabetes

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Background and aims: ITCA 650, an injection-free glucagon-like peptide-1 (GLP-1) receptor agonist delivers continuous subcutaneous (SC) exenatide for up to 12 months from a single sub-dermal placement of the osmotic minipump.
Materials and methods: This first pivotal Phase 3 study randomized (1:1:1) type 2 diabetes (T2DM) subjects with HbA1c ≥7.5 to ≤10% on diet and exercise or oral antidiabetics to ITCA 650 40 or 60 mcg/d or placebo (PBO), starting with 20 mcg/d for 13 weeks, then 40 or 60 mcg/d for 26 wks. HbA1c, body weight, and % achieving HbA1c <7% were tested hierarchically.
Results: Baseline characteristics were similar in the 3 groups (n=460): mean HbA1c 8.5%; BMI 33.5 kg/m2, diabetes duration 9 years. Efficacy endpoints were statistically significant for both doses vs PBO. Mean reduction in HbA1c from baseline was -1.1% (97.5% CI: -1.29, -0.71) and -1.2% (97.5% CI: -1.37, -0.80) with 40 and 60 mcg/d (p=0.001 vs PBO) at Week 39 (last observation carried forward). Greater reductions in HbA1c (-1.7%) occurred in subjects not taking sulfonylureas (SUs), most of whom were on metformin monotherapy. Progressive weight loss was dose dependent (Figure). ITCA 650 60 mcg/d resulted in greater weight loss, more subjects achieving HbA1c <7%, and less need for rescue therapy. Gastrointestinal adverse events consistent with the GLP-1 class were most common and decreased over time. ITCA 650 was well tolerated with a low rate of discontinuation for adverse events.
Conclusion: ITCA 650 ensured consistent SC delivery of exenatide over 39 weeks and resulted in meaningful reductions in HbA1c and body weight in inadequately controlled T2DM.
Clinical Trial Registration Number: NCT01455857
Supported by: Intarcia Therapeutics, Inc.

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Once a month treatment with HM11260C improves glycaemic control in type 2 diabetes mellitus: interim data from a 16-week study

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Background and aims: HM11260C (HM) is a novel ultra-long acting GLP-1R agonist with a $T_{1/2}$ of~158 hrs. This 16-week, randomized, placebo (PBO) controlled, double-blind parallel group study was designed to investigate efficacy, safety, and tolerability of a range of once monthly (QM) HM doses in subjects with T2DM.
Materials and methods: We report interim data from 86 patients (mean age 56 yrs, BMI 32.1 kg/m^2, T2DM duration 95.4 months) out of 209 patients with unsatisfactory glycemic control while on a stable dose of metformin (≥3 months) before entering this study. Patients were randomized to one of three HM QM doses (8, 12 and 16 mg) or PBO, followed by a 4-week titration period.
Results: Overall, all HM doses produced remarkable reductions in HbA1c, fasting plasma glucose, and 7-point daily glucose (Table 1). Percentage of patients on HM achieving A1c Target<7% was 73.3% (8 and 16 mg) and 64.3% (12 mg) vs. 22.2% on PBO. With HM, the reduction in body weight ranged from 2.16% to 2.80% vs. 1.24% on PBO. The most frequent AEs in HM-treated patients were mild or moderate gastrointestinal events. No increase in heart rate was recorded.
Conclusion: All QM doses of HM demonstrated clinically meaningful improvement in blood glucose and body weight loss. The current results will have to be confirmed upon completion of the trial and warrant further studies to evaluate the long-term efficacy and safety of a monthly regimen with HM in T2DM.

Table 1. Summary of efficacy and safety measures after 16 weeks (Interim analysis)

	Placebo (n=22)	HM11260C 8 mg/month [c] (n=21)	HM11260C 12 mg/month [c] (n=22)	HM11260C 16 mg/month [c] (n=21)
Baseline characteristics (Safety set)				
HbA1c, %	7.90 (0.575)	8.01 (0.693)	7.61 (0.852)	7.73 (0.657)
Body weight, kg	93.967 (20.877)	95.233 (15.320)	91.732 (17.038)	91.000 (10.110)
Efficacy measures (Full Analysis Set)				
HbA1c change, % [a]	-0.30 (0.171)	-1.26* (0.178) (p=0.0002)	-0.81 (0.170)	-1.03 (0.180)
% of subjects with HbA1c < 7.0% [b]	22.2	73.3	64.3	73.3
% of subjects with HbA1c ≤ 6.5% [b]	5.6	46.7	57.1	53.3
Fasting plasma glucose change, mmol/L [a]	0.20 (0.418)	-1.31 (0.445)	-0.74 (0.440)	-0.81 (0.443)
Mean daily glucose change (7-point SMBG), mmol/l [a]	0.065 (0.364)	-0.998 (0.427)	-0.638 (0.393)	-1.228 (0.393)
Body weight change, kg [a]	-0.866 (0.698)	-2.039 (0.743)	-2.271 (0.705)	-2.375 (0.754)
Body weight change, % [a]	-1.24 (0.740)	-2.32 (0.788)	-2.16 (0.747)	-2.80 (0.800)
Safety measures (Safety Set)				
Incidence of Nausea, %	0	28.6	40.9	57.1
Incidence of Vomit, %	4.5	9.5	9.1	28.6
Heart rate change, BPM [d]	-1.6 (5.97)	-2.1 (7.21)	-2.4 (10.32)	2.2 (7.34)

[a] MMRM, statistics indicate LS Mean (SE), MMRM includes treatment, visit and their interaction as factors to explain change in HbA1c and baseline HbA1c as a covariate with unstructured covariance matrix across all visits. Type I error rate was adjusted using O'Brien-Fleming boundary for interim that is 0.0035.
[b] Fisher's exact test
[c] Titration phase consists of HM 4 mg once a week for 4 weeks, HM 8 mg once during the next week and assigned doses once a month for 2 months.
[d] Mean (SD)
* p-value <0.0035 vs. placebo

Clinical Trial Registration Number: NCT02081118

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Efficacy and safety of liraglutide added to insulin therapy in patients with type 1 diabetes: the Lira-1 study

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Background and aims: Weight gain and hypoglycaemia are common side effects to insulin therapy in type 1 diabetes (T1D). The combination of insulin and glucagon-like peptide-1 receptor agonist (GLP-1RA) therapy has proven effective in reducing weight gain and insulin dose in type 2 diabetes and may have similar effects in patients with T1D.
Materials and methods: This 26-week trial is the first randomised, double-blinded, placebo controlled study to evaluate efficacy and safety of GLP-1RA treatment in poorly controlled, overweight patients with T1D. In total 100 patients with T1D, HbA1c>64 mmol/mol and BMI>25 kg/m^2, were randomised to liraglutide 1.8 mg once daily or placebo added to intensive insulin therapy.
Results: Mean baseline characteristics were similar between groups (liraglutide±SD;placebo±SD) age 47±13;49±12 years (p=0.355), HbA1c 73±8;73±8 mmol/mol (p=0.827), total daily insulin dose 60±23;61±21 IU/day (p=0.813) and bodyweight 93.4±14.2;94.0±12.5 kg (p=0.828) except diabetes duration 20±12;25±12 years (p=0.049). After 12 weeks of treatment, liraglutide reduced HbA1c, bodyweight and daily insulin dose compared with placebo. At the end of treatment no difference in HbA1c between groups was found. Bodyweight and daily insulin dose remained reduced in the liraglutide group compared to placebo. Frequency of hypoglycaemia did not differ between groups. Heart rate increased with liraglutide treatment, while no differences in systolic or diastolic blood pressure was found by 24-hour ambulatory blood pressure monitoring (table). Gastrointestinal AEs occurred more frequently with liraglutide than placebo, i.e. nausea (48% vs.7%).
Conclusion: In conclusion, liraglutide added to insulin treatment in overweight and poorly controlled patients with T1D reduced bodyweight and daily insulin dose, but increased heart rate and did not improve HbA1c compared with placebo at end of treatment.

Key Results: Liraglutide 1.8 mg vs. placebo as add-on to intensive insulin treatment

	Liraglutide, n=46 [95% CI]	Placebo, n=44 [95% CI]	Difference	*P* value
Change in HbA1c at 12 weeks (mmol/mol)	-6.35 [-7.77; -4.92]	-2.57 [-4.40; -0.73]	-3.78	0.001
Change in HbA1c at 26 weeks (mmol/mol)	-6.02 [-7.79; -4.25]	-4.23 [-5.94; -2.52]	-1.79	0.146
Change in bodyweight at 12 weeks (kg)	-5.02 [-5.84; -4.20]	-0.07 [-0.92; +0.78]	-4.95	<0.0001
Change in bodyweight at 26 weeks (kg)	-5.89 [-6.97; -4.82]	+0.23 [-0.63; +1.08]	-6.12	<0.0001
Change in daily insulin dose at 12 weeks (units)	+3.15 [+1.20; +5.10]	+11.77 [+7.91; +15.63]	-8.62	<0.0001
Change in daily insulin dose at 26 weeks (units)	+4.04 [+1.94; +6.15]	+13.61 [+9.92; +17.30]	-9.57	<0.0001
Change in hypoglycemic episodes at 26 weeks (%)*	+0.08 [-3.85; +4.01]	+2.92 [-1.49; +7.33]	-2.84	0.349
Change in heart rate at 26 weeks (beats per minute)	+4.74 [2.93; 6.55]	+0.24 [-2.35; 2.83]	+4.50	0.005
Change in systolic blood pressure at 26 weeks (mmHg)	+0.55 [-3.51; +4.62]	-1.29 [-6.80; +4.23]	+1.84	0.585
Change in diastolic blood pressure at 26 weeks (mmHg)	+1.55 [-0.40; +3.50]	-0.91 [-3.95; +2.13]	+2.46	0.169

*Glucose < 3.9 mmol/L assessed by blinded continuous glucose monitor.

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OP 20 Diabetes and pregnancy: long-term implications for mother and child

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A reduced incretin effect can be detected in non-diabetic women with previous gestational diabetes even before the development of diabetes

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Background and aims: The incretin effect is decreased in patients with type 2 diabetes, which prompted us to study whether reduced incretin effect can be detected in high-risk individuals such as women with previous gestational diabetes mellitus (GDM).
Materials and methods: One hundred and two non-diabetic women with previous GDM were examined on two separate occasions: 1) 4-hour 75 g OGTT and 2) isoglycaemic i.v. glucose infusion (IIGI). Based on the fasting plasma glucose (FPG) and the 2-hour plasma glucose concentrations of the OGTT, the women were classified as having normal glucose tolerance (NGT) or prediabetes (impaired fasting glucose and/or impaired glucose tolerance) (WHO 2006).
Results: Sixty three of the women (62%) were diagnosed with prediabetes (age: 38±5 years (mean ± SD); BMI: 32±4 kg/m^2; waist:hip ratio: 0.9 ±0.1; HbA_{1c}: 34±4 mmol/mol; insulin resistance according to homeostatic model assessment 2 ($HOMA2_{IR}$): 1.8±0.8), and 39 (38%) had NGT (age: 39±5 years; BMI: 31±5 kg/m^2; waist:hip ratio: 0.9±0.1; HbA_{1c}: 33 ±4 mmol/mol; $HOMA2_{IR}$: 1.9±1.1). Women with prediabetes had higher FPG (5.5±0.5 vs. 5.2±0.4 mmol/l, p=0.002) and 2-hour plasma glucose during OGTT (9.2±1.1 vs. 7.0±0.8 mmol/l, p<0.0001) as well as lower insulin sensitivity measured by Matsuda Index (2.6±1.3 vs. 3.3±1.8 p<0.005) compared to women with NGT. The incretin effect was calculated from insulin responses during the two experimental days [100%×($AUC_{insulin,OGTT}$ - $AUC_{insulin,IIGI}$) / $AUC_{insulin,OGTT}$] and amounted to 41±18% and 54±13% in women prediabetes and NGT, respectively (p=0.0003) (Figure). The groups were similar with respect to age, BMI, waist:hip ratio, HbA_{1c}, $HOMA2_{IR}$, fasting insulin (110±58 vs. 96±60 pmol/l, p=0.078), number of previous GDM-pregnancies (1.2 ±0.4 vs. 1.1±0.3, p=0.448) and duration since index pregnancy (5.2±2.7 vs. 4.9±2.5 years, p=0.658).
Conclusion: Our results show that prediabetes is prevalent in women with previous GDM, and alterations in the incretin effect can be detected in these high-risk individuals even before the development of type 2 diabetes.

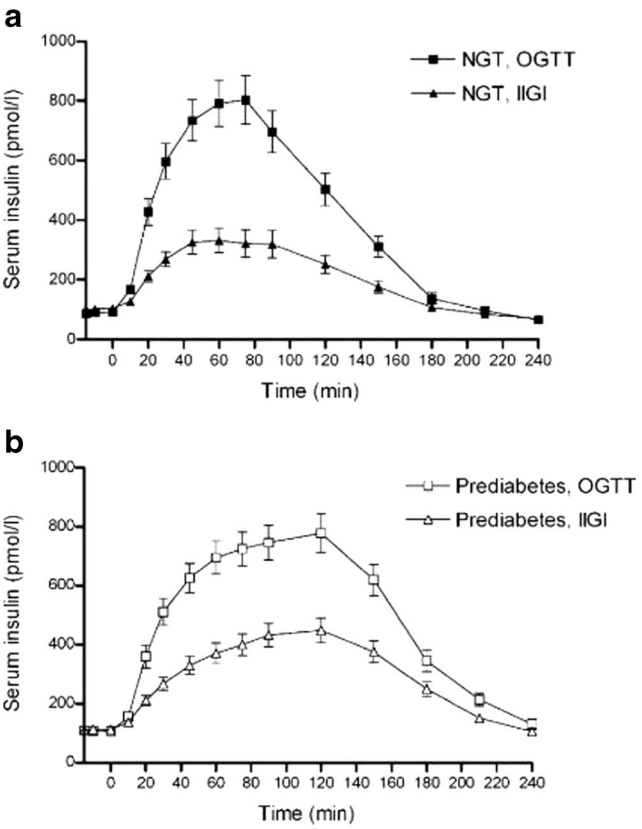

Figure: Insulin response during OGTT and isoglycaemic i.v. glucose infusion (IIGI) presented as mean ± SEM. A) Normal glucose tolerant women (NGT): Incretin effect [100% × ($AUC_{insulin, OGTT}$ - $AUC_{insulin, IIGI}$) / $AUC_{insulin,OGTT}$] amounted to 54 + 2% (mean ±SEM). B) Women with prediabetes: Incretin effect amounted to 4 1 ⊥ 2%.

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Insulin resistance and large amounts of visceral fat characterise women with previous gestational diabetes and non-alcoholic fatty liver disease

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Background and aims: Non-alcoholic fatty liver disease (NAFLD), a condition with fat accumulation in the liver in the absence of significant

alcohol consumption, is the most common liver disease in Western countries. The disease spectrum spans from simple steatosis to liver cirrhosis. In type 2 diabetes, NAFLD is linked to cardiovascular disease and hepatocellular carcinoma, which can occur in patients with or without cirrhosis. Because of their high risk of type 2 diabetes, women with previous gestational diabetes (GDM) may have a high risk of developing NAFLD.
Materials and methods: Non-diabetic women with previous GDM (n= 98, age: 38±5 years) underwent 75 g OGTT, dual energy X-ray absorptiometry whole body scan and ultrasound scan and elastography of the liver to evaluate steatosis (NAFLD). Elastography is a novel, non-invasive way of measuring steatosis. The median of ten representative elastographic measurements is provided as the E-median index. Insulin resistance was evaluated by the HOMA2-IR. Values are mean ± SD for age and BMI and mean±SEM for the remaining variables.
Results: Twenty-six (27%) women had NAFLD based on the ultrasound scan and 72 (73%) were without steatosis. None had cirrhosis. When comparing the two groups, women with NAFLD had significantly higher BMI (34±5 vs. 31±4 kg/m^2, p= 0.001) and android-to-gynoid fat-ratio (1.2±0 vs. 1.1±0, p= 0.021). Women with NAFLD also had larger visceral fat mass (1,488±136 vs. 1,009±60 g, p= 0.003), were more insulin resistant (2.5±0 vs. 1.7±0, p=0.019), had higher fasting C-peptide (672± 61 vs. 496±21 pmol/l, p= 0.011), E-median index (1.7±0 vs. 0.9±0, p= 0.001), and liver enzyme levels (alanine aminotransferase: 34±4 vs. 23± 1 U/l, p= 0.017; aspartate aminotransferase: 31±2 vs. 26±1 U/L, p= 0.018) and lower plasma high density lipoprotein cholesterol (1.1±0 vs. 1.3±0 mmol/l, p= 0.019). Multivariable logistic regression analysis showed that visceral fat mass (p=0.0011) and the aspartate aminotransferase level (p= 0.001) were independent predictors of NAFLD.
Conclusion: The results show that a large proportion of women with previous GDM and insulin resistance have NAFLD at a young age. We have identified predictors to be used for early identification of these women for best possible management of comorbidities in order to prevent future liver disease and cardiovascular events.
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Decreased gene expression of leptin, adiponectin and resistin in subcutaneous adipose tissue biopsies from adult offspring of women with diabetes in pregnancy

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Background and aims: Offspring exposed to intrauterine hyperglycaemia are at increased risk of developing adiposity and type 2 diabetes (T2D). Adipose tissue secretes adipokines such as leptin, adiponectin, and resistin, all of which play important roles in lipid and carbohydrate metabolism, as well as in the development of adiposity and T2D. We hypothesized that the plasma levels, and/or expression levels of the genes encoding these adipokines in subcutaneous adipose tissue (SAT), may be altered in adult offspring of women with diabetes in pregnancy compared to unexposed controls.
Materials and methods: We obtained a total of 166 biopsies from subcutaneous adipose tissue (SAT) in adult offspring of women with gestational diabetes mellitus (O-GDM (n=60)) or type 1 diabetes in pregnancy (O-T1D (n=63)), and offspring of women (controls) from the background population (O-BP (n=43)). All subjects were born between 1978 and 1985 and data was collected in 2012-2013. The subjects underwent clinical examinations with anthropometric measurements and 2-hour 75 g oral glucose tolerance tests (OGTT's), as well as a dual energy x-ray absorptiometry (DEXA) whole-body scanning. Women with T1D were characterized by increased average plasma glucose levels during pregnancy compared with women with GDM during pregnancy. Gene expression of leptin, adiponectin and resistin in SAT was examined by real-time PCR and plasma levels were measured by Meso Scale Discovery.
Results: No significant differences were found in average BMI, waist-hip ratio, or body composition between the three groups. Compared to O-BP, 2-hour plasma glucose was significantly higher in O-GDM (p=0.019) and O-T1D (p=0.001). Plasma levels of leptin were increased in O-GDM and in O-T1D compared to O-BP, but only the difference between O-T1D and O-BP reached statistical significance (p=0.05). Measurement of plasma adiponectin and resistin levels are ongoing. Interestingly, significantly lower levels of gene expression of all three adipokines were found in O-GDM compared with O-BP: leptin (1.18 vs. 1.63; p=0.003), adiponectin (1.32 vs. 1.99; p=0.0003) and resistin (1.07 vs. 7.01; p<=0.0001). Similarly, in O-T1D compared with O-BP, significantly decreased levels of adiponectin (1.56 vs. 1.99; p=0.03) and resistin (3.56 vs. 7.01; p=0.001) were found. The gene expression of leptin in O-T1D was similar to that in O-BP.
Conclusion: Despite borderline increased plasma leptin levels, the gene expression of leptin in SAT was markedly reduced in O-GDM compared with O-BP controls. Furthermore, the gene expression of the two other adipokines, adiponectin and resistin, was also significantly reduced in O-GDM compared with O-BP. O-T1D was exposed to a higher degree of intrauterine hyperglycaemia, yet the adipokine gene expression differences in O-T1D compared with O-BP were quantitatively less pronounced, indicating that the quantitatively more pronounced changes seen in O-GDM compared with O-BP cannot be explained by intrauterine hyperglycaemia alone.
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DNA methylation and gene expression of PPARGC1A in muscle and fat biopsies from adult offspring of women with diabetes in pregnancy

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Background and aims: Fetal exposure to intrauterine hyperglycemia is associated with increased risk of type 2 diabetes (T2DM) later in life, but the underlying mechanisms are unknown. Epigenetics has been proposed as a link between environmental exposures and the phenotype. Methylation of CpG-sites in the DNA is the most studied epigenetic marker. Increased CpG methylation and reduced gene expression of the metabolic regulator peroxisome proliferator-activated receptor gamma co-activator 1 alpha (PPARGC1A) in skeletal muscle and subcutaneous adipose tissue (SAT) has been reported in patients with T2DM. We aimed to investigate DNA methylation and gene expression of PPARGC1A in skeletal muscle and SAT from adult offspring of women with diet-treated gestational diabetes (O-GDM, N=83) or with type 1 diabetes (O-T1DM, N=67) and from the background population (O-BP, N=58) (Total N=208).
Materials and methods: The adult offspring (26-35 year) underwent a 2-hour 75 g OGTT and tissue sampling from the vastus lateralis muscle and abdominal SAT. PPARGC1A promoter DNA methylation was studied by pyrosequencing at three CpG sites. PPARGC1A gene expression was assessed using real-time quantitative PCR.

Results: Plasma glucose during OGTT was significantly higher for both groups exposed to intrauterine hyperglycemia compared to O-BP (O-GDM: [30 min: p=0.005] and [120 min: p=0.019]; O-T1DM: [30 min: p=0.001]). Average PPARGC1A promoter DNA methylation was higher in O-GDM in muscle (p=0.05) and SAT (p=0.02) compared to O-BP, while no differences neither in muscle or SAT was found between O-T1DM and O-BP. PPARGC1A gene expression in muscle was significantly lower in O-GDM compared to O-BP (p=0.0003), while no difference was found between O-T1DM and O-BP. PPARGC1A gene expression in SAT was similar in all three groups.
Conclusion: Both groups of adult offspring of women with diabetes in pregnancy had elevated glucose levels during OGTT. O-GDM presented significantly altered DNA methylation in both skeletal muscle and SAT and decreased PPARGC1A gene expression in skeletal muscle. The findings in O-GDM may contribute to the increased risk of developing T2DM, but our negative findings in O-T1DM despite fetal exposure to more severe hyperglycemia indicate that other mechanisms than only exposure to intrauterine hyperglycaemia or other mechanisms in terms of epigenetic markers may be involved in fetal programming of adult adverse metabolic health.
Supported by: The Research Found of Rigshospitalet

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Effects of a hyperglycaemic intrauterine environment on offsprings epigenome and risk of cardio-metabolic disease later in life
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Background and aims: Offspring of women with Gestational Diabetes Mellitus (GDM) are at high risk of developing Type 2 Diabetes (T2D) later in life, but the putative epigenetic mechanisms underlying this association remain unknown. Using an epigenome-wide approach in a subcohort of the Danish National Birth Cohort, we aimed to explore whether DNA methylation in blood differed in 9-14 year old offspring of GDM women versus matched controls.
Materials and methods: We recruited 623 GDM- and 617 control offspring. DNA from 95 GDM offspring and 95 controls were analyzed for genome-wide DNA methylation profiles using the Infinium HumanMethylation450 BeadChip.
Results: BMI, fat percentage, fasting glucose, insulin and c-peptide levels were higher among GDM offspring compared to controls (P≤0.04). We did not find any differentially methylated positions at the genome-wide significance level (Bonferroni adjusted), but identified 1,828 sites potentially differentially methylated between the groups (P≤0.01, unadjusted). When performing a differential methylated region (DMR) analysis, we identified a small non-coding RNA, VTRNA2-1, that reached genome-wide significance, covering 16 CpG sites overall presenting lower methylation in the GDM group (P=0.02, adjusted). Multiple examples of differential methylation within the same gene were noted, suggesting a coordinated change in methylation, and several localized to genes previously associated with DNA methylation changes, in earlier studies of GDM cord blood and placenta samples. From the top ten probes based on P-value, PTCH1 was identified with decreased methylation in GDM offspring and associated with increased glucose levels among the offspring (P=0.02).
Conclusion: Our data suggests that offspring of GDM women exhibit pre-diabetes traits at preadolescent age. A substantial proportion of methylation differences observed in previous GDM cord blood/placenta studies are likely to resolve during childhood, thus, the persistent marks identified in this study may serve as interesting sites in investigation of causal relation to the increased T2D risk later in life. Validation studies of the identified 1,828 sites are in progress in the entire cohort of 1,240 children.

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Epigenetic effects of maternal type 1 diabetes: placental DNA methylations, miRNAs and their effect on gene expression
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Background and aims: Offspring of mothers with type 1 diabetes (T1D) have a lower risk of T1D than offspring of fathers with T1D. This could be explained by intrauterine, epigenetic effects, detectable already at the time of birth. Our aim was to assess the effect of maternal T1D on the placental expression of miRNAs and DNA methylation and their effect on gene expression.
Materials and methods: Samples of the maternal and foetal sides of the placenta were obtained from women with T1D and type 2 diabetes (T2D), women whose partner had T1D and controls matched for age and gestational age. Five "pools" of 8-10 samples of maternal and 5 of foetal placenta were analysed. DNA methylation patterns (Methylation-specific digital karyotyping) were analysed and compared. Genes in which high methylation (fold-change of tags<-1) levels coincided with a reduction in the mRNA expression level (fold-change<-1.5) were identified. Massive sequencing of miRNAs was performed. Potential target genes of novel miRNAs were sought (simulation, miRDB). We selected miRNAs that showed a difference in expression between groups (p<0.1) and a difference (in the opposite direction) in the expression levels of the target mRNA (fold-change below-0.5/above0.5). We report the changes identified in the foetal side of the placentas in T1D
Results: A total of 38 women with T1D and 32 with T2D, 15 women whose partners had T1D and 59 controls have been included [age 32.3(5.9), 32.3(5.8), 28.4(5.1) and 29.8 (5.8), years; gestational age 38.7(1.6), 38.5(1.5), 38.4(1.3) and 39.5 (1.4) weeks, respectively); pregestational HbA1c in the women with diabetes 7.3(1.9) and 7.0(1.2)% and third trimester HbA1c 6.4(0.9) and 6.1(0.7) %]. We identified 13 genes with relevantly different methylation in the foetal placenta of mothers with T1D, as well as 5 new and 8 known miRNAs (See table). The latter have consequences on the expression of genes involved in immune response, aminoacid and protein metabolism and insulin signalling.
Conclusion: Epigenetic effects of maternal T1D can be detected at birth and the most relevant seem to be due to hyperglycaemia, rather than autoimmunity, as suggested by a lack of difference with the T2D group. The implications of these preliminary findings are still to be clarified.

T1D compared with	Methylation increased and gene expression reduced in T1D	Methylation increased and gene expression reduced in comparison group
T2D		
Control	NUMBL, SPTB, DTNB, CCND1, KCNQ1*	KIF2A, NIPA1
Partner with T1D	KCNQ1*, FAT4, TRPM4, PTGS2	TAF15, PPOX, PIGH

T1D compared with	Differently expressed miRNAs	Affected genes	Novel miRNAs (sequence)
T2D			
Control	mir-16-5p** miR-19a-3p** miR-204-5a miR-20a**	CCND1, GSTM4 HARS2, HSPA18 HSPA1A, IOP4 ITGA2, PISD UBE2S, UBE4A WNT3A, CCND1 CDH11, CCND1	CHR1_749 TGAGACTCTGGGTCAGTCTA# CHR17_245 GGGGGCCGGCGGCGGCGGCG
Partner with T1D	miR-1 miR-127-3p miR-135a-5p miR-145-5p	ANP32B, GCH1 GNPNAT1, IQGAP3 KIF2A, LZTFL1* SLC25A22, BCL6 APC, JAK2* IRS1*	CHR10_580 ATATATAGTATATGTGCATGTA CHR1_923 TGAGACTCTGGGTCAGTCTA# CHR11_307 GAGGGGGCGGGCTCCGG CHR19_396 CCCGCCGCCCCTCCTCCTCCC

Identical miRNAs, *previously related to diabetes, **previously related to preeclampsia

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OP 21 The multifaceted danger of hypoglycaemia

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The risk of severe hypoglycaemia events in working-age adults and use of basal insulins NPH, glargine and detemir: a nationwide register study in Finland

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Background and aims: Hypoglycaemia is a common and costly side effect of insulin treatment. The risk of hypoglycaemia increases with the duration of treatment and with advancing age. In comparison to the earlier generation basal insulin NPH, modern, longer acting basal insulins glargine and detemir have shown a lower incidence of hypoglycaemia in clinical studies. In real-life care the benefits have not been fully demonstrated, especially among the working-age population, for whom the target levels of glucose control are strict than for older persons. We evaluated the risk of severe hypoglycaemia events among new users of NPH, glargine and detemir in a real-world setting among working-age adults in Finland.
Materials and methods: Persons aged 18-65 years with diabetes and recently prescribed with basal insulin (NPH, glargine, detemir) during 2006-2009 were identified from the Finnish national prescription register. During this period all three insulins were fully reimbursed for patients with diabetes. The study patients were followed up until the first event of severe hypoglycaemia or death by the end of 2009, utilizing the nationwide hospital discharge register and cause of death register. The Poisson model was used to evaluate the absolute risk (with 95% confidence intervals) for severe hypoglycaemia events for new users of NPH, glargine or detemir during follow-up. The risk between the study insulins was compared with Cox's proportional hazards model adjusting for age, gender, type of diabetes, use of sulfonylurea, use of other than study insulin, history of hospitalizations due to severe hypoglycaemia, and switch of study insulin. Several sensitivity analyses were performed to test the robustness of the results with respect to the exposure definitions and possible confounders.
Results: During the follow-up of up to four years, (mean 1.8 years) there were 16,985 persons (2,604 with type 1, 13,254 with type 2, and 1,127 with unclassified diabetes mellitus) who initiated basal insulin treatment. 5,586 patients initiated NPH, 7,499 glargine, and 3,900 detemir. During the 27,815 person-years of follow-up, 2,730 (16%) people switched insulin at least once, and 536 (3%) people were hospitalized due to an event of severe hypoglycaemia with 190, 222 and 76 during exposure to insulin NPH, glargine or detemir, respectively. The type of insulin in use was not specified for 48 events. The absolute rate (per 1000 patient-years) of severe hypoglycaemia was 20.6 (95% CI 17.9, 23.8) for NPH, 17.8 (15.6, 20.3) for glargine, and 12.4 (9.9, 15.5) for detemir initiators. With NPH as reference, the adjusted hazard ratio (HR) for the risk was 0.92 (95% CI 0.74, 1.15, p=0.47) for glargine, and significantly lower 0.70 (0.52 0.94, p<0.05) for detemir. The corresponding risk was also significantly lower (HR 0.76 [0.58, 0.99, p <0.05]) for detemir compared to glargine.
Conclusion: We observed significant differences in the risk of severe hypoglycaemia between different basal insulins among working-aged adults with diabetes in Finland. Use of insulin detemir was associated with a significantly lower risk of severe hypoglycaemia requiring hospital care compared to glargine and NPH. The results suggest that in routine practice the burden of hypoglycaemia may be reduced by the choice of basal insulin.
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Reactive hypoglycaemia after Roux-en-Y gastric bypass in type 2 diabetic subjects

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Background and aims: Reactive hypoglycaemia (Hypo) is a well-recognised complication of RYGB surgery in non-diabetic subjects. In type 2 diabetes (T2DM), RYGB improves glucose metabolism, but whether this improvement is related to the later development of reactive hypoglycaemia is not known. Our aim was to investigate presence and mechanisms of postprandial Hypo in T2DM undergoing RYGB.
Materials and methods: 32 obese T2DM subjects treated with RYGB received a 5-hr OGTT before and 12-18 months after surgery. Hypo was defined as a plasma glucose ≤3.3 mmol/L in subjects off hypoglycaemic agents. Insulin sensitivity was assessed by Matsuda index and ß-cell function by modelling analysis of the C-peptide response to the OGTT.
Results: Hypo occurred in 10 of 32 RYGB patients. Age did not discriminate Hypo from non-Hypo (NH) subjects. Presurgery BMI was lower in Hypo than NH (42.8±6.4 vs 46.0±5.9 kg/m2, p=0.03). Pre-surgery fasting glycaemia was similar in both groups, as was mean plasma glucose during OGTT (11.0±2.1 vs 10.4±2.0 mM, p=ns). Pre-surgery mean insulin during OGTT and insulin secretion rate (basal and total) were similar in both groups. Post-surgery, mean insulin was reduced to a similar extent in both groups (p=0.003), whereas insulin secretion was unchanged. Baseline peripheral insulin sensitivity was higher in Hypo than NH and after surgery it improved more in Hypo than in NH (4.3±2.3 vs 9.0±3.1 and 2.8±1.1 vs 5.5±2.6 mL.min-1.m-2, p<0.0001 for time, p=0.05 for interaction time*Hypo). Baseline ß-cell glucose sensitivity was similar in the two groups (29±22 vs 25±34 pmol.min-1.m-2.mM-1), but after surgery it increased more in Hypo than NH (82±49 vs 34±23 pmol.min-1.m-2.mM-1, p=0.0018 for interaction time*Hypo). Baseline insulin clearance was similar, but post-surgery it improved in Hypo (p=0.004 interaction time*Hypo). No differences in fasting or postglucose GLP1 and glucagon were found between Hypo and NH, whereas fasting plasma PYY concentrations before surgery were significantly higher in Hypo compared to NH (p=0.04). In logistic regression, presurgery insulin sensitivity (positive, p=0.03) and fasting PYY (positive, p=0.04) were the only predictors of reactive hypoglycaemia after surgery.
Conclusion: A better insulin sensitivity and higher fasting PYY concentrations before surgery are associated with a higher risk of post-surgery reactive hypoglycaemia in T2DM subjects. Independently of diabetes remission, improvement in ß-cell glucose sensitivity in association with peripheral insulin sensitivity is responsible for postprandial hypoglycaemia after RYGB.
Supported by: EFSD

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Hypoglycaemia association with hospitalisation and mortality in older patients with type 2 diabetes mellitus initiating Basal Insulin (BI) in a US medicare advantage population

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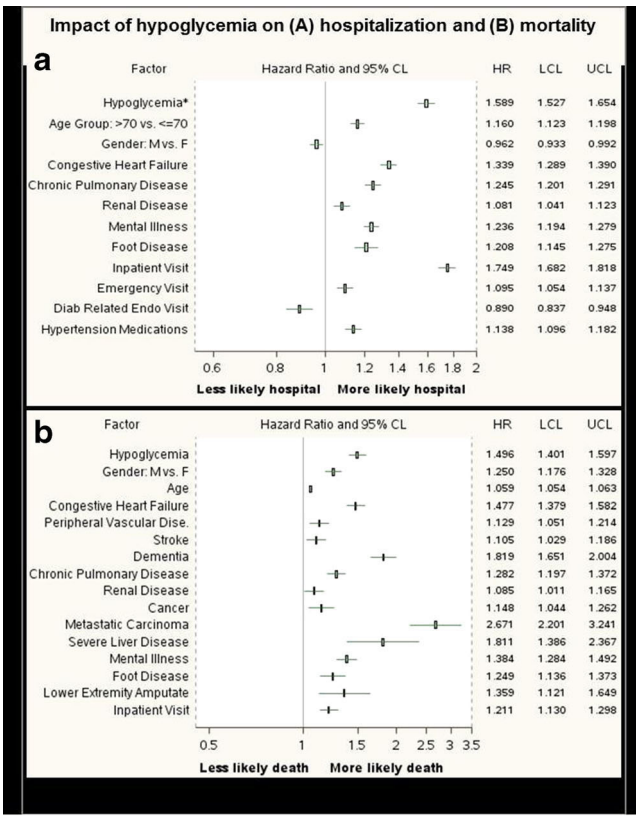
Background and aims: Hypoglycemia has been associated with an increased risk of cardiovascular (CV) events and all-cause mortality. Older patients initiating basal insulin are at particular risk of experiencing severe hypoglycemic events given the observed increase in hypoglycemic rates in this population, and the established risk between insulin and hypoglycemia. We performed a retrospective US health insurance claims database study to assess the current burden of hypoglycemia in older patients initiating basal insulin treatment, investigating whether there is an association between hypoglycemia, hospitalization, and all-cause mortality.
Materials and methods: The Clinformatics® Data Mart Medicare Advantage database was used to identify patients with T2DM who initiated Basal insulin between January 2007 and December 2013. All patients had a minimum of one year continuous health coverage prior to and after basal insulin initialization. A Cox proportional hazard model was employed to evaluate the risk of all-cause hospitalization and death, with medically attended hypoglycemia (HG) as a time-varying covariate adjusting for demographic, comorbidities and medication history.
Results: Of the total 31,035 patients analyzed, 16,534 (53%) experienced hospitalization and 4414 (14%) died during a mean follow-up of 2.9 year. A greater proportion of patients with HG recorded during the first-year post BI initiation required hospitalization than those without HG (75.6% *vs* 50.8%), experiencing 1.43 and 0.65 hospitalization episodes/patient-year, respectively. Patients with HG were more likely to die than those without HG (21.1% *vs* 13.4%). After accounting for other risk factors, HG was associated with an elevated risk of all-cause hospitalization (HR 1.60; 95%CI: 1.53-1.65) and death (HR 1.50; 95%CI: 1.40-1.60). (Figure: Hazard ratio analysis for all-cause hospitalization and death)
Conclusion: In a large US Medicare Advantage population with T2DM, HG is a strong independent risk factor for hospitalization and death following BI initiation, suggesting the need for a new BI with a favorable HG profile in this older population.



*Hypoglycemia: indicator of medically attended hypoglycemia occurring **before** hospitalization or death. HR, hazard ratio; LCL, lower confidence limit; UCL, upper confidence limit.

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Reduced cognitive function in people with type 1 diabetes and impaired awareness of hypoglycaemia

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Background and aims: Impaired awareness of hypoglycaemia (IAH) implies an increased risk of severe hypoglycaemia (SH) in type 1 diabetes (T1D). Recurrent SHs may impair brain function, and the hippocampus is especially vulnerable. We aimed to investigate whether T1D patients with IAH have reduced cognitive function compared to T1D patients without IAH.
Materials and methods: In total 105 participants (33 T1D with IAH (Gold score≥4), 35 T1D with normal awareness (NAH; Gold score≤2), and 37 healthy controls) completed a web-based neuropsychological test battery assessing verbal-, spatial-, and working memory, executive function, processing speed and pattern separation. Raw scores were transformed to z-scores using the results of the healthy controls as reference.
Results: As shown in Table 1, age (~47 years) and gender distribution was similar in the IAH, NAH and healthy control groups. In the IAH and NAH subjects, diabetes duration (~30 years), mean HbA1c (~8%), and percentage of subjects with no retinopathy (48.% vs. 45.7%) did not differ between groups (Student t-test and Chi square tests p-values 0.984-0.194). No participant had severe hypoglycaemia the day before testing or biochemical hypoglycaemia during testing (Table 1). IAH-subjects had lower z-scores than NAH-subjects in tests of verbal memory delayed recall ($t(62) = 2.4$, $p = 0.018$, $d = 0.60$), spatial memory immediate recall ($t(57.5) = 2.1$, $p = 0.043$, $d = 0.51$), pattern separation ($t(59) = 2.8$, $p = 0.007$, $d = 0.72$), and in processing speed and executive function ($t(60) = 2.2$, $p = 0.032$, $d = 0.57$).
Conclusion: T1D participants with IAH had lower performance in neuropsychological tests than NAH participants. These differences were most pronounced in tests associated with hippocampal function.

Table 1. Characteristics of the participants[1]

	IAH (n=33)	NAH (n=35)	Control (n=37)
Gender (M/F)	19 / 14	21 / 14	21 / 16
Age (years)	46.9(10.3)	46.7(9.8)	47(10.8)
Education[2,3]	2 (1-4)	2 (1-4)	3 (1-4)
HbA_{1c}(mmol/mol)	63.2(17.8)	64(12.1)	34.7(2.7)
HbA_{1c}(%)	7.9(1.6)	8.0 (1.1)	5.3(0.2)
Diabetes duration	30.1(9.6)	30.2(10.4)	
Pre test blood glucose level (mmol/L)	10.4(2.9)	10.3(3.9)	
Post test blood glucose level (mmol/L)	10.0(3.2)	10.0(4.0)	
No retinopathy[4]	16 (48.5)	16 (45.7)	
Self-measurement of blood glucose ≥ 4 times per day[4]	17 (51.5)	17 (48.6)	
Insulin regimen			
Rapid + long-acting insulin analogues[4]	18 (54.5)	16 (45.7)	
Rapid acting insulin analogue + NPH insulin[4]	7 (21.2)	7 (20.0)	
Insulin pump[4]	8 (24.2)	11 (31.4)	
Biphasic insulin[4]	0	1 (2.9)	

[1]Mean (SD) unless otherwise stated
[2]Median (range)
[3]Education level: 1=primary school and lower secondary level; 2= upper secondary school; . 3= ≤ 4 years at university or college; 5=> 4 years at university or college
[4]n (%)

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Interim results on the relationship between mild-moderate and severe hypoglycaemia and cardiovascular disease in a cohort of sulfonylurea users

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Background and aims: Severe hypoglycemia is associated with higher risk for cardiovascular disease (CVD), but most hypoglycemic events are non-severe. Little is known about the CVD effects of mild-to-moderate hypoglycemia, which is less likely to be encoded in administrative databases. We used electronic health records (EHR) to identify a large cohort of sulfonylurea (SU) users and examine the association between baseline hypoglycemia by severity and CVD outcomes at follow-up.
Materials and methods: Patients with type 2 diabetes prescribed SUs were identified from the Humedica Research Database from January 2009 to March 2014. This database includes EHRs on over 30 million patients throughout the United States. Hypoglycemia occurrence was identified using an algorithm incorporating International Classification of Diseases, Ninth Revision (ICD-9) diagnostic codes and natural language processing (NLP) of free text clinical notes to improve capture of non-severe hypoglycemic events. We identified mentions of hypoglycemia and synonyms within the free text from note sections likely to indicate the patient's current status/experience (History of Present Illness, Diagnoses, Problems, and Patient Complaints) and did not include records of patient counseling about the risk of hypoglycemia or other false-positive scenarios. Mentions of hypoglycemia associated with negations or a non-confirmatory sentiment (e.g. denies hypoglycemia) were not considered. We categorized hypoglycemic events as serious, mild-moderate, or unknown based on descriptors within the free text and by site of care, treatments, and comorbidities. Frequency of hypoglycemia events, by seriousness, was assessed as a determinant of CVD outcomes, acute myocardial infarction (AMI) and congestive heart failure (CHF), identified from diagnoses within the EHR. We compared the relative rates (RR) and 95% confidence intervals (CI) of CVD across categories of baseline hypoglycemia with adjustment for baseline covariates via Poisson regression models.
Results: Of the 82,321 eligible patients, 4,922 (6%) had at least one hypoglycemia event in the year before cohort entry. In the follow-up period (Median: 164 days; 25th and 75th percentiles: 20 days, 601 days), 47% had an observed diagnosis for any CVD, 2% had a diagnosis for AMI, and 15% had a diagnosis for CHF. Severe hypoglycemia frequency was positively associated with CHF (RR per episode: 1.69, CI 1.55-1.84) and AMI (RR 1.66, CI 1.31-2.09). Mild-moderate hypoglycemia and hypoglycemia of unknown seriousness were more strongly associated with CHF (Mild-Moderate RR 1.48, CI 0.93-2.34; Unknown Seriousness RR 1.43, CI 1.37-1.48) relative to AMI (Mild-Moderate RR 0.96, CI 0.15-6.3; Unknown Seriousness RR 1.19, CI 1.06-1.35). Restricting to patients with active sulfonylurea use at the time of hypoglycemia assessment yielded comparable results.
Conclusion: The association between hypoglycemia and CVD may be dependent on the subtype of CVD and the seriousness of hypoglycemia. Hypoglycemia characterized as mild-moderate or unknown had attenuated associations with AMI. Limitations of this study include differential documentation of hypoglycemia, lack of validation of the NLP algorithm, incomplete capture of non-serious hypoglycemic events and residual confounding.

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Cardiac autonomic regulation during experimental hypoglycaemia in type 1 diabetes with impaired awareness of hypoglycaemia

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Background and aims: Impaired awareness of hypoglycaemia (IAH) affects 25% of patients with type 1 diabetes (T1DM) and increases the risk of severe hypoglycaemia (SH). Previous studies report decreased vagal outflow during hypoglycaemia in T1DM, potentially increasing vulnerability to cardiac arrhythmias. Our aim was to investigate the effect of experimental hypoglycaemia on cardiac autonomic regulation in patients with IAH.
Materials and methods: Ten participants with T1DM and intact hypoglycaemia awareness, eight with IAH (Gold score≥4), six with previously impaired but restored awareness (Gold score<4) and ten without diabetes underwent a stepped hyperinsulinaemic hypoglycaemic clamp study. Glucose was stabilised at 5 mmol/l at time 20 minutes (T20) and reduced step-wise to a nadir of 2.4 mmol/l at time 180 minutes (T180). Heart rate variability was measured during each glucose step.
Results: An increase in heart rate (HR) and a corresponding reduction in high frequency (HF) power, suggesting vagal withdrawal was seen in participants without diabetes with recovery by T180 (HR at T20 65.3; T100 68.7, p=0.057; T180 62.8). HR increased in subjects with diabetes and intact awareness of hypoglycaemia without signs of recovery by T180 (HR T20 72.2; HR T180 80.7, p=0.008). Increases in normalised low frequency (LFnorm), indicating elevated sympathetic contribution, were higher in participants with diabetes than subjects without diabetes. Participants with IAH had a peak heart rate at T100 with evidence of recovery by T180 (HR T20 79.43, T100 83.61, T180 81.70 p=0.041). This was accompanied by small, non-significant reductions in HF power and increases in LFnorm which recovered by T180. Participants with restored awareness showed early changes in heart rate variability with a peak in heart rate at time 60 minutes (T60) and recovery by 140 minutes. Reductions in HF power (p=0.02) and LFnorm (p=0.013) peaked at T60 with similar patterns of recovery. Rises in plasma metanephrine were lower in participants with IAH and restored awareness of hypoglycaemia (Table 1).
Conclusion: Changes in cardiac autonomic regulation during experimental hypoglycaemia are time dependent and differ according to self-reported awareness of hypoglycaemia in T1DM. In subjects with IAH, hypoglycaemia had a diminished effect on sympathetic activation, blunting hypoglycaemia recognition through autonomic symptoms but, potentially providing cardiovascular protection against arrhythmias. This corresponds to a reduction in metanephrine response to hypoglycaemia.

	40 minutes (Glucose 5.0 mmol/L)	200 minutes (Glucose 2.4 mmol/L)	Fold Change	p value (paired t test)
Non-diabetic	175	590	3.4	<0.0001
Intact awareness	213	517	2.4	<0.0001
IAH group	200	342	1.7	0.038
Restored awareness	275	447	1.6	0.086

Table 1: Plasma metanephrine (pg/mL) levels at T40 and T200.

Clinical Trial Registration Number: 52164803
Supported by: Diabetes UK

OP 22 Management of cardiovascular risk factors

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Therapeutic inertia for glycaemic and blood pressure control in patients with type 2 diabetes mellitus and the cardiovascular consequences

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Background and aims: The delay or failure to intensify treatment for better glycaemic and blood pressure (BP) control (therapeutic inertia, TI) in patients with poor glucose and BP control, the risk burden and the effects of TI on cardiovascular (CV) risk in newly diagnosed T2DM patients have not been explored. The aims of this longitudinal cohort study were to evaluate the prevalence of TI, the extent of potentially avoidable glycaemic and BP burden, and the association of TI and TI-associated glycaemic and BP burden with the risk of composite of myocardial infarction (MI), heart failure (HF) and stroke.
Materials and methods: A cohort of 1,145,979 patients, aged≤70 yrs with diagnosis of T2DM from 2000 were selected from the Centricity EMR Database of USA, with follow-up data available till September 2014. CEMR provides longitudinal patient-level data on over 38 million insured and non-insured patients from about 35,000 care providers (70% primary care providers) derived from clinical practices across 49 US states. Longitudinal follow-up data on HbA1c, BP, prescriptions and doses for anti-diabetes drugs (ADDs) and anti-hypertensive drugs (AHDs), and CV events with dates of events were extracted. TI for glucose and BP control were defined as failure to take at least 2 ADDs (ADD2) and at least 2 AHDs (AHD2), respectively, in patients with HbA1c and BP persistently above target for 2 years. 474,429 patients had a minimum of 2 yrs of follow-up data post diagnosis of diabetes.
Results: Patients were 49% male, mean (SD) age 58 (11) yr, HbA1c 8.4 (2)%, SBP 132 (18) mmHg, 59% had HbA1c≥7.5%, and 29% had SBP≥ 140 mmHg at diagnosis. The proportions of patients with ischemic heart disease, hypertension and chronic kidney disease prior to diagnosis of diabetes were 1.2%, 12 .3% and 0.8% respectively. In patients with HbA1c≥7.5% at diagnosis, more than 47%/29% had HbA1c consistently above 7%/7.5% during 2 yrs post diagnosis. In patients with HbA1c consistently above 7%/7.5% over 2 yrs, 40%/36% never received ADD2, only 46%/51% received ADD2 within 2 yrs, and the median time to intensification with ADD2 in these patients were 15/13 months. Only 7% patients had SBP≥140 mmHg consistently over 2 yrs, 9% of these did not receive AHD2, and the median time to intensification by AHD2 was 7 months. During 4 yrs of median follow-up, 0.82% had MI , 0.67% had HF, 1.72% had stroke, and 3.1% experienced at least one of these events. Adjusting for age, sex, ethnicity, smoking status, renal diseases, stratified by year of diagnosis of diabetes , in patients without history of CVD, TI for glycaemia and blood pressure was associated with 38% (95% CI of HR: 1.14, 1.66) and 20% (95% CI of HR: 1.05, 1.38) increased risk of a composite of MI, HF and stroke, respectively. Those with HbA1c consistently above 7.5% over 2 yrs, independently had 80% (95% CI of HR: 1.69, 1.93) increased risk of composite CV events, compared to those with HbA1c below 7.5%.
Conclusion: This study shows that in a large population of people with newly diagnosed diabetes, aged≤70 yrs, and with minimal prior CVD, there was a high prevalence of TI, a significant glycaemic burden over two years even with intensified treatment , and an association of TI with an increased CV risk over 4 years. Greater efforts are needed to understand, identify and appropriately manage this group of patients.

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Increased risk of cardiovascular-related events associated with sulfonylureas compared to other antihyperglycaemic drugs: a Bayesian meta-analysis of survival data

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Background and aims: Studies suggest different glucose-lowering treatments can have different, and even divergent, effects on cardiovascular risk and mortality. We conducted a systematic review involving studies that compared sulfonylurea to placebo/no intervention or other anti-diabetic drugs.
Materials and methods: Since events occur at differing time points among included studies, we employed a complementary-log-log link model in the Bayesian framework to obtain comparative hazard ratios (HR) between interventions. Conventional pairwise meta-analyses were used to pool adjusted hazard ratios for observational studies.
Results: We identified 91 RCTs including 36,573 patients and 26 observational studies including 1,553,856 patients. Analyses of RCT data demonstrated an increased risk of all-cause mortality and cardiovascular-related mortality for sulfonylureas compared to all treatments combined (HR 1.26 [1.10; 1.44] and 1.46 [1.21; 1.77], respectively). These results were confirmed in the analyses of the observational studies. The risk of myocardial infarction was significantly higher for sulfonylureas compared to DPP-4 inhibitors (HR 2.54 [1.14; 6.57]) and elevated compared to SGLT-2 inhibitors HR 41.80 [1.64; 360.4]), but not other classes of treatments. Observational studies confirmed an increased risk of myocardial infarction for sulfonylureas compared to all other treatments combined. The RCTs showed that the risk of stroke was significantly higher for sulfonylureas compared to DPP-4 inhibitors, GLP-1 agonists, TZDs and insulin (HR 9.40 [3.27; 41.9], 45.40 [1.99; 362.7], 1.75 [1.20; 2.69], and 1.46 [1.01; 2.14], respectively). No observational evidence was available on the risk of stroke.
Conclusion: Our findings in RCTs demonstrate a higher risk of cardiovascular-related events associated with sulfonylureas compared to other anti-diabetic drugs that increases over time. The analyses on the observational studies confirmed our results. These findings should inform guidelines for patients with elevated risk of CV events.
Supported by: MSD Ltd

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Second-line treatment with sulfonylurea compared to DPP4 inhibitors is associated with risk of cardiovascular disease, all-cause mortality and severe hypoglycaemia

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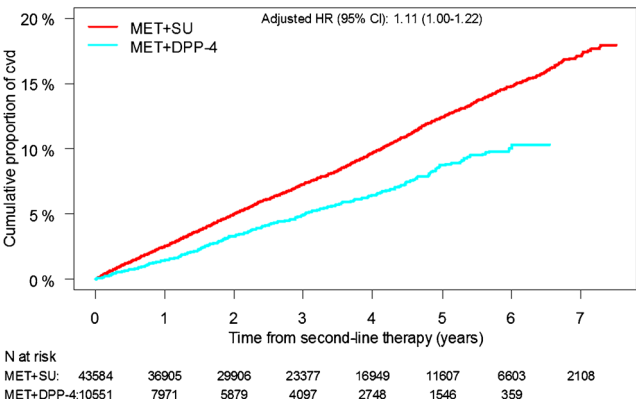
Background and aims: To compare the risk of cardiovascular disease (CVD), all-cause mortality and severe hypoglycaemia in type 2 diabetes (T2DM) patients who initiate second-line treatment with either sulfonylurea (SU) or DPP4 inhibitors (DPP4i).
Materials and methods: T2DM patients who were treated with one non-insulin antidiabetic drug (NIAD) and that added a second glucose lowering drug during 2006_2013 were identified in the Swedish Prescribed Drug Register and linked with National Patient-, and Cause of Death registers. Index date was defined as the date of patients second NIAD dispense after monotherapy on NIAD. Cox survival models, adjusted for age, gender, time on first-line treatment, previous CVD and fragility (3 or more days in hospital the year prior to index) were used to estimate event risks during follow-up. Cardiovascular disease was defined as hospitalization due to unstable angina, myocardial infarction or embolic stroke, or cardiovascular related death; severe hypoglycemia as any hospitalisation with diabetic hypoglycemia diagnosis.
Results: In the 68,351 patients who were started on dual NIAD treatment; the two most frequent dual-combination were metformin+SU (64%) followed by metformin+DPP4i (15%). Metformin+SU vs metformin+DPP4 were similar in age (65 vs 61 years), gender (59 vs 62% were men), had similar history of cardiovascular-, (32 vs 28%) and microvascular disease (18 vs 15%). Blood pressure-, lipid lowering and low dose aspirin treatment were also similar in the two groups. Add-on with SU compared with DPP4 in second-line treatment was associated with higher risk of subsequent fatal/nonfatal CVD-, all-cause mortality and severe hypoglycemia; hazard ratios (95% CI): 1.11 (1.00-1.22), 1.20 (1.07-1.33) and 1.62 (1.11-2.36) respectively.
Conclusion: In T2DM patients, second-line treatment with sulfonylurea compared to DPP4i was associated with an increased risk of subsequent cardiovascular events, all-cause mortality and, severe hypoglycemia. Ongoing and future randomized trials will be important to elucidate possible causal relationships between treatments and the reported complications.

Figure: Adjusted risk of fatal-/nonfatal CVD in patients who were either initiated on second-line treatment with metformin (MET)+sulfonylurea (SU) or MET+DPP4.



Supported by: AstraZeneca

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Treatment characteristics and outcomes associated with sulphonylurea versus metformin therapy in incident type 2 diabetes mellitus patients: results of the German CREST study

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Background and aims: The aim of this study was to analyse (1) characteristics of incident T2DM-patients having started either a MET or SU monotherapy and (2) whether these therapies are associated with different mortality, T2DM-related hospitalizations and macrovascular events (MACE).
Materials and methods: This was a cohort study using a German AOK PLUS-claims data set covering the years 2010-12. Incident T2DM patients having started either a MET/SU monotherapy between 01/07/2010-31/12/2011 without having received any prior antidiabetic medication during the preceding 180 days were included. Observation started with the first MET/SU prescription. Follow-up-time for each patient was at least 12 months, or, in case of death or therapy discontinuation (treatment gap>180 days or prescription of another agent), until that date. Event rates per 1,000 patient years were compared between the two patient groups using incidence rate ratios (IRR), Kaplan Meier curves, and Log

Rank tests. Furthermore, a Upropensity score matched (PSM) comparison of event rates between the two groups and a multivariate Cox regression analysis were carried out.
Results: We observed 35,661 incident T2DM- patients. Of them, 904 (2.5%)/7,874 (22.1%) started a SU/MET monotherapy, respectively. A mean age of 70.1/61.4 years ($p<0.001$), 54.6%/50.3% ($p<0.001$) female gender and a mean Charlson Comorbidity Index excluding age factor of 2.23/1.44 ($p<0.001$) were observed in the respective SU/MET groups. In the unmatched patient sample comparisons, the mortality rate (all rates per 1,000 patient-years) in the SU/MET groups was 82.5/25.5, respectively (IRR=3.24, $p<0.001$), for MACE 94.3/56.9 (IRR=1.66, $p<0.001$), and for T2DM-hospitalizations 32.1/11.2, respectively (IRR=2.87, $p<0.001$). In the PSM comparison which included 1,460 patients (730 patients per group), the mortality rates in the SU/MET groups were 56.3/40.3, respectively (IRR=1.40, $p>0.100$), for MACE/T2DM-related hospitalizations they were 82.5/61.1 (IRR=1.35, $p>0.100$), and 30.1/6.9 (IRR=4.34, $p<0.005$), respectively. In the multivariate Cox regression analysis, SU monotherapy was associated with higher T2DM-hospitalization risk (HR 2.822; $p<0.001$), higher MACE risk (HR 1.342; $p<0.050$), higher T2DM-related hospitalization risk (HR 2.014; $p<0.001$), and higher composite event risk (HR 1.776; $p<0.001$).
Conclusion: Among incident T2DM patients initiating first line treatment, those receiving SU therapy are older, more likely to be female, more comorbid, and discontinue their monotherapy earlier than patients treated with MET; a substantial number may also face MET contraindications. Taking these differences in patient characteristics into account, patients treated with SU seem to be at increased risk of death, MACE and T2DM-hospitalization compared to MET treated patients in Germany. Residual confounding cannot be excluded as at least a partial explanation to these findings.
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Dual antiplatelet therapy after acute coronary syndrome in patients with and without diabetes: a Swedish nationwide population-based cohort study
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Background and aims: Diabetes mellitus confers a high risk for recurrent ischemic events, including death, in patients with acute coronary syndromes (ACS). Patients with diabetes have platelet hyper-reactivity, as well as impaired platelet inhibition, in response to clopidogrel, suggesting that they might receive a greater benefit from more potent antiplatelet regimens. To characterize patients with ACS with and without diabetes and describe the dual antiplatelet therapy (DAPT; $P2Y_{12}$ antagonist and acetylsalicylic acid) regimen in these populations.
Materials and methods: This observational cohort study linked morbidity, mortality, and medication data from Swedish national registries from patients with ACS alive after discharge from the hospital in 2013. Patients were categorized by diagnosis of diabetes or not. Chi-squared test was used to test for statistically significant differences between groups.
Results: In total, 17,135 patients with ACS were identified, 4122 with diabetes and 13,013 without diabetes, with a median age of 72 vs. 71 years, respectively. Patients with diabetes more frequently had a history of more than one myocardial infarction than non-diabetics (20% vs. 11%). Patients with diabetes underwent revascularization less often compared with patients without diabetes (61% vs. 66%). Among patients with diabetes, 50% were treated with percutaneous coronary intervention (PCI) vs. 59% of patients without diabetes. However, 11% of patients with diabetes were treated with coronary artery bypass graft (CABG) surgery vs. 7% of patients without diabetes. In total, 71% of patients with diabetes received DAPT vs.76% of patients without diabetes. Among patients receiving DAPT, a larger proportion of patients with diabetes were treated with clopidogrel (44%), compared with patients without diabetes (36%). Consequently, 56% of patients with diabetes received a newer $P2Y_{12}$ antagonist (prasugrel or ticagrelor) vs. 64% of diabetic patients without diabetes. All reported differences between groups were statistically significant. There was no difference in DAPT duration between patients with and without diabetes.
Conclusion: Patients with ACS and diabetes were less often revascularized and less often prescribed DAPT, compared with ACS patients without diabetes. Among patients receiving DAPT, patients with diabetes less often received newer $P2Y_{12}$ antagonists. Possibly, patients with diabetes could benefit from more frequent revascularization and from receiving more potent DAPT to prevent future ischemic events.

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No difference in Major Adverse Cardiovascular Events (MACE+) with Basal Insulin peglispro (BIL) vs comparator insulins in patients with type 1 or type 2 diabetes
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Background and aims: Diabetes mellitus is associated with an increased risk for cardiovascular disease (CVD). Whether insulin use contributes to CVD risk is still uncertain. In order to identify possible differences in CV risk among different insulin therapies, we performed pre-specified meta-analyses across the clinical program for BIL, in which patients were randomised to treatment with either BIL or comparator insulin (glargine [GL] or NPH).
Materials and methods: One Phase 2 (12-week) and 6 Phase 3 (26–78-week) studies of BIL compared to GL or NPH were included, where more patients were randomised to BIL. The participants were diverse with respect to demographics, baseline glycaemic control, and concomitant disease or medications. For any potential CV or neurovascular event, relevant medical information was collected and provided to an external clinical events committee, where each instance was prospectively adjudicated by blinded, independent experts. Cox regression analysis was used to calculate the estimated risks. The primary endpoint was a composite of adjudicated MACE+ (CV death, nonfatal MI, nonfatal stroke, or hospitalization for unstable angina).
Results: The pooled population included 5872 patients. Mean age (SD) was 54.1 (13.3) years; 56% were male, 16% were Hispanic or Latino, and 5%, 5%, and 88% were Asian, black, or white, respectively. Baseline demographic and clinical characteristics, including use of statins or other lipid-lowering drugs, were comparable between BIL and comparators. A total of 83 patients experienced at least 1 MACE+ and 70 patients experienced at least 1 MACE (CV death, nonfatal MI, or nonfatal stroke). Overall, there were no treatment-associated differences in time to MACE+ (hazard ratio [HR] for BIL vs comparator insulin [95% CI]: 0.82 [0.53, 1.27]) or MACE (HR [95% CI]: 0.83 [0.51, 1.33]). Although there were only 12 MACE+ in patients with T1D, the HR favoring BIL was statistically significant (table). There were no differences in the incidence rates for MACE+, MACE and all-cause death between BIL and comparators. Sub-group analyses did not identify any population with increased treatment-associated risk.
Conclusion: Treatment with BIL vs comparator insulin in patients with T1D or T2D was not associated with increased risk for major CV events.

	Pooled		T1D		T2D	
	GL or NPH	BIL	GL	BIL	GL or NPH	BIL
N	2284	3578	608	957	1676	2621
Patient-years	2016	3278	666	1046	1351	2231
HR[a] for MACE+	0.82 (0.53, 1.27)		0.24 (0.07, 0.85)*		1.02 (0.63, 1.65)	
	Events: n (events/100 patient years)					
MACE+	36 (1.8)	47 (1.4)	9 (1.4)	3 (0.3)	27 (2.0)	44 (2.0)
CV death	9 (0.4)	13 (0.4)	2 (0.3)	0 (0.0)	7 (0.5)	13 (0.6)
Nonfatal MI	18 (0.9)	15 (0.5)	5 (0.8)	1 (0.1)	13 (1.0)	14 (0.6)
Nonfatal stroke	7 (0.3)	14 (0.4)	1 (0.2)	2 (0.2)	6 (0.4)	12 (0.5)
Unstable angina	6 (0.3)	7 (0.2)	1 (0.2)	0 (0.0)	5 (0.4)	7 (0.3)
MACE	30 (1.5)	40 (1.2)	8 (1.2)	3 (0.3)	22 (1.6)	37 (1.7)
All-cause death	10 (0.5)	19 (0.6)	3 (0.5)	0 (0.0)	7 (0.5)	19 (0.9)

[a]HR: hazard ratio for BIL vs comparator insulin (95% CI). *p=.027

Clinical Trial Registration Number: NCT01027871, NCT01435616, NCT01790438, NCT01468987, NCT01481779, NCT01454284, NCT01582451

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OP 23 Novel model systems in beta cell research

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Recruitment of beta cells during glucose stimulation in mouse pancreas tissue slices

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Background and aims: Beta cells in the islet of Langerhans respond to glucose with a tonic increase in intracellular calcium concentration ([Ca2+]i), with superimposed high frequency [Ca2+]i oscillations. The pattern of activity is aligned across different cells due to gap-junctional coupling between cells. We recently introduced functional multicellular [Ca2+]i imaging (fMCI) to study [Ca2+]i changes in a large number of beta cells from all layers of an islet in acute pancreas tissue slices. In the present paper, we describe how stimulus strength (i.e., glucose concentration) modulates parameters of the [Ca2+]i response in populations of beta cells in islets of Langerhans of NMRI mice.

Materials and methods: [Ca2+]i patterns in beta cells were obtained using confocal fMCI imaging in acute mouse pancreas tissue slices. Islets of Langerhans were loaded with the fluorescent calcium indicator Oregon Green 488 BAPTA-1 and stimulated with different concentrations of glucose in the form of pulses of a single stimulatory concentration and staircase stimulations with increasing or decreasing concentrations. Regions of interest were selected based on cell morphology and further analyzed for cell activity off-line.

Results: Glucose dependence of the [Ca2+]i response is very steep. Beta cells in an islet start to activate at 7 mM glucose and all cells are active at 10 mM glucose (EC50=8 mM glucose). The onsets of [Ca2+]i responses are delayed relative to the beginning of stimulation and this delay depends on glucose concentration. During stimulation with 7 mM glucose, all cells that become active at this concentration are recruited over a period of 25 minutes. Increasing the concentration improves the synchrony of the response onsets. At 12 mM glucose, the last cells that respond to this concentration responded 7 minutes after stimulation onset. Similarly to the increase in basal [Ca2+]i, the superimposed high frequency oscillations depend on concentration of glucose. Two crucial parameters of the superimposed oscillatory behavior, namely frequency and duration of oscillations, respond to an increasing concentration of glucose such that the former gradually increases from 7 to 12 mM glucose and declines beyond this concentration (16 mM), whereas the latter continuously increases with increasing glucose concentrations.

Conclusion: Combining fMCI and the acute tissue slice technique, we were able to study the reponses of large populations of beta cells to different stimulatory concentrations of glucose in situ. Glucose modulates both the basal [Ca2+]i response and the superimposed [Ca2+]i oscillations. Increasing glucose concentrations recruit cells within an islet in a concentration and time dependent manner. The dose-response curve is very steep, with an EC50 of 8 mM. In addition the frequency of superimposed [Ca2+]i oscillations as well as the duration of oscillations increase across the physiological range of concentrations (7-12 mM). Taken together, this indicates that at low levels of stimulation (7-9 mM), recruitment of beta cells significantly contributes and is arguably the most important mechanism of response to increasing concentrations of glucose, whereas at higher concentrations (9-16), the increased frequency and duration of oscillations are the predominate. Our findings might have important implications for responsiveness under pathological conditions when concentrations of glucose progressively increase.

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Porcine neonatal islets with eGFP-labeled beta cells as novel tool in diabetes research

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Background and aims: Due to the easy availability of pancreata of pigs and high similarity between porcine and human islets, porcine islets might be of great interest for studying beta-cell biology and for screening compounds affecting beta-cell proliferation and function. Further, availability of efficient tools of genetic modification in pigs enables specific labeling of beta-cells by fluorescent reporter gene expression for *in vivo* imaging of islets and FACS sorting of beta-cells.
Materials and methods: We established a routine procedure for isolation of porcine neonatal islet cell clusters (NICCs). Briefly, pancreata of piglets (<8 days old) were minced and shortly digested with collagenase. The suspension was sieved through a 500-μm mesh and exocrine cells were removed within the first three days in culture. Best culture conditions proved to be recovery media of Ham's F12/M199 with additives of protease inhibitors, antioxidants and additional nutrients for the first three post-isolation days followed by maturation media of Ham's F10 with IBMX. After *in vitro* maturation, NICCs were characterized by immunofluorescence (IF) analysis, glucose stimulated insulin secretion (GSIS) assays, and proliferation assays. To generate islets donor pigs with eGFP labeled beta-cells, porcine kidney cells were transfected with a porcine INS promoter-eGFP expression vector, pools of stable transfected cell clones were used for somatic cell nuclear transfer (SCNT), and cloned embryos were transferred to recipient gilts. Offspring were genotyped by PCR and Southern blot analysis and characterized for reporter gene expression.
Results: Our isolation protocol facilitated a yield of 10-30 thousand NICCs per donor piglet (culture day 5). IF analysis demonstrated that NICCs contained similar proportions of insulin- and glucagon-positive cells. GSIS assays revealed on average 3.2-fold increased insulin secretion in high- vs. low-glucose medium. NICC derived beta-cell proliferation was significantly ($p<0.001$) increased by a GSK3β inhibitor and significantly ($p<0.001$) impaired by a CDK4 inhibitor. A total of 12 live INS-eGFP transgenic founder piglets were born, with a total of 10 different transgene integration patterns. Fluorescence microscopy and immunohistochemistry for GFP in sections of pancreas and a panel of other tissues demonstrated islet-specific transgene expression. Consistent with these results, isolated NICCs of the transgenic founder pigs showed different levels of eGFP fluorescence (1 strong, 1 weak, all others intermediate). Double-immunofluorescence analysis identified eGFP expression specifically in the beta-cells; eGFP was neither detected in glucagon positive alpha-cells nor in somatostatin positive delta-cells. Two founders with high and intermediate beta-cell specific eGFP expression were successfully reproduced by SCNT to set up breeding lines of donor pigs for large-scale NICC isolation.
Conclusion: Porcine islets expressing beta-cell specific eGFP marker protein will enable *in vivo* imaging of islets, e.g. in the setting of islet transplantation. Further, eGFP-labeled beta-cells enable efficient and specific FACS sorting of beta-cells which may provide a useful screening system for compounds affecting beta-cell proliferation and function.
Supported by: Sanofi-Aventis

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Electrophysiological characterisation of the human beta cell line EndoC-βH2

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Background and aims: We have characterised the novel human beta cell- line EndoC-βH2 in terms of glucose-induced insulin secretion (GISS), depolarization-evoked exocytosis and properties of voltage-gated ion channels.
Materials and methods: Insulin secretion was tested by static incubations. Secretion rates have been normalised to insulin content. Cytosolic calcium levels ($[Ca^{2+}]_i$) were monitored in cells transfected with the genetically encoded calcium sensor GCaMP5. Exocytosis was assessed by whole-cell capacitance measurement. Membrane potential and currents were recorded using the perforated patch or standard whole-cell configuration of the patch-clamp technique. Membrane currents were isolated using specific blockers.
Results: Basal insulin secretion at 1 mmol/l glucose amounted to 2%. Glucose (6 or 20 mmol/l) stimulated insulin secretion 1.5- ($p<0.05$) to 2-fold ($p<0.01$), respectively. This was associated with initiation of action potential firing and elevation of $[Ca^{2+}]_i$. The effects of glucose on insulin secretion, electrical activity and $[Ca^{2+}]_i$ were mimicked by tolbutamide (0.5 mmol/l) and reversed by diazoxide (0.2 mmol/l), consistent with a key role of ATP-regulated potassium channels. Forskolin potentiated GISS evoked by 6 mmol/l glucose ~3-fold. Exocytosis, evoked by a train of ten 500 ms depolarizations, was biphasic with the largest responses occurring during the initial 3 depolarizations with subsequent depolarizations eliciting progressively smaller responses. The cumulative increase of capacitance is of $15fF.pF^{-1}$ (increase in cell capacitance normalized to cell size, n=9). The contribution of different voltage-gated calcium channels to calcium entry was evaluated using an action potential-like voltage-clamp command (based on spontaneous glucose-induced action potentials recorded in EndoC-βH2 cells). The inward current evoked by the action potential waveform amounted to $8pA.pF^{-1}$ (n=7) of which 10% was due to tetrodotoxin (TTX)-sensitive sodium channels, 10% due to SNX482-sensitive R-type calcium channels with the remaining 80% attributable to isradipine-sensitive and ω-agaotoxin-sensitive L- and P/Q-type calcium channels.
Conclusion: The complement of voltage-gated ion channels in EndoC-βH2 is very similar to that seen in primary human beta-cells. We conclude that EndoC-βH2 cell line is good glucose-sensitive model of insulin secretion and electrical activity of primary human beta-cells.
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Human pancreatic microislets as a standardised model for glucose-stimulated insulin and glucagon secretion

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Background and aims: Isolated human pancreatic islets represent a useful tool to study islet function and hormone secretion in vitro. Nevertheless, insulin response to glucose is known to vary considerable between different donor preparations, and glucagon secretion is challenging to measure in primary human islets. In addition to this, both donor characteristics and isolation procedures add inconsistency regarding islet size and function. The aim of this study was to evaluate InSight™ microislets from InSphero as an alternative model to primary human islets for the assessment of insulin and glucagon secretion.
Materials and methods: The assay-ready 3D InSight™ Human Pancreatic Microislets are produced from dispersed human islets using the hanging-drop technique and delivered with one microislet per well in a

96-well GravityTRAP™ plate. Three different lots/donors were evaluated with the focus on insulin and glucagon secretion. The microislets' ability to regulate insulin and glucagon release in response to glucose was measured at different occasions up to three weeks after delivery. The possibility to re-use the microislets was also evaluated. Insulin and glucagon levels were measured after two hours of stimulation with different glucose concentration (2.8-22.2 mM), with or without addition of the glucagon-like peptide 1 (GLP-1) analogue Exendin-4 (10 nM), and measured using hormone specific ELISA kits (Mercodia). In order to expose the islets to a diabetogenic milieu, high glucose (22.2 mM) and interleukin 1β (IL-1β, 20 ng/ml) were added. Glucose-stimulated insulin secretion (GSIS) was measured after 24 hours of incubation in high glucose conditions. In parallel experiments human primary islets were exposed to the same conditions and GSIS measured. Briefly, five islets/well were transferred into a 96 well plate and insulin secretion was measured after 1 h incubation in 2.8 or 11.1 mM glucose.

Results: Both insulin and glucagon secretion could be readily measured in all three lots of microislets. The half maximal effect of glucose (EC_{50}) in stimulation of insulin secretion was 7.1 mM (n=6) while the EC_{50} for inhibition of glucagon secretion was 2.1 mM (n=3). The microislets responded to Exendin-4 with a modest but stable 1.4±0.05 fold increase in insulin secretion (11.1 mM glucose). The microislet plates could be re-used for hormone secretion experiments at least two times during the three week period tested, with similar insulin and glucagon responses to glucose. Diabetogenic culture conditions reduced the insulin secretion stimulation index (11.1 mM glucose/2.8 mM glucose) from 16.6±1.4 in microislets cultured at 5.8 mM glucose, to 1.9±0.3 after cultured in high glucose (22.2 mM) and to 1.6±0.3 in microislets with high glucose + IL-1β pre-treatment. This was comparable to a similar experiment using primary human islets with corresponding stimulation indices; 17.4, 1.7 and 1.5. In both preparations, the decreased stimulation index was a consequence of both increased basal secretion (2.8 mM) and decreased capacity to secrete insulin upon glucose stimulation (11 mM).

Conclusion: The human pancreatic microislets constitute a robust and reproducible tool for islet research where both insulin and glucagon secretion can easily be measured in a 96-well plate format in the same experiment. There was no apparent difference in GSIS between the microislets compared to primary human islets.

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Defining the islet 'GPCR peptidome': quantification and functional modelling of all GPCR peptide ligand signalling pathways in human and mouse islets

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Background and aims: Human islets express 293 G-protein coupled receptors (GPCRs), and 110 of these GPCRs (37.5%) have polypeptides ('peptides') as ligands. Autocrine GPCR signaling in islets, where islet cells express both a receptor and its cognate ligand, plays an important role in regulating islet function. Some islet autocrine signaling pathways are well characterised (e.g. glucagon/glucagon receptor, serotonin/serotonin receptors) but no systematic study has been undertaken to define the human and mouse islet 'GPCR peptidomes' and to define how similar the human and mouse GPCR peptidomes are. The aim of this study is to quantify the expression of all known human GPCR peptide ligands and their mouse orthologs and to create an atlas describing all known islet GPCR/peptide ligand signaling pathways in human and mouse islets.

Materials and methods: Human islets were isolated from heart beating donors at our islet isolation centre. Mouse islets were isolated from 8 week old male ICR mice using collagenase digestion and handpicked. mRNA was extracted from islets using the TRIzol method and reverse transcribed into cDNA. Peptide ligand mRNA expression was quantified relative GAPDH by quantitative real-time PCR. An atlas of all GPCR/peptide ligand signaling pathways was constructed by manually extracting data from guidetopharmacology.org and PubMed.

Results: The expression of 138 GPCR peptide ligand genes (encoding 212 mature GPCR peptide ligands) was quantified in human islets, and mRNAs of 114 (82.6%) GPCR peptide ligand genes (encoding 173 mature GPCR peptide ligands) were found to be expressed in human islets. Mouse equivalents exist of 126 (91.3%) of the 138 human GPCR peptide ligands, as the remaining 12 (8.7%) GPCR peptide ligand genes are absent from the mouse genome. mRNAs of 102 (81%) GPCR peptide ligand genes, encoding 163 different GPCR peptide ligands, were expressed in mouse islets. The 10 most abundant GPCR peptide ligand mRNAs in human islets are: glucagon (GCG)>somatostatin (SST)>amylin (IAPP)>pancreatic polypeptide (PPY) >>neuropeptide Y (NPY)>interleukin-8 (CXCL8)>ghrelin (GHRL)>Wnt protein 4 (WNT4)>Complement 3a (C3)>angiotensin (AGT). In mouse islets, the 10 most abundant GPCR peptide ligand mRNAs are: glucagon (Gcg)>amylin (Iapp)>somatostatin (Sst)>pancreatic polypeptide (Ppy) >>peptide YY (Pyy)>urocortin 3 (Ucn3)>R-spondin-4 (Rspo4)>dynorphin (Pdyn)>neuropeptide Y (Npy)>Wnt protein 4 (Wnt4). A detailed islet pathway atlas outlining all auto- and exocrine signaling pathways involving GPCRs and peptide ligand in human and mouse islets was constructed. 323 GPCR/peptide signaling pathways were identified in human islets, consisting of 239 (74%) autocrine and 84 (26%) exocrine signaling pathways. In mouse islets, 283 GPCR/peptide signaling pathways were identified, made up of 249 (88%) autocrine and 34 (12%) exocrine pathways.

Conclusion: Human and mouse islets express a large number of peptide ligands of G-protein coupled receptors. Although the human and mouse islet GPCR peptide ligand repertoires seem similar at a first glance, there are fundamental differences in both peptide mRNA expression levels and auto- and exocrine signaling pathways. These differences are very likely to influence islet function, so great caution is warranted when translating mouse islet functional data to a human islet context.

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Reduced glucose-stimulated insulin release from human type 2 diabetic islets associates with multiple metabolomic changes in the secretome

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Background and aims: Beta cell insulin secretory failure is the hallmark of type 2 diabetes (T2D). The associated molecular changes are still largely unclear in the beta cell.

Materials and methods: We evaluated glucose-stimulated insulin release (by the batch sequential incubation method - 45 min), metabolomic features in the secretome (using a screening metabolomic platform), and gene expression profiles (with beta cells obtained by laser capture microdissection) of islets from 17 non-diabetic (ND; 10 males/7 females; age: 68±17 yrs; BMI: 26.0±3.4 Kg/m2) and 14 T2D (12 males/2 females; age: 74±7 yrs; BMI: 26.0±3.1 Kg/m2) organ donors.

Results: Insulin release (μU/ml) at 3.3 mmol/l glucose was 35±19 with ND and 41±42 with T2D islets (NS), and increased respectively to 188±95 and 47±52 in response to 16.7 mmol/l glucose ($p<0.01$). Accordingly, stimulation index was 5.6±1.4 with ND and 1.1±0.1 with T2D islets ($p<0.01$). Compared to ND islets, T2D islets showed a significant ($p\leq0.05$) retention of glucose and release of aminoacids (glycine, alanine, phenylalanine, serine). Furthermore, T2D islets released significantly ($p=0.02$) more citrate into the incubation medium. Array data from ND and T2D beta cells, showed significantly decreased expression of enolase 1, mitochondrial aconitase 2, ATP citrate lyase, and dihydrolipoamide

dehydrogenase, and increased expression of aldolase B, lactate dehydrogenase A, phosphoenolpyruvate carboxykinase and oxoglutarate dehydrogenase.
Conclusion: Taken together, these results are compatible with metabolic overload and alterations of the late glycolytic and TCA cycle pathways in human T2D beta cells. If corrected, these changes could lead to improved glucose-stimulated insulin secretion.

OP 24 Brain reward and satiety

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Brain reward-system activation in response to anticipation and consumption of palatable food is altered by GLP-1 receptor activation in humans

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Background and aims: It has been suggested that obese individuals have increased brain reward system activation while anticipating food intake, which may lead to cravings for food, and decreased reward system activation during actual food consumption, which may induce overeating. Gut-derived hormones, such as glucagon-like peptide-1 (GLP-1), are likely involved in the regulation of food intake. GLP-1 receptor agonists improve glycaemic control and reduce food intake and body weight. We hypothesised that food intake reduction following GLP-1 receptor activation is mediated through brain areas regulating anticipatory and consummatory food reward.
Materials and methods: Obese T2DM patients (n=16; mean ± SD; BMI 34±4 kg/m2; HbA1C 6.9±0.9%, 8 males), age- and gender-matched normoglycaemic obese (n=16; BMI 33±3 kg/m2) and lean individuals (n=16; BMI 23±1 kg/m2) were studied in a randomised, crossover, placebo-controlled trial. Using functional MRI, we determined the acute effects of GLP-1 receptor activation on brain responses to anticipation and receipt of chocolate milk vs. tasteless solution. All subjects underwent three functional MRI sessions at separate visits with intravenous infusion of A) the GLP-1 receptor agonist exenatide, B) exenatide with prior GLP-1 receptor blockade by exendin 9-39 or C) placebo; during somatostatin pituitary-pancreatic clamps (glucose 5 mmol/L). We assessed the effects of BMI and GLP-1 receptor activation on brain responses in areas regulating reward, appetite and motivation (putamen, caudate nucleus, amygdala, insula and orbitofrontal cortex).
Results: BMI was negatively correlated with brain responses to receipt of chocolate milk and positively correlated with anticipation of receipt of chocolate milk in brain areas regulating reward, appetite and motivation ($P<0.005$). Exenatide vs. placebo increased brain responses to receipt of chocolate milk and decreased anticipation of receipt of chocolate milk ($P<0.005$). Exenatide vs. placebo reduced caloric intake during a subsequent choice buffet by 23% in lean subjects, by 24% in obese subjects and by 14% in obese T2DM patients. The exenatide-induced effects on brain activations and caloric intake were largely prevented by exendin 9-39.
Conclusion: GLP-1 receptor activation decreased anticipatory food reward, which may reduce cravings for food, and increased consummatory food reward, which may prevent overeating. Our findings provide novel insights into the mechanisms by which GLP-1 regulates food intake and how GLP-1 receptor agonists induce weight loss.
Clinical Trial Registration Number: NCT01281228
Supported by: Eli Lilly / BMS / AstraZeneca

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Insulin sensitisation normalises brain processing of satiation in non-obese insulin resistant men

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Background and aims: Altered activity of brain networks involved in appetite regulation are implicated in the aetiology of diabetes and obesity, conditions associated with insulin resistance. We characterised the effects of early systemic insulin resistance and the impact of insulin sensitisation on pre- and post-prandial brain activity in non-obese men.
Materials and methods: 16 healthy right-handed insulin resistant (IR) and 17 insulin sensitive (IS) men (HOMA2-IR 1.6±0.3, 0.5±0.1, age 34.9±10.2, 33.3±7.1 years, BMI 26.2±2.3, 24.2±1.9 kg/m^2, respectively) were studied twice in random order. Regional cerebral blood flow (CBF), a surrogate marker of resting neuronal activity, was measured using continuous arterial spin labelling magnetic resonance imaging in a 1.5 T MRI scanner before (-14 to -8 minutes) and after (0 to +6, +8 to +14, and +34 to +40 minutes) consuming 50 ml water or 630 kcal mixed meal after an overnight fast. Subjective measures of appetite were collected on a visual analogue scale after each scan. IR then completed 3 months metformin therapy with lifestyle advice before repeating MRI studies. Statistical parametric analysis of whole-brain CBF was performed using SPM-8. A cluster-forming uncorrected threshold of $Z > 2.31$ was used, with clusters surviving statistical significance ($p<0.05$), corrected for multiple comparison.
Results: Within-group analyses showed no differences between pre-water and pre-meal CBF. Compared to water, food reduced CBF in centres that provide inhibitory control of eating (dorsolateral prefrontal cortex, DLPFC) in IR and in visual processing centres (lingual gyrus) in IS. Between-group analyses showed greater CBF in regions involved in hedonic processing (orbitofrontal, anterior cingulate cortices) in IR than IS before the meal and throughout the water study. After food, IR showed lower CBF in the default mode network (precuneus) and greater CBF in regions involved in processing interoceptive awareness (insula) than IS. In IR, insulin sensitisation improved HOMA2-IR (1.34±0.46, vs pre-treatment $p=0.02$) and increased fullness ($p=0.02$). Post-treatment, IR no longer showed a fall in DLPFC CBF post-meal compared to post-water. Compared to pre-treatment, the intervention was associated with decreased pre-ingestion CBF in the default mode network (posterior cingulate cortex) and in regions involved in memory and energy regulation (hippocampus) and increased it in lingual gyrus and reward processing centres (striatum), with similar findings post-water, whilst post-meal CBF decreased in insula and increased in striatum. Regression analyses showed a negative association between changes in HOMA-IR and post-meal CBF in striatum, where greatest improvements in insulin sensitivity were associated with greatest increases in striatal CBF.
Conclusion: In non-obese men with systemic insulin resistance, altered responses of brain regions involved in reward processing and hedonic evaluation, inhibitory control and interoception in anticipation of and in response to meal ingestion may promote excessive eating behaviours. Strategies that improve systemic insulin resistance ameliorate these alterations in ways that could improve satiation, enhancing appetite control. This mechanism may offer a target for preventing weight gain and diabetes.
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Differences in brain responses to visual food stimuli among monozygotic twins discordant for BMI

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Background and aims: It has been suggested that increased central reward and satiety responses to food stimuli play an important role in the development of obesity. Since our genetic variation has not changed substantially in the past decades, the recent increase in obesity rates is assumed to be driven by changes in the environment. Therefore, we aimed to investigate to what extent the previously observed increases in central reward and satiety responses to food cues in obese individuals are influenced by environmental factors.
Materials and methods: Monozygotic twins discordant for BMI provide a unique opportunity to investigate the effects of environmental factors on obesity, since these subjects share an identical genetic background. We included fifteen rare female monozygotic twin pairs from the Netherlands Twin Register, with a mean age of 49.0±9.7 and a mean BMI difference of 4.18±1.9 kg/m2 in a cross-sectional study. We measured neural activity in reward and satiety related brain areas in response to visual food stimuli, using functional magnetic resonance imaging (fMRI), after an overnight fast. Subjects were presented pictures of high-calorie food, low-calorie food and non-food items. Imaging data were analysed using SPM8 and activation contrasts were computed (food versus non-food and high-calorie food versus non-food).
Results: In all subjects the viewing of food versus non-food pictures and high-calorie versus non-food pictures resulted in activation of limbic brain areas, such as amygdala, insula and orbitofrontal cortex, measured as changes in blood oxygen level dependent (BOLD) signal. When comparing leaner and heavier co-twins, no differences in brain activation were found when watching food versus non-food pictures. However, when watching high-calorie food versus non-food pictures, the heavier co-twins showed decreased, not increased, brain activation compared to their leaner co-twins within right orbitofrontal cortex, left insula and left caudate nucleus.
Conclusion: Contrary to previous studies in singletons, we found no increased brain activation in response to food versus non-food pictures in heavier compared to leaner individuals as measured with fMRI in a co-twin control study using monozygotic twins discordant for BMI. Indeed, in response to high-calorie food versus non-food pictures a decreased, not increased activation was found in heavier versus leaner co-twins. The increased activation in obese individuals observed in previous studies may reflect an influence of genetic factors on brain responses to food. The contradictory higher activation in leaner versus heavier co-twins in responses to high-calorie versus non-food pictures may be explained by an acquired higher attention to foods that are consciously being avoided in lean individuals in attempt to remain thin despite one's inherited risk of weight gain. Future studies should investigate the influence of genetic factors on previously observed increased brain responses to food in obese individuals.
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Leptin-substitution increases connectivity in reward-related brain areas in patients with congenital lipodystrophy

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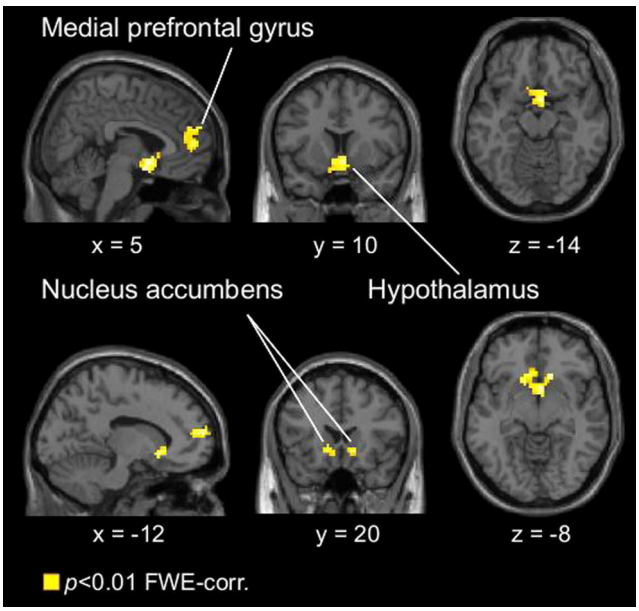
Background and aims: In patients with congenital lipodystrophy (LD) leptin serum concentrations are reduced, serum triglycerides high and patients often suffer from a severe diabetes with high insulin demands. Furthermore, LD patients can develop a disturbed hunger/satiety regulation: after the consumption of a meal, satiety lasts only a short period of time, leading to short intervals between meals. To assess long term effects of leptin substitution on eating behavior and related brain connectivity in treatment naïve patients with congenital LD, we performed resting-state functional MRI (fMRI) before and during the initial 52 weeks of treatment.
Materials and methods: The study included eight leptin-treatment naïve LD patients (six females, age 36±4 y, BMI 27±2 kg/m^2). MRI scannings were performed before leptin supplementation, and after one, four, 12, 26, and 52 weeks of treatment. In each session, patients consumed a meal consisting of 20% of their daily energy requirements. Before and after the meal, patients rated satiety feelings using 100 mm visual analog scales (VAS, 0 mm=no satiety; 100 mm=extreme satiety).
Results: Glycated haemoglobin (HbA1c) decreased by 17.1% after 52 weeks of leptin treatment (before leptin treatment 8.3±0.6%, after 52 weeks 6.7±0.5%, p=.13). Satiety ratings after the standard meal increased from 65±10 mm to 93±2 mm, p=.04 after 26 weeks and 71±11 mm, p=.55 after 52 weeks. FMRI analysis showed connectivity increases in nucleus accumbens bilaterally (T=4.17/4.38), medial frontal gyrus (T=4.77), and hypothalamus (T=4.85) over the 52 weeks of treatment (Figure).
Conclusion: After leptin substitution, patients anecdotically described longer periods of satiety and reduced meal frequencies. Consistently, leptin treated patients showed increased satiety ratings in the VAS after a meal. Under leptin treatment, we also observed an increase of functional connectivity in nucleus accumbens, an important player in the dopaminergic reward system, suggesting a modulation of this network by leptin. Furthermore, we found an increased connectivity of the hypothalamus, the homeostatic control center of the brain, where humoral information from the body converges (Figure).



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Specific deletion of lipoprotein lipase in ventromedian hypothalamus induces obesity and impairment of glucose homeostasis in mice

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Background and aims: The Lipoprotein Lipase (LPL) is the key enzyme of triglycerides (TG) hydrolysis of chylomicrons and VLDL, allowing the storage of the TG in the adipose tissue. It also has a crucial role in the Central Nervous System (CNS) in the control of the energy balance: a neuronal deletion of LPL leads to the onset of obesity and a modification of locomotor activity and energy expenditure. We studied here its specific deletion in the ventro-median hypothalamus (VMH), an area known to control food intake and peripheral glucose homeostasis via the autonomous nervous system.
Materials and methods: We injected in the VMH of LPL lox/lox mice (and controls) an Adeno Associated Virus, expressing the fusion protein Cre-GFP, to get a specific and localized deletion of LPL. We measured food intake, time course of body weight, body composition, and metabolic parameters (by indirect calorimetry) and performed glucose and insulin tolerance tests. Tests and tissues sampling were performed at 10 days post injection (10d, before the onset of obesity) or 12 weeks post injection (12w, massive obesity).
Results: Compared to WT, LPL VMH -/- mice have a significant increase of body weight 15 days post injection, without any hyperphagia. Obesity is massive at 12w and associated with insulin resistance and glucose intolerance. They show already at 10d post injection a significant decrease of locomotor activity, a decreased respiratory quotient during

24 h of fasting, and an increased food intake during refeeding. Early deregulation of insulin sensitivity is also present at 10d. Analysis of gene expression shows an early modulation of enzymes of ceramides metabolism and of FATP1 transporter.
Conclusion: A decrease of LPL hypothalamic activity leads to obesity associated with deregulation of glucose homeostasis. This phenotype is not linked to hyperphagia but likely to a modulation of locomotor activity and energy metabolism. Fine modulations of hypothalamic nervous activity (potentially due to intracellular variations, quantitative or qualitative, of cerebral ceramides content) could explain this phenotype.
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GLP-1R activation by exendin-4 prevents neuronal loss in the piriform cortex of type 2 diabetic rats

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Background and aims: Type 2 diabetes (T2D) accelerates the progression of cognitive decline and neurodegenerative diseases such as Alzheimer's (AD) and Parkinson's (PD). Impaired olfactory function, which is an early symptom of AD and PD has also been associated with T2D. The piriform cortex is a crucial area involved in the regulation of olfaction that has been shown to be vulnerable in AD and PD. However, olfactory dysfunction in T2D has not been previously linked to a specific brain neuropathology on histological and quantitative level. Aim of this study was to determine potential neuronal cell loss in the piriform cortex of T2D rats along aging. Others and we have recently shown that antidiabetic drugs targeting the Glucagon-like receptor 1 (GLP-1) are neuroprotective and can counteract neurodegeneration. Thus, we determined whether GLP-1R agonist Exendin-4 was able to counteract the T2D-induced neuronal loss.
Materials and methods: Young 3-month-old and middle-aged 13-month-old T2D Goto-Kakizaki (GK) rats were compared to age-matched Wistar rats (n=6 in each group). Additional groups of GK rats have been treated with Exendin-4 or vehicle for 4 weeks before sacrifice. Neuropathological assessments have been performed in the piriform cortex using stereology methods after immunohistochemistry of the specific neuronal marker NeuN and of the specific interneuronal marker Calbindin 28 KDa.
Results: The results show a significant decrease of the total neuronal number in the piriform cortex of GK versus Wistar rats along aging as assessed by NeuN staining (mean=48,207.4 in GK middle-aged vs. mean=66,256.9 in Wistar middle-aged; $p \leq 0.01$). We also demonstrate that interneurons positive for Calbindin 28 KDa are particularly vulnerable to the neuronal loss induced by T2D (mean=2,866.5 in GK middle-aged vs. mean=6,165.2 in Wistar middle-aged; $p \leq 0.001$). Interestingly, the GLP-1R agonist Exendin-4 substantially counteracted the neuronal loss observed in the piriform cortex of T2D animals as assessed by both NeuN (mean=47,560.6 in vehicle vs. mean=60,184.0 in Ex-4; $p \leq 0.001$) and Calbindin 28KDa (mean=3,890.2 in vehicle vs. mean=7,439.2 in Ex-4; $p \leq 0.0001$) staining.
Conclusion: Our results indicate neuronal pathology in the piriform cortex of the T2D brain along aging. These results could provide the neural anatomical basis of olfactory deficits in T2D. In addition, our results reveal a potentially novel finding at the basis of the detrimental effect of T2D in neurodegenerative diseases. Finally, by showing that neuronal loss in the piriform cortex can be pharmacologically counteracted by GLP1-R activation, our findings support recent research promoting the use of GLP-1R agonists against neurodegeneration.
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OP 25 Diabetic pregnancy from A to Z

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Prepregnancy care for a region: exploring the clinical and economic effects

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Background and aims: Prepregnancy care (PPC) has the potential to reduce the risk of adverse pregnancy outcomes in the setting of pregestational diabetes. Our aims were to implement and examine the effectiveness of a regional PPC program on pregnancy outcomes and to determine whether the additional costs of delivering the program are balanced against potential savings from averted complications.
Materials and methods: This prospective cohort study included 414 women with type 1 and type 2 diabetes attending five antenatal centers along the Irish Atlantic Seaboard between January 2006 and December 2014. PPC was offered to all women age 18-40 years via a diabetes register. Primary outcomes included: neonatal outcomes [shoulder dystocia, congenital malformation, stillbirth/neonatal death, admission to the neonatal intensive care unit (NICU)], hypertensive disorders of pregnancy, cesarean delivery, use of prepregnancy folic acid and glycemic control in pregnancy. Comparisons were made between those that did and did not attend PPC. A cost analysis took into account the direct cost of PPC and antenatal care delivery along with management of infant complications during the first 6 months post delivery.
Results: A total of 149 (36%) women attended and 265 (64%) did not attend PPC. Those women who attended were more likely to take preconceptual folic acid (97.3% vs 57.7%, p<0.001), have a lower 1st trimester HbA1c (6.8% vs 7.7%, p<0.001) and not smoke during pregnancy (91.3% vs 83.4%, p=0.03). Their offspring had lower rates of congenital malformations (0.81% vs 5.24%, p=0.04) and fewer admissions to the NICU (44.3% vs 62%, p=0.02). There were no significant differences between groups in relation to the other primary outcomes. Table 1 demonstrates the difference in average cost of complication treatment for those who did and did not attend PPC. A delivery in the PPC group was associated with an average saving of €3251. Although the major component of the difference is that associated with malformations, exclusion of any single component still yields a significant difference in total cost on the rank-sum test. When adjusted for age, ethnicity, BMI, smoking and parity, PPC remains significantly associated with a reduced total cost of €2578. This fully offsets the average cost of PPC delivery per pregnancy of €449.
Conclusion: A successful regional PPC program was implemented. Women who attended were better prepared for pregnancy and their offspring experienced significantly lower rates of congenital anomalies and NICU admissions. A significant cost saving is expected per pregnancy, even in the short-term.

Table 1 Average complication costs (€) of those who did and did not attend PPC

		Group mean			p-value	
		No PPC	PPC	Diff.	t-test	rank-sum
N		265	149	414		
Total Cost	mean	10,055.76	6,804.66	-3,251.10	0.001	0.010
	(std.err.)	(874.34)	(480.67)	(1,220.96)		
Stillbirth	mean	59.71	26.55	-33.16	0.237	0.287
	(std.err.)	(20.83)	(18.71)	(31.13)		
Mode of delivery	mean	3,802.00	3,707.52	-94.47	0.660	0.647
	(std.err.)	(127.61)	(172.42)	(213.72)		
NICU admissions	mean	2,374.43	1,624.60	-749.83	0.036	0.003
	(std.err.)	(233.61)	(268.82)	(371.11)		
Hypertensive disorders	mean	1,829.31	1,445.99	-383.32	0.230	0.241
	(std.err.)	(201.60)	(247.09)	(326.53)		
Malformations	mean	1,990.32	0.00	-1,990.32	0.010	0.023
	(std.err.)	(762.83)	(0.00)	(1,017.86)		

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The prevalence and prognosis of gestational diabetes mellitus after assisted reproductive technology

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Background and aims: Pregnancies after assisted reproductive technology (ART) are associated with poor prognosis, including more gestational diabetes mellitus (GDM), perhaps due to confounding factors as age or twin pregnancies. Whether ART confers a worse prognosis for GDM is unknown.
Materials and methods: We analyzed the prevalence of GDM and of GDM-related events (preeclampsia or birth weight ≥4000 g or shoulder dystocia) in the 18.305 women without known diabetes who delivered in our hospital between January 2002 and December 2010 after natural pregnancies (n=17.697) or ART (n=608: 155 ovulation inductions, 453 procedures).
Results: The greater rate of GDM after ART than after natural pregnancies (17.6 vs 14.2%, p<0.05) was driven by ART procedures (18.3%) rather than by ovulation inductions (15.5%). In multivariate analysis taking into account the parameters associated with GDM in univariate analyses (French risk factors for GDM including age, body mass index, familial history of diabetes, history of GDM or macrosomic child; history of miscarriage; multi-parity; smoking before pregnancy; hypertension; twin pregnancy; origin/ethnicity), pregnancies after ART procedures as compared with pregnancies after natural pregnancies or ovulation induction were still associated with more GDM (odds ratio 1.32 [95% confidence interval 1.03-1.69], p<0.05). In women without twin pregnancies, the rate of GDM-related events were different across groups: no GDM 9.0%; natural pregnancies with GDM 14.8%; GDM after ovulation induction 13.0%; GDM after ART procedure 11.4% (p<0.001); as the rate of caesarian deliveries: 20.3; 27.7; 21.7 and 28.8%, respectively (p< 0.001).
Conclusion: Independently of confounding factors, GDM is more usual during pregnancies following ART procedures. However, prognosis of GDM appears to be similar or even better after ovulation induction and ART procedures as/than after natural pregnancies.

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Predictors of need for insulin therapy in gestational diabetes mellitus

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Background and aims: The incidence of Gestational Diabetes Mellitus (GDM) is increasing, due to an increase in obesity in pregnant women and more stringent guidelines for screening and detection. Hyperglycaemia during pregnancy may cause peri- and postnatal complications for both mother and newborn. This risk may be reduced by achieving adequate glycaemic control with dietary intervention and/or insulin therapy. The aim of this study was to identify relevant factors which predict the necessity of additional insulin therapy during pregnancy.
Materials and methods: Retrospective cohort study of singleton pregnancies of all women with GDM between January 2011 and September 2014 in a teaching and an academic hospital. Patients underwent an 75-gram OGTT if they had risk factors for GDM or symptoms suggestive of GDM (e.g. polyhydramnion, large for gestational age). GDM was

diagnosed if fasting plasma glucose was ≥7.0 mmol/l and/or 2-hour glucose ≥7.8 mmol/l. After GDM diagnosis, instruction for self-measurement of blood glucose (SMBG) and dietary advice was provided. Insulin therapy was started if with SMBG fasting glucose was≥5.3 mmol/l or if 1-hour postprandial glucose values were≥7.8 mmol/l, despite dietary intervention.

Results: A total of 820 women with GDM were referred for treatment. Their mean (±SD) age was 32±5 years, BMI was 27.7 [IQR 24.0-31.9] kg/m2. Of them, 460 women (56%) were able to maintain adequate glycaemic control with dietary restrictions only, while 360 (44%) required exogenous insulin. Of the women who required exogenous insulin, 142 women (39%) received thrice daily pre-prandial rapid-acting insulin, 164 women (46%) received basal-bolus insulin therapy and 39 women (11%) received NPH insulin once daily at bedtime. Insulin dose varied from 2 to 80U (median 22 [IQR 12-42]U). Logistic regression analysis showed the following significant predictors for the need of insulin therapy: history of GDM, a previous newborn with birthweight>4500 gram or>P95, first-degree relative with type 2 diabetes, multiparity, mediterranean ethnicity, pre-pregnancy BMI≥30, and both increased fasting and 2-hour blood glucose during OGTT. The strongest predictor for insulin therapy was a fasting blood glucose of≥5.5 mmol/l (RR 6.81 (CI 4.03-11.5, p<0.001). Maternal age, smoking during pregnancy, history of IUFD, chronic hypertension and other ethnic groups did not predict insulin need. The prediction model had an explained variance of 16%.

Conclusion: This study developed a prediction model to identify GDM patients with an increased likelihood for the need for exogenous insulin therapy. In GDM, fasting glucose≥5.5 mmol/l is the strongest predictor for the need of insulin therapy.

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Pregnancy outcomes of women with untreated GDM (according to the WHO 2013 diagnostic criteria)

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Background and aims: Based on the HAPO (Hypoglycemia and Adverse Pregnancy Outcomes) study results, WHO issued new recommendations for the diagnosis of gestational diabetes mellitus (GDM) in 2013. While the use of the new diagnostic criteria (not yet applied in Hungary) approximately doubles the prevalence of GDM, there are no Hungarian data on the pregnancy outcomes of untreated WHO 2013 GDM women. We aimed to compare pregnancy outcomes of women with untreated GDM (according to WHO 2013, WHO-GDM) and women with normal glucose tolerance (NGT).

Materials and methods: During a universal screening program in a Western Hungarian region 4677 pregnant women (age: 29.6±5.6 years; mean ± SD) had a 75 g OGTT with the determination of fasting, 60-minute and 120-minute glucose determination between 16/JAN/2009 and 10/APR/2013. NGT was diagnosed in n=4113, untreated WHO-GDM in n=445 cases.

Results: Untreated WHO-GDM women were older (1.6, SE 0.3 yrs), had higher fasting (difference: 1.0, SE 0.02), 60-minute (1.4, SE 0.11), and 120-minute (0.8, SE 0.07 mmol/l) blood glucose, and blood pressure (2.2, SE 0.5 / 1.4, SE 0.4 mmHg). No difference in marital status, education, and residency was found (all p>0.05). While weight gain was similar in both groups (13.1, SE 0.3 vs. 13.0, SE 0.1 kg, p=0.90), GDM newborns had a higher birthweight (116, SE 26 g). Hypertension during pregnancy was more frequent in the GDM group (OR 1,44, 95%CI 1,02-2,03), as well as induced delivery(OR 1.34, 95%CI 1.10-1.64), forceps or vacuum use (OR 1.31, 95%CI 1.08-1.60), acute cesarian section (OR 1.34, 95%CI 1.10-1.64), and macrosomia (>4000 g, OR 1.64, 95%CI 1.23-2.20). No difference in the risk of preeclampsia and malformations (p>0.25) was found.

Conclusion: According to the present analysis, the pregnancy outcomes of women diagnosed with GDM according to the novel WHO criteria were worse compared to normal glucose tolerant women. Randomized controlled trials are required to prove that the treatment of these women would improve their outcomes.

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The effect of professional continuous glucose monitoring on glycaemic control and hypoglycaemia in insulin-requiring gestational diabetes mellitus

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Background and aims: Good glycaemic control in gestational diabetes mellitus (GDM) improves pregnancy outcomes and reduces perinatal morbidity. Continuous Glucose Monitoring (CGM) is ideal for monitoring glucose levels where tight glycaemic control without hypoglycaemia is required for short periods of time. CGM has been shown to improve glycaemic control and pregnancy outcomes in women with pre-gestational diabetes. However the same has not been established in GDM. Aim: To determine if professional CGM improves glycaemic control with less hypoglycaemia in insulin-requiring GDM.

Materials and methods: In this prospective, open-label trial , women with insulin-requiring GDM, gestation <28 weeks, were randomized to 2 groups. Group 1(CGM) (n=25) underwent professional CGM using the iPro2 Enlite 6-day sensor at 28, 32 and 36 weeks gestation while Group 2(control)(n=25) did not. Patients in both groups performed 7 point finger-stick glucose profiles 3×/week, recorded hypoglycaemic events(symptoms without glucose readings or finger-stick fasting glucose<3.5/non-fasting glucose<4.0 mmol/l) and were reviewed at 1-2 week intervals. In Group 1, both CGM and finger-stick data were used to manage diabetes while women in Group 2 were managed based on finger-stick data alone. HbA1c was measured at weeks 28, 33 and 37. Euglycaemia on CGMS was defined as 3.5-6.7 mmol/l.

Results: No significant difference was seen in mean age, pre-pregnancy BMI, HbA1c and total insulin dose between groups at baseline.The control group had a significant rise in mean HbA1c from week 28(5.27±0.52%) to 37(5.57±0.57%), while mean HbA1c in the CGM group did not significantly change from week 28(5.08±0.32%) to 37(5.17±0.41%). The control group also showed a significantly higher mean HbA1c at 33 and 37 weeks compared with the CGM group (5.42±0.58 vs 5.08±0.37%; 5.57±0.57 vs 5.17±0.41%). There was a significant difference in mean change in HbA1c between the groups from week 28 to 37(CGM +0.09%, control +0.31%, p=0.027). 87.5% of patients in the CGM group had HbA1c <5.6% at 37 weeks compared to only 52% of controls (p=0.012). Although 58.8% of patients in the CGM group had increased frequency of hypoglycaemia from week 28 to 37 compared to 37.5% in controls, mean increase in hypoglycaemia frequency was negligible (<1 in CGM group, not significantly different from control group). Within the CGM group, mean time spent in euglycaemia significantly rose from the 1st to 3rd CGM (84.6±9.3 vs 88.8±7.0, p=0.016) with significant decrease in time spent in hyperglycaemia (12.7±9.9 vs 8.3±6.3, p=0.017). This was not accompanied by an increase in mean time spent in hypoglycaemia(2.7±5.0 vs 2.9±3.2, p=NS). There was no significant difference in mean total insulin dose at 37 weeks between the groups. Offspring from the CGM group had lower mean birth weight compared to controls (2842.4±448.6 vs 2976.0±473.5, p=NS).

Conclusion: Women who underwent CGM had significantly better glycaemic control compared to those who received usual antenatal care based on finger-stick glucose alone. Our findings suggest that use of CGM is associated with improvement in maternal glycemic control and may prove beneficial in management of GDM.
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Supported by: Endocrine research grant

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Characteristics and risk factors for large-for-gestational age infants in a large cohort of pregnant women with type 1 diabetes
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Background and aims: In women with type 1 diabetes (T1DM) the prevalence of large-for-gestational age infants (LGA) remains high, despite strict glycaemic control and intensive obstetrical follow-up. Our aim was to evaluate the prevalence and risk factors for LGA in T1DM in our university hospital during the last 3 decades.
Materials and methods: Retrospective analysis of the medical files of pregnant women with T1DM attending our hospital form 01-01-1992 till 31-07-2014. Clinical and biochemical characteristics associated with LGA were analyzed. Excessive weight gain during pregnancy was defined according to the 2009 Institute of Medicine recommendations. Lipid levels were measured before pregnancy or in the first trimester. The generalized mixed model was used to adjust for several pregnancies over time in the same women and to adjust for confounders such as weight, weight gain and Hba1c in de different trimesters.
Results: Over a 22-year period, 259 pregnancies in 180 T1DM women were identified. Mean diabetes duration of women was 13.7±7.1 years, with a mean age at delivery of 29.5±5.2 years. Of these women, 33.9% were overweight, 10.9% were obese before pregnancy and 35.1% had excessive weight gain. Despite an active policy of pregnancy planning, 10.0% of women had unplanned pregnancies. HbA1c before pregnancy was 6.9±0.9%, dropping to 6.6% ±0.8, 5.9% ±0.7 and 6.1% ±0.6 in the first, second and third trimester respectively. Insulin pumps were used by 84.6% of women. Comparing the first versus third decade, diabetes duration at conception increased (15.4±8.39 vs. 11.7±5.8, p=0.002), women were older (30.4±4.9 vs. 28.7±3.9, p=0.03), more women were overweight (29.3% vs. 23.3%, p=0.009) and more women had excessive weight gain (37.7% vs. 20.3%, p=0.019). Macrosomia (>4 Kg) was present in 16.2% of women, LGA was present in 45.2% and 67.2% received a cesarean section. These numbers did not change over time (resp. p=0.92, p=0.19 and p=0.70). Compared to women with a non-LGA baby, women with a LGA baby had a higher weight at delivery (84.1±11.1 vs. 80.4±10.8, p=0.016), had more often excessive weight gain (45.3% vs. 25.2%, p=0.003) and were more often multiparous (61.3%vs.46.2% , p=0.01). Glycaemic control was also less strict in the first and third trimester in women with a LGA baby (resp. 6.7% ±0.9 vs. 6.5% ±0.8, p=0,01 and 6.2% ±0.5 vs. 6.0% ±0.6, p=0.01), without difference in the frequency of non-planned pregnancies (9.4% vs. 10.6%, p=0.65). No differences in BMI before pregnancy (25.2±3.7 kg/m^2 vs. 25.3±3.8 kg/m^2, p=0.80), duration of diabetes (13.7±6.7 years vs. 13.7± 7.4 years, p=0.99), age at delivery (29.5±4.1 years vs. 29.6±4.3 years, p=0.92), triglycerides levels (79±36 mg/dl vs. 85±41 mg/dL, p=0.60), HDL cholesterol (66±16 mg/dL vs. 67±17 mg/dl, p=0.47) and LDL cholesterol (87±28 mg/dl vs. 91±28 mg/dL, p 0.70) were seen between LGA and non-LGA groups. In the forward multivariate analysis, excessive weight gain (p=0.013) and HbA1c level in the first trimester (p= 0.006) remained independent predictors for LGA, after adjustment for confounders.
Conclusion: LGA remains a frequent complication in T1DM. Excessive weight gain and HbA1c in early pregnancy are important risk factors for LGA in our population.
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OP 26 Optimising the insulin treatment paradigm

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Basal insulin peglispro in combination with oral agents provides similar glycaemic control when given once daily in the morning or at bedtime in insulin naïve type 2 diabetic patients

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Background and aims: Basal insulin peglispro (BIL) is a novel basal insulin with a flat profile which has a hepato-preferential action resulting from reduced peripheral effects. In clinical trials BIL treatment consistently improved glycaemic control and reduced nocturnal hypoglycaemia (hypo) compared to insulin glargine or NPH. Since the other BIL Phase 3 comparator trials were conducted using bedtime administration, this prespecified secondary objective was intended to evaluate if administration of BIL in the morning (AM) was non-inferior (margin=0.4%) to bedtime administration (PM) in reducing HbA1c after 26 weeks of treatment.
Materials and methods: This secondary objective was part of a 26-week, Phase 3, open-label, treat-to-target study to compare the safety and efficacy of BIL to NPH when added to prestudy oral agents in 641 patients with type 2 diabetes mellitus. Changes to prestudy oral agents and doses were not permitted during the study. This analysis involves the 428 patients treated with BIL who were randomised to either AM (n=213) or PM (n=215) administration.
Results: After 26 weeks both AM and PM BIL administration significantly reduced HbA1c, with no significant difference between the AM and PM treatment groups (AM: 6.7 vs PM: 6.8%; treatment difference [95% CI]:
-0.13% [-0.29, 0.03%]; p=0.109). A similar proportion of patients in both groups achieved HbA1c <7.0%. No significant differences were found in fasting glucose, weight gain, or insulin dose between groups. However, significantly greater reductions in self-monitored blood glucose (BG) values were observed for the AM group at midday pre-meal (p=0.038), evening pre-meal (p=0.003), and bedtime (p=0.048) compared to the PM group (see table). There was no significant difference between groups in the percentage of patients reporting missed treatment doses. Total hypo rates (events/patient/30 days) were similar for AM vs PM dosing, as were rates of nocturnal hypo. A similar proportion of patients achieved HbA1c <7.0% without nocturnal hypo. However, there were higher rates of non-nocturnal (daytime) hypo in the AM group compared to the PM group (AM: 1.32 vs PM: 1.01; p=0.048). There was one case of severe hypo in each group.
Conclusion: These analyses showed that glycaemic control, as measured by HbA1c and fasting glucose, was similar regardless of whether BIL was administered in the morning or at bedtime, with no significant difference in mean insulin dose. However, morning administration of BIL was associated with lower daytime BG and a tendency for more daytime hypo in the absence of OAM adjustment, suggesting that individualised management of insulin and/or oral agents may be warranted.

Outcomes after 26 Weeks of Treatment

Outcome	AM (N=213[a])		PM (N=214[a])		Between Treatment (AM vs PM)	
	Baseline	26 Weeks	Baseline	26 Weeks	Difference or RR	p-value[b]
HbA1c (%)	8.5±0.07	6.7±0.06	8.5±0.07	6.8±0.06	-0.13	0.109
HbA1c Change from baseline	-1.8±0.06		-1.7±0.06			
HbA1c <7.0%, n (%)	7 (3.3)	137 (68.8)	9 (4.3)	121 (63.7)	-	0.238
HbA1c<7.0% and no nocturnal hypo, n (%)	6 (2.8)	85 (42.7)	7 (3.3)	67 (35.3)	-	0.082
FSG from laboratory (mmol/L)	9.6±0.18	6.3±0.12	9.8±0.18	6.2±0.12	0.14	0.420
Body weight change from baseline (kg)	NA	2.19±0.22	NA	1.85±0.23	0.34	0.287
Basal insulin dose (U/kg)	NA	0.40±0.02	NA	0.39±0.02	0.00	0.828
Patients with no missing treatment doses, n (%)	NA	165 (78.2)	NA	161 (75.6)	-	0.565
Total hypo rate[c]	0.26±0.09	1.62±0.15	0.19±0.08	1.31±0.12	RR: 1.24	0.112
Nocturnal hypo rate[c]	0.14±0.06	0.31±0.06	0.14±0.08	0.32±0.05	RR: 0.97	0.912
Non-nocturnal hypo rate[c]	0.12±0.05	1.32±0.12	0.06±0.03	1.01±0.11	RR: 1.31	0.048
Severe hypo[d], n (%)	0	1 (0.5)	0	1 (0.5)	-	>0.999
Self-Monitored Blood Glucose (mmol/L)						
Morning pre-meal	9.4±0.16	6.2±0.09	9.2±0.16	6.1±0.09	0.14	0.287
Midday pre-meal	10.0±0.21	6.7±0.14	9.8±0.21	7.1±0.14	-0.40	0.038
Evening pre-meal	9.9±0.20	7.0±0.14	9.8±0.20	7.6±0.14	-0.57	0.003
Bedtime	10.8±0.21	7.8±0.16	10.7±0.21	8.2±0.15	-0.43	0.048
3:00 AM	9.2±0.19	6.5±0.12	9.0±0.19	6.4±0.12	0.05	0.794
Next day morning pre-meal	9.2±0.15	6.2±0.09	8.9±0.15	6.1±0.09	0.06	0.645

Data are LS Mean ± SE unless otherwise indicated
[a] Patients taking at least one dose of study drug (full analysis set).
[b] p-values are for between treatment differences at week 26.
[c] Group mean events/patient/30 days.
[d] Number of patients with severe hypoglycaemia.
CI=confidence integral; FSG=fasting serum glucose; hypo=hypoglycaemia; NA=not applicable; RR=relative rate, AM/PM; U=units

Clinical Trial Registration Number: NCT01790438
Supported by: Eli Lilly and Company

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Assessment of real-world efficacy and safety of basal insulin plus rapid-acting insulin vs basal insulin treatment among patients with type 2 diabetes in the UK

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Background and aims: Basal insulin alone and basal insulin plus rapid-acting insulin (RAI) are commonly used as add-on therapies for patients with type 2 diabetes (T2DM) who have inadequate glycemic control with oral antidiabetic drugs. The objectives of this study were to evaluate the real world efficacy and safety of basal insulin plus RAI (basal+RAI) therapy and basal insulin alone therapy.
Materials and methods: Patients with a diagnosis of T2DM and a claim for basal insulin and/or RAI, regardless of daily dosing frequency (index event) were identified during 1/1/2004 to 12/31/2012 from The Health Improvement Network (THIN) U.K. database. T2DM patients were grouped into 2 cohorts; one treated with basal+RAI and the other treated with basal insulin alone. Patient characteristics, clinical outcomes, and healthcare resource use were evaluated and compared during a 12-month baseline period. Generalized linear models were used to control for key patient characteristics and evaluate adjusted treatment outcomes.
Results: Using THIN database, 3,186 T2DM patients were identified with basal+RAI usage and 7,277 were identified with basal alone usage. At baseline the mean age of the basal+RAI cohort was less than the basal alone cohort (61 vs. 67 years, p<0.001). Also, the basal+RAI cohort had a greater mean baseline weight (92.4 vs. 88.3 kg, p<0.001) and a higher mean HbA1c level (9.24% vs. 8.36%, p<0.001). After controlling for key patient characteristics, the mean change in HbA1c was -0.29% (greater reduction) (p<0.001) for the cohort treated with basal+RAI compared to

the cohort treated with basal alone. The frequency of hypoglycemia did not significantly differ among study cohorts. Using the regression adjustment, the incremental number of healthcare utilizations (4.1, p<0.0001) and number of outpatient only healthcare utilizations (3.0, p<0.001) for all causes during the follow-up period were greater for the cohort treated with basal+RAI in comparison to the cohort treated with basal alone.
Conclusion: In the real-world setting, while basal+RAI treatment is effective in reducing Hb HbA1c, the additional change in HbA1c (-0.29%) associated with basal+RAI vs. basal alone treatment was relatively low. Titration of RAI remains one of the challenges of treatment of patients with T2DM.
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Effectiveness of intensification therapy in patients with type 2 diabetes who used basal insulin only
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Background and aims: Limited data exist on the glycemic benefit of different intensification therapies in type 2 diabetes (T2D) patients who use basal insulin only in a routine clinical setting. We examined the effect of different intensification therapies on glycemic control among T2D patients who received basal insulin only therapy.
Materials and methods: We identified a cohort of all individuals with a first basal insulin only prescription in Northern Denmark, 2000-2012 and identified all add-on intensification therapy with bolus insulin, premixed insulin, or GLP-1 receptor agonists. We used Poisson regression to compute adjusted relative risks (aRRs) of reaching a HbA1c target value of <7.0% adjusted for age, gender, comorbidities, and baseline HbA1c.
Results: We included 6,114 patients with a first basal insulin prescription and HbA1c measurements. Of these 2,156 (35.3%) received intensification therapy after a median of 11 months: 59.2% (n=1276) were intensified with premixed insulin, 27.5% (n=592) with bolus insulin, 11.8% (n=255) with GLP-1 agonists, and only 1.5% (n=33) received more than one add-on regimen simultaneously. Overall, 25.9% attained an HbA1c target of <7% within 3-6 months after intensification. Reductions in median HbA1c were 0.9 percentage points (pp) for premixed insulin, 0.4 pp for bolus insulin, 0.9 pp for GLP-1 agonists, and 1.1 pp for patients with >1 intensification drug. Compared with the large group of premixed insulin intensification as reference, aRRs of attaining an HbA1c <7% were 1.01 (95% CI 0.84-1.21) for bolus insulin, and 1.28 (95% CI 1.02-1.60) for GLP-1 agonists.
Conclusion: In this population-based study one fourth of T2D patients reached a target HbA1c <7% within 3-6 months after intensification of their basal insulin only therapy. GLP-1 agonists were associated with higher target attainment than premixed insulin intensification.
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The significance of obtaining single patient evidence for the best insulin treatment in patients with type 1 diabetes with recurrent severe hypoglycaemia
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Background and aims: The scientific evidence for the benefit of insulin analogues on a single patient level in type 1 diabetes is sparse. We made a post-hoc analysis of data from the HypoAna cross-over trial to test the hypothesis that obtaining single-patient evidence for the superior treatment can improve overall treatment outcomes compared to grouped treatment approach, based on either human insulin or analogue insulin.
Materials and methods: In a two-year, multicentre, prospective, randomised, open, blinded endpoint (PROBE) trial, 114 patients with type 1 diabetes and recurrent severe hypoglycaemia were treated with basal-bolus therapy based on analogue (detemir/aspart) and human (NPH/regular) insulin in a balanced cross-over design aiming at maintenance of baseline HbA1c. For each patient the superior treatment was considered that resulting in fewest events of severe hypoglycaemia defined by ADA criteria, or in case of similarity, that eventually resulting in a more than 0.4% lower HbA1c.
Results: A higher proportion of the patients (n=56; 49%) had superior outcome with analogue insulin compared with equal outcome (n=30; 26%) and better outcome on human insulin (n=28; 25%) (p=0.0016). The overall rate of severe hypoglycaemia during the superior treatment for each patient of 0.67 episode per patient-year was lower compared to the 1.1 and 1.6 episode per patient-year with analogue insulin and with human insulin, respectively (p<0.0001). There was no clinically significant difference in HbA1c between the three groups.
Conclusion: In patients with type 1 diabetes and recurrent severe hypoglycaemia insulin treatment selected according to single-patient evidence may result in clinically significant improvement of outcomes compared to a grouped treatment approach based on either human or analogue insulin.
Clinical Trial Registration Number: NCT00346996
Supported by: Novo Nordisk

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Insulin pump in difficult to control type 2 diabetes mellitus
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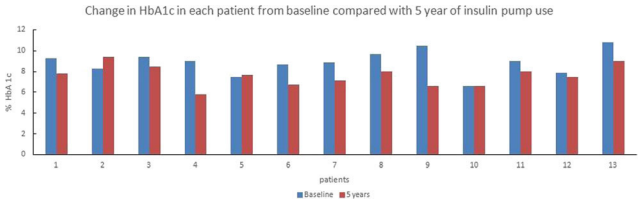
Background and aims: The prevalence of type 2 diabetes mellitus is increasing worldwide. Despite the availability of a number of anti-hyperglycemic agents, large percentage of diabetics remains poorly controlled. Due to the progressive loss of beta-cell functions, most type 2 diabetics eventually require intensification of insulin therapy. Delivering high doses of insulin by multiple daily injections present a therapeutic challenge. Continuous subcutaneous insulin infusion (CSII) through an insulin pump has been successfully used in type 1 diabetics; however, long term data is lacking in type 2 diabetes. OpT2mise trial tested CSII in type 2 diabetics, and showed a decrease of mean glycated hemoglobin (HbA1c) by 1·1% in the pump treatment group compared to 0·4% in the multiple daily injections (MDI). Nevertheless, all the previous trials including OpT2mise have a very short experience with the insulin pump (6-12 months). The long-term efficacy and safety of CSII in patients with type 2 diabetes has not been reported. We hereby, aim to present our five years experience with the use of insulin pumps in type 2 diabetics.
Materials and methods: Our study was single centered, outpatient-based, retrospective, un-blinded, and non-randomized chart review of all the patients with type 2 diabetes treated via insulin pump between 2005 and 2013. Inclusion criteria were, age between 18-75 years, with type 2 DM, HbA1c of more than 7% at initiation, and on insulin pump for more than 5 years. Additionally, all patients had one of the problems with the insulin therapy: dose more than 100 units, more than 4 injections, wide glycemic excursions, and/or intractable hypoglycemia. Exclusion criteria were type 1diabetics. A total of 13 patients were identified. The primary endpoint was change in HbA1c from baseline to five years. HbA1c was assessed at baseline, at 3 months, 6 months, 9 months, 12 months, and at 5 years. We also reviewed the change in body mass index (BMI), basal insulin requirement, and hypoglycemia from baseline to one year. All oral anti-diabetic agents were discontinued with the exception of metformin. Data are presented as the mean (SD) and were

analyzed using unpaired t test. A p-value of less than 0.05 was considered statistically significant.
Results: At baseline, mean HbA1c was 8.89±1.15 (Range 6.6-10.8). At 3, 6, 9, and 12 months the mean HbA1c was 7.58%, 7.55%, 8.33%, and 7.96% respectively. The HbA1c at 5 years was 7.7231 compared to baseline HbA1c of 8.89 (p value equals 0.007). These results were encouraging as in historical data from patients on multiple daily injections; there is a progressive increase of HbA1c. However, in our data after initial increase the patients have sustained decrease in HbA1c even at five years of follow up. Furthermore, we did not find any documented episode of severe hypoglycaemia. Moreover, CSII was not associated with significant increase in BMI, or basal insulin requirements.
Conclusion: The use of insulin pumps in patients with difficult to treat type 2 diabetes is safe and effective, with the beneficial effects persisting for five years of follow-up.



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The dual-wave bolus provides better glycaemic control of the high-protein meal in type 1 diabetic children treated with insulin pumps: randomised cross-over study
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Background and aims: Insulin pumps apart from normal boluses have also the ability to deliver the insulin over a time by use of a dual-wave or square-wave bolus. It is well known that carbohydrates need to be cover with normal boluses as well as the fat content meals need dual-wave boluses. There is a lack of studies estimating the need for additional insulin for high-protein (H-P) meals. The aim of the study was to assess the differences in the postprandial glycemic excursions following a high-protein, low-fat meal after administration of insulin by normal versus dual-wave bolus.
Materials and methods: We performed a randomized, double-blind, two-way crossover study. There were 38 patients included,14 (37%) boys and 24 (63%) girls, aged 15.1±1.7 years (range 11-18) with mean diabetes duration 9.2±2.7 years, mean HbA1c 8.3±1.1%, mean daily insulin dose 0.75±0.26 u/kg. Subjects were randomly assigned into two groups: group A consisted of patients who applied normal bolus or group B included subjects who applied dual wave bolus. All patients had a standardized (H-P) breakfast for 2 subsequent days. Insulin in normal bolus was calculated based on the number of carbohydrate units. One carbohydrate unit was defined as 10 grams of carbohydrate. Insulin in dual-wave bolus was calculated based on the number of carbohydrate units and protein-fat units. One protein-fat unit was defined as a 100 kcal from protein and/or fat. The same insulin-ratio was for one carbohydrate unit and one protein-fat unit. The primary outcome was postprandial glycemia (PPG) based on continuous glucose monitoring system and self-blood glucose measurements assessed in 60, 120 and 180 minutes after the meal bolus. Secondary outcome was the frequency of hypoglycemia (<65 mg/dl) during 3-h follow-up.
Results: Postprandial glycemia assessed in 180 min was significantly higher in group A compared to group B: 157 vs 115 mg/dl ($p<0.0001$). There were no statistically significant differences in blood glucose levels between both groups during first 2 h after the meal bolus: 109 vs 104 mg/dl at the baseline ($p=0.35$); 152 vs 151 mg/dl in 60 min ($p=0.57$); and 177 vs 168 mg/dl ($p=0.43$) in 120 min. 3 hours after the meal bolus we observed 1 (2%) hypoglycemic episode in the group A and 6 (15%) hypoglycemic episodes in the group B ($p=0.2$).
Conclusion: We demonstrated that applying the dual wave bolus for high protein meal improve postprandial glycemic profile compared to normal bolus in type 1 diabetic children treated with insulin pumps. Reduction of postprandial hyperglycemia was not associated with increased risk of hypoglycemia.
Clinical Trial Registration Number: NCT02276859
Supported by: Medtronic

OP 27 Lowering lipids in diabetes

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Evaluation of the one-year efficacy, safety and glycaemic effects of evolocumab (AMG 145) in 4,802 subjects with, at high risk for, or at low risk for, diabetes mellitus

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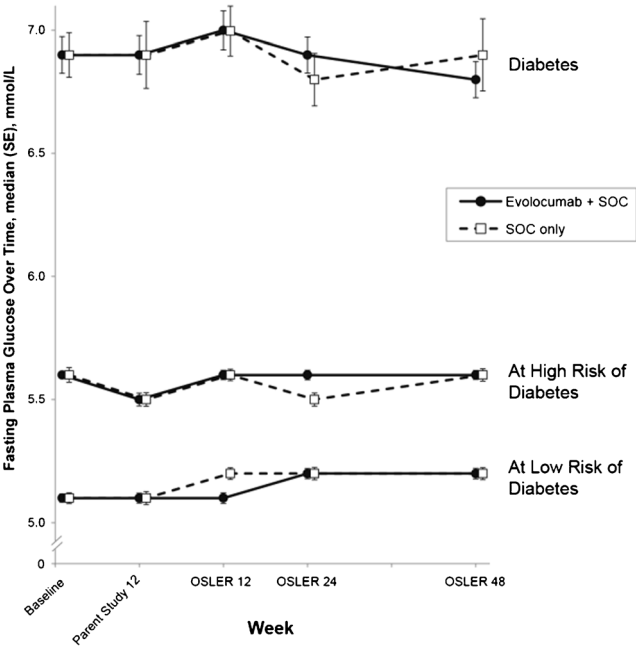
Background and aims: Statins reduce cardiovascular risk but increase the risk of developing diabetes. We investigated the effects of the PCSK9 inhibitor evolocumab (AMG 145), a fully human monoclonal antibody to PCSK9, on measures of glycaemia and adverse event (AE) rates in subjects stratified by glycaemic status.
Materials and methods: In two open-label trials (OSLER-1 and OSLER-2), 4,802 subjects completed one of 13 phase 2 or 3 parent studies of evolocumab and were randomly assigned in a 2:1 ratio to receive either evolocumab 140 mg every 2 weeks or 420 mg monthly plus standard of care (SoC) or SoC alone. SoC included statin use for some patients. Changes in fasting plasma glucose (FPG), HbA1c, and AEs were evaluated over 48 weeks in three subject groups: 852 with type 2 diabetes mellitus (T2DM), 2,432 at high risk of developing T2DM (defined as metabolic syndrome, IFG, HbA1c >6% or BMI >30 kg/m2), and 1,518 at low risk of developing T2DM.
Results: LDL-C reductions for evolocumab + SoC compared with SoC were comparable across the 3 subgroups (57% to 60%). No notable differences were seen in median (SE) change in FPG from baseline to 48 weeks in patients on evolocumab + SOC compared with SoC alone (see Figure). Mean (SE) HbA1c changes at week 48 in patients on evolocumab + SoC and in patients on SoC alone were +0.16 (0.05) and +0.23 (0.06)% in patients with diabetes; +0.05 (0.01) and +0.06 (0.01)% in patients at high diabetes risk; and +0.06 (0.01) and +0.07 (0.01)% in patients at low risk of diabetes. Results were similar irrespective of parent-study drug assignment. Rates of AEs in patients on evolocumab + SoC vs. SoC alone were: 64% and 63% (T2DM); 69% and 64% (high diabetes risk); and 69% and 63% (low diabetes risk).
Conclusion: Evolocumab showed encouraging safety with no measurable effect on glycaemic parameters despite reducing LDL-C levels markedly.

Figure. Median (SE) Fasting Plasma Glucose Over Time



Clinical Trial Registration Number: NCT01439880, NCT01854918
Supported by: Amgen Inc.

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Efficacy and safety of alirocumab in individuals with diabetes: analyses from the ODYSSEY LONG TERM study

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Background and aims: Diabetes (DM) increases risk for cardiovascular disease (CVD) and is often complicated by dyslipidaemia. The efficacy and safety of the PCSK9 inhibitor alirocumab (ALI) for lowering LDL-C in individuals with DM vs those without DM is unknown. We compared the effects of ALI in individuals with and without DM according to medical history at baseline in ODYSSEY LONG TERM.
Materials and methods: LONG TERM included 2341 individuals (832 with DM; 35.5%) at high CVD risk and LDL-C ≥70 mg/dL on maximally tolerated statin±other lipid-lowering therapy, randomized (2:1) to ALI 150 mg or placebo (PBO) Q2W (as 1 mL injection) for 78 weeks. We report prespecified efficacy analyses at Week 24 and safety including all available data from baseline to Week 78 (all individuals to at least Week 52). Final study data will be presented at EASD.
Results: At baseline, mean LDL-C and HDL-C levels were slightly lower in individuals with DM than those without; individuals with DM also had higher TGs than those without DM (Table). LS mean (95% CI), PBO-corrected, reductions in LDL-C of 59% (63.2 to 54.8) were observed in ALI-treated individuals with DM at Week 24 (Table); LDL-C was reduced by 63.4% (66.5 to 60.3) in individuals without DM. Decreases in TGs and increases in HDL-C levels were also seen with ALI compared with PBO. These treatment effects were similar in individuals with or without DM. Adverse events occurring in ≥5% of individuals in all subgroups were nasopharyngitis, URI and UTI, including those treated with

both ALI and PBO. In individuals with DM, injection site reactions (ISRs) occurred in 3.4% of ALI individuals vs 3.2% in PBO; myalgia occurred in 3.1% vs 2.9%, respectively. In those without DM, ISRs occurred in 7.0% (ALI) vs 4.9% (PBO); myalgia occurred in 5.9% vs 3.1%, respectively. Treatment-emergent CV events were positively adjudicated in 5.1% (ALI) and 7.2% (PBO) of individuals with DM; for those without DM, CV event rates were 3.4% (ALI) and 2.9% (PBO). In *post-hoc* analyses, rates of major CV events were 1.8% (ALI) and 4.3% (PBO) for those with DM and 1.2% (ALI) and 2.4% (PBO) for those without DM; hazard ratios (95% CI) vs PBO were 0.41 (0.18-0.96) [with DM] and 0.51 (0.23-1.13) [without DM].

Conclusion: Significant LDL-C reductions with ALI vs PBO were seen in individuals with DM; these reductions were similar to individuals without DM. ISRs and myalgia occurred more frequently with ALI than PBO in individuals without DM; in those with DM, rates were similar for ALI and PBO. Treatment with ALI for potential reduction of CV events is under evaluation in the large ongoing ODYSSEY OUTCOMES study which will include a sizeable proportion of individuals with DM.

Table. Summary of efficacy in response to treatment

Efficacy analysis (ITT)	Individuals with DM (N=818)		Individuals without DM (N=1492)		Interaction p-value*
	Alirocumab 150 mg Q2W (n=545)	Placebo Q2W (n=273)	Alirocumab 150 mg Q2W (n=985)	Placebo Q2W (n=507)	
Calculated LDL-C					
Mean (SE) baseline (mg/dL)	116.6 (1.6)	117.1 (2.2)	126.1 (1.5)	124.6 (1.9)	
LS mean % difference (95% CI) alirocumab vs placebo (Week 24)	-59.0 (-63.2 to -54.8)		-63.4 (-66.5 to -60.3)		0.0957
HDL-C					
Mean (SE) baseline (mg/dL)	48.3 (0.5)	48.8 (0.8)	50.7 (0.4)	50.6 (0.5)	
LS mean % difference (95% CI) alirocumab vs placebo (Week 24)	3.2 (1.0 to 5.3)		5.4 (3.8 to 7.0)		0.1078
TGs					
Combined estimate for mean (SE) baseline (mg/dL)[†]	172.0 (4.4)	163.8 (5.0)	137.8 (2.2)	144.0 (3.3)	
Combined estimate for adjusted mean % difference (95% CI) alirocumab vs placebo (Week 24)[††]	-18.5 (-23.1 to -13.9)		-16.7 (-20.1 to -13.3)		0.5430

*Interaction p-value for treatment effect in individuals with DM versus those without; calculated for efficacy parameters only.
[†]Baseline combined estimate and SE are obtained by combining baseline means and SE of the different imputed data sets; [††]Combined estimate obtained by combining adjusted means and SE from robust regression model analyses of the different imputed data sets (multiple imputation).
CI, confidence interval; DM, diabetes mellitus; HDL-C, high-density lipoprotein cholesterol; ITT, intent-to-treat; LDL-C, low-density lipoprotein cholesterol; LS, least squares; Q2W, every 2 weeks; SE, standard error; TGs, triglycerides.

Clinical Trial Registration Number: NCT01507831
Supported by: Funded by Sanofi and Regeneron Pharmaceuticals, Inc.

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Efficacy of one year of treatment with the PCSK9 inhibitor evolocumab (AMG 145) in 4,802 subjects with or without type 2 diabetes

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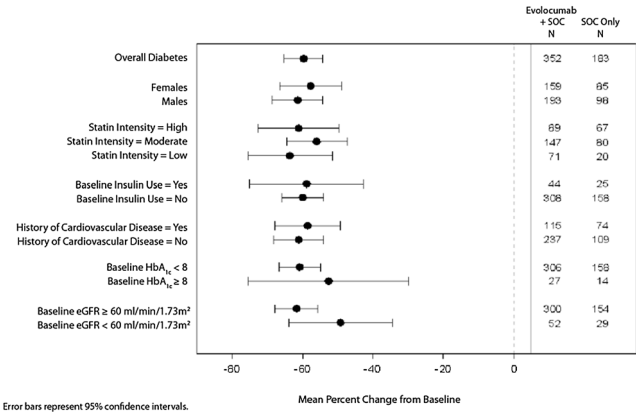
Background and aims: Lipid-lowering therapy is a mainstay of treatment for subjects with type 2 diabetes mellitus (T2DM), who are at elevated risk for cardiovascular disease. In a pooled analysis of subjects with T2DM from 2 open-label extension trials, we evaluated the efficacy of one year of subcutaneous evolocumab (AMG 145) treatment added to standard of care (SoC) in subjects with or without T2DM at baseline.
Materials and methods: In total, 4,802 subjects completed one of 13 phase 2 or 3 studies and continued into OSLER-1 (phase 2 extension) or OSLER-2 (phase 3 extension). Subjects were randomly assigned 2:1 to either evolocumab 140 mg biweekly or 420 mg monthly plus SoC or SoC alone; randomization was irrespective of parent-study assignment. Changes in LDL-C (calculated) and other lipids were evaluated at week 48 in subjects with or without T2DM. LDL-C changes within T2DM subgroups (including low-, moderate-, or high-intensity statin; insulin or no insulin treatment; presence or absence of CVD; HbA1c≥or< 8.0%; and eGFR≥or<60 mL/min/1.73 m2) were also evaluated.
Results: In total, 563 subjects with and 2,637 without T2DM (defined by history, glycemic criteria, or baseline diabetes medication) received evolocumab plus SoC in the open-label extension phase of the study. In subjects on evolocumab plus SoC, mean percent changes in LDL-C from baseline versus SoC at one year were -60% and -58% in those with and without T2DM, respectively. Similar changes in LDL-C were observed between treatment arms in T2DM subgroups (Figure). Changes in lipoprotein (a) (-30% and -29%) and TG (-14% and -11%) were also comparable in evolocumab plus SoC-treated subjects with and without T2DM, respectively, compared with SoC alone.
Conclusion: One year of treatment with evolocumab resulted in marked and comparable reductions in LDL-C in subjects with and without T2DM, and these changes were similar across T2DM subgroups, irrespective of baseline characteristics. Beneficial changes in other lipids were also observed in those receiving evolocumab plus SoC.

Figure. SoC-Adjusted Percent Change from Baseline in LDL-C at One Year in Subjects with Diabetes Mellitus



Error bars represent 95% confidence intervals.

Clinical Trial Registration Number: NCT01439880 and NCT01854918
Supported by: Amgen Inc.

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Lipid changes during 26-week treatment with the novel basal insulin peglispro (BIL) vs insulin glargine or NPH insulin in 6 IMAGINE Trials

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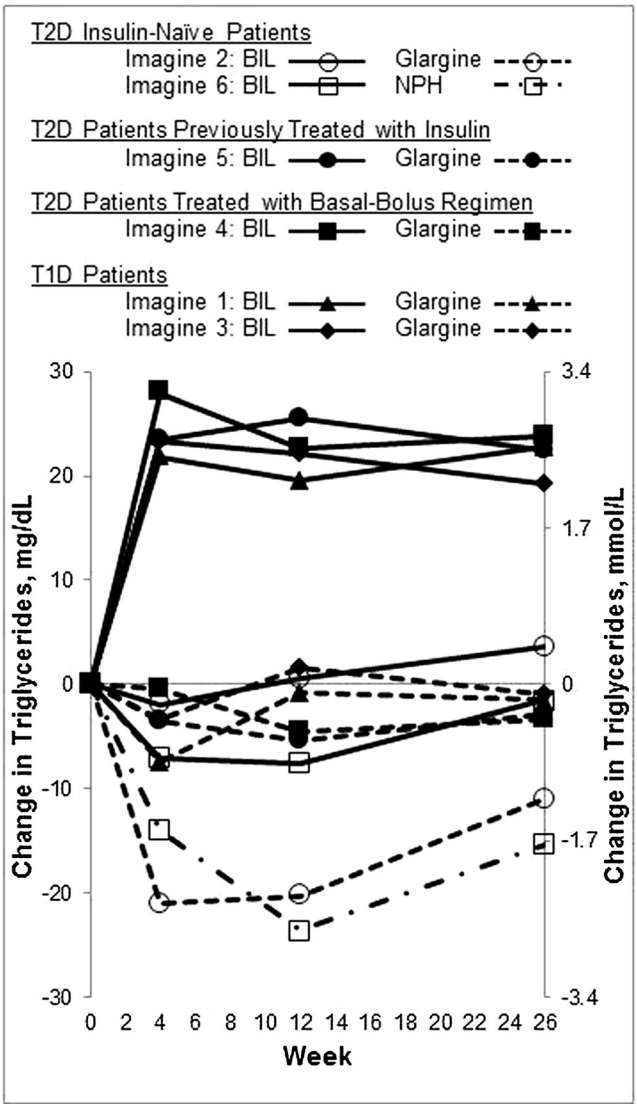
Background and aims: Basal insulin peglispro (BIL) is a novel basal insulin with a flat activity profile which has a hepato-preferential action resulting from reduced peripheral effects. Lipid changes observed with BIL in Phase 3 studies differ from those observed with the comparators insulin glargine and NPH insulin. To better understand these differences, we analysed fasting lipid data from the first 26 weeks of treatment from 6 Phase 3 trials of BIL. Five of these trials had subgroups from which apolipoproteins and free fatty acid data were collected.
Materials and methods: Trials were divided into 4 categories for analysis: trials of a basal insulin regimen in insulin-naïve patients with type 2 diabetes (T2D); trials of a basal insulin regimen in patients with T2D previously treated with insulin; trials of a basal-bolus insulin regimen in patients with T2D previously treated with insulin; and trials of a basal-bolus insulin regimen in patients with type 1 diabetes (T1D) previously treated with insulin.
Results: By week 26, in insulin-naïve patients with T2D, triglycerides decreased with glargine and NPH (-.12 mmol/L [-11 mg/dL] and -.17 mmol/L [-15 mg/dL], p<.05) but were unchanged from baseline with BIL (range: -.02 mmol/L [-1 mg/dL] to .04 mmol/L [4 mg/dL], p=NS). In

patients with T1D or T2D previously treated with insulin, triglycerides were stable with glargine (p=NS) and increased .21 to .27 mmol/L (19 to 24 mg/dL; 15 to 25%) with BIL (p<.001). After BIL discontinuation triglycerides decreased to pre-study levels. In all trials, with BIL, HDL-cholesterol (C) changed 0 to -.12 mmol/L (0 to -5 mg/dL); LDL-C changed 0 to .17 mmol/L (0 to 7 mg/dL). With glargine, HDL-C and LDL-C changed 0 to -.04 mmol/L (0 to -1 mg/dL) and .03 to .12 mmol/L (1 to 5 mg/dL), respectively. Apolipoproteins A1 and B were mostly unchanged with BIL. Among patients previously treated with insulin, there were no treatment differences in free fatty acids (p=NS); in insulin-naïve patients with T2D, free fatty acids decreased more with glargine than BIL (p=.003).

Conclusion: Lipids, free fatty acids and apolipoproteins A1 and B had little or no change with BIL in insulin-naïve patients with T2D; in contrast, GL and NPH lowered triglycerides. When BIL replaced conventional insulin treatments, increases in triglycerides were observed. Increased hepatic de novo lipogenesis, increased adipose tissue release and hepatic flux of free fatty acids, or decreased adipose tissue lipoprotein lipase activity, either alone or in combination, may also be the basis for differences in triglyceride concentrations between BIL and other insulins.



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RESEARCH: superior effect of ezetimibe was sustained on LDL-C level and the rate of achievement of target value in a 52-week analysis

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Background and aims: Lowering cholesterol levels decreases the risk of atherosclerotic diseases. Low-density lipoprotein cholesterol (LDL-C), other atherogenic lipid parameters, especially small dense LDL-C (sd-LDL) have been shown to increase the risk of coronary artery diseases(CAD). We evaluated long-term ezetimibe add-on therapy in type 2 diabetic (DM) patients with hypercholesterolemia not achieving LDL-C target value.
Materials and methods: A total of 109 DM patients not attaining LDL-C target value despite usual-dose statin therapy in Japan were recruited. We investigated the difference in cholesterol lowering effect between ezetimibe add-on statin (E) group and double-dose statin (S) group. Changes of lipid parameters were assessed in a randomized, multicenter, 52 weeks and open label study. We determined sd-LDL levels by the precipitation method. The participants were randomly assigned to 2 groups, E group (n=31) with ezetimibe 10 mg + atorvastatin 10 mg or pitavastatin 1 mg combination and S group (n=38) with atorvastatin 20 mg or pitavastatin 2 mg.
Results: At the baseline(0 week), there was no difference between the groups in age, body mass index (BMI), sex ratio, HDL-C, triglyceride (TG), hemoglobin A1c (A1c) and high-sensitivity C-reactive protein (hs-CRP) . However, sd-LDL and nonHDL-C were significantly higher in D group than in E group. Then, both of the groups showed significant decrease in LDL-C, sd-LDL and non HDL-C during the study. LDL-C, sd-LDL and non HDL-C were significantly lower in the E group than in the S group at the end of the study.
Conclusion: In the present 52-week long-term period, ezetimibe add-on therapy showed a robust advantage in lowering LDL-C and in attaining target LDL-C values compared with the doubling of statin dose. Moreover,it's meaningful that sd-LDL, powerfully atherogenic lipoprotein, was decreased prominently by ezetimibe add-on therapy. DM patients with hypercholesterolemia are at high risk for CAD, and adding ezetimibe onto usual-dose statin treatment in Japan has been suggested as the first-line therapy for those DM patients who failed to attain the target LDL-C value.

	0 week			⊿(52w − 0w)		
	E	S	p *	E	S	p ***
	Mean±SD			Least square mean±SE		
Age (years)	61.4±10.3	61.3±8.8	0.647			
Sex (Male/Female)	20/11	24/14	0.907**			
BMI (kg/m^2)	25.4±5.6	27.0±6.2	0.173			
A1c (%)	7.38±0.66	7.28±1.00	0.471	−0.07±0.12	−0.02±0.11	0.748
hs-CRP (mg/dl)	0.86±0.91	1.01±1.36	0.801	−0.31±0.25	−0.17±0.22	0.700
TG (mg/dl)	130±62	171±90	0.059	−2±10	2±9	0.793
HDL-C (mg/dl)	57.7±14.0	54.7±7.9	0.571	−3.7±1.4	−1.0±1.2	0.162
LDL-C (mg/dl)	130±17	135±20	0.437	−38±4	−12±3	<0.001
sd-LDL (mg/dl)	44.1±14.6	54.4±17.4	0.007	−15.0±2.2	−4.3±2.0	0.001
Non HDL-C (mg/dl)	152±20	164±23	0.025	−42±4	−12±4	<0.001
RLP-C (mg/dl)	5.6±3.4	6.6±4.3	0.265	−1.3±0.7	−0.0±0.6	0.204

* Mann-Whitney test ** chi-square test *** ANCOVA adjusted for TG, Non HDL-C and sd-LDL

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Efficacy and safety of liraglutide versus sulphonylurea both in combination with metformin during Ramadan in subjects with type 2 diabetes (LIRA-Ramadan): a randomised trial

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Background and aims: There are approximately 50 million Muslims worldwide with diabetes who fast during Ramadan, 79% of which are estimated to have type 2 diabetes (T2D). Fasting during Ramadan leads to a 5- and 7.5-fold increased risk of severe hyper- and hypoglycaemia, respectively. Furthermore, adjustment of glucose-lowering medication and closer follow-up during Ramadan may be necessary. The effect of liraglutide vs sulphonylurea (SU), both + metformin (Met), on change in glycaemic control in subjects with T2D who fasted during Ramadan was examined.
Materials and methods: In this up to 33-week, open-label trial, adults (HbA1c 7-10%; BMI >20 kg/m^2; stable SU + Met; intent to fast during Ramadan) were randomised to either switch to once-daily liraglutide 1.8 mg (N=172) or continue pretrial SU (N=171), both + Met. After 3-week dose escalation, a 6-19-week maintenance period preceded Ramadan. Primary endpoint was change in fructosamine from start to end of Ramadan (liraglutide N=151; SU N=165) (Table).
Results: During Ramadan, despite lower fructosamine and HbA1c at start of Ramadan in the liraglutide arm, a similar reduction in fructosamine and HbA1c with liraglutide and SU was seen. Confirmed hypoglycaemic episodes appeared to be lower with liraglutide and fewer subjects withdrew during Ramadan (liraglutide 3, SU 11). At end of Ramadan, more subjects reached the composite endpoint of HbA1c <7%, no weight gain and no confirmed hypoglycaemia with liraglutide (51.3% vs 17.7%; odds ratio 4.90; 95% confidence interval 2.79-8.62; p<0.0001). For changes in blood pressure during Ramadan, reduction in systolic blood pressure was greater with liraglutide than SU (estimated treatment difference: -4.01 mmHg; p= 0.0061); there was no difference for diastolic blood pressure. AE frequencies appeared at similar levels: liraglutide 23.7%; SU 20.9%. Gastrointestinal AEs were more common for liraglutide (10.5%; SU 3.7%). A low incidence of SAEs was observed (liraglutide 1.3%; SU 0%).
Conclusion: During Ramadan, liraglutide showed similar improvements in glycaemic control from lower fructosamine and & HbA1c levels compared to SU with a similar number of AEs, fewer confirmed hypoglycaemic episodes and better weight control.

Baseline (BL)	Liragl utide N=171	SU N=170	ENDPOINT	Liragl utide	SU	Treatment difference or ratio (95% CI; p*)
Fructosamine, μmol/L, mean (SD), BL	320.3 (53.6)	316.0 (58.3)	Fructosamine (μmol/L), mean change: during Ramadan	-12.8	-16.4	3.51 (-5.26, 12.28; 0.4311)
At start of Ramadan, mean (SD)	291.8 (54.9)	301.6 (56.7)	from BL to end of Ramadan	-39.6	-29.3	-10.3 (-18.7, -1.89)
Body weight, kg, mean (SD)	81.0 (17.1)	83.1 (16.1)	Body weight (kg) mean change: during Ramadan	-1.43	-0.89	-0.54 (-0.94, -0.14)
			from BL to end of Ramadan	-5.40	-1.46	-3.94 (-4.54, -3.33)
HbA1c, %, mean (SD)	8.3 (0.9)	8.2 (0.9)	HbA1c (%), change from BL to end of Ramadan	-1.24	-0.65	-0.59 (-0.79, -0.38)
			HbA1c <7%, at end of Ramadan**	57.1%	26.4%	3.71^ (2.18, 6.30)
			HbA1c <7% and no hypoglycaemic episodes, at end of Ramadan**	53.9%	23.5%	3.80^ (2.24, 6.46)
Age, years, mean (SD)	54.9 (9.27)	54.0 (9.33)	During Ramadan, Confirmed hypoglycaemic episodes, % of subjects	2.0%	4.3%	Not done
Duration of diabetes, years, mean (SD)	8.0 (5.3)	7.2 (4.4)	Events per 1000 PYE	246	623	

SD, standard deviation; Confirmed hypoglycaemia, subject unable to treat themselves and/or plasma glucose <3.1 mmol/L (56 mg/dL) with or without symptoms; *p<0.05 unless noted otherwise;**based on number of subjects entering Ramadan; ^odds ratio; PYE=patient years of exposure

Clinical Trial Registration Number: NCT01917656
Supported by: Novo Nordisk

OP 28 Renal rounds: diabetes and the kidney

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SGLT2 inhibitor dapagliflozin ameriorates diabetic nephropathy by inhibiting high-glucose-induced oxidative stress in a mouse model of type 1 diabetes

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Background and aims: Previously, we demonstrated that dapagliflozin, a sodium glucose cotransporter 2 (SGLT2) inhibitor, protects against diabetic nephropathy; however, it was unclear whether it is mediated by a direct action on kidney. Therefore, we investigated the effect of dapagliflozin on early stage of diabetic nephropathy using a mouse model of type 1 diabetes and cultured murine proximal tubular epithelial cells.
Materials and methods: Eight-week-old Akita mice were treated with 1.0 mg/kg of dapagliflozin (n=5) or 10-20 unit/kg/day of insulin (n=5) for 12 weeks. Control C57BL/6 mice (n=6) and Akita mice (n=6) received saline for 12 weeks. Murine proximal tubular epithelial (mProx24) cells were cultured and treated with dapagliflozin and transfected with *Slc5a2* (encoding SGLT2) siRNA.
Results: Compared with control Akita mice, dapagliflozin and insulin equally decreased blood glucose and hemoglobin A1c levels (dapagliflozin: 6.8±0.2%; insulin: 6.4±0.2%; Akita: 9.2±0.2%). Urinary albumin excretion (UAE) was higher in Akita group compared with that in the C57BL/6 group, and was significantly lower in both dapagliflozin and insulin groups. Moreover, dapagliflozin was more effective than insulin in reducing UAE. ROS production, which was detected by dihydroethidium, was higher in the cortex of Akita group than in that of C57BL/6 group, and it was suppressed by insulin and more efficiently by dapagliflozin. Similarly, Nox4, a subunit of NADPH oxidase, was upregulated in the cortex of Akita group compared with the C57BL/6 group, but it was attenuated in the dapagliflozin group and in the insulin group, though less effectively by the latter. As demonstrated by qRT-PCR analysis of kidney tissue, expression of Nox4 was significantly suppressed by dapagliflozin, but not by insulin. Moreover, Western blotting showed that dapagliflozin, but not insulin, suppressed the Nox4 protein expression. Collectively, oxidative stress induced by diabetes was suppressed by glycemic control using insulin, and was more strongly suppressed by dapagliflozin. These data indicate that dapagliflozin directly reduced oxidative stress in diabetic kidney. To further investigate the effect of dapagliflozin on high-glucose-induced ROS production in kidney, we performed dichlorofluorescin diacetate staining and FACS analysis using cultured mProx24 cells. High-glucose medium increased ROS production in mProx24 cells, which was significantly attenuated by dapagliflozin treatment in a dose-dependent manner. To evaluate whether or not the suppressive effect of dapagliflozin depends on the inhibition of SGLT2, we performed RNAi against *Slc5a2* in mProx24 cells. Knockdown of *Slc5a2* also suppressed the expression of these inflammatory genes to similar levels as dapagliflozin did. These results show that dapagliflozin inhibited oxidative stress by inhibiting SGLT2 in proximal tubular epithelial cells.
Conclusion: In conclusion, our study demonstrated that inhibition of SGLT2 by dapagliflozin improved hyperglycemia and ameliorated diabetic nephropathy directly by inhibiting oxidative stress in Akita mice.

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The effect of SGLT-2 inhibitor, tofogliflozin on the urinary albumin excretion rate by the degree of albuminuria in Japanese type 2 diabetes

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Background and aims: Diabetic nephropathy is a major microvascular complication of diabetes characterized by albuminuria and progressive loss of kidney function. The renal sodium glucose transporter-2 (SGLT2) is specifically expressed on the proximal tuble as well as on the mesangial cells in the literature. However, there were a few reports that showed that SGLT2 inhibitors improved albuminuria. The aim of this study was to investigate the effect of the SGLT2 inhibitor, tofogliflozin (TOFO), on the urinary albumin excretion rate (UACR) by the degree of albuminuria in not so obese Japanese type 2 diabetic patients from TOFO phase 3 studies.
Materials and methods: Total number of subjects was 1044 type 2 diabetics(male:698, female:346, age:mean 58.3 years, duration of DM:6.9 years, BMI:25.6 kg/m2) who were rolled from TOFO phase 3 four studies except two subjects with eGFR less than 30. They were divided into three groups by the degree of UACR. Normal albuminuria group(G1:UACR<30 mg/gCr) was 701 subjects(placebo:40, TOFO:661). Microalbuminuria group(G2:UACR;30 to <300) was 290 subjects(placebo:12, TOFO:278). Macroalbuminuria group(G3:UACR≧300) was 53 subjects(placebo:4, TOFO:49). Glucose and physiological parameters were followed with urinary indexes(UACR, urinary beta-2 microglobulin to creatinine ratio[u-MG] and urinary N-acetyl-beta-D-glucosaminidase to creatinine ratio[u-NAG]). The changes of variables from baseline to week 24 (last observation carried forward [LOCF] procedure) were analyzed by Wilcoxon sign rank test. Urinary indexes were log-transformed in the correlation analysis.
Results: TOFO made significant reduction in HbA1c(mean:-0.76 to -0.89%) and weight(-2.72 to -2.85 kg) similarly in the three groups. A little reduction in UACR(median) was observed in G1(-0.60 mg/gCr, $p < .05$ vs baseline), but its clinical significance was obscure. On the contrary, clinical significant reduction in UACR was observed in G2(-26.10, $p < .0001$) and G3(-323.30, $p < .0001$). UACR reduction was positively correlated with the changes in fasting plasma glucose(r=0.30), u-MG(r=0.28), u-NAG(r=0.27), weight(r=0.19) and diastolic blood pressure(r=0.17) in G2. On the contrast, UACR reduction in G3 was prominent and correlated only with urinary indexes, u-MG(r=0.60) and u-NAG(r=0.32).
Conclusion: SGLT2 inhibitor, TOFO, reduced UACR in Japanese type 2 diabetic patients preserved renal function with micro- and macroalbuminuria. Although UACR reduction might be attributed to improvement of glucose and physiological parameters by SGLT2 inhibition in microalbuminuric stage, other direct mechanism, for example, inhibition of SGLT2 on tubule or mesangial cells was considered in the cases with macroalbuminuria.

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Linagliptin decreases the growth of immortalised human podocytes through the SDF-1-CXCR4/CXCR7 axis

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Background and aims: Linagliptin is a potent and selective dipeptidyl peptidase (DPP)-4 inhibitor, approved as an oral treatment for patients with type 2 diabetes mellitus. In contrast to its effects on glucose homeostasis, the effects of linagliptin on diabetic nephropathy have been less well investigated, although mounting data indicate a therapeutic potential. Stromal cell-derived factor (SDF)-1 is a physiological substrate of DPP-4, and has been postulated to mediate the effects of DPP-4 inhibitors. However, due to complex interactions involving two chemokine receptors (CXCR4 and CXCR7), the role of SDF-1 remains controversial. The aims of this study were to evaluate whether linagliptin exerts direct effects on human glomerular cells, and determine the role of the SDF-1-CXCR4/CXCR7 axis in mediating these effects.
Materials and methods: Expression of DPP-4, SDF-1α, CXCR4, and CXCR7 was evaluated in human immortalised podocytes and mesangial cells at the mRNA (RT-PCR analysis) and protein levels (western blot and ELISA). DPP-4 activity was assessed in cell extracts by measuring the cleavage of the DPP-4 substrate H-Ala-Pro-7-amido-4-trifluoromethylcoumarin. Cell growth was measured using an MTT assay. The role of SDF-1-CXCR4/CXCR7 signalling pathways was investigated by analysing the effects of the bicyclam AMD3100, which is a non-peptide CXCR4 competitive antagonist and CXCR7 allosteric modulator.
Results: Compared with mesangial cells, the expression and activity of DPP-4 was significantly higher in podocytes ($p<0.001$). The DPP-4 activity in podocyte extracts was abolished by linagliptin (0.01–100 nM; $pIC_{50}=8.9$). Moreover, podocyte growth, which is a pathophysiological feature of activated podocytes, was decreased by linagliptin in a time- and concentration-dependent manner; maximal effect (mean±SEM) at 5 days was 25.1±1.6% ($p<0.01$ vs cells exposed to vehicle alone, and $pEC_{50}=8.8$). CXCR4 and CXCR7 were expressed in podocytes, and mean±SEM levels of SDF-1α increased from 0.82±0.18 ng/ml (day 0) to 7.76±0.17 ng/ml (day 5; $p<0.001$). The effects of linagliptin on podocyte growth were mimicked by AMD3100, and a synergistic interaction was observed when linagliptin and AMD3100 were combined.
Conclusion: Our *in vitro* data suggest that linagliptin could exert direct effects on human podocytes. In particular, it may promote the maintenance of a more favourable quiescent phenotype of this cell type, which is essential for preserving the integrity of the glomerular filtration barrier. These effects may depend on inhibiting DPP-4-mediated processing of SDF-1.
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Comparable effectiveness of real-world dual combination therapies across different levels of renal function in patients with type 2 diabetes

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Background and aims: Dipeptidyl Peptidase-4 (DPP-4) inhibitors are widely used in the management of type 2 diabetes mellitus (T2DM). They have demonstrated utility in patients with normal renal function, but have additional advantages for patients with renal impairment (RI). These include reduced need for complex dose titration and lack of increased risk of hypoglycaemia. Another advantage may be comparable efficacy across all levels of renal function, ages and duration of diabetes that may not be seen with other drug classes, and especially in comparison vs. sulphonylureas (SUs). To further explore this hypothesis in a 'real-world' setting, we compared the effectiveness and glycaemic response of a DPP-4 inhibitor, vildagliptin vs. SUs as add-on second-line dual therapy in patients with different degrees of RI enrolled in an observational, non-interventional study, EDGE, which included T2DM patients with failing antidiabetes monotherapy, worldwide.
Materials and methods: In this exploratory post-hoc analysis we used descriptive statistics and adjusted multivariate analysis to assess the effectiveness by the absolute change in HbA1c and the proportion of patients responding to treatment (HbA1c <7%) without tolerability issues

such as weight gain (≥3%) or hypoglycaemia for 12 months. The estimated glomerular filtration rate (eGFR) values were available for a subset of EDGE patients (optional as per original study protocol) in whom vildagliptin or SU for treatment intensification was chosen by the investigators based on clinical judgment.

Results: MDRD-eGFR values were available for 20,822 patients; 2,532 of whom (1,649 in vildagliptin vs. 883 in SU group) had an eGFR value <60 ml/min/1.73 m^2 denoting the presence of RI. Participants with RI were older (65 vs. 57 years), had a lower BMI or absolute body weight and had a longer duration of diabetes (7 vs. 5 years) than those with an eGFR >60 ml/min/1.73 m^2. In multivariate analysis, the change in HbA1c in response to add-on therapy was associated with baseline HbA1c, weight, and assignment to receive vildagliptin (all $p<0.001$). However, the HbA1c response to either vildagliptin or SUs was not significantly influenced by the presence or absence of renal impairment ($p=0.7$). Overall, patients treated with vildagliptin reached their glycaemic targets more often without weight gain or hypoglycaemia vs. those treated with SUs (OR 1.766, 95%CI 1.646, 1.896, $p<0.001$). The response rates (HbA1c <7% without tolerability issues) were modestly lower in those patients with an eGFR <60 vs. those with an eGFR >60 ml/min/1.73 m^2 in both patients receiving vildagliptin (36 vs. 38%) vs. SUs (22 vs. 26%), respectively. In patients with an eGFR <60 ml/min/1.73 m^2, weight gain appeared to be the main reason for the reduced tolerability ($p=0.002$), and this issue was greater with SU add-on therapy.

Conclusion: Diabetes management is complicated and costly in an ever increasing number of patients with RI. This exploratory analysis from EDGE suggests that, DPP-4 inhibitors may have the potential to retain and simplify glycaemic control even in a heterogeneous, 'real-life' setting, without tolerability issues such as hypoglycaemia.

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LEADER-6: variation of renal function status and risk markers across geographic origin, race and ethnicity in a high CV risk type 2 diabetic population: baseline results of the trial

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Background and aims: Cardiovascular disease (CVD) correlates with the risk of chronic kidney disease (CKD) in patients with type 2 diabetes (T2D) but it is not clear whether 'prior CVD' or having multiple risk factors for CVD increase the risk of CKD, i.e. the direction of causality is unclear. Also ethnic, racial, geographical or demographic differences of CKD have not been extensively studied. Our objective was to examine prevalent CKD and potential risk factors in a study designed to evaluate the long-term cardiovascular safety of liraglutide in a multi-racial and multi-ethnic T2D population with high CVD risk.

Materials and methods: The Liraglutide Effect and Action in Diabetes: Evaluation of cardiovascular outcome Results (LEADER) trial included 9,340 participants with T2D from 32 countries. The majority of the participants (81.3%, n=7,592) had 'prior CVD'. In this group, CKD at baseline was estimated by calculation of glomerular filtration rate (eGFR) using the MDRD formula and CKD was defined as eGFR <60 mL/min/1.73 m^2 and/or an albumin-to-creatinine ratio (ACR) >2.0 mg/mmol. We used descriptive statistics and the association of baseline characteristics with prevalent CKD was examined using multivariable logistic regression analysis.

Results: Of the LEADER participants with prior CVD, 34.5% had normal renal function (eGFR ≥90 mL/min/1.73 m^2). For the remaining participants with renal impairment, there were 38.8%, 24.4%, and 2.3% with mild (eGFR 89-60 mL/min/1.73 m^2), moderate (eGFR 59-30 mL/min/1.73 m^2), and severe (eGFR <30 mL/min/1.73 m^2), respectively. The prevalence of CKD differed between regions and was highest in participants from Asia (80%), lowest in patients from Europe or the Middle East (47.5% and 47.6%, respectively) and 60% in participants from Australia and the Americas. In multivariate logistic regression analysis, the prevalence of CKD in participants with prior CVD was associated with increasing age (OR per SD: 1.40, p<0.0001), increasing HbA1c (1.30, p<0.0001), diabetes duration (1.23, p<0.0001), systolic BP (1.34, p<0.0001), heart rate (1.10, p=0.0290) and serum triglycerides level (1.21, p<0.0001). Furthermore, number of antihypertensive medications (1.54, p<0.0001); region of living being Asia (4.78, p<0.0001), N. America (1.50, p<0.0001), Australia (1.48, p=0.0211), Africa (1.47, p=0.0176) and S. America (1.40, p=0.0004) were associated with CKD at baseline (reference region Europe). In addition, male gender (1.28, p=0.0001), current smoking (1.33, p=0.0010), being on insulin only (1.71, p=0.0002), no history of angina (1.71, p<0.0001), myocardial infarction (1.41, p<0.0001), and stroke (1.39, p<0.0001) were factors that increased the likelihood of having CKD at baseline. In contrast, diastolic BP (0.91, p=0.0121) and no diuretic treatment (0.75, p<0.0001) was associated with a reduced likelihood of prevalent CKD.

Conclusion: The prevalence of CKD is high among T2D patients with 'prior CVD'. Particularly, Asian patients are at highest risk. Age, diabetes control, comorbidities and medications used are the most important factors associated with prevalence of CVD. However, these data do not infer causality. Nonetheless, screening for CKD in T2D patients with prior CVD or high risk of CVD and priority inclusion of patients at high risk of CKD into preventive programs may be of great importance.

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Albumin creatinine ratio and cardiovascular outcomes in the SAVOR-TIMI 53 trial

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Background and aims: Amongst type 2 diabetic patients (pts) albumin creatinine ratio (ACR) is an important marker both for the risk of renal function deterioration and for cardiovascular adverse (CV) events. However, the exact threshold of ACR at which CV risk is increased is unknown.

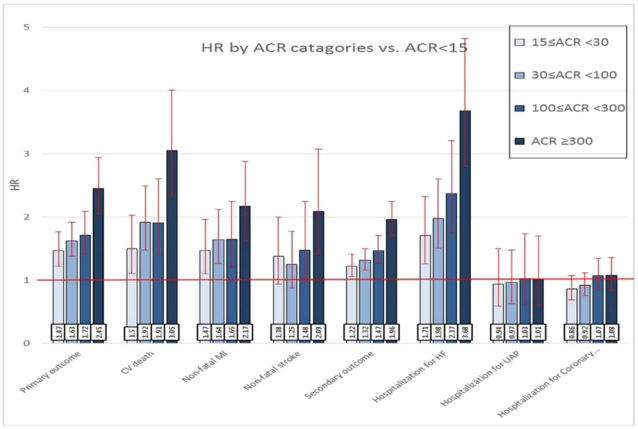
Materials and methods: We studied the CV outcomes of the 9,696 (58.8%) pts with ACR<30 mg/G, 4,426 (26.8%) pts with microalbuminuria (30≤ACR<300 mg/g) and 1,638 (9.9%) pts with macroalbuminuria (ACR≥300 mg/g) at baseline that participated in the SAVOR-TIMI 53 trial. We further subdivided ACR groups into ACR< 15 [N=7,445 (45.1%)], 15≤ACR<30 [N=2,251 (13.7%)], 30≤ACR< 100 [N=2,893 (17.5%)] and 100≤ACR<300 [N=1,533 (9.3%)] mg/g. Pts. were followed prospectively for a median of 2.1 years. Cox proportional hazard ratio for time to first CV event was calculated with stratification for baseline eGFR and CV risk group and for treatment arm, ACR category, sex, age, diabetes duration, smoking, hypertension, hyperlipidemia, BMI and HbA1c as model terms.

Results: ACR>15 mg/g was associated with increased risk for most adverse CV events (figure). In the ACR≤15 mg/g group, every increase in ACR by 1 mg/g was associated with increased HR_{adj} for the primary composite endpoint (1.06 (95% CI 1.02-1.09)) and its components: CV death (1.05 (95% CI 1.00-1.11)) and myocardial infarction (1.05 (95% CI

1.00-1.10)) ($p<0.05$). Such an association could not be demonstrated in the 15≤ACR<30 mg/G group.

Conclusion: Within the normoalbuminuric range, higher ACR is associated with increased CV risk. The risk for CV events increases rapidly in the ACR≤15 mg/dl range and continues to increase gradually thereafter.

Multivariable cox proportional hazard ratio for the association of ACR categories with time to first CV event: primary and secondary composite endpoints and their components



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Stimulation of insulin secretion by cannabinoid ligands LH-21 and Abn-CBD in mouse and human islets: evaluation of the role of GPR55

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Background and aims: The cannabinoid receptors CB1 and GPR55 belong to the highly specialised G-protein coupled receptor (GPCR) family, and we and others have reported that ligands for these receptors regulate insulin secretion from islet beta cells. This study aims to investigate the effect on insulin secretion of the CB1 neutral antagonist LH-21 and the GPR55-selective agonist Abn-CBD to gain new insights into the signaling pathways operating downstream of CB1 and GPR55 in human and mouse islets.

Materials and methods: Islets isolated from WT C57BL6/J and GPR55 KO mice and from human pancreas were perifused with a physiological salt solution supplemented with 2 mM glucose followed by 20 mM glucose in the absence or presence of LH-21 or Abn-CBD at 37°C, and insulin secretion was quantified by radioimmunoassay. For assessment of downstream signalling mouse and human islets and MIN6 beta-cells were treated with Abn-CBD and/or LH-21 (0.1-10 μM) at 20 mM glucose for 30 min, after which phosphoCREB, total CREB and β-actin expression were evaluated by electrophoresis and western blotting with specific antibodies.

Results: Abn-CBD (1 μM) had no significant effect on glucose-stimulated insulin secretion from WT mouse islets, but it unexpectedly increased insulin secretion from GPR55 KO mice islets (control: 8±1.5 pg/islet/min, Abn-CBD: 14±0.9, n=3-4, $p<0.0001$). In contrast, LH-21 at 0.1 and 1 μM increased insulin secretion from WT mouse islets, but no changes in secretion were observed with 10 μM LH-21 (control: 9±1.7 pg/islet/min; 0.1 μM LH-21: 25±5.6, $p<0.0001$; 1 μM LH-21: 15±0.2, $p<0.01$; 10 μM LH-21: 10±2.1, $p>0.2$). The stimulatory effects of 0.1 μM LH-21 on insulin secretion were abolished in islets from GPR55 KO mice (WT: 27±5.4 pg/islet/min, KO: 11.4±7.4, n=3-4, $p<0.01$). 1 μM LH-21 also potentiated glucose-induced insulin secretion from isolated human islets (188±16.7 pg/islet/min vs 124±9.3 pg/islet/min at 20 mM glucose, n=3-4, $p<0.0001$) as did 0.1 and 1 μM Abn-CBD (225±14.1 and 230±8.7 pg/islet/min, respectively, $p<0.0001$ vs 20 mM glucose alone). 10 μM Abn-CBD increased CREB phosphorylation by 20% in WT mouse islets and in MIN6 beta-cells, as revealed by densitometry analysis of the western blot signals, but it did not alter phosphoCREB expression in human islets. 0.1 μM LH-21 also promoted CREB phosphorylation in MIN6 beta-cells.

Conclusion: Our results indicate that LH-21 potentiates glucose-stimulated insulin secretion from mouse and human islets and the data obtained using islets from GPR55 KO mice imply that it exerts these stimulatory effects by activating GPR55. In contrast, the apparently selective GPR55 agonist Abn-CBD stimulated insulin secretion in a GPR55-independent manner. Both ligands were capable of inducing small increases in CREB phosphorylation, suggesting a potential role in beta-cell mass expansion. A broader knowledge of these novel mechanisms of action of LH-21 and Abn-CBD might offer new approaches to treat type 2 diabetes.

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Diet-derived short chain free fatty acids stimulate insulin secretion from mouse and human islets

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Background and aims: Short chain fatty acids (ScFA) are saturated 2-6 carbon fatty acids originating from fermentation of undigested carbohydrates and fibre by gut microbiota, which act as endogenous ligands of G-protein coupled receptors FFA2 and FFA3. Diets rich in fermentable carbohydrates improve glucose homeostasis, but it is not known if direct action of ScFA at islets via FFA2 and FFA3 contributes to this. FFA2 couples to Gi and Gq, while FFA3 is Gi-coupled.
Materials and methods: We have studied the effects of sodium acetate (SA; C2) and sodium propionate (SP; C3), the main circulating ScFAs, on insulin secretion from human (HI) and mouse islets (MI) via dynamic insulin profiling, with quantification of secreted insulin by radioimmunoassay. Changes in intracellular Ca2+ in Fura-2-loaded islet cells following ScFA treatment were measured by single cell calcium microfluorimetry.
Results: Perifused human islets (HI) responded with a significant and reversible increase in insulin secretion upon exposure to SA (0.1-1 mM) in the presence of 20 mM glucose, with 0.1 mM being as effective as 1 mM (20 mM glucose: 25.9±3.4 pg/islet/min; +0.1 mM SA: 110.7±25; +1 mM SA: 118.0±12.9, n=5, p<0.001). Insulin secretion from human islets was also potentiated by 1 mM SP, albeit to a lesser extent than with 1 mM SA (20 mM glucose: 24.8±1.9 pg/islet/min; +1 mM SP: 40.3±6.4, n=5, p<0.05). Potentiation of glucose-induced insulin secretion was also observed in response to SA and SP in perifusion experiments using mouse islets (MI), although the magnitude of response was lower than that seen in human islets (20 mM glucose: 8.0±0.7 pg/ islet/min; +1 mM SA: 11.5±1.6, n=3, p<0.01; +1 mM SP: 25.2±4.8, n=4 p<0.001). Despite the reversible potentiation of insulin secretion observed upon exposure to SA and SP in perifused human and mouse islets, neither ScFA significantly affected insulin secretion during one hour static incubation experiments of both HI and MI (HI, 20 mM glucose: 1.8±0.2 ng/islet/h; +1 mM SP: 1.9±0.2, n=8, P>0.2; MI, 20 mM glucose: 1.0±0.3 ng/islet/h; +1 mM SP: 1.0±0.1, n=8, P>0.2). Dispersed MI loaded with Fura-2 responded with reversible increases in free intracellular Ca2+ concentrations in the presence of 20 mM glucose upon exposure to SA or SP (SA basal to peak difference 0.069±0.025 n=10, p<0.05; SP basal to peak difference 0.033±0.015, n=12 p<0.05)
Conclusion: Despite reports of an inhibitory role for ScFAs on insulin secretion via FFAs, our dynamic insulin and calcium profiling experiments depict a different picture, with a stimulatory role of SA and SP, possibly acting via the Gq-coupled FFA2. These data implicate the ScFA receptor system as a potential pharmacological target in the treatment of type 2 diabetes.
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Potentiation of glucose-stimulated insulin secretion by GPR40-PLC-TRPC pathway in pancreatic beta cells

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Background and aims: G-protein coupled receptors (GPCRs) are expressed in pancreatic beta cells. G protein-coupled receptor 40 (GPR40) contributes to medium- or long-chain fatty acids-induced amplification of glucose-stimulated insulin secretion (GSIS). Thus, its agonists are promising therapeutic target in type 2 diabetes. Recently we demonstrated that GLP-1, a ligand of pancreatic GPCR, activates a class of nonselective cation channel (NSCC) and enhances GSIS. The aim of the current study is to determine whether GPR40 signal interacts to NSCCs and to identify what type of channel is related to the NSCC.
Materials and methods: Islets of Langerhans were isolated by collagenase digestion from male Wister rats. Measurement of insulin secretion was performed with static incubations. Perforated whole-cell clamped currents were recorded at the holding potential of -70 mV in the presence of 100 μM tolbutamide or at -80 mV without tolbutamide. The latter potential was used to exclude the influence of KATP-channel current on NSCC-current changes induced by GPR40 agonist.
Results: GPR40 agonist (fasiglifam 10 μM) potentiated GSIS at 16.7 mM glucose(control vs. fasiglifam 10 μM; 19±0.3 vs. 26.2±0.8 ng/mL/10 islets/hour), but not at 2.8 mM glucose (5.0±0.1 vs. 6.1±0.2). NSCC current was activated by fasiglifam at 5.6 mM glucose with 100 μM tolbutamide at the holding potentialof -70 mV(control vs. fasiglifam 10 μM; -1.8±0.3 pA/pF vs. -3.8±1.2 pA/pF, p=0.016, n=6) and the consistent results were obtained at 5.6 mM glucose without tolbutamide at -80 mV. The current increase was prevented by the presence of 2-APB (a nonspecific NSCCs blocker) or BTP2 (transient receptor potential canonical; a TRPC channel blocker). Then we examined the relationship between GPR40 stimulation and phospholipase C (PLC) downstream of the GPR40 signal. A PLC inhibitor U73122 inhibited the increase in GSIS of 16.7 mM glucose (fasiglifam vs. fasiglifam+U73122; 29.7±1.7 vs. 22.9±0.7 ng/mL/10 islets/hour, p<0.05) and the NSCC-current (fasiglifam vs. fasiglifam+U73122; -1.4±0.3 pA/pF vs. -1.4±0.9 pA/pF, p=0.99). Fasiglifam did not increase cytosoic cyclic adenosine 3′,5′-monophosphate (cAMP) concentration of islets. Protein kinase A inhibitor H89 did not affect the potentiation of GSIS by fasiglifam at 16.7 mM glucose.
Conclusion: The present study demonstrates a novel mechanism underlying potentiation of GSIS by GPR40 in pancreatic beta-cells. Stimulation of GPR40-PLC-TRPC channel pathway potentiates GSIS by depolarization of the beta-cell plasma membrane.

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Extracellular ATP affects stimulus-secretion coupling of mouse pancreatic beta cells by activating Ca^{2+}-dependent K^+ channels

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Background and aims: ATP acts as an autocrine signal in beta-cells which affects stimulus-secretion coupling. The signalling cascade downstream the activation of purinergic receptors that modulates electrical activity and the cytosolic Ca^{2+} concentration ($[Ca^{2+}]_c$) as well as the consequences for insulin secretion are still a matter of debate.
Materials and methods: Membrane potential (V_m) and Ca^{2+} currents (I_{Ca}) were measured using the perforated patch-clamp technique; $[Ca^{2+}]_c$ was determined by fluorescence technique. Experiments were carried out with clusters of beta-cells from C57Bl/6N wild type and BK knockout mice.
Results: In 10 mM glucose ATP (100 μM) led to a hyperpolarization of V_m (Δ-24±4 mV, n=7, p≤0.05) which was reduced by blocking SK_4 channels or knock-out of BK channels (Δ-12±1 mV, n=5 and Δ-10±1 mV, n=9, respectively, p≤0.05). ATP caused a transient peak in $[Ca^{2+}]_c$ after blocking L-type Ca^{2+} channels with D600 (100 μM) (Δ24±2 nM, n=31, p≤0.05). This transient Ca^{2+} release was inhibited by either the $P2_{x/y}$ antagonist suramin (200 μM) or emptying Ca^{2+} stores of the endoplasmic reticulum with thapsigargin (1 μM). Surprisingly, ATP (100 μM) only transiently decreased glucose-induced (10 mM) Ca^{2+} oscillations for (235±32 sec, n=24, p≤0.05) to basal values. This effect was not altered by preincubation with thapsigargin (1 μM). The time interval of the transient decrease was reduced by blocking SK_4 channels or knock-out of BK channels (149±21 sec, n=17, 128±

21 sec, n=37, respectively, p≤0.05). Moreover, ATP induced a decline in the maximum amplitude of the Ca^{2+} oscillations ($[Ca^{2+}]_{cMax}$) (Δ-285± 47 nM, n=24, p≤0.05). Both ATP effects on $[Ca^{2+}]_c$ described above were blocked by suramin (400 μM) and the effect on $[Ca^{2+}]_{cMax}$ was mimicked by the P_{2X} agonist α,β-Methylene ATP (100 μM) (Δ-340± 34 nM, n=16, p≤0.05). The reduction in $[Ca^{2+}]_c$) is probably owing to an ATP-induced decrease in voltage-dependent Ca^{2+} currents (I_{Ca}) (Δ-13±4 pA, n=9, p<0.05) which were elicited by voltage steps from -70 mV to 0 mV.

Conclusion: ATP appears to act as a negative autocrine signal in beta-cells and seems to exert this effect through ionotropic purinergic P_{2X} receptors. Obviously, this leads to activation of SK_4 and BK channels as well as a direct inhibition of I_{Ca} through L-Typ Ca^{2+} channels culminating in changes of beta-cell electrical activity and $[Ca^{2+}]_c$.

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The calcium sensor sorcin lowers G6PC2 expression to protect against obesity-induced beta cell failure

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Background and aims: Pancreatic beta cell dysfunction is central to the pathogenesis of Type 2 diabetes. We have recently reported that the calcium-sensor sorcin (SRI) prevents glucose-induced nuclear shuttling of the transcription factor ChREBP (Carbohydrate-responsive element-binding protein). Moreover, SRI overexpression in pancreatic beta cells in transgenic mice preserves whole body glucose homeostasis during high fat feeding, and alleviates endoplasmic reticulum (ER) stress. Here, we demonstrate a role in SRI signalling for *G6pc2 (Glucose-6-phosphatase 2)*, a regulator of fasting blood glucose in human.

Materials and methods: Transgenic male mice bearing one (SRI-1) or ten (SRI-10) copies of SRI cDNA, resulting in SRI overexpression specifically in beta cells, and their littermate controls (CTRL), were maintained on a high-fat diet (60%; HFD). Glucose-stimulated insulin secretion (GSIS) was assessed *in vivo* and *ex vivo*. Real-time intracellular calcium imaging was measured using Fura-2 for cytosolic calcium ($[Ca^{2+}]_{cyt}$) and D4ER for ER calcium ($[Ca^{2+}]_{ER}$), and changes in gene expression was assessed by quantitative RT-PCR on isolated islets.

Results: SRI-10 mice displayed enhanced GSIS *in vivo* (30 min plasma insulin (mmol/L): 0.60±0.06 for SRI-10, 0.43±0.04 for CTRL, n=5-7 mice, p<0.05) and *ex vivo* (% secreted insulin, high glucose condition: 0.57±0.06 for SRI-10, 0.37±0.02 for CTRL, n=3 mice, p<0.01). Concomitantly, SRI-1 and SRI-10 islets displayed higher $[Ca^{2+}]_{cyt}$ in response to high glucose than control islets (AUC (a.u.): 499.1±7.3 for SRI-1, 471.4±7.8 for CTRL, n=3 mice, p<0.05; 165.6±7.1 for SRI-10, 144.3 ±5.4 for CTRL, n=3 mice, p<0.05). Moreover, as assessed using acetylcholine to fully deplete the ER calcium stores, SRI-10 islets had a greater $[Ca^{2+}]_{ER}$ compared to controls (fall (%) : -6.1±0.5 for SRI-10, -4.2±0.3 for CTRL, n=3-4 mice, p<0.01). Furthermore, islets from both SRI-1 and SRI-10 mice showed lowered expression of *G6pc2* with respect to those from littermate controls (fold-change normalised to beta-actin: 0.6±0.06 for SRI-1, n=3 mice, p<0.05; 0.4±0.05 for SRI-10, n=5 mice, p<0.01). G6PC2 is a negative regulator of GSIS whose inactivation in mice leads to increased $[Ca^{2+}]_{cyt}$ and decreased fasting blood glucose. Similar changes in fasting blood glucose were observed in our SRI-10 mice on a HFD (0 min glycaemia (mmol/L): 8.7±0.4 for SRI-10, 10.9±0.8 for CTRL, n=9-11 mice, 8-week-old, p<0.05).

Conclusion: SRI appears to preserve beta cell function during high fat feeding at least in part by lowering *G6pc2* expression. This is likely to increase glucose 6-phosphate levels, elevate ER calcium, lower ER stress, and potentiate GSIS. Such changes may avert or delay the decline in insulin secretion which underlies progression towards Type 2 diabetes.

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CAMTA1 - Calcium-Calmodulin Transcriptional Activator 1, a new player in the regulation of microRNAs and insulin secretion

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Background and aims: CAMTAs are calmodulin-binding transcriptional activators highly conserved across a wide range of multicellular eukaryotes, involved in cell differentiation in response to environmental stresses. Mammalian CAMTA1 and CAMTA2 came to our attention as putative transcription factors involved in miR-212/132 expression, a cluster which responds to glucose and cyclic AMP in rat insulin secreting β-cells and is upregulated in islets of the diabetic Goto-Kakizaki (GK) rat model. Our aim was to examine the role of CAMTAs in insulin secretion and the regulation of miR-212/132 expression in islets and the INS-1 832/13 insulin secreting beta-cells.

Materials and methods: Human and rat islets and INS-1 832/13 cells were conditioned for hours or days at various glucose concentrations, cyclic AMP stimulation and/or alpha-2 adrenergic stimulation. Insulin secretion was determined by radioimmunoassay. Gene knockdowns were performed with siRNA. Gene expression was determined by qPCR. Luciferase assay was performed using a construct containing the promoter of miR-212/132.

Results: In GK rat islets incubated for 24 h at 16.7 mM glucose or 6 days at 5 mM glucose CAMTA1 and CAMTA2 expression was markedly reduced compared to Wistar control: 24 h at 16.7 mM glucose (n=4), CAMTA1 (p=6.7E-4), CAMTA2 (p=6.9E-3); six days at 5 mM glucose (n=4), CAMTA1 (p=5.4E-4), CAMTA2 (p=0.028). In human islets CAMTA1 and CAMTA2 are both upregulated after 72 h at 16.7 mM glucose compared to 5 mM glucose (n=7): CAMTA1 (p=4.0E-4); CAMTA2 (p=0.023). Knockdown of CAMTA1 reduced insulin secretion (p=9.0E-3, n=3) in INS-1 832/13 cells and upregulated miR-132 (p=0.032, N=3). Luciferase expression from miR-212/132 promoter was upregulated after knockdown of CAMTA1 (p=7.0E-4, n=3). In INS-1 832/13 cells expression of CAMTA1 was reduced after 2 h in Forskolin/IBMX (p=9.8E-3, n=3) and after 48 h of 50 nM clonidine (p=4.1E-3, n=3).

Conclusion: This data show that CAMTA1 expression is modulated not only by glucose and cyclic AMP in beta-cells but also by alpha-2 adrenergic stimulation and is involved in the regulation of insulin secretion and expression from the miR-212/132 promoter. We believe that CAMTA1 is therefore a new player involved in the pathways that lead to reduced insulin secretion in the diabetic GK model.

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OP 30 The genetic landscape of diabetes in high resolution

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Elucidating the role of protein-coding variants in type 2 diabetes susceptibility

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Background and aims: Changes in protein-coding sequence have been hypothesised to be particularly enriched for variants of large-effect on complex human diseases. Such variants are more likely to have functional impact and support more direct biological interpretation than non-coding alleles. To evaluate the contribution of coding variants to type 2 diabetes (T2D) risk, we combined exome array data from 208,465 individuals (50,760 cases and 157,705 controls) in 39 studies from five ancestry groups: European (78.1%), South Asian (11.7%), African American (5.6%), Hispanic (3.0%), and East Asian (1.5%).
Materials and methods: Within each study, we tested single variants for association with T2D, with/without body-mass index (BMI) adjustment, using a linear mixed model to account for relatedness and population structure. We combined association summary statistics across studies in a fixed-effects z-score effective sample size weighted meta-analysis. In established genome-wide association study (GWAS) loci, we investigated the relationship between coding variants and previously reported lead SNPs (where available on the exome array) through approximate conditional analyses implemented in RareMetal.
Results: A total of 36 coding variants, mapping to 22 loci, were associated with T2D at exome-wide significance ($P < 5\times10\text{-}7$) in European only or trans-ethnic meta-analysis. All but three were common, with minor allele frequency (MAF) >5% in the ancestry driving the association. Eleven variants were located outside established T2D GWAS loci. These included common variants in ZZEF1 (L1972P, $P=4.6\times10\text{-}9$ and I2014V, $P=1.3\times10\text{-}8$), POC5 (H36R, $P=3.4\times10\text{-}7$), PNPLA3 (I148M, $P=5.7\times10\text{-}8$), and a low-frequency variant (MAF=1%) in FAM63A (Y285N, $P=6.1\times10\text{-}8$). Of these, POC5 maps to a known BMI GWAS locus and the PNPLA3 variant has been implicated in fatty liver disease. The other 25 variants mapped to established T2D susceptibility loci. These included nine variants in SLC30A8, MACF1, GCKR, PPARG, KCNJ11-ABCC8, and PAM-PPIP5K2, which were confirmatory of previously-reported coding alleles that drive GWAS association signals. However, the 16 remaining variants have not previously been directly implicated in T2D susceptibility, and not reported to drive the non-coding association signal. Amongst novel variants that were distinct from the GWAS signal was GIPR E354Q, for which T2D association observed only after BMI adjustment ($P=2.4\times10\text{-}8$), but which was not eliminated after conditioning on the previously reported inter-genic lead SNP ($Pcond=3.6\times10\text{-}6$). Conversely, at the CILP2 locus, a coding variant in TM6SF2 (E167K; $P=1.0\times10\text{-}10$) was indistinguishable from the previously reported lead SNP ($Pcond=0.11$), suggesting that the GWAS signal is mediated through this gene.
Conclusion: Our results indicate that low-frequency and rare coding variants of large effect do not make a major contribution to T2D risk. However, these analyses implicate several novel genes in T2D pathogenesis, and provide direct insight into the underlying biology of the disease.

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A meta-analysis of Japanese genome-wide association studies identified seven novel susceptibility loci to type 2 diabetes

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Background and aims: Genome-wide association studies (GWAS) have been extensively performed in diverse ethnic groups, and have identified over 80 loci for susceptibility to type 2 diabetes; ~50 loci from European GWAS and ~30 loci from non-European or trans-ethnic GWAS meta-analysis. Although many loci have been shown to be common loci for conferring susceptibility to type 2 diabetes across different ethnic groups, the joint effects of these variants could explain only less than 10% of the heritability for type 2 diabetes. To identify novel genetic loci associated with susceptibility to type 2 diabetes further, we expanded Japanese GWAS by combining a new Japanese GWAS data (Stage-1; 9,817 cases and 6,763 controls, BioBank Japan) with a GWAS data in previously reported case-control samples (Stage-2; 5,646 cases and 19,420 controls, BioBank Japan).
Materials and methods: We analyzed ~7.5 million single nucleotide polymorphisms (SNPs) data obtained from genotype imputation performed by mini-mac using directly genotyped data (Study-1; Omni-express exome, Study-2; Illumina 610 K SNP array) and reference data in the 1000 genomes (JPT+CHB+CHS, n=275), and combined the results of the 2 GWAS with a meta-analysis using the inverse variance method. As a result, we have identified 17 novel loci showing suggestive evidences for the association with type 2 diabetes ($p<1\times10^{-6}$) in Japanese populations. These loci were further evaluated in an independent Japanese case-control study (Stage-3; 7,936 cases and 6,478 controls, multi-center) using *de novo* genotyping data obtained by the multiplex PCR-invader assay.
Results: After combining all the association data (Stage-1, Stage-2 and Stage-3) for the 17 loci by a meta-analysis, the association of 7 loci reached to a genome-wide significant level ($p<5\times10^{-8}$); rs1116357 on chromosome (Ch) 2: $p=9.20\times10^{-11}$, OR (95%CI)=1.10 (1.07-1.13), rs147538848 on Ch12: $p=2.96\times10^{-10}$, OR (95%CI)=1.11 (1.08-1.15), rs1575972 on Ch9: $p=1.14\times10^{-9}$, OR (95%CI)=1.19 (1.13-1.26), rs67839313 on Ch15: $p=2.05\times10^{-9}$, OR (95%CI)=1.09 (1.06-1.13), rs9309245 on Ch2: $p=4.21\times10^{-9}$, OR (95%CI) =1.11 (1.07-1.14), rs7107784 on Ch11: $p=5.97\times10^{-9}$, OR (95%CI)=1.15 (1.10-1.20), rs67156297 on Ch1: $p=4.30\times10^{-8}$, OR (95%CI)=1.13 (1.08-1.18). The effect sizes (odds ratio) of these 7 SNPs were similar before and after adjusting for age, sex, and BMI.
Conclusion: By performing the largest Japanese GWAS meta-analysis, we have identified 7 new loci associated with susceptibility to type 2 diabetes in the Japanese population.
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High-density imputation and trans-ethnic association analysis reveals a novel locus for type 2 diabetes susceptibility

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Background and aims: The Resource for Genetic Epidemiology Research on Adult Health and Aging (GERA) is a large multi-ethnic

population-based cohort, created for investigating the genetic and environmental basis of common age-related diseases, including type 2 diabetes (T2D). Participants were genotyped using one of four custom arrays, designed to maximise coverage of common and low-frequency variants in non-Hispanic white, East Asian, African-American and Latino ethnicities. With these data, this study aimed to discover loci for T2D susceptibility through high-density imputation and trans-ethnic association analysis and to evaluate the evidence for heterogeneity in allelic effects on the disease between ancestry groups.

Materials and methods: After quality control, 71,604 unrelated participants, including 9,747 T2D cases, were retained for analysis and imputed up to the multi-ethnic reference panel (Phase 3, October 2014 release) from the 1000 Genomes Project Consortium. High-quality imputed variants were tested for association with T2D in a logistic regression model, after adjustment for sex and eigenvectors from principal components analysis to account for trans-ethnic and ancestry-specific population structure.

Results: Common lead SNPs at ten loci attained genome-wide significant evidence ($p<5\times10^{-8}$) of association with T2D, including a novel signal mapping to *TOMM40* ($p=2.8\text{x}10^{-9}$). This gene has been previously implicated in Wolfram Syndrome, a neurodegenerative disorder characterised by diabetes mellitus. Across lead SNPs for the ten loci, nominal evidence of heterogeneity in allelic effects between ethnicities was observed only at *TCF7L2* ($p_{HET}=0.012$), where the risk allele had the lowest T2D odds-ratio for participants of East Asian ancestry.

Conclusion: This study provides further evidence for the homogeneity of allelic effects of common T2D risk variants across diverse populations, and highlights the benefits of trans-ethnic analysis for the discovery of novel loci associated with the disease.

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Genome-wide association study imputed to 1000 Genomes Project reference panels reveals 17 novel associations with type 2 diabetes

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Background and aims: Genome-wide association studies (GWAS) using imputation based on the HapMap 2 reference panel (analysis of 2.5 M single nucleotide variants (SNVs)), have identified more than 70 loci associated with type 2 diabetes (T2D). These loci are exclusively common, with minor allele frequency (MAF)>5%. The aim of current work was to detect additional common (MAF≥5%) and low-frequency (0.5%≤MAF<5%) T2D associated loci using the denser 1000 Genomes (1000G) Project imputation reference panel.

Materials and methods: We performed imputation using the March 2012 release of the 1000G "all ancestries" reference panel in 26,676 T2D cases and 132,532 controls from 18 European GWAS. The 1000G panel provides more comprehensive coverage than HapMap for imputation of both common and low frequency variants. Amongst more than 12 million SNVs in meta-analysis of which 2.9 M were low frequency, we followed up those achieving $p<5\times10^{-5}$ in an additional 14,545 cases and 38,994 controls of European ancestry genotyped with Metabochip.

Results: In combined meta-analysis, we identified 17 novel loci achieving genome-wide significant ($p<5\times10^{-8}$) associations with T2D. All lead SNVs were common with MAF>10%, including loci mapping to/near *PLEKHA1* ($p=1.8\times10^{-12}$), *APOE* ($p=1.5\times10^{-10}$), and *CENPW* ($p=5.9\times10^{-10}$). On the basis of the meta-analysis, we defined 99% credible sets of variants that were most likely to drive the association signal in established and novel loci. The 99% credible set contained fewer than 20 SNVs at 17 loci. Within these 17 credible sets, we were able to find coding variants for three genes (*HNF1A, CTRB2, ADCY5*), which were not present in HapMap2.

Conclusion: Our results highlight the potential for the identification of novel T2D loci through imputation up to 1000G reference panels, and highlight functional mechanisms driving association signals at three loci through coding variants that were not present in HapMap2.

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Genome-wide association studies on kidney complications in patients with type 1 diabetes

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Background and aims: Diabetic nephropathy is a common and serious complication of type 1 diabetes (T1D), but few genetic risk factors have been identified. Our aim was to detect genetic risk factors by performing genome-wide association studies (GWAS) on a variety of renal phenotypes in four European studies with 5,156 T1D patients from the SUMMIT Consortium.

Materials and methods: We employed seven different case-control definitions based on the severity of diabetic nephropathy (DN; micro-/macro-albuminuria and/or end stage renal disease (ESRD)), chronic kidney disease (CKD; estimated glomerular filtration rate (eGFR) <60 ml/min), or a combination. The proportion of phenotypic variance explained by the GWAS SNPs, the narrow-sense heritability, was estimated in 3,415 Finnish patients (GCTA). Genotype imputation with the 1000 Genomes (March 2012) resulted in 8,578,867 variants (minor allele frequency ≥0.01) found in at least two studies. Association analysis with an additive model was adjusted for sex, diabetes duration, age at diabetes onset, and kinship matrix (EMMAX). Meta-analyses were performed using a sample size weighted method (METAL). Associations with $p<5\times10^{-6}$ were *in silico* replicated in six additional GWAS with 7,041 T1D patients and data on renal complications.

Results: Heritability of DN was estimated to be 35% ($p=0.006$), or 50% after adjusting for sex, duration of T1D, and age at T1D onset ($p=2.5\times10^{-4}$). Strongest heritability was estimated for the combined CKD and DN vs. no CKD and normal AER phenotype (59%, $p=0.0011$). A total of 101 loci reached $p<5\times10^{-6}$ in GWAS meta-analyses. After joint meta-analysis with the *in silico* studies, no locus reached genome-wide significance, but three reached a suggestive $p<10^{-6}$: on chromosome (chr) 7q36.1 near *CNTNAP2* for the combination of DN and CKD ($p=6\times10^{-7}$); on chr4q21.3 near *PTPN13* for ESRD ($p=6\times10^{-8}$); and on chr2q11.2 in *AFF3* for ESRD ($p=3\times10^{-7}$). In a look-up from the SUMMIT meta-analysis of 5,717 patients with type 2 diabetes (T2D), only the *AFF3* signal showed any corroboration for kidney phenotypes (CKD+DN: T2D $p=0.002$, T1D+T2D $p=2\times10^{-7}$). These three SNPs, and a SNP near *NRG3* with $p<0.05$ for DN in replication, were further genotyped in up to 1,800 T1D patients. However, the associations were not significant and did not improve the overall p-values.

Conclusion: Although no locus was robustly associated with renal complications in T1D, these analyses support the role of *AFF3*, and suggest a notable genetic contribution, especially for the more extreme phenotypes.
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Synergistic effect of genetic susceptibility and poor glycaemic control on renal decline in patients with type 1 diabetes and proteinuria

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Background and aims: The key phenotype of diabetic nephropathy, the renal decline, is a progressive loss of glomerular filtration rate that eventually leads to end-stage renal disease (ESRD). Its genetic background is not well understood. Glycemic control may be an important modifier of genetic susceptibility to renal decline.
Materials and methods: As a part of search for genes determining time to onset of ESRD in type 1 diabetes (T1D) patients with proteinuria we sought for common genetic variants associated with renal decline, whose effect on the slope of renal decline is modified by glycemic control. We examined 544 patients with T1D and proteinuria, followed for up to 24 years. Serial serum creatinine determinations were obtained during the follow-up. We used a joint longitudinal-survival model, which accounts for informative censoring in those who rapidly progress to ESRD. We tested for an interaction with HbA_{1c} in strata below and above values of 8.0% or 63.9 mmol/mol (1st quartile), 9.1% or 76.0 mmol/mol (median) and 10.5% or 91.3 mmol/mol (3rd quartile). The patients were genotyped with the Illumina HumanCoreExome Beadarray 1.0.
Results: We identified two variants associated with renal decline with genome-wide significant effect modification by HbA_{1c}. A minor allele of rs10997250 polymorphism (minor allele frequency 7.2%) in patients with fair glycaemic control (HbA_{1c}<10.5%) was not associated with a significant change in the slope of renal decline (1.0 ml/min/1.73 m^2/year less decline per one additional minor allele, p=0.24), while in patients with poor glycaemic control (HbA_{1c}≥10.5%) an additional minor allele was associated with a 9.1 ml/min/1.73 m^2/year steeper renal decline than in those with fair glycaemic control ($p=2.2\times10^{-8}$ for the interaction). Similarly, a minor allele of rs12877579 (minor allele frequency 14.8%) in patients with fair glycaemic control was associated with 0.8 ml/min/1.73 m^2/year less decline (p=0.20), but in patients with poor glycaemic control an additional minor allele resulted in a 6.9 ml/min/1.73 m^2/year steeper renal decline than in the patients with fair glycaemic control ($p=4.2\times10^{-8}$ for the interaction). This gene-environment interaction can be also presented as estimated mean slopes for each genotype and HbA_{1c} stratum. For the variant rs10997250 the mean slopes of renal decline in patients with fair glycemic control in major homozygotes, heterozygotes and minor homozygotes were -5.7, -4.5 and -7.5 ml/min/1.73 m^2/year, respectively. In those with poor glycemic control they were -8.9, -17.2 and -23.8 ml/min/1.73 m^2/year. The respective means for the rs12877579 variant were -5.8, -4.7, -5.7 and -8.3, -15.0, -17.7 ml/min/1.73 m^2/year. No significant interactions were found for HbA_{1c} strata formed by thresholds 8.0% and 9.1%.
Conclusion: The minor alleles of variants rs10997250 on chromosome 10 and rs12877579 on chromosome 13 have strong deleterious effect on renal decline only in patients with poor glycaemic control. Genetic susceptibility and poor glycemic control seem to have synergistic effect on the risk of renal decline. This phenomenon should be accounted for in studies of genetics of diabetic nephropathy.
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OP 31 Understanding the effects of SGLT inhibitors

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Empagliflozin reduces HbA_{1c} with lower insulin doses in patients with type 1 diabetes: a 4-week placebo-controlled trial (EASE-1)

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Background and aims: In the EASE-1 study, we investigated the pharmacodynamics, efficacy and safety of empagliflozin (EMPA) as adjunct to insulin in patients with type 1 diabetes.
Materials and methods: In this Phase II, double-blind trial, patients with type 1 diabetes were randomized to placebo (PBO; n=19), EMPA 2.5 mg (n=19), EMPA 10 mg (n=19) or EMPA 25 mg (n=18) as adjunct to insulin (multiple daily injections) for 28 days. Insulin dose was to be kept as stable as possible for 7 days and was freely adjustable thereafter. The primary exploratory endpoint was change from baseline in 24-h urinary glucose excretion (UGE) at day 7.
Results: EMPA significantly increased 24-h UGE vs PBO at days 7 and 28. At day 28, EMPA significantly reduced HbA1c vs PBO, with reductions in fasting plasma glucose, and a significant reduction in recorded total daily insulin use. EMPA significantly reduced weight vs PBO at day 28. Adverse events (AEs) were reported in 94.7%, 89.5%, 78.9% and 100.0% of patients on PBO, EMPA 2.5 mg, 10 mg and 25 mg, respectively. AEs consistent with urinary tract infection were reported in one patient (on EMPA 25 mg). No AEs consistent with genital infection were reported. In PBO, EMPA 2.5 mg, 10 mg and 25 mg groups, respectively, 19, 8, 10, and 13 symptomatic hypoglycemic episodes with glucose ≤54 mg/dL not requiring assistance were reported. One hypoglycemic episode requiring assistance was reported (on PBO).
Conclusion: In patients with type 1 diabetes, EMPA for 28 days as adjunct to insulin increased UGE, improved HbA1c and reduced weight with lower insulin doses vs PBO and was well tolerated. EMPA is not yet approved for use in patients with type 1 diabetes.

		PBO (n=19)	EMPA 2.5 mg (n=19)	EMPA 10 mg (n=19)	EMPA 25 mg (n=18)
24-hour UGE (g/24 h)	Baseline, mean (SE)	20.3 (4.0)	21.4 (4.7)	14.0 (3.7)	13.4 (2.6)
	Change from baseline at day 7†	-3.3 (6.2)	72.8 (6.3)***	103.1 (6.2)***	101.5 (6.4)***
	Change from baseline at day 28†	0.2 (8.0)	79.1 (8.1)***	114.9 (8.1)***	109.4 (8.3)***
HbA1c (%)	Baseline, mean (SE)	8.18 (0.15)	8.35 (0.17)	8.28 (0.18)	8.15 (0.13)
	Change from baseline at day 28†	-0.18 (0.09)	-0.53 (0.09)**	-0.54 (0.09)**	-0.67 (0.10)***
Total recorded insulin dose, (U/kg)	Baseline (weekly mean), mean (SE)	0.66 (0.06)	0.65 (0.04)	0.71 (0.04)	0.65 (0.05)
	Change from baseline within the fourth week of treatment†	-0.01 (0.02)	-0.08 (0.02)*	-0.10 (0.02)*	-0.09 (0.02)*
Weight (kg)	Baseline, mean (SE)	79.8 (3.2)	75.9 (3.3)	87.1 (3.1)	76.9 (3.4)
	Change from baseline at day 28†	0.2 (0.3)	-1.4 (0.3)***	-1.6 (0.3)***	-1.7 (0.3)***

All analyses were exploratory. †Adjusted mean (SE) based on ANCOVA in patients who received ≥1 dose of study medication and had a 24-h UGE value at baseline and at day 1 or day 7, with last observation carried forward imputation.
*p<0.05, **p<0.01, ***p<0.001 for difference versus placebo.

Clinical Trial Registration Number: NCT01969747
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Sotagliflozin, a dual SGLT1 and SGLT2 inhibitor, improves glycaemic control in type 1 diabetes mellitus in a randomised, placebo-controlled, double-blind study

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Background and aims: Dual inhibition of both SGLT1 and SGLT2 may provide additional benefit above that achieved with SGLT2 inhibition alone when used as adjunctive therapy to insulin in T1DM. Sotagliflozin (LX4211) is a dual inhibitor of sodium-glucose cotransporters SGLT1 and SGLT2. In T1D we hypothesized that inhibition of SGLT1, the major intestinal glucose transporter, would reduce glucose absorption in the gastrointestinal tract, with an increase in incretins. SGLT2 inhibition reduces renal glucose reabsorption.
Materials and methods: This 29-day study evaluated 33 subjects with T1D (ages 21-57 years, duration of diabetes 3-42 years) with screening A1C of 7.0%-9.0%, treated with insulin pump or multiple daily injection therapy who were randomized to double-blind treatment with sotagliflozin 400 mg or placebo once daily.
Results: All participants completed the study. Raw means are displayed; p-values for change from baseline and between group effects are based on least squares means, unless otherwise noted.
Conclusion: Sotagliflozin a next generation SGLT inhibitor, significantly reduced A1C levels, daily bolus and total daily insulin dose, postprandial blood glucose, and body weight, and increased PYY in T1D with no increase in hypoglycemia risk. Larger studies of a longer duration are needed to confirm these findings.

Results	Placebo N = 17	Sotagliflozin N = 16	p-value Sotagliflozin vs. Placebo
Efficacy			
A1C change from baseline (%)	-0.06	-0.55 *	**0.002**
Fasting plasma glucose change from baseline assessed at Day 29 (mg/dL)	+39.0	-18.6	0.15
Daily bolus insulin change from baseline assessed at Days 3-27 (%)	-6.4	-32.0 *	**0.007**
Daily basal insulin change from baseline assessed at Days 3-27 (%)	+0.2	-2.4	0.53
Total daily insulin change from baseline assessed at Days 3-27 (%)	-0.7	-15.3 *	**0.029**
Mean body weight change from baseline assessed at Day 29 (kg)	+0.5	-1.7 *	**0.005**
Post-meal urinary glucose (g/3 hr) at Day 29 †	9.2	29.1	**0.025**
Post-meal plasma glucose AUC (mg•hr/dL over 3 hr) at Day 29 †	761	595	**0.005**
PYY Post-meal AUC change from baseline assessed at Day 29 (pmol/L•hr over 3 hr)	-0.7	+6.0 *	**0.018**
Seated systolic blood pressure change from baseline assessed at Day 29 (mm Hg)	-3.9	-4.9	0.45
Safety			
Patients with any treatment-emergent AE (%)	12 (71)	14 (88)	N/A
Patients with SAE (both with DKA‡)	0	2	N/A
Hypoglycemic events (SMBG ≤70 mg/dL, baseline-Day 36)	354	304	N/A
Documented symptomatic hypoglycemia (SMBG ≤70 mg/dL, baseline- Day 36)	185	162	N/A
Asymptomatic hypoglycemia (SMBG ≤70 mg/dL, baseline-Day 36)	117	80	N/A
Severe hypoglycemia	0	0	N/A
Hypoglycemia (SMBG ≤70 mg/dL, PPD) change from baseline at Days 3-27	-0.4 *	-0.7 *	0.77
Hypoglycemia (CGM ≥10 continuous min <70 mg/dL, PPD) change from baseline assessed at Days 3-27	-0.15	-0.09	0.75
Laboratory values associated with volume status:			
Serum sodium (mmol/L),change from baseline at Day 29 (36)	-1.00 (-0.53)	-0.50 (1.50)	N/A
Serum creatinine (μmol/L), change from baseline at Day 29 (36)	-0.53 (+1.53)	+2.63 (+0.63)	N/A
Serum BUN (mmol/L), change from baseline at Day 29 (36)	+0.41 (+0.11)	+1.02 (-0.41)	N/A
Hematocrit, change from baseline at Day 29 (36)	-1.4 (0)	+2.1 (+1.5)	N/A

For laboratory values, change from baseline was assessed at Day 29 the last day of therapy and (Day 36, 1 week off therapy), unless otherwise specified
DKA = diabetic ketoacidosis, N/A = Not Applicable, PPD = events per patient per day
*p<0.05 Change from baseline; † Day 1 is not a true "baseline", therefore p-values are calculated from 2-sample t-tests using the observed means; ‡ both were assessed as due to insulin pump and deemed not drug related.

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Dapagliflozin modulates SGLT1 and GLUT2 expression and glucagon secretion in a SGLT2-independent manner in murine alpha cells

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Background and aims: SGLT2 inhibitors reduce plasma glucose levels via a forced glycosuria through inhibition of glucose uptake by the kidney, with a fully insulin-independent mechanism. However, recent studies show increased glucagon concentrations after acute or chronic administration of SGLT2 inhibitors. Whether or not such effect is consequence of a series of systemic metabolic variations rather than due to a direct effect on pancreatic α-cells is unclear.
Materials and methods: We tested this hypothesis by treating for different times (30, 45, 60 min and 12 h) murine pancreatic α-cell line (alpha-TC1) with 100 ng/ml dapagliflozin (Dapa), and measuring its effect on the expression of glucose transporters, molecular mediators of hormone secretion and glucagon and GLP-1 release.
Results: SGLT2 was not detectable in these cells even by digital PCR technique. SGLT1, was quite abundantly represented and was significantly increased by Dapa at 30' and 45'(+65±21% and + 16±3% vs untreated, $p<0.001$ and 0.05, respectively). GLUT1 expression was substantially unaffected by Dapa (-5% at 30'and -7% at 45'), while GLUT2 was down-regulated (-24±60% at 30' and -19±23% at 45', both $p<0.005$). At 12 h, the expression of both glucose transporters returned to baseline levels. Similarly, Dapa did not induce variations in the expression of several molecules involved in the modulation of glucagon release or α-cell phenotype (fold induction at Dapa 30' vs untreated=CHGA: 1.10± 0.17; PAX6 1.13±0.14; PCSK2: 1.20±0.18; PCSK1: 0.99±0.18; SYP: 1.14±0.23, all p=ns). Moreover, GLP-1 receptor expression did not differ in α-cells treated or untreated with Dapa. Accordingly, glucagon release minimally changed in cells pretreated with Dapa (at 30': 822±76 vs 828± 85; at 45': 1198±127 vs 1515±112 pg/ml/μg protein, both p=ns). Of note, at 12 h, glucagon release was significantly lower in Dapa-treated cells (3524±254 vs 2446±157 pg/ml/μg protein, $p<0.005$). GLP-1 did not significantly change after Dapa (at 30': 610±76 vs 640±52; at 45': 842±96 vs 1000±118pmol/ml/μg protein, both p=ns). At 12 h, GLP-1 was 3533±216 in untreated cells and 3604±270 pmol/ml/μg in Dapa-treated cells (p=ns). Low (5.5 mM) or high (25 mM) glucose pulses did not affect SGLT1, GLUT1 or GLUT2 expression, while glucagon (2210 ±125vs 1725±117 pg/ml/μg protein, $p<0.05$) and GLP-1 release (2671± 139 vs 2090±154 pmol/ml/μg protein, $p<0.01$) secretion were slightly but significantly reduced by high glucose.
Conclusion: Dapa acutely upregulates SGLT1 and downregulates GLUT2 expression in pancreatic α-cells, while SGLT2 was not detectable in these cells. In addition, glucagon release was significantly reduced after long-term (12 h) treatment with Dapa. These data suggest that the glucagon raise reported in type 2 diabetes patients treated with Dapa does not reflect the direct action of this molecule on pancreatic α-cells.
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Dapagliflozin potentiates glucagon secretion at high glucose: experimental findings and mathematical simulation

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Background and aims: Inhibiting glucose re-absorption in the kidney by blocking the Na+/glc co-transporters SGLT has been proven to be a safe and effective treatment for diabetes. However, recently it was discovered that SGLT inhibition raises hepatic glucose output and plasma glucagon

levels. It is plausible that the former is caused by the latter and we thus attempted to investigate the direct effect of the SGLT2 blocker Dapagliflozin on glucagon secretion from pancreatic islets.α-cells respond to low glucose levels with increased glucagon secretion which is directly regulated by the electrical activity of the cell. The current generated by the electrogenic SGLT-transporters is thus expected to affect glucagon secretion by changes of the membrane potential. We constructed a mathematical model of α-cell electrical activity based on data from human α-cells which is able to explain our experimental findings.

Materials and methods: Mice islets were prepared by pancreas collagenase digestion and for human islets assay ready 3D InSight™ plates were used. Islets were kept for 1 h under the indicated conditions and glucagon levels determined using ELISA-kits from Merocodia. For each preparation secretion was measured in 6-8 wells and median secretion calculated. Values were normalized to secretion at 1 mM glc and data are presented as average from indicated number of preperations. A Hodgkin-Huxley type model of electrical activity was constructed including KATP, Nav, T-, L- and P/Q-type Cav, A-type and delay-rectifier Kv-channels based on published human A-cell electrophysiological data. A previously published 6-state model of SGLT Na+/glc co-transport was included to analyze the effect of SGLT currents on α-cell electrophysiology. Simulations were performed in XPPAUT using the cvode solver.

Results: In mouse islets raising the glucose concentration to 11 mM glc reduced glucagon secretion to 17 +/- 2.6% (n=16) compared to 1 mM glc. Dapagliflozin (10 nM) had no effects at 1 mM glc but increased secretion from 17% to 59.1 +/-22% (n=13) at high glucose. In human islets Dapagliflozin had similar effects (19.2+/-3.5% at 11 mM glc, 32.7 +/- 7.3% with 11 mM glc + Dapagliflozin n=5). We then simulated the effects of glucose and Dapagliflozin on the membrane potential. High (11 mM) and low (1 mM) glucose was simulated by changing the extracellular glucose concentration (Go) in the SGLT submodel and varying the KATP-channel conductance (G-KATP) between 0.19 nS and 0.17 nS, which resulted in action potentials reaching 10 mV and -12 mV, respectively. Inhibition of SGLT2 at Go and G-KATP corresponding to low glucose had almost no effect whereas at high glucose it increased action potential peaks from -12 mV to 15 mV.

Conclusion: Our α-cell model reproduces the effects of glucose on action potential firing seen in membrane potential recordings in rodent α-cells. Dapagliflozin directly stimulates glucagon secretion from pancreatic islets at high glucose. Since glucagon secretion is strongly dependent on the maximal depolarization reached during an action potential, the increased action potential height upon SGLT2 inhibition in our model fits well to our secretion data. These findings potentially explain the observation that diabetic subjects treated with SGLT-blockers experience elevated glucagon levels and increased hepatic glucose output.

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Dapagliflozin reduces albuminuria on top of renin-angiotensin system blockade in hypersensitive patients with diabetes

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Background and aims: Hypertension (HTN) and albuminuria are risk factors for cardiovascular (CV) and renal disease in patients with type 2 diabetes mellitus (T2DM). Glycaemic, blood pressure (BP) and albuminuria control are critical to reducing CV and renal risk. Dapagliflozin (DAPA) reduces HbA1c and BP in patients with T2DM. The aim of the study was to characterise the effect of DAPA on albuminuria and estimated glomerular filtration rate (eGFR).

Materials and methods: Data was pooled from 2 studies for patients with T2DM, micro- or macroalbuminuria and HTN, who were assigned to DAPA 5 mg (n=87), 10 mg (n=167) or placebo (PBO; n=189). All patients were on stable angiotensin-converting enzyme inhibitor or angiotensin receptor blocker therapy.

Results: At 12 weeks, DAPA resulted in greater reductions from baseline vs PBO in albuminuria (as assessed by albumin:creatinine ratio [ACR]). Reductions in HbA1c and systolic BP (SBP) vs PBO were also observed (Table). There was a decrease in eGFR with DAPA vs PBO, which was readily reversed 1 week after the last dose. DAPA's ACR-reducing effect was also present after adjusting for changes in HbA1c, SBP and eGFR. There were no increases in serum creatinine≥1.5×baseline for DAPA 5 or 10 mg vs PBO (0%, 1.2%, 1.1%, respectively). Potassium≥6 mEq/L values were also similar for DAPA 5 or 10 mg vs PBO (1.2%, 2.4%, 2.2%, respectively). There were no serious renal-related adverse events (AEs) in any group.

Conclusion: DAPA reduces ACR in T2DM patients with HTN using renin-angiotensin system blockade without increasing serious renal AEs.

	PBO N=189	DAPA 5 mg N=87	DAPA 10 mg N=167
ACR			
Baseline, mg/g (SD)	320.3 (674.8)	380.7 (843.1)	419.8 (948.7)
Adjusted % change from baseline* (95%CI)	−18.9 (-29.5, -6.7)	−47.4 (−57.3, −35.3)	−45.8 (−53.1, −37.3)
Difference vs PBO, (95% CI)		−35.2 (−49.5, −16.8)	−33.2 (−45.4, −18.2)
eGFR,mg/mL/1.73 m²			
Baseline (SD)	85.8 (21.0)	85.3 (24.3)	82.1 (19.7)
Adjusted change from baseline (95% CI)	−0.3 (−2.1,1.5)	−1.5 (−4.2, 1.3)	−3.1 (−5.0, 1.2)
1 week after cessation	−0.9 (−2.8,1.1)	0.9 (−2.0, 3.7)	0.7 (−1.4, 2.7)
HbA1c, %			
Baseline (SD)	8.1 (0.9)	8.1 (0.9)	8.1 (1.0)
Adjusted change from baseline (95% CI)	0.01 (−0.1, 0.1)	−0.5 (−0.7, −0.3)	−0.5 (−0.6, −0.4)
SBP, mmHg			
Baseline (SD)	151.4 (8.0)	150.6 (7.3)	151.9 (9.0)
Adjusted change from baseline (95% CI)	−6.3 (−8.0, 4.6)	−12.5 (−15.0, 10.0)	−9.8 (−11.6,−8.0)

*Analysis based on log-transformed values.

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Canagliflozin reduces both HbA_{1c} and body weight in patients with type 2 diabetes on background dipeptidyl peptidase-4 inhibitors or glucagon-like peptide-1 agonists

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Background and aims: Canagliflozin (CANA), a sodium glucose co-transporter 2 (SGLT2) inhibitor approved for the treatment of type 2 diabetes mellitus (T2DM), has been shown to provide reductions in HbA1c and body weight (BW) compared with placebo (PBO) over 18 weeks in subgroups of patients on background dipeptidyl peptidase-4 (DPP-4) inhibitors or glucagon-like peptide-1 (GLP-1) agonists from the CANagliflozin cardioVascular Assessment Study (CANVAS), an ongoing, randomised, double-blind, study in patients with T2DM and a history or high risk of cardiovascular disease; this post hoc analysis evaluated the proportion of patients who had decreases in both HbA1c and BW with CANA versus PBO at Week 18.

Materials and methods: Of the 4,330 patients enrolled in CANVAS, 316 patients were taking DPP-4 inhibitors (mean baseline HbA1c, 8.1%; BW, 90.7 kg) and 95 were taking GLP-1 agonists (mean baseline HbA1c, 8.1%; BW, 108.8 kg). Patients were randomised to receive CANA 100 or 300 mg or PBO. Using individual patient data, the proportion of patients achieving both change from baseline in HbA1c <0% and BW <0 kg was assessed at Week 18.

Results: At Week 18, least squares (LS) mean changes in HbA1c with CANA 100 and 300 mg and PBO were -0.46%, -0.64%, and 0.10%, respectively, in patients taking DPP-4 inhibitors; LS mean percent

changes in BW were -3.2%, -3.9%, and -0.9%, respectively. In patients taking GLP-1 agonists, LS mean changes in HbA1c at Week 18 with CANA 100 and 300 mg and PBO were -0.83%, -0.89%, and 0.17%, respectively; LS mean percent changes in BW were -3.0%, -3.7%, and -0.4%, respectively. In the DPP-4 inhibitor subgroup, a greater proportion of patients had reductions in both HbA1c and BW at Week 18 with CANA 100 and 300 mg versus PBO (65.3%, 78.1%, and 29.2%, respectively; PBO-subtracted differences [95% confidence interval (CI)] of 36.2% [22.2, 50.2] and 48.9% [35.9, 62.0]). A greater proportion of patients in the GLP-1 agonist subgroup also had reductions in both HbA1c and BW at Week 18 with CANA 100 and 300 mg versus PBO (82.4%, 85.7%, and 24.1%, respectively; PBO-subtracted differences [95% confidence interval (CI)] of 58.2% [34.9, 81.6] and 61.6% [37.8, 85.3]). CANA was generally well tolerated in both subgroups with a safety profile similar to that seen in previous Phase 3 studies of CANA.
Conclusion: CANA provided greater attainment of reduction in both HbA1c and BW versus PBO at Week 18 and was generally well tolerated in patients with T2DM as add-on to DPP-4 inhibitors or GLP-1 agonists.
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OP 32 Insights into lifestyle and diabetes

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Is access to the outdoors associated with childhood overweight and obesity?

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Background and aims: Overweight and obese children are at increased risk of becoming overweight and obese adults and therefore an important risk factor for developing type 2 diabetes. Being overweight or obese is associated with environmental, parental and socioeconomic status (SES) characteristics. The aims of this study were to assess the association of environmental characteristics during ages 3-5 on being overweight or obese at age 7. Furthermore we assessed if parental behaviors moderate or mediate this influence. In the final stage of our analysis we assessed if the influence of SES on the derived model the previous aims was assessed.
Materials and methods: The analysis used the Millennium Cohort Study; a nationally representative study of around 19,000 children born in the UK between 2000-2001 who are followed over time. We use measures from the surveys carried out at age 9 months, 3 years, 5 years and 7 years. Logistic regression was used to test the initial association between the outcome measure overweight/obese (according to the age specific Cole criteria) and determinants: amount of green space in the neighborhood, having access to a garden and the condition (i.e. shabbiness) of the neighborhood. Subsequently, parental determinants consisting of variables summarizing: food consumption, physical activity, rules and regularity were evaluated as moderators or mediators of the initial association. Lastly SES related variables, namely education level, housing tenure and poverty were tested as moderators or mediators of the associations.
Results: Statistically significant associations ($p \leq 0.05$) were found between low levels green space, not having access to a garden, shabbiness of the neighborhood and childhood obesity (OR (95% CI) respectively: 1,14 (1,02-1,27), 1,35 (1,16-1,58), 1,22 (1,05-1,42)). Parental determinants were related to the environmental determinants and childhood overweight/obese but did not moderate or mediate the association between the latter two. Therefore no parental variables were left in the model. For SES, the highest level of education in the household did diminish the magnitude of the associations found between the environmental determinants and being overweight/obese. In the final model the remaining significant associations with childhood overweight/obese were no garden access for lower educated households and shabbiness of the neighborhood for higher educated households (OR (95% CI) respectively: 1,41 (1,09-1,84), 1,38 (1,12-1,70)).
Conclusion: Not having access to a garden at age 3 - 5 for lower educated households increased childhood overweight/obesity at age 7. Also the combination of a more dilapidated neighborhood and higher education increased childhood overweight/obesity. To conclude, we showed that limits on access to outdoors space is associated with future childhood overweight/obesity although moderated by education level. More research is needed to see how we can deploy these findings in the prevention of type 2 diabetes.

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Body mass index during early adulthood in relation to risk of diabetes in later life among Chinese men and women

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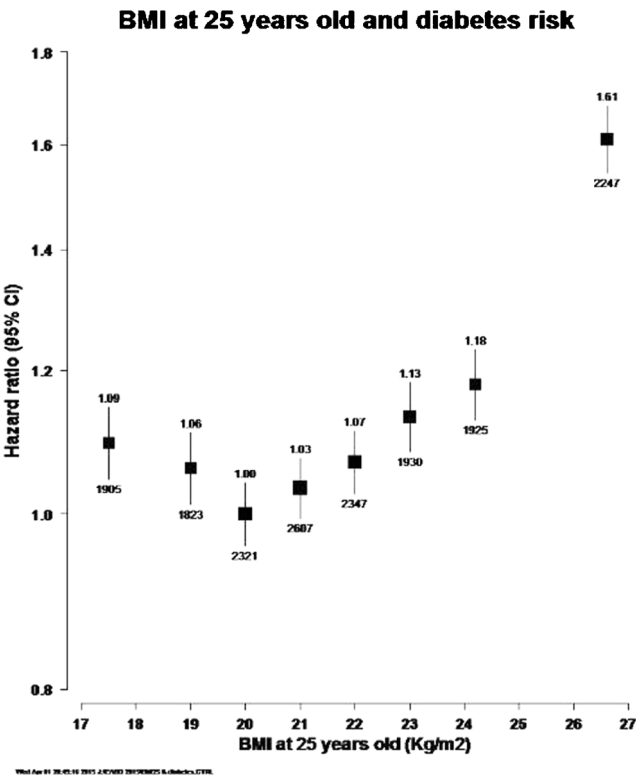
Background and aims: Although adiposity has been established as a strong risk factor for diabetes, the independent effects of body mass index (BMI) in early adulthood on risk of diabetes in middle and older age remains unclear, particularly in China.
Materials and methods: The study population includes 430,074 participants (57% women, mean baseline age 51 years) enrolled into the China Kadoorie Biobank between 2004-8 from 10 diverse localities across China, for whom self-reported data on weight at 25 years old (weight25) were available. Information on socio-demographics, lifestyle and personal & family disease history was collected using an electronic questionnaire. Data on diabetes incidence was collected through electronic linkage with mortality and morbidity registries and the national health insurance system. Height at 25 years old (height25, in meters) was estimated according to the age trend of baseline measured height, and body mass index (BMI) at 25 years (BMI25) was calculated using the standard formula. The Pearson correlation coefficient of weight25 recalled at baseline and at resurvey (~3 years after baseline, n=14,452) was calculated to assess its reproducibility. Cox regression models were used to yield hazard ratios (HRs) relating BMI25 to risk of incident diabetes (between 35-79 years of age), adjusting for age, sex, region, smoking, alcohol consumption, education, income, baseline values of BMI and waist circumference, and family history of diabetes.
Results: The correlation coefficient of weight25 reported at baseline and resurvey was 0.80. Mean (SD) BMI25 was ~2.5 kg/m2 lower [21.4 (2.5) kg/m2] than BMI at baseline [23.8 (3.4) kg/m2] and the correlation coefficient between the two was 0.32. During approximately 7-years of follow-up, 17,105 incident diabetes cases were documented. A J-shaped association was observed between BMI25 and diabetes, with risk lowest at BMI25 around 20 (19.5-20.5) kg/m2. Between 20 and 25 kg/m2, diabetes risk increased by 3-6% per unit BMI25 increase. However, at BMI25>25 kg/m2 the HR of diabetes increased substantially to 1.61 (95% CI: 1.54-1.68) (Figure). BMI25 lower than 19.5 was also associated with a 6-9% elevated risk of diabetes. Although a similar J-shaped association was observed in both genders and different age groups, stronger associations were observed in men than in women, and in younger than in older people.
Conclusion: Among Chinese, overweight in early adulthood is associated with a greatly elevated risk of diabetes in late adulthood. This association is independent of family history of diabetes and adulthood adiposity, highlighting the importance of lifelong body weight control.



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Objectively measured sedentary behaviour patterns according to diabetes status: The Maastricht Study

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Background and aims: Studies have shown that physical activity (PA) plays a role in the development and management of type 2 diabetes mellitus (T2DM). Next to the role of PA, there has been growing interest in the role of sedentary behaviour. However, large-scale studies with objectively measured sedentary behaviour in a population with T2DM are scarce. Therefore, we examined differences in objectively assessed sedentary behaviour patterns between participants with T2DM, with prediabetes (impaired glucose metabolism) and without T2DM.
Materials and methods: We included 2,529 participants (aged 40-76 years) of The Maastricht Study who wore an activPAL accelerometer 24 h/day for 7 consecutive days on the front right thigh. We calculated percentage sedentary time (ST) of waking time, mean number of breaks in ST per day (≥1 minute of ST followed by ≥1 minute activity), and mean step frequency per day (measure for PA). To determine glucose metabolism status, all participants underwent an oral glucose tolerance

test. Associations between glucose metabolism status and percentage ST were examined using linear regression analysis.
Results: 726 participants (28.7%) had T2DM, 391 (15.5%) had prediabetes, and 1,412 (55.8%) had no T2DM. Participants provided on average 6.4 valid days (defined as ≥14 h of monitoring), and 91% wore the device 6 or more days. Participants with T2DM spent on average (mean ± SD) 10.2±1.8 hours per day sedentary, as compared to on average 9.5±1.8 in participants with prediabetes and 9.1±1.6 in participants without T2DM (P<0.001). Mean number of sedentary breaks per day was 35.8±9.3 among participants with T2DM, and 37.4±8.8 and 38.0±8.5 among participants with prediabetes and participants without T2DM (P<0.001). After adjustment for demographic factors (sex, age, level of education), health factors (smoking status, body mass index, mobility limitation, history of cardiovascular diseases and chronic obstructive pulmonary disease), and mean step frequency, participants with T2DM had on average 22.5 minutes more ST per day (95%CI=16.8 - 28.2; P<0.001) compared to those without T2DM.
Conclusion: Having T2DM was significantly associated with on average 22.5 sedentary minutes more per day. The results suggest that next to PA, sedentary behaviour could be an important determinant of the development and management of T2DM.
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Overall dietary characteristics of individuals with high consumption of beverages previously associated with risk of type 2 diabetes
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Background and aims: Consumption of several beverages has been associated with risk of type 2 diabetes; high coffee and tea consumption has been associated with a decreased risk and high consumption of sugar-sweetened beverages (SSB) with an increased risk. Regarding juice and artificially sweetened beverages (ASB), the results are inconclusive. As beverages are part of our overall diet and lifestyle we hypothesized that high consumption of these beverages (SSB, ASB, juice, coffee and tea) may be associated with certain characteristics of the overall diet that could be difficult to take into account when analysing associations between beverage consumption and disease.
Materials and methods: Analyses were performed among 25 112 individuals (60% women, 45-74 years, mean BMI=25.6) without prevalent diabetes, cardiovascular disease or cancer from the population based Swedish Malmö Diet and Cancer Cohort. Intake of beverages, macronutrients and 24 food groups were obtained from a modified diet history method including a 7-day food record and a 168-item questionnaire. To examine food intakes across five intake groups of the different beverages, we used linear regression adjusted for age, sex, season, method, BMI, leisure time physical activity, total energy intake, smoking, education and alcohol intake. Bonferroni correction was applied to handle multiple testing and therefore statistical significance was assumed at p<0.002 (0.05/28=0.002).
Results: We observed a high consumption of SSBs to be significantly associated with lower intakes of foods generally perceived as healthy; the largest intake differences between high and low consumers of SSBs were seen for fruits, vegetables, yoghurt, breakfast cereals, fiber rich bread and fish (all p-values for trend<0.002). In contrast, high consumption of both tea and juice was significantly associated with higher intakes of foods perceived as healthy; largest differences were seen for fruits, vegetables and yoghurt (all p-values for trend<0.002). High consumption of ASBs was significantly associated with higher intakes of low fat products; low fat milk and margarines (both p-values for trend<0.002). High consumption of coffee associated with higher intakes of meat and high fat margarine and a lower intake of breakfast cereals (all p-values for trend<0.002).
Conclusion: We observed that high consumption of beverages that have previously been associated with risk of type 2 diabetes strongly associates with characteristics of the overall diet. This might be of importance when interpreting future studies investigating beverage-disease associations.
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Maternal smoking during pregnancy is associated with poorer aerobic fitness of offspring in young adulthood
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Background and aims: In Finland pregnant women are offered a cost-free health care at municipal maternity welfare clinics and health counselling is being given incl. adequate/excessive gestational weight gain (GWG) and maternal smoking. Our aim was to investigate the association of maternal pre-pregnancy body mass index (BMI), GWG and maternal smoking with aerobic fitness and weight in young men aged 19-28.
Materials and methods: Data consisted of 508 mothers and their offspring in the Northern Finland Birth Cohort (NFBC 1986) who entered military service at the Sodankylä Jaeger Brigade in 2005. The associations of weight, 12-minute running test result (Cooper test),smoking among offspring on entry to military service were evaluated with antenatal factors including maternal smoking, pre-pregnancy BMI and GWG in this prospective cohort study. Physical activity at the 15-16 years age was evaluated from questionnaires.
Results: Weight of conscripts whose mothers were overweight and gained excessive weight during pregnancy was higher (84.4 kg, SD 18.1) than those of overweight mothers whose weight gain was considered appropriate (73.5 kg, SD 12.5) (p=0.005). Offspring of mothers who smoked>10 cigarettes / day during pregnancy also had a lower Cooper test result (2237 m, 95% CI 1930-2544) compared to the offspring of mothers did not smoke during pregnancy (2537 m, 95% CI 2499-2574). Multivariate linear regression analysis showed that maternal smoking, low level of physical activity at 15-16 years age, conscripts own smoking and own BMI, however not maternal pre-pregnancy BMI or GWG, were independently associated with worsening of Cooper test result. According to mediation analysis, conscript's own BMI mediated the association of maternal pre-pregnancy BMI and GWG with the Cooper test result. Direct effect of maternal pre-pregnancy BMI on the Cooper test result of the offspring was 28% (regression coefficient of direct effect - 4.07, p=0.394) and that of GWG was 60% (regression coefficient of direct effect - 5.51, p=0.092). No association of birth weight and the Cooper test result was observed.
Conclusion: Maternal smoking during pregnancy had a long-term negative impact on the aerobic fitness of the offspring, observed during a 19-year follow-up. This study highlights the need of health-counsel for pregnant women concerning non-smoking and appropriate weight gain during pregnancy. Supervised exercise and smoking cessation groups for pregnant women via maternity clinics could be one way for attaining beneficial circumstances for pregnancy.

Table 1. Association of maternal pre-pregnancy body mass index, excessive gestational weight gain, maternal smoking, physical activity of offspring at 15-16 years age, conscript's body mass index and smoking with the change in the Cooper test result.

Variable	*B* (SE)	β	p
Intercept	2740.30 (44.00)		<0.001
Maternal prepregnancy BMI *	3.13 (6.31)	0.03	0.620
Gestational weight gain	-1.21 (3.90)	-0.02	0.757
Maternal smoking			
Cessated smoking	-110.30 (48.00)	-0.11	0.022
1-10 cigarettes/day	-162.30 (68.30)	-0.12	0.018
> 10 cigarettes/day	-273.40 (131.00)	-0.10	0.038
Physical activity of offspring			
Active	-111.80 (55.60)	-0.12	0.045
Moderately active	-143.80 (52.40)	-0.17	0.006
Lightly active	-218.80 (54.80)	-0.25	<0.001
Inactive	-275.50 (96.40)	-0.15	0.005
BMI of offspring *	-40.20 (4.70)	-0.43	<0.001
Smoking of offspring	-124.00 (36.60)	-0.16	0.001

Effect size is *B* coefficient (standard error) and standardized beta (β), calculated by linear regression. *Centered for mean of the study population (maternal BMI 22.3 kg/m^2, own BMI 23.6 kg/m^2)

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The association between sleep duration and insulin sensitivity: the EGIR-RISC study

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Background and aims: In the past 10 years, over three-dozen studies reported a relationship between short sleep and disturbed glucose metabolism. Studies with insulin sensitivity assessed according to the gold standard hyperinsulinemic-euglycemic clamp are however still missing. We therefore aimed to evaluate the cross-sectional association between sleep duration and insulin sensitivity in the European Relationship between Insulin Sensitivity and Cardiovascular Disease (EGIR-RISC) study.
Materials and methods: We used data from the baseline measurements of the European, multi-centre EGIR-RISC study, which included 1259 clinically healthy men and women. Sleep and physical activity were measured objectively using a single-axis accelerometer, which was worn for at least 3 days and up to 8 days. Insulin sensitivity was measured by hyperinsulinemic-euglycemic clamp (M/I). Other markers of metabolic syndrome included levels of fasting plasma glucose, insulin, 2-h plasma glucose and insulin as well as BMI, waist circumference and blood pressure.
Results: In our current analysis, we included 797 participants (57% women, age 44±8 years). A weak U-shaped association between hours of sleep and M/I was observed: after adjustment for age and sex, M/I was 10.7 (-4.4- 25.9),3.6 (-10.2-17.4), -0.8 (-15.9-14.3) and 4.7 (-14.3-23.9) μmolmin^{-1}kgffm^{-1}nM^{-1} higher for the 6-7 hour group, 7-8 hour group, 8-9 and the >9 hour group, respectively, compared to the<6 h group. Additionally, we observed a J-shaped association for basal glucose: after adjustment for age and sex, glucose levels were 0.13 (0.02-0.24), 0.12 (0.02-0.22), 0.16 (0.05-0.27) and 0.19 (0.05-0.33) mmol/l higher for the 6-7 hour group, 7-8 hour group, 8-9 and the >9 hour group, respectively, compared to the<6 h group. Similar J-shaped associations were observed for basal insulin levels and 2-h glucose and insulin levels. No associations were observed for BMI, waist circumference or blood pressure.
Conclusion: Short and long sleep duration are weakly associated with insulin sensitivity and markers of glucose homeostasis in a healthy population. Prospective studies are needed to study the temporal associations in a healthy population.
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OP 33 Devices changing treatment paradigm

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Six year follow-up after CSII initiation: HbA_{1c} is significantly reduced without weight gain

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Background and aims: Meta analyses show that treatment with continuous subcutaneous insulin infusion (CSII) lowers HbA1c by 0.3% compared to mulitiple daily injections (MDI) in adult populations. The longest observation period in the included RCTs was one year. An observational study included 331 patients that were observed for 5.5 years in ten clinics. In CSII treated patients, HbA1c decreased 0.42% after 1 and 2 years, but only 0.2% after 5 years, when compared to time-matched MDI patients. In our clinic, we have previously found that the HbA1c of 505 patients was reduced by 0.7% (females) and 0.5% (males) one year after CSII initiation. In the present study, our aim was to investigate the long term effect of shift from MDI to CSII on HbA1c and weight.
Materials and methods: All patients were analysed for age, T1D duration, weight (kg), and HbA1c (IFCC mmol/mol and DCCT %) four years before and six years after initiation of CSII. Data are mean±standard deviation. A p-value below 0.05 was considered statistically significant.
Results: Since 2002, 856 patient initiated CSII at our center. Mean age and T1D duration at CSII initiation were 39±14 years and 21±13 years. 622 patients that had data two years before CSII were included in the analysis. There was no significant change in weight or in HbA1c prior to minus 2 years (Fig. 1). HbA1c decreased by 1.6 mmol/mol during the ensuing period prior to initiation (1.1 mmol/mol between minus 2 years and minus 1 year ($p<0.0002$), and 0.5 mmol/mol between minus 1 year and initiation ($p<0.02$)). This reduction was concomitant to the patients receiving education on carbohydrate counting. The total reduction in HbA1c from minus 2 years to one year after initiation was 8.3 mmol/mol ($p<0.0001$). The reduction in HBA1c from initiation to six years after was 7.8 mmol/mol (n=183, $p<0.0001$). There was no significant rise or decline in HbA1c between one and six years after CSII initiation.
Conclusion: Our one year data show a significant HbA1c reduction after CSII initiation which is comparable, or better, than results from RCTs. Previous studies have suggested a diminishing effect of CSII on HbA1c over time. By contrast, here we found a stable reduction of HbA1c in the six year follow-up period after CSII initiation. Importantly, contrasting the effect of most other effective glucose-lowering strategies the effect was obtained without an increase in body weight.

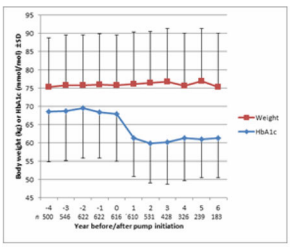

Figure 1. HbA1c (mmol/mol) and total body weight (kg) development over time in adult subjects transferring from multiple daily injection therapy to continuous subcutaneous insulin infusion. Patients with HbA1c data available two years prior to pump initiation were included (n=622). Number of patients for each data point is given below the graph. Year "0" represents the HbA1c closest to initiation (-13 ± 26 days); the others represent averages of all data available for each patient during that year.

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Effects on glycaemic variability and glyco-metabolic control of an insulin pump integrated with continuous glucose monitoring system in type 1 diabetic patients

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Background and aims: Often, insulin pump therapy is not enough to reach an adequate glycemic control; this is particularly true in type 1 diabetic patients, where glycemic fluctuations are very high. For this reason, in the latest years, continuous glucose monitoring system (CGMS) has become available. The aim of this study was to evaluate the effects of an insulin pump integrated with continuous glucose monitoring system (CGMS) compared to insulin pump alone on glycemic control, and blood glucose variability in type 1 diabetic patients, in a case-control clinical trial. We also evaluated the degree of patients' satisfaction to treatment.
Materials and methods: We enrolled 38 type 1 diabetic subjects, with an inadequate glycemic control (glycated hemoglobin >7.5%), with an insulin pump from at least three months. At baseline, patients were instructed to use a CGMS able to communicate with an insulin pump in an integrated system. The function of suspension of insulin delivery, in case of hypoglycemia, was activated. We evaluated: anthropometric measurements, glycated hemoglobin, fasting plasma glucose (FPG), postprandial glucose (PPG). These parameters were recorded at baseline, at 3 and 6 months after the placement of the device. Diabetes Treatment Satisfaction Questionnaire (DTSQ) was used to assess patients' satisfaction toward treatment. The primary analyzed variable was glucose measured by CGMS and evaluated using area under curve. Percentage of time spent (based on CGMS records) in different ranges of glucose was also analyzed. These two variables were analyzed using Wilcoxon signed rank test, and stepdown Bonferroni (Holm's) correction of p-values was applied to adjust p-values for multiple testing. The following variables were analyzed using Wilcoxon signed rank test: M value, glycated hemoglobin, mean blood glucose, number of hypoglycemia during CGMS (blood glucose≤70 mg/dL), and standard deviation (SD) of glucose measured by CGMS. Mean amplitude of glycemic excursions (MAGE) measured by CGMS was analyzed using t-test.
Results: After 6 months since the placement of the integrated system, there was a decrease of glycated hemoglobin, FPG and PPG compared to baseline ($p<0.05$ after 3 months, and $p<0.01$ after 6 months). The daily blood glucose variability, expressed as MAGE, was reduced after the placement of the integrated system. Mean blood glucose, as calculated from CGMS values, was significantly higher with insulin pump alone compared to insulin pump + CGMS (172.4±60.4 vs 153.1±51.3, $p<0.05$). M value with insulin pump + CGMS was lower compared to insulin pump alone between 7-8, 10-11, 17-18, and 23-24 h. The SD, and the MAGE values were significantly lower with insulin pump + CGMS compared to insulin pump alone ($p<0.05$). The MODD value at 7-8, and 10-11 h was lower with insulin pump + CGMS compared to insulin pump alone ($p<0.05$). Insulin pump + CGMS gave less time spent with glycemia<70 mg/dl and more time with glycemia between 70 and 180 mg/dL compared to insulin pump alone. There was a greater degree of satisfaction for the insulin pump integrated with CGMS according to the DTSQ.
Conclusion: The insulin pump integrated with CGMS better improved glycemic control, glycemic variability and patients' satisfaction in type 1 diabetic patients compared to insulin pump alone.

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Medical imaging for performance characterisation of a novel continuous subcutaneous insulin infusion set

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Background and aims: Insulin pump therapy for Continuous Subcutaneous Insulin infusion (CSII) is a highly effective method of glycemic control for diabetic patients. However, CSII set performance is often characterized as the "Achilles heel" of an otherwise sophisticated control system. An investigational flow-stabilizing infusion set with 28G, 6 mm side-ported polymer catheter has been designed to reduce infusion delivery pressures, stabilize flow performance, and minimize insulin flow stoppages that may occur without triggering pump alarms (e.g. "silent occlusions"). Medical imaging can be highly informational but is not a routine testing procedure for delivery devices. The objective of this study was to utilize various imaging techniques to characterize the *in vivo* flow and deposition performance of the investigational CSII set against control 6 mm and 9 mm commercial polymer sets in both animal and human studies.
Materials and methods: *In vivo* fluoroscopy was performed using both static and dynamic imaging during bolus (10U) infusion of iodinated contrast media on anesthetized swine from the investigational set and commercial controls. Images were analyzed for location of device placement in the tissue, bolus depot patterning, and occurrence of delivery faults such as incomplete insertion and leakage. A corollary human MRI study with saline placebo was performed in 8 non-diabetic subjects across multiple bolus volumes (2-20U) and various routine insulin infusion sites (arm, thigh, abdomen, and gluteus). The addition of MR contrast enhancers was not required for effective depot visualization.
Results: For each imaging modality, contrast-filled polymer catheters were visible *in situ*. Depots were clearly visible after bolus administration, even at low bolus volumes. The investigational ported set typically exhibited more diffuse subcutaneous (SC) depot patterns in contrast to denser more localized depots observed from marketed sets. Side-port flow was typically most readily observed during stepwise boluses of increasing volume (Figure 1). The investigational set demonstrated effective SC placement and placebo delivery at all anatomical sites tested sites during the human trial.
Conclusion: Both fluoroscopic and MR imaging techniques provide effective characterization of CSII set insertion and bolus delivery performance at relevant anatomical sites and may enable better comparative device performance assessment. The investigational catheter set porting appears to create an auxiliary tissue flow pathway that may reduce occlusion and stabilize flow.

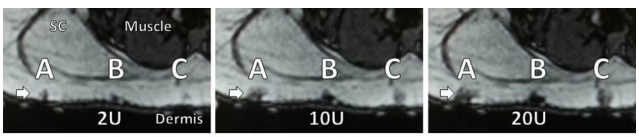

Figure 1. Human MR tranverse images of SC infusion from 6mm CSII sets (A, C ported investigational sets; B commercial device) in upper gluteal region at increasing bolus volumes (2, 10, 20U). Arrow highlights side-port delivery on device A. Investigational sets show increased depot diffusion.

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Effect of sensor augmented pump treatment on fear of hypoglycaemia and quality of life in subjects with dysregulated type 1 diabetes

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Background and aims: To investigate the one year effect of sensor augmented pump (SAP) treatment on glycaemic control and quality of life of subjects with dysregulated type 1 diabetes (DT1D) and/or problematic hypoglycaemia.
Materials and methods: As part of quality assurrance we studied 64 patients on insulin pump (32 male and 32 female) with DT1D (HbA1c >69 mmol/mol for >12 months), or problematic hypoglycaemia (unawareness or≥one severe episode during the preceding year) who were offered SAP. Primary endpoints were decrease in HbA1c and effect on quality of life as evaluated by the Hypo Fear Scale (HFS), Problem Areas in Diabetes (PAID), and WHO5. Non-parametric statistical analyses were used to compare responses before and one year after SAP was initiated. Data are expressed as mean±sem.
Results: The average age was 44±1.7 years. Baseline HbA1c was 75.3±1.5 mmol/mol (n=64), HFS 38±3 (n=53), PAID 26.6±2.6 (n=54); 19.4±2.7 and 35.0±4.1 for males (n=29) and females (n=25), respectively (p=0.004), and WHO5 15.1±0.7 (n=59). More than five episodes of severe hypoglycaemia had been experienced by 33%. Any degree of hypoglycaemia unawareness was significantly more common in this group (p=0.02). After one year 48 patients (75%) were still using SAP. These were significantly older than those who stopped (45±2 vs. 39±3, p=0.04) but there was no difference in duration of diabetes or time on pump. Average sensor use was 9.0±0.6 out of 12 months; 34 patients (71%) reporting a use greater than 2/3 of the time. HbA1c decreased in both SAP users and in those who stopped using SAP (from 73.8±1.6 to 66.1±1.7 [n=48; p<0.0001] and 80.3±3.5 to 72.9±3.2 [n=14; p=0.001], in users and non-users, respectively). There was no correlation between time on SAP and HbA1c lowering. By 12 months HFS had decreased significantly (from 38.1±3.2 to 28.9±3.4; n=39, p=0.001), as had PAID (from 25.2±2.6 to 20.9±2.6; n=41, p=0.007), whereas there was no difference in WHO5. PAID>30, a level indicating high diabetes related distress, was found in 62% of those who stopped using sensor, compared to 31% for the SAP users, (p=0.047). The influence of SAP on the top three PAID score questions at baseline are shown in Table 1. WHO5 score<13, indicating declined wellbeing, was found in 16% of the SAP users and 43% of patients who stopped using sensor (p=0.035).
Conclusion: Additional to a significant improvement in HbA1c, SAP seems to offer specific support concerning the fear of hypoglycaemia and diabetes related distress to subjects with T1D who are prone to hypoglycaemia with no negative impact on overall wellbeing of the patients from the use of sensors.

Which of the following diabetes issues is currently a problem for you?	Baseline	12 mth	P
9. Worrying about low blood sugar reactions? (n = 44)	1,47	0,93	0,004
12. Worrying about the future and the possibility of serious complications? (n = 46)	2,15	1,82	0,01
20. Feeling "burned out" by constant effort needed to manage diabetes? (n=46)	1,37	1,05	0,03

Table 1. Top three PAID score questions at baseline.

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HbA_{1c} improvement with less hypoglycaemia in patients with type 1 diabetes wearing an artificial pancreas for two months from dinner to breakfast

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Background and aims: Wearable artificial pancreas (AP) at home from dinner to breakfast appears a safe concept for first routine use in patients

with Type 1 diabetes (T1D). We explored this concept in the first AP trial with sufficient length to assess HbA_{1c}.

Materials and methods: Thirty-five adult T1D patients with insulin pumps and an HbA_{1c} between 7.5 and 10% were enrolled in a randomized crossover trial comparing AP use from dinner to wake-up time and sensor augmented pump (SAP) during day-time vs. all day SAP under free-living conditions. The design included a run-in of 2 weeks with SAP and a wash-out of 4 weeks between 2 periods of 2 months. AP was achieved by connecting a continuous glucose monitor (CGM) and insulin pump to a smart-phone which ran a Model Predictive Control algorithm. CGM data from the last 6 weeks of each period were analyzed on an intention-to-treat basis with % time in target [70-180 mg/dL] from 20:00 to 08:00 as primary endpoint. HbA_{1c} change was analysed with an ANOVA model with patient, period and treatment as factors.

Results: Three patients dropped out, 1 before and 2 shortly after randomization, due to poor system acceptance. 18 (56%) of 32 analysed patients were female, HbA_{1c} was 8.2±0.6% and age 47.0±11.2 years. From 20:00 to 08:00, time in target was higher with AP: 67.0 vs. 57.8% (paired difference 8.2%, IQR 5.0-14.4, $p<0.0001$), through a reduction in %time both >180 mg/dL and <70 mg/dL: 31.8 vs. 38.6% (paired difference 6.7%, 95%CI 3.7-9.8, $p<0.0001$) and 1.7 vs. 3.0% (paired difference 1.3%, IQR 0.5-3.0, $p<0.0001$). Also over 24 hrs, higher time in target was seen with AP (63.7 vs. 58.9%, paired difference 5.0%, IQR 1.7-7.8, $p=0.00017$), again through a reduction in %time both >180 mg/dL (33.5 vs. 36.9% paired difference 4.3% IQR 0.7-7.4, $p=0.00179$) and <70 mg/dL (2.2 vs. 3.0%, paired difference 1.0% IQR 0.3-1.7, $p=0.00013$). HbA_{1c} was reduced 0.18% more on AP (95%CI 0.00 to 0.36, $p=0.047$).

Conclusion: AP from dinner to wake-up time increases % time in target range during this time period and also over 24 hours, due to a decrease in time both above and below target and with a significant decrease in HbA_{1c}. These results support AP from dinner to breakfast as a safe and beneficial option for moving to the market.

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Delivery of exenatide by subdermal placement of ITCA 650 in a nonclinical model demonstrates 1 year of continuous exposure and tolerability

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Background and aims: ITCA 650 is a mini osmotic pump designed to deliver exenatide as a continuous zero-order subdermal infusion. In an ongoing 2-year carcinogenicity study, rats received ITCA 650 at exenatide doses of 0, 3, 10 (females), 20 (males) and 40 mcg/d. The low and high doses were selected to provide approximately 4-fold and 25-fold exposure multiples of the anticipated clinical dose (60 mcg/d).

Materials and methods: On Day 1, an ITCA 650 osmotic minipump or placebo was placed subdermally in each rat. At 6-month intervals, devices were removed and replaced with another of the same dose at the same site to provide continuous exposure during the 2-year study. Plasma exenatide was measured by a validated ECL assay in toxicokinetic group animals on Day 365/366.

Results: There was no evidence of systemic toxicity and survival was not adversely affected by treatment. As expected from the pharmacology of exenatide, a decrease in body weight gain (up to 40% vs control) was noted in ITCA 650-treated groups. Macroscopic evaluation of tissues local to the placement site revealed a minimal tissue response to the device. Despite repeated removal and replacement of devices during the study, microscopic observations were minimal to mild in all groups, with minimal inflammation in some animals. Exenatide exposures increased as the dose increased from 3 mcg/d to 40 mcg/d. Comparing Day 365 AUC24h with human AUCs at the clinical dose (60 mcg/d) showed that this study achieved exposure multiples of at least 7.3-fold at the mid dose and 18.1-fold at the high dose.

Conclusion: These results are relevant to the human experience as prolonged exposure and repeat minipump placements did not result in any local or systemic toxicologic differences between the active and control groups.

OP 34 Biomarkers of beta cell autoimmunity

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Non-invasive determination of the beta cell mass with ^{111}In-exendin-3 in rodent models for spontaneous type 1 diabetes

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Background and aims: The beta cell mass (BCM) plays a key role in the development and progression of diabetes, but the onset of beta cell loss and the relationship between loss of beta cell mass and beta cell function still needs to be unraveled. A non-invasive method to determine the BCM *in vivo* allows measurement of the BCM over time during the development and progression of type 1 and 2 diabetes. Exendin-3 is a stable analogue of glucagon-like peptide 1 (GLP-1) and has high affinity for the GLP-1 receptor, which is specifically expressed on beta cells. Recently, targeting of the GLP-1 receptor on beta cells was proven to be a successful method to determine the beta cell mass *in vivo* in a rat model of chemically induced beta cell loss. Furthermore, in a first clinical proof-of-principle study these preclinical data were confirmed. In this study we investigated the potential of ^{111}In-exendin-3 to determine the BCM *in vivo* in a mouse and rat model that closely mimic the development of type 1 diabetes in humans: Non-obese Diabetic (NOD) mice and BioBreeding Diabetes Prone (BBDP) rats. In these models the potential of SPECT, the relationship between the BCM, exendin uptake in the pancreas and insulitis were investigated.
Materials and methods: NOD mice (n=78) of 7-21 weeks old and BBDP rats (n=45) of 4-18 weeks of age were injected intravenously with 15 MBq ^{111}In-exendin-3. SPECT/CT Images were acquired 1 h p.i. and the animals were euthanized after SPECT/CT acquisition. The pancreas and relevant organs were dissected, weighed and the radioactivity in the organs was measured in a gamma counter. The pancreas was fixed in formalin, embedded in paraffin and sections were used for histology, determination of the beta cell mass by morphometric analysis and degree of insulitis.
Results: With SPECT the pancreas in both rats and mice was visualized. Biodistribution showed high uptake of ^{111}In-exendin in the pancreas in healthy mice of 7 weeks of age (15.1±2.5%ID/g) and a significantly reduced uptake in diabetic mice of 21 weeks old (10.1±3.8%ID/g, p= 0,0379). In BBDP rats a reduction in pancreatic uptake of more than ninety percent was found. In BBDP rats and NOD mice the correlation (Pearson) between the BCM (mg) and pancreatic uptake (%ID) was 0.80 ($p<0,0001$) and 0.42 ($p<0,0002$), respectively. *Ex vivo* autoradiography showed high uptake in the exocrine pancreas in mice, but not in rats, which might explain the difference in correlation between BCM and uptake between both models. In severely diabetic rats, with no insulin-positive cells, the pancreas was undetectable by autoradiography. Furthermore, insulitis did not have an influence on the uptake of exendin in the pancreas.
Conclusion: In conclusion, the BCM can be determined after injection of ^{111}In-exendin-3 in a mouse and rat model for type 1 diabetes, closely mimicking the human development of type 1 diabetes. Furthermore, the pancreas can be visualized with SPECT in both models. These results indicate that ^{111}In-exendin-3 is a promising tracer to determine the BCM during the development of type 1 diabetes, without interference of severe insulitis or insulin treatment. However, due to exocrine uptake of exendin in mice, rat models would be more suitable than mouse models when studying the GLP-1R in diabetes.
Supported by: JDRF

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Immune subsets profile through disease onset, remission phase and final establishment in type 1 diabetic children

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Background and aims: Type 1 diabetes (T1D) is an autoimmune disease with regulatory/effector T cell imbalance. Its autoinflammatory nature makes it possible to develop new therapies based on immune-modulatory strategies. Immunologic characterization along the natural history of the disease (onset, remission and established T1D) is essential for better targeting the immune treatment and to identify key immunological markers that will allow monitoring future interventions towards immune balance restoration. The aim of this study was to evaluate the dynamics of T cell subsets from T1D onset to final establishment, in children.
Materials and methods: T1D children (n=24) were followed along 3 time points of the disease (t1 - onset, t2 - remission, t3 - establishment). Peripheral blood (PB) samples from patients and matched controls were analyzed by flow cytometry. Treg were identified as the CD4+CD25+CD127dim subset within CD3+T cells. Th1, Th17, Tc1 and Tc17 cells were identified through the production of IFN-γ and IL17 within CD4 and CD8 T cells, after a 5 h stimulation with PMA+Ionomycin, at 37°C, 5% CO2.
Results: Compared to controls, in t1, T1D children presented a tendency for lower % of Treg, recovering in t2. At t3, Treg % and absolute counts were significantly impaired, also compared to t1 and t2 ($p<0.05$). Again in t1, a significantly lower % of IFN-γ secreting cells (Th1 and Tc1) was observed ($p<0.05$) compared to t2 and t3. Despite this tendency for higher values in the final t2 and t3 evaluations, none of the time points significantly differ from controls. In contrast, compared to controls, Th17 cells were significantly impaired in all time points ($p<0.05$), in particular t1. Tc17 present the same profile, but their % recover to levels similar to controls in t3.
Conclusion: To our knowledge, this is the first study evaluating the immune profile throughout T1D stages. Our data points to a more pro-inflammatory profile as the disease evolves, with loss of Treg, and increasing IFN-g, as well as Il-17, producing T cells. Although the status of Treg in T1D onset is controversial, our study shows a significant Treg imbalance only after disease establishment. The normal values of Treg in remission may be related to the apparent control of the immune response at this stage. The increasing Th1/Tc1 compartments observed in T1D establishment are in accordance with the less effective presence of suppressive Treg. Also, remission corresponds to regeneration of β-cells and normalization of glycaemia, which can lead to new inflammatory events with subsequent Th1/Tc1 differentiation and activation. A more prominent Th17 response in T1D has been suggested. We report lower circulating Th17 cells in T1D patients, though recovering at established disease. Accordingly, recent studies report increased Th17 cells in pancreatic lymph nodes (PLN), but not in PB, speculating the migration of Th17 to target organs later in the disease. In our study, circulating Th17 and Tc17 were impaired in early stages of T1D, when massive destruction of pancreatic tissue occurs, which suggests an early migration of Th17 into target tissues, associated with tissue damage events. The chemotactic capacity of Th17 towards neutrophils can reinforce our data, as PB neutrophils were impaired in T1D patients, mostly in t1 and t2. PLN immune profile evaluation would help the interpretation of our data on the quest for immune markers and therapeutic targets for T1D.
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Relationship between islet specific autoantibodies at the diagnosis and the first autoantibody marking initiation of the autoimmune process in children with type 1 diabetes

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Background and aims: Serum autoantibodies specific for islet antigens characterize the onset of type 1 diabetes and also predict development of the disease. Demographic and genetic associations with the presence of specific autoantibodies have been described suggesting immunological heterogeneity in the immunology of the disease. Prospective follow-up studies collecting samples from children at genetic risk starting from birth on have recently made it possible in many cases to detect the first islet autoantibody to appear. This has revealed patterns associated with the specificity of this autoantibody. In the present study we aimed to compare islet autoantibodies detected at the diagnosis of overt diabetes with the first autoantibodies initiating the diabetes associated autoimmunity.
Materials and methods: Antibody data from the children participating in the follow-up cohort of the Finnish Diabetes Prediction and Prevention (DIPP) study who had developed clinical type 1 diabetes were analysed. A single first islet autoantibody out of three (IAA, GADA and IA-2A) could be identified in 135 of 233 (57.9%) children who had developed type 1 diabetes.
Results: The results are summarized in the Table. The most common initializing autoantibody was IAA followed by GADA and IA-2. However, when the presence of various antibodies was compared at diagnosis, IA-2A was the autoantibody most often detected followed by IAA and GADA.
Conclusion: These results can be used to estimate to what extent the presence of various autoantibodies at diagnosis reflects the initiating autoantibody. Because of antigen spreading and disappearance of some autoantibodies (especially in the case of IAA), during the prediabetic period autoantibody status at diagnosis gives limited information on the development of islet autoimmunity. In addition antibody status at diagnosis is largely affected by the variable proportion of different first autoantibodies at various ages.

Table. Autoantibodies present at diagnosis divided according to the first autoantibody initialising the autoimmunity and first antiboy to appear in children positive for different autoantibodies at diagnosis

First autoantibody to appear	Autoantibodies present at diagnosis					
	IAA		GADA		IA2-A	
	N	%	N	%	N	%
IAA (N=82)	60	73.2	36	43.9	61	74.4
GADA (N=37)	16	43.2	33	89.2	29	78.4
IA-2A (N=16)	7	43.8	4	25	16	100

Autoantibody present at diagnosis	First autoantibody to appear					
	IAA		GADA		IA2-A	
	N	%	N	%	N	%
IAA (N=83)	60	72.3	16	19.3	7	8.4
GADA (N=73)	36	49.3	33	45.2	4	5.8
IA-2A (N=106)	61	57.5	29	27.4	16	15.1

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Functional defects of regulatory T cells and the influence of age in naïve and memory T cells in new-onset type 1 diabetes and first degree relatives

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Background and aims: T cells are key mediators of autoimmune diseases like type 1 diabetes (T1D) and regulatory T cells (Tregs) are a critical subset that plays an indispensable role in maintaining immunotolerance. In the present study, we aimed to assess number and function of Tregs as well as naïve and memory T cell population in children and adults with new-onset T1D compared to first degree relatives (FDR) and heathy controls and we evaluated age effects on T cell subsets.
Materials and methods: Peripheral blood cells from 14 adult T1D (age: 35±12ys), 29 juvenile T1D patients (age: 12±4ys), age matched FDR (20 children, 38 adults) and healthy controls (17 children, 55 adults) were quantified by a multi-parameter FACS analysis. Apoptosis and suppressive capacity of Tregs were measured in vitro with FACS sorted Tregs and stimulated autologous effector T cells.
Results: The suppressive capacity of Tregs was decreased in children with T1D (mean±SEM; 20.6±6.6% vs 39.4±4.2% vs 43.8±6.4% p= 0.024) and adult T1D patients (7.5±8.1% vs 34.5±3,6% vs 39.7±2.0% p<0.001) in comparison to FDR and healthy. Apoptosis of Tregs was only significantly increased in children with T1D (2.83±0.68% vs 1.24± 0.31% vs 0.67±0.19%, p=0.026) but apoptosis of effector T cells were increased (p=0.001) in adults with T1D. Number and function of Tregs did not change with age within the group of new onset T1D, FDR or healthy controls but naïve $CD4^{+}CD25^{hi}$ decreased and memory $CD4^{+}CD25^{hi}$ T cells increased with age in each group.
Conclusion: Our data show that Tregs function is impaired in juvenile and adult new-onset T1D, which is already seen in FDR. Furthermore age influences the frequencies of naïve and memory $CD4^{+}CD25^{hi}$ T cells and the functional differences in peripheral Tregs suggest an age dependent defect of Tregs function in the pathogenesis of T1D.
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Autoantibodies to posttranslationally modified insulin in type 1 diabetes

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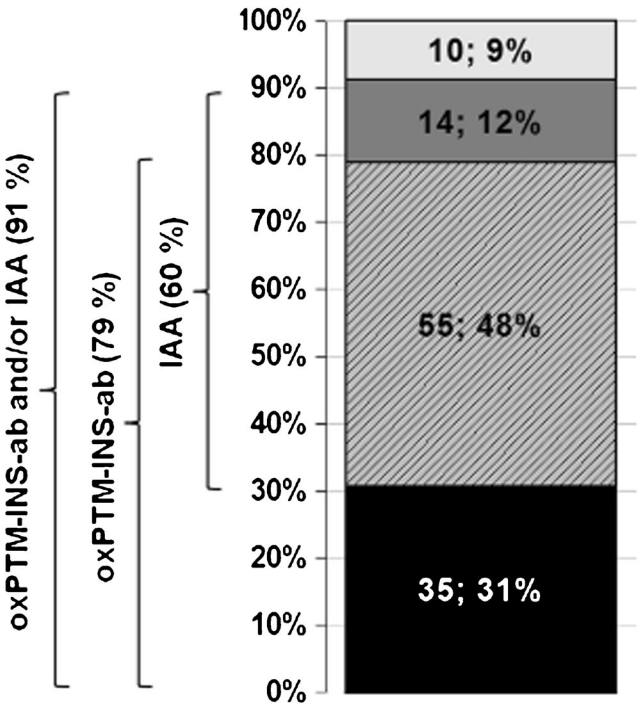
Background and aims: Insulin is the most specific antigen for pancreatic beta-cells and a primary potential autoantigen in type 1 diabetes. Although insulin autoantibodies (IAA) are the earliest marker of beta-cell autoimmunity only half of children with newly-diagnosed type 1 diabetes and fewer patients diagnosed in adult age are positive to IAA. The aim of this investigation was to determine if insulin, posttranslationally modified by reactive oxidants (oxPTM) present within the inflamed beta-cells microenvironment, could generate neoantigenic epitopes.
Materials and methods: oxPTM of insulin were induced by incubation with reactive oxidants such as ribose and various reactive oxygen species (ROS). Modifications were analysed by polyacrylamide gel elecrophoresis, 3-dimensional fluorescence and mass spectrometry. Reactivity to posttranslationally modified insulin by ROS (oxPTM-INS)

was measured by ELISA, radioimmunoassay and Western blotting, using sera from 114 patients with newly-diagnosed type 1 diabetes and 113 healthy controls.

Results: Mass spectrometry mapped oxPTM of insulin to chlorination of Tyr16 and 26; oxidation of His5, Cys6 and Phe24 and glycation of Lys29 and Phe1 in B-chain. Significantly higher binding to oxPTM-INS vs native-insulin was observed in type 1 diabetic individuals ($P<0.0001$). oxPTM-INS autoantibodies showed better sensitivity than IAA (79 vs. 60% with the same 99% specificity) and were able to detect 31% of IAA-negative patients. The combined measurement of IAA and oxPTM-INS autoantibodies raised the detection of insulin autoimmunity to 91% of subjects with new-onset type 1 diabetes. Binding to oxPTM-INS did not correlate with blood glucose or age and was directed toward oxPTM-INS fragments with slower mobility than native insulin.

Conclusion: These data suggest that oxPTM-INS is an autoantigen in type 1 diabetes and that antibodies to oxPTM-insulin are present in the large majority of new-onset type 1 diabetes patients. Overall, over 90% of individuals with new-onset type 1 diabetes presented autoimmunity to insulin, either IAA or oxPTM-INS autoantibodies.



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Delivery of recombinant human soluble receptor for advanced glycation end products delays autoimmune diabetes in the NOD mouse model

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Background and aims: Like other autoimmune diseases in developed nations, the risk of developing type 1 diabetes (T1D) is increasing, which remains unexplained, and is postulated to occur as a result of environmental triggers. Recently, circulating advanced glycation end products (AGEs) and their receptor, receptor for AGEs (RAGE), have been shown to be altered in children prior to T1D diagnosis. The soluble RAGE (sRAGE) isoform is thought to bind RAGE ligands within the circulation. We aimed to reduce AGEs early in life in the NOD mouse model by delivering a short pulse of recombinant human sRAGE, and to determine the therapeutic effect on diabetes incidence, beta cell function and immune cell infiltration.

Materials and methods: Groups of female NOD mice were randomized to either vehicle (PBS) or recombinant human sRAGE (25 μg twice daily) via intraperitoneal injection from day 50-64 of life. To determine the effect of sRAGE therapy on the immune system, glucose homeostasis and diabetes incidence, mice were followed until 0 (n=6), 2 (n=14) or 22 (n=15) weeks post-treatment, respectively.

Results: Two weeks of sRAGE therapy afforded protection from diabetes at day 221 of life compared to mice given the vehicle control only (13% vs 53%; p=0.01). This reduction in diabetes associated with a progressive decrease in non-fasted ($p<0.0001$) and fasted blood glucose levels (p=0.02) after 22 weeks post sRAGE treatment compared to vehicle control. Although oral glucose tolerance tests revealed no change in the area under the glucose curve between groups (835 vs 899 mmol/L.120 min; p=0.19), there was an increase in the area under the insulin curve demonstrating an increase in insulin secretion (29.8 vs 37.1 ng/mL.120 min; p=0.02). Flow cytometry analysis confirmed an increase in splenic numbers of $CD11b^{+}CD11c^{+}CD8^{-}RAGE^{+}$ dendritic cells (2.7-fold; p=0.008), as well as classical ($F4/80^{+}CD11c^{-}Ly6C^{+}$; 2-fold; p=0.04) and non-classical ($F4/80^{+}CD11c^{-}Ly6C^{-}$; 1.9-fold; p=0.02) macrophages directly after sRAGE therapy compared to vehicle alone.

Conclusion: These results demonstrate for the first time that short-term, recombinant human sRAGE therapy prediabetes, has long lasting effects that improve insulin secretion and autoimmune diabetes incidence. The mechanism responsible for these improvements by sRAGE remain to be elucidated, but may involve changes to antigen presenting cells such as macrophages and dendritic cells.

Supported by: JDRF Innovative Grant

OP 35 Assembling adiposity: building fat tissue

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Intrinsic depot-specific differences in the angiogenic potential of ASC

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Background and aims: Fat body distribution is known to be an important factor for the development of metabolic and cardiovascular diseases. Regional differences include variation in the residing cell populations and the vasculature. Fat cell progenitors (adipose-derived stem cells, ASC) are considered one of the most abundant (15-50% of total cells) and important cellular population within adipose tissue (AT) because their active role in the modulation of tissue expansion and homeostasis, producing paracrine factors and metabolic signals. The expansion of AT is closely associated with the development of its vasculature and the regulation of AT angiogenesis is emerging as a promising therapeutic target in obesity. Our objective was to explore the role of ASC isolated from the two main adipose depots, subcutaneous and omental visceral fat, in the modulation of AT angiogenesis.
Materials and methods: ASC were isolated from omental (omASC) and subcutaneous (subASC) fat paired samples obtained from metabolically-healthy morbidly obese (ob, n=13, 6 M/7 F, BMI=43.3±5.1 kg/m^2, age: 33.4±9.5 years) and metabolically-healthy control individuals (n-ob, n=6, 5 M/1 F, BMI=28.7±2.7 kg/m^2, age: 43.7±10.5 years). ASC were in vitro expanded, and gene expression and protein levels were evaluated by real-time PCR and WB, respectively.
Results: The gene expression level of a set of pro-angiogenic and anti-angiogenic molecules was analyzed: VEGFA, VEGFB, VEGFC, TSP1, and TGFβ1. The genes encoding for VEGFB, TSP1 and TGFβ1 showed a similar expression pattern and expression levels in ob and n-ob individuals, being significantly up-regulated in subASC compared with omASC, with fold-changes ranging from 2 to 3 for VEGFB and TGFβ1, and reaching 10-fold up-regulation for TSP1, further confirmed at protein level. For VEGFA and VEGFC, we only observed depot-specific differences in ob individuals. The expression of VEGFA in subASC was 2.4-fold higher than in omASC, and 2-fold higher than in the counterpart form n-ob individuals. Moreover, correlation analysis revealed that the expression levels of VEGFA in omASC, but not in subASC, negatively correlated with serum cholesterol levels (r=-0.454, p=0.05). VEGFC was the only angiogenic factor with higher expression in omASC than in subASC, showing a slight (1.2-fold up-regulation) but significant (p=0.012) differential expression. A range of endothelial cell (EC) markers (CD105, vWF, VEGFR1, and VEGFR2) was explored. We did not find depot-specific differences in the expression of CD105 and vWF. Interestingly, high significant differences, and opposite pattern, were observed in the expression of VEGFR1 and VEGFR2, in both ob and n-ob. The expression of VEGFR1 was higher in subASC compared with omASC (2.55±1.96 arbitrary units (AU) vs 0.22±0.18 AU, p<0.001, ob; 1.33±0.57 AU vs 0.29±0.22 AU, p<0.01, n-ob), whereas VEGFR2 showed the opposite trend, moderate gene expression in omASC (1.76±1.40 AU, ob; 1.45±1.39 AU, n-ob) and almost undetectable expression in subASC, further confirmed at protein level. Unexpectedly, omASC did not show in vitro EC differentiation capacity.
Conclusion: The results show a more active role of subASC producing pro and anti-angiogenic paracrine factors than omASC. VEGFA and VEGFC appear as important factors in obesity. Due to the differential expression of VEGFR2 and VEGFR1, there is a depot-specific role for VEGFA in ASC that is independent of EC differentiation potential.
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The role of BMP2 in the pathophysiology of obesity

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Background and aims: Besides its known functions in the embryonic development the Bone Morphogenetic Protein 2 (BMP2) may affect adipogenesis. Our aim was to investigate the potential role of BMP2 in the pathophysiology of obesity and related traits and to uncover genetic variants determining the variability in circulating BMP2 levels.
Materials and methods: Circulating BMP2 levels were measured by commercially available ELISA in serum samples from subjects derived from two phenotypically well characterized cohorts: the Sorbs (n=638) and the Leipzig cohort (n=489). We measured *BMP2* mRNA expression by qRT-PCR in 632 paired samples of human subcutaneous and visceral adipose tissue. We also conducted a Genome Wide Association Study (GWAS) for circulating BMP2 in the Sorbs using the GenAbel Package of R for HapMap imputed genotypes generated by Affymetrix Gene chip arrays (500 K and 6.0) (n=580).
Results: BMP2 serum levels were significantly lower in obese (BMI> 30 kg/m^2, n=248) compared to lean subjects (BMI<25 kg/m^2, n=250; p <0.05 adjusted for age and sex). Consistently, they correlated negatively with waist circumference, Waist- to Hip- Ratio (WHR) and Waist- to-Height- Ratio (WHtR) (adj. p<0.05). Furthermore, *BMP2* mRNA expression in visceral and subcutaneous adipose tissue correlated positively with BMI (n=620; adj. p<0.001) and related traits like waist circumference (adj. p<0.001) and circulating blood lipids (n=110; adj. p<0.05). After adjusting for sex, age, BMI and inflation factor, 45 single nucleotide polymorphisms (SNPs) showed association with circulating BMP2 at p <1×10-5, including rs918604 (p=1.77×10-7) in RGS7BP (Regulator of G-Protein Signalling 7 Binding Protein), rs17111592 (p=6.5×10-7) in USP24 (Ubiquitin Specific Peptidase 24) and rs7799603 (p=1.75×10-6) in CNTNAP2 (Contactin-associated Protein-like 2) locus.
Conclusion: Our data suggests that BMP2 is related to the pathophysiology of obesity and highlights the role of genetic variation in the regulation of circulating BMP2 levels.
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Reduced SIRT1 and SIRT2 expression in visceral adipose stem cells may promote visceral fat expansion in human obesity

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Background and aims: Adipose tissue expands as a consequence of hypertrophy of preexisting fat cells and hyperplasia by recruitment of adipose stem cells (ASC) into the adipogenic program. SIRT1 and SIRT2 have been involved in regulation of adipocyte differentiation in rodent cells. In this study, we investigated the role of SIRT1 and SIRT2 in human adipocyte differentiation in obesity.
Materials and methods: Subcutaneous (Sc)- and visceral (V)-ASC were isolated from fat biopsies of 30 obese (Ob) and 30 non-obese (n-Ob) donors and differentiated in vitro. Cell cultures were analyzed by immunofluorescence with Nile Red/DAPI to quantify triglyceride-containing cells (adipogenesis) and Oil-Red-O staining followed by spectrophotometry to measure accumulated triglycerides (lipogenesis). mRNA

expression of marker genes for early (PPARγ, SREBP1c, C/EBPalpha) and terminal (FAS and adiponectin) phases of adipogenesis was evaluated after selective overexpression of SIRT1 or SIRT2 by adenoviral-mediated gene transfer in V-ASC. Assessment of SIRT1 and SIRT2 mRNA levels in human Sc and V fat biopsies from 149 donors with a wide BMI range was also carried out.

Results: No differences in either adipogenesis or lipogenesis were found in Sc-ASC from Ob vs. n-Ob donors. By contrast, both the number of triglyceride-containing cells and extent of triglyceride accumulation were ~2.0-fold higher in V-ASC from Ob compared to n-Ob donors ($p<0.05$). These cellular phenotypes correlated with changes in SIRT1 and SIRT2 mRNA and protein levels, which were not different in Sc-ASC from n-Ob and Ob, and 50% lower in V-ASC from Ob compared to n-Ob subjects ($p<0.05$). Noteworthy, the selective overexpression of SIRT1 or SIRT2 in Ob V-ASC resulted in downregulation of genes involved in early adipogenesis, such as PPARγ, SREBP1c, C/EBPalpha ($p<0.05$), as well as genes marking terminal adipocyte differentiation, such as FAS and adiponectin ($p<0.05$), and in a significant 45% decrease in both lipogenesis and adipogenesis ($p<0.05$). Finally, assessment of SIRT1 and SIRT2 mRNA levels in human Sc and V fat biopsies from 149 donors with a wide BMI range showed a significant negative relationship between SIRT1/SIRT2 expression in V fat and BMI, both in males ($R=-0.448$, $p<0.05$) and females ($R=-0.535$, $p<0.05$), while no correlation was found between SIRT1/SIRT2 expression in Sc fat and BMI.

Conclusion: Reduced SIRT1 and SIRT2 expression in V-ASC from obese individuals promotes enhanced lipogenesis and adipogenesis, representing a new mechanism for expansion of V fat in human obesity.

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TMEM18 is a regulator of adipogenesis and involved in PPARG signalling in vivo

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Background and aims: Polymorphisms in *TMEM18* are associated with obesity in children and adults. We have reported previously that *TMEM18* is a regulator of human adipogenesis *in vitro*. The aim of this study was to investigate the role of *TMEM18* during adipose tissue (AT) accumulation *in vivo*.

Materials and methods: We used the zebrafish as an *in vivo* model for AT development. In addition, we analysed *TMEM18* expression in whole AT samples, isolated adipocytes and cells of the stroma-vascular fraction (SVF) from lean and obese children of our Leipzig Childhood AT cohort, and addressed associations with *PPARG* expression and measures of obesity and glucose homeostasis.

Results: Using whole mount *in situ* hybridisation on 9 day old zebrafish larvae, we showed co-expression of *tmem18* and *pparg* in a visceral region where also first adipocytes are detectable by nile red staining. Morpholino-mediated inhibition of *tmem18* expression resulted in a reduction in the number of visceral adipocytes but did not affect zebrafish development *per se*. There was no effect of *tmem18* knockdown on eating behaviour suggesting that the inhibition of adipocyte formation was not mediated by a central effect. Tmem18-mediated inhibition of adipogenesis was accompanied by a significant down-regulation in *pparg* expression. Using luciferase reporter assays in 3 T3-L1 cells, we detected a significant activation of the *PPARG* promoter in presence of Tmem18 indicating that Tmem18 is an upstream regulator of *PPARG* signalling. In line with these data, *TMEM18* expression correlated with *PPARG* expression in whole AT and isolated adipocytes but not in SVF cells of children included in our Leipzig AT cohort. Similar to *PPARG*, *TMEM18* expression was down-regulated in adipocytes of obese children compared to lean children and correlated with adipocyte diameter, macrophage infiltration, serum adiponectin levels and HOMA-IR as a measure of insulin resistance.

Conclusion: Our findings may indicate a potential role of *TMEM18* as a regulator of *PPARG*-driven adipogenesis and obesity progression *in vivo*.

Clinical Trial Registration Number: NCT02208141
Supported by: BMBF IFB AdiposityDiseases

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The impact of birth weight on epigenetic and transcriptional regulation of adipose-derived stem cells

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Background and aims: Low birth weight (LBW) is associated with dysfunctions of adipose tissue contributing to an elevated risk of developing metabolic diseases in adulthood. We hypothesized that altered epigenetic and transcriptional regulation of adipose-derived stem cells (ADSCs) could play a role in programming adipose tissue dysfunctions in LBW subjects.

Materials and methods: ADSCs were isolated from adipose tissue of 13 normal birth weight (NBW) and 13 LBW adult males. Global DNA methylation and gene expression patterns were analysed in cultured ADSCs during proliferation and after differentiation into mature adipocytes.

Results: Immature ADSC from LBW subjects exhibit differential expression of 506 gene transcripts after correction for multiple testing, including genes involved in spliceosomal function and cell cycle regulation. Principal component analyses confirmed that the transcriptional and epigenetic profiles differed more between the birth weight (BW) groups in the immature ADSC compared with only subtle differences in the mature adipocytes. Although differentiation of ADSCs into matures adipocytes was associated with multiple and within the BW groups converging changes in gene expression and DNA methylation, the dynamics of specific gene groups varied. Construction of gene interaction networks suggest a key role for the transcription factor CCNT2 in the diverse gene regulation patterns seen between BW groups during adipocyte differentiation.

Conclusion: Our findings support the notion that the differential epigenetic and transcriptional changes in LBW subjects are most pronounced in immature ADSCs that in turn may program more permanent structural and physiological characteristics of the mature adipocytes influencing risk of metabolic diseases.

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Increased brown fat and insulin sensitivity in obese mice overexpressing WISP2 in the adipose tissue

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Background and aims: WISP2 is highly expressed in, and secreted by, mesenchymal precursor cells. It is an important early regulator of mesenchymal precursor cell commitment to the adipogenic lineage through dual effects; by forming a BMP4-regulated complex with the PPARγ

transcriptional activator ZFP423 in the cytosol preventing its nuclear translocation and; as a secreted protein activating the canonical WNT pathway and inhibiting differentiation of the precursor cells. To examine the in vivo effect of secreted WISP2 by the adipose tissue we generated a transgenic (Tg) mouse overexpressing WISP2 under an aP2 promoter.

Materials and methods: The mice were fed a control or high fat diet (HFD) for 17 weeks. Metabolic phenotyping included hyperinsulinemic euglycemic clamps combined with [C14] 2-deoxyglucose uptake and body composition analysis with DEXA. Samples were collected for blood chemistry and several tissues were analyzed with immunohistochemistry, protein and gene profiling. Conventional statistical methods were used to analyse results.

Results: Tg mice on HFD showed unchanged body weight compared to wildtype (wt) mice but had markedly increased and hypercellular brown adipose tissue (BAT) (weight increased≈100%, $p<0.05$), hypercellular white adipose tissue with smaller cells (42% increase in number of cells; $p<0.05$) and increased lean body mass (≈8% $p<0.05$). In spite of same body weight, Tg mice were markedly more insulin-sensitive, verified by hyperinsulinemic euglycemic clamps (GIR +237% at steady-state, $p<0.01$) and had increased glucose uptake by the adipose tissue and skeletal muscle, increased adipose tissue adiponectin mRNA and circulating levels (150%, $p<0.01$) and increased GLUT4 in both adipose tissue and skeletal muscles (≈140% $p<0.05$). Insulin-stimulated glucose uptake ex vivo was also increased in adipose cells and skeletal muscle (≈160%, $p<0.05$). Interestingly, de novo lipogenesis, known to be elevated when GLUT4 is increased in the adipose tissue, and serum levels of the novel fatty acid-hydroxy fatty acids (FAHFAs) were also increased in Tg compared to wt mice on HFD (≈175% $p<0.05$). The mesenchymal tissue hypercellularity suggests that WISP2 increases mesenchymal precursor cell growth and this was also verified in vivo in BAT. Furthermore, serum from Tg mice increased the proliferation of both BAT precursor cells and the mesenchymal stem-like CH3T101/2 cells and this effect was inhibited by adding specific anti-WISP2 monoclonal antibodies to the serum.

Conclusion: WISP2 is a novel circulating adipokine which targets mesenchymal precursor cells leading to increased lean body mass, hypercellular BAT and hyperplastic healthy white adipose tissue with increased GLUT4 and circulating levels of adiponectin. Lipogenesis is increased leading to higher serum levels of the novel FAHFAs and improved insulin sensitivity. Thus WISP2 is a promising target to prevent obesity-related complications and Type 2 diabetes.

Supported by: Research Council, Torsten Söderberg, Novo Nordisk Foundation, EFSD

OP 36 Diabetic neuropathy: from top to toe

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A multi-national normative dataset for corneal nerve morphological parameters with using corneal confocal microscopy for early diagnosing diabetic neuropathy

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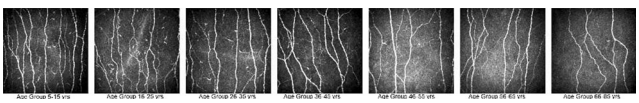
Background and aims: Corneal confocal microscopy (CCM) is a novel diagnostic technique for the detection of nerve damage and repair in a range of peripheral neuropathies, in particular diabetic neuropathy. To enable clinical translation and wider use of this technique, normative reference values need to be defined. We have therefore undertaken a multicenter collaboration to provide worldwide age adjusted normative values of corneal nerve fibre parameters.

Materials and methods: 1965 corneal nerve images from 343 healthy volunteers were pooled from six clinical academic centers across the world (Manchester, Brisbane, Calgary, Düsseldorf, Utah and Toronto). All subjects underwent examination with the Heidelberg Retina Tomograph corneal confocal microscope. Images of the sub-basal nerve plexus were acquired by each centre using a standard protocol and analyzed by manually with using Semi-automated software (CCMetrics) and also by automated software (ACCMetrics). Age-trends were established using simple linear regression, and normative corneal nerve fiber density (CNFD), corneal nerve fiber branch density (CNBD), corneal nerve fiber length (CNFL) reference values were calculated using quantile regression analysis for each method of measurements.

Results: There was a significant linear age-dependent decrease in CNFD (-0.164 no/mm2 per year for men, $P<0.01$ and -0.161 no/mm2 per year for women, $P<0.01$) that determined with both analysing systems. There was no change with age in CNBD (+0.192 no/mm2 per year for men, $P=0.26$ and -0.050 no/mm2 per year for women; $P=0.78$). CNFL decreased in men (-0.045 mm/mm2 per year, $P=0.07$) and women (-0.060 mm/mm2 per year, $P=0.02$). Height, weight and BMI did not influence the 5th percentile normative values for any corneal nerve parameter. Significant correlations for CNFL, CNBD, CNFL were observed between 2 methods of measurements.

Conclusion: This study provides robust worldwide normative reference values for corneal nerve parameters to be used in research and clinical practice in the study of diabetic and other peripheral neuropathies.



Supported by: NIH/ JDRF

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Obesity related neuropathy and the effects of bariatric surgery

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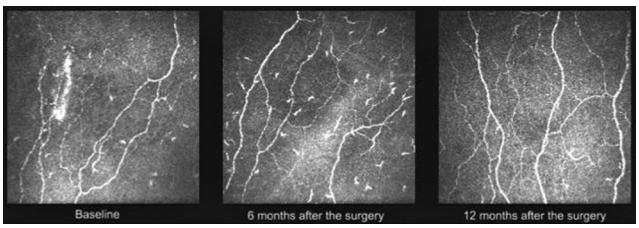
Background and aims: Obese subjects have risk factors for the development of neuropathy. Bariatric surgery normalises these risk factors, however its benefits on neuropathy have not been assessed.
Materials and methods: 50 morbidly obese subjects and 30 age-matched controls underwent assessment of: sural nerve conduction velocity (SNCV) and amplitude (SNAP), peroneal nerve conduction velocity (PMNCV) and amplitude (PNA), Vibration Perception Threshold(VPT), cold(CT) and warm(WT) threshold, corneal nerve fibre density(CNFD), branch density (CNBD) and fibre length(CNFL). 19 subjects were re-assessed 6 months post-bariatric surgery
Results: Obese subjects had a higher BMI (50.5±2.2 v 27.9±1.9, P< 0.000), HbA1c (55.1±4.4 v 38.5±0.8, P=0.002) with no difference in blood pressure (mmHg)(133.2±4.7/73.0±2.6 v 125.8±2.9/74.1±2.4, P= 0.69/0.37). They had a significant reduction in SNAP (mV)(11.4±1.4 v 20.5±1.9, P<0.001), PNA (mV)(3.65±0.3 v 5.5±0.42, P<0.001), PMNCV (m/s)(45.7±0.8 v 47.8±2.0, P=0.005) and CT (0C)(24.1±1.4 v 28.7±0.3, p=0.02) with an increase in WT (0C)(40.0±0.7 v 36.7±0.79, P=0.02). CNFD (no/mm2)(23.3±1.3 v 37.7±2.0,p<0.000), CNBD(no/mm2)(32.0±3.1 v 37.9±2.6, p<0.000) and CNFL (mm/mm2)(14.2±0.6 v 26.9±2.0,p<0.000) were significantly reduced. Paradoxically, CNBD (38.5±5.7 v 23.9±2.8, p=0.03) and CNFL (15.1±1.1 v 13.2±0.5,p= 0.05) were significantly lower in the non-diabetic (n=35) compared to the diabetic (n=14) group. In the 19 subjects assessed post-operatively at 6 months, BMI (52.2±3.5 v 34.6±2.1, P=0.004), BP (112±2.3/71.5± 0.4, P=0.03), CIP (7.3±1.6 v 16.5±4.0, P=0.05) and CNFD (23.0±1.6 v 25.3±0.9, P=0.05) and (CNFL (14.3±1.0 v 16.0±1.0, P=0.005) improved with no change in other parameters
Conclusion: Obese subjects have large and small fibre neuropathy. Paradoxically those with diabetes have less severe small fibre damage, presumably driven by factors other than hyperglycaemia. Bariatric surgery improves small fibre neuropathy as shown by CCM within 6 months.



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Disrupted resting-state attentional networks in type 2 diabetes mellitus patients

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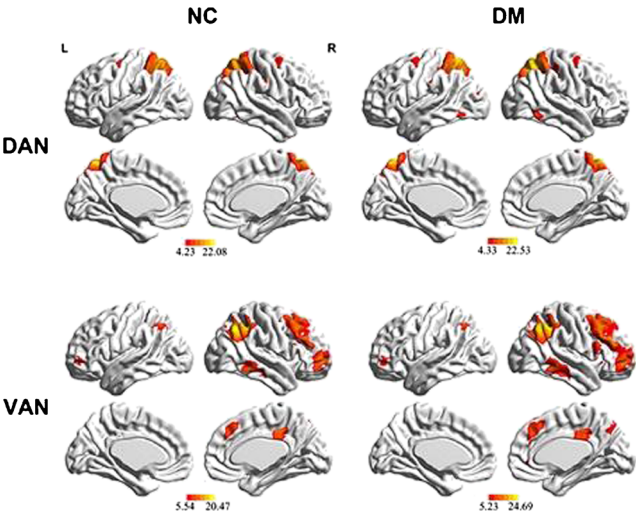
Background and aims: Although Type 2 diabetes mellitus (T2DM) is a well-recognized risk factor for dementia, the neural mechanisms that underlie cognitive impairment in T2DM remain unclear. In addition to memory deficits, attention is compromised in patients with T2DM. Our study aims to examine attentional network alterations in T2DM patients and their relationships to impaired cognitive performance using resting-state functional magnetic resonance imaging (fMRI).
Materials and methods: For the first time, data-driven independent component analysis was applied to resting-state fMRI data from 38 T2DM patients and 32 healthy controls to identify the dorsal attention network (DAN) and ventral attention network (VAN). Correlations were then determined among the resting-state functional connectivity (rsFC), clinical data, and neuropsychological scores.
Results: Typical significant spatial patterns of the DAN and VAN in both the controls and T2DM patients were revealed (p<0.01, corrected;figure). When compared with the healthy controls, the T2DM patients exhibited decreased rsFC in the left middle frontal gyrus (MFG) and bilateral inferior parietal lobe (IPL) of the DAN, as well as the left IPL and right MFG/IFG of the VAN. In addition, the rsFC of the left MFG was inversely correlated with the Trail Making Test-B scores (r=-0.533, p=0.001); the rsFC of the left IPL was positively correlated with the Digit Span Test scores but negatively correlated with HbA1c (r=0.403, p=0.012; r=-0.370, p=0.022, respectively).
Conclusion: T2DM patients exhibit disrupted functional integration within attention-related brain networks. Several core regions in the DAN, especially the IPL, likely underlie the attentional deficits in T2DM patients. We suggest T2DM affects resting-state attentional networks, which may be related to reduced attention and a hyperglycemic state. Our data may potentially support resting-state fMRI as a promising tool to detect attentional changes in T2DM and provide new insights into the neural deficits present in T2DM patients.



Clinical Trial Registration Number: ChiCTR-ONRC-13003095
Supported by: WS; XW

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Thalamic neuronal dysfunction and patterns of sensory loss in diabetic neuropathy: a proton magnetic resonance spectroscopy study

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Background and aims: Although we have previously demonstrated thalamic neuronal dysfunction in diabetic neuropathy (DN) using proton magnetic resonance spectroscopy (1H-MRS), the relationship between quantitative sensory testing of large and small fibre function (QST) and thalamic neuronal dysfunction is not known.
Materials and methods: 37 subjects with T1DM (12 No-DN and 25 DN) and 14 healthy volunteers (HV) underwent QST using the German DFNS protocol. All subjects were right handed and aged between 45 and 60 years (with no significant difference between the groups).1H-MRS examination was performed at 3 T (Ingenia, Phillips Netherlands). Single

voxel spectra were obtained from a 2.25 cm3 (15×10×15mm) cubic volume of interest within the L. thalamus. TE=135 ms, TR=1600 ms, NSA=256 using point resolved (PRESS) technique. Fitted metabolite area ratios were calculated for choline (Cho) at 3.2 ppm, Creatine (Cr) at 3.0 ppm, and N-Acetyl Aspartate (NAA) at 2.02 ppm, and mean area under the curve values were compared. ANOVA was performed for group means and correlations between QST parameters and 1H-MRS ratios were calculated using Pearson's correlation.

Results: Subjects with DN had significantly lower NAA:Cr [1.74(0.22)] compared to HV [1.96(0.3)] and No-DN [1.90(0.26); ANOVA p=0.036]. There was a significant negative correlation between NAA:Cr and small fibre assessments of heat pain threshold r=-0.287(p=0.04) and warm detection thresholdr=-0.289 (p=0.043). There was no significant correlations between assessments of large fibre dysfunction and NAA:Cr.

Conclusion: The thalamus plays an important role in processing ascending somatosensory inputs to the cerebral cortex. Reduction in NAA:Cr as assessed using 1H-MRS indicates abnormal thalamic neuronal function in DN. In this preliminary study we have demonstrated that thalamic neuronal dysfunction relates to abnormalities of small fibre function on detailed QST. NAA ratios associated with changes in the thalamus may be linked to specific patterns of sensory abnormalities. Further investigation of the relationship between these findings and underlying structural and perfusion abnormalities, may provide a physiological mechanism for this novel finding.

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Vibration sensitivity violation evaluation for diabetic distal polyneuropathy early diagnosis in patients with type 2 diabetes

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Background and aims: Distal sensorimotor polyneuropathy (DSPN) is a severe complication of type 2diabetes. According to the German National Disease Management Guidelines for neuropathy adults with type 2 diabetes mellitus (T2DM) should be screened for distal sensorimotor polyneuropathy (DSPN) at diagnosis set up and yearly thereafter. This study aimed to assess the prevalence of vibration sensitivity disorders in T2DM patients using Vibratip device which can be used as an alternative test for vibration sensation evaluation for DSPN early diagnosis.

Materials and methods: 4303 patients with T2DM (2757 women, 1546 men, average age: 59,2±10,4 years, average body mass index (BMI) 32,2 ±5,6 kg / m2) were examined. The study was conducted within the framework of the campaign "Approach Optimization in Diabetic polyneuropathy Early Diagnostics and Prevention Improvement". All participants with inclusion criteria (T2DM duration≤1 year with average duration of DM: 0,8±0,3 years) had a clinical examination and were examined with Vibratip on both feet. Vibratip is new device using standardized vibration for DSPN detection. Vibratip is applied to the patients feet, testing 2 sites on both feet (1st metatarsal head on the plantar surface and hallux pump)-once whilst non-vibrating and once whilst vibrating and the patient (with their eyes closed) is asked to indicate when they feel the vibration. Vibration sensitivity disorder was defined in condition when patients were answering twice "yes" to the question, "Are you able to feel vibration on any site of both feet during the test?"

Results: Clinical symptoms of DSPN were prevalent in 3781 (88%) participants with T2DM, 775 (18%) of whom were suffered from pain, 706 (16.4%) - from burning, 1144 (26.6%) - numbness, 921 (21.4%) - feeling of pins and needles, 235 (5.5%) - feeling of electric shock. Patients with clinical symptoms such as numbness (26.6%) and feeling of pins and needles (21.4%) had the highest prevalence of clinical impairment of feet. Vibration sensitivity disorders feet were found in 24,1% (1036) of diabetic patients: 15,3% (656) women, 8,8% (380) men using Vibratip device.

Conclusion: Our findings showed a high prevalence of vibration sensitivity disorders among patients with T2DM with disease duration≤1 year using Vibratip device, suggesting inadequate attention to diabetic foot prevention practice.

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Oxidative and carbonyl stress in relation to polyneuropathy in subjects with recently diagnosed type 1 and type 2 diabetes

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Background and aims: Oxidative stress resulting from enhanced free-radical formation, reduced antioxidative defense, and reactive carbonyls which react with proteins to form advanced glycation endproducts (AGEs) have been implicated in the pathogenesis of diabetic neuropathy. We sought to determine whether biomarkers of systemic oxidative stress, defective antioxidative defense, and carbonyl stress are altered in relation to diabetic sensorimotor polyneuropathy (DSPN) in recently diagnosed diabetes patients.

Materials and methods: We assessed serum concentrations of thiobarbituric acid reactive substances (TBARS) as a marker of oxidative stress, extracellular superoxide dismutase (SOD3) and reduced glutathione (GSH) as markers of antioxidative defense, and methylglyoxal as a marker of carbonyl stress in 111 type 1 and 223 type 2 diabetic subjects from the baseline cohort of the German Diabetes Study (GDS) (type 1/type 2 diabetes (T1D/T2D): age: 34.3±13.0/52.8±11.0 [SD] years; male: 57/65%; BMI: 24.6±4.1/31.7±6.0 kg/m^2, diabetes duration: 7.2±3.2/6.1±3.0 months; HbA1c: 6.8±1.4/6.5±1.0% [50.5±15.2/47.3±10.4 mmol/mol]) and 52 healthy control subjects (age: 40.3±16.4 years; male: 56%). Neurophysiological and clinical measures included motor and sensory nerve conduction velocity (MNCV, SNCV), Neuropathy Symptom Score (NSS), and Neuropathy Disability Score (NDS). DSPN was defined by electrophysiological and clinical criteria (Toronto Consensus, 2011). All comparisons were adjusted for sex, age, and BMI.

Results: TBARS levels were higher in T1D (3.1±2.0 μM) and T2D (3.2±1.8 μM) than in controls (0.9±0.3 μM) as was methylglyoxal (T1D: 404±150, T2D: 412±160, controls: 154±69 nM, while SOD3 concentrations were reduced in T1D (38.9±14.9 ng/ml) and T2D (32.7±16.3 ng/ml) compared to controls (52.4±16.6 ng/ml) as was GSH (T1D: 1.6±0.6, T2D: 1.7±0.8, controls: 2.7±1.2 μM) (P< 0.0001 for T1D and T2D vs controls for all markers). Subclinical and clinical DSPN confirmed by nerve conduction studies were present in 21% of subjects with T1D and 29% of those with T2D. Multiple linear regression analysis revealed that low SOD3 levels were associated with the presence of DSPN in both T1D subjects (ß=-0.319, P=0.002) and those with T2D (ß=-0.151, P=0.030), while low methylglyoxal concentrations were associated with DSPN in T2D subjects (ß=-0.153, P<0.022). No association with DSPN was noted for TBARS and GSH.

Conclusion: Patients with recently diagnosed T1D and T2D show evidence of systemic oxidative and carbonyl stress despite good glycaemic control, but only lower SOD3 concentrations in both T1D and T2D and lower methylglyoxal levels in T2D were linked to the presence of polyneuropathy. The predictive value of these markers for the development and progression of diabetic neuropathy remains to be determined in prospective studies.

Clinical Trial Registration Number: NCT01055093
Supported by: Ministry of Science/Research NRW (MIWF NRW), German BMG, BMBF to DZD e.V.

OP 37 Oldies but goodies? Update on traditional anti-diabetic drugs

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Sulfonylurea is still useful in type 2 diabetic patients with sulfonylurea failure

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Background and aims: A large number of type 2 diabetic patients cannot achieve good glycemic control with maximum dosage of sulfonylurea (SU), so called sulfonylurea failure (SUF). Some guidelines suggest to continue SU whereas some suggest to stop SU once insulin therapy is started in patients with SUF. This study was aimed to evaluate whether SU is still useful in patients with SUF.

Materials and methods: Type 2 diabetic patients who had A1c≥8% while receiving recommended maximum dosage of SU and metformin were studied. Beta-cell function and insulin resistance status were assessed by plasma glucose and insulin response to oral glucose tolerance (OGTT) method. OGTT were performed 3 times including while receiving maximum dose of SU (max-SU), after discontinuation of SU for 4 weeks (discont-SU), and at 2 weeks after restarting the same SU at 25% of max-SU (25%-SU). During each OGTT, plasma insulin and glucose levels were measured at 0, 30, 60, 90 and 120 minutes. Beta-cell function was assessed by area under the curve (AUC) of plasma insulin divided by AUC of plasma glucose. Insulin sensitivity and insulin resistance were assessed by Matsuda index and HOMA-IR, respectively. The patients were assigned to continue the same dosage of metformin throughout the study and to measure fasting capillary blood glucose at home (CBG) at least 3 times per week to assess glycemic control and for safety monitoring during the intervals between OGTT.

Results: Twenty-five patients with SUF were recruited. Median duration of diabetes was 12 years (range, 4-38). Beta-cell function during max-SU (median 0.1, range 0.02-0.56) was significantly higher than that of during discont-SU (0.06, range 0.01-0.22), ($p<0.001$) and also higher but not significant ($p=0.269$) than that of during of 25%-SU (0.09, range 0.01-0.32). Beta-cell function was also higher during 25%-SU (0.09, range 0.01-0.32) compared with during discont-SU (0.06, range 0.01-0.22), ($p<0.001$), suggesting that SU is still able to stimulate insulin secretion in SUF in dose dependent manner. Among the 3 OGTT, there was no significant different in Matsuda index (OGTT-1 1.97±1.14, OGTT-2 1.93 ±1.1 and OGTT-3 1.98±1.31, $p=0.759$) and HOMA-IR (OGTT-1 11.48 ±8.17, OGTT-2 12.13±6.7 and OGTT-3 11.24±7.42, $p=0.179$), suggesting that SU has no significant effect on insulin sensitivity or insulin resistance. For the glycemic control, the mean CBG measured during max-SU and 25%-SU were lower than that of during discont-SU (186.2 ±43.5 vs. 222.3±60.6 vs.256.1±66.7 mg/dL, respectively, $p<0.05$).

Conclusion: Discontinuation of SU results in a decrease in insulin secretion and worsening glycemic control. The findings lead to the clinical implication that SU is still useful and should be continued, if not contraindicated, in diabetic patients with SUF who need insulin therapy.

Supported by: Siriraj Research Development Fund (Managed by Routine to Research: R2R)

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The OCT1 reduced-function polymorphisms are associated with common metformin-induced gastrointestinal side-effects

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Background and aims: Metformin is the first-line drug for treatment of type 2 diabetes, and the most widely used antidiabetic agent. However, 20-30% patients treated with metformin develop gastrointestinal (GI) side-effects, which may result in suboptimal drug dosage and non-adherence. About 5% of patients are not able to tolerate metformin due to severity of these GI symptoms. The mechanism of GI side-effects, as well as their considerable interindividual variability, is unknown. We have recently shown that organic cation transporter 1 (OCT1) polymorphisms are associated with severe intolerance to metformin, namely the discontinuation of metformin and switching to another oral hypoglycaemic agent in the first months of metformin treatment. The aim of this study was to examine the association OCT1 reduced-function alleles with common metformin-induced GI adverse effects in T2D patients.
Materials and methods: This prospective observational study included 92 newly diagnosed T2D patients who were prescribed metformin as their initial hypoglycaemic therapy. Patients were monitored during the first six months of metformin treatment for development of adverse GI outcomes. Two common loss-of-function variants in the OCT1 gene (*SLC22A1*), R61C (rs12208357) and M420del (rs72552763) were genotyped using TaqMan genotyping assays. The association of OCT1 reduced-function diplotypes with GI side-effects was analysed using logistic regression. Age, gender, weight, and the concomitant use of medications known to inhibit OCT1 activity, were added as covariates.
Results: A total of 43 patients (47%) experienced GI side-effects during first six months of metformin treatment. The effects were ranging from mild/moderate to severe GI symptoms. The most frequent symptom was diarrhoea. Female gender and lower weight showed a trend of association with the presence of adverse effects. Interestingly, the number of OCT1 reduced-function alleles was significantly associated with over two-fold higher odds of metformin-induced GI side-effects (OR=2.31, 95% CI 1.07-5.01, p=0.034).
Conclusion: In this study we showed for the first time the association of OCT1 reduced-function variants with the incidence of common metformin-induced GI side-effects in T2D patients. Our results confirm the recent findings related to the severe cases of metformin intolerance, and suggest that a high interindividual variability seen in mild to moderate, as well as severe GI intolerance, share common underlying mechanism, despite considerably different intensity and duration of GI symptoms. Importantly, our data could lead to more personalised and safer treatment with metformin.
Supported by: Federal Ministry of Education and Science B&H, EFSD Albert Renold Fellowship

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The acute glucose-lowering effect of metfomin in patients with type 2 diabetes is partly glucagon-like peptide-1-dependent

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Background and aims: Metformin has been suggested to increase glucagon-like peptide-1 (GLP-1) secretion. By the use of the GLP-1 receptor antagonist exendin 9-39 (Ex9-39), we evaluated whether the acute effect of metformin on postprandial glucose excursions is GLP-1-dependent.
Materials and methods: Twelve patients with type 2 diabetes (age: 60.8±8.8 years (mean ± SD); BMI: 29.8±3.0 kg/m^2; HbA_{1c}: 6.5±0.5% (48±6 mmol/mol)) were included in this placebo-controlled, double-blinded study. On 4 separate days, after 1-week washout of antidiabetic drugs, the patients received metformin (1.5 g) or placebo suspended in a 302-kcal liquid meal with 1.5 g paracetamol (for evaluation of gastric emptying) with i.v. infusion of Ex9-39 (450 pmol×kg body weight^{-1}×min^{-1}) or saline. At baseline and during 240 min blood was sampled, gallbladder volume was evaluated by ultrasound and appetite was evaluated by visual analogue scale. At the end of each day ad libitum food intake was measured.
Results: On the 2 days of Ex9-39 infusion, mean basal concentrations of plasma glucose were higher compared with the 2 days of saline infusion (9.8±0.58 mmol/l vs. 8.7±0.52 mmol/l, $p<0.05$). Metformin + saline increased plasma GLP-1 incremental AUC (iAUC) compared with placebo + saline (1985±365 pmol/l×min vs. 1156±244 pmol/l×min, $p<0.05$). Ex9-39 increased GLP-1 iAUC after both metformin and placebo, but GLP-1 iAUC remained significantly higher with metformin compared to placebo (3218±563 pmol/l×min vs. 1798±333 pmol/l×min, $p<0.05$). Metformin + saline reduced the postprandial plasma glucose iAUC by 58% vs. placebo + saline (145±68 mmol/l×min vs. 350±73 mmol/l×min, $p< 0.05$), and while the difference was still significant, metformin + Ex9-39 only reduced iAUC by 25% vs. saline + Ex9-39 (467±112 mmol/l×min vs. 614±124 mmol/l×min, $p< 0.05$). C-peptide:glucose ratios were significantly higher after metformin + saline vs. placebo + saline (18225±2703 pmol/mmol × min vs. 12345±1766 pmol/mmol×min, $p<0.05$) whereas the difference was no longer significant with Ex9-39 infusions (13233±3243 pmol/mmol×min vs. 9863±2505 pmol/mmol×min, $p=0.07$). Metformin did not affect gastric emptying, plasma cholecystokinin concentrations and gallbladder contraction vs. placebo, irrespective of Ex9-39. None of the interventions affected appetite perceptions or ad libitum food intake.
Conclusion: We show that acute administration of metformin increases plasma GLP-1 secretion and reduces postprandial plasma glucose excursions vs. placebo, and that a substantial part of the glucose-lowering effect appears to be GLP-1-dependent.
Clinical Trial Registration Number: NCT02050074

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Metformin, methylmalonic acid and the risk of neuropathy: a randomised placebo-controlled trial

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Background and aims: Metformin lowers serum vitamin B12 (B12) and increases serum methylmalonic acid (MMA), the gold standard

biomarker for tissue B12 deficiency. However, the clinical relevance of metformin-associated decreases in B12 is still controversial, since relevant clinical outcomes are lacking. Current guidelines mention B12 deficiency as a disadvantage of metformin, but do not yet give recommendations on the detection and prevention of B12 deficiency, waiting for more clinical evidence. Therefore, we studied whether the increase in MMA was associated with the onset or deterioration of neuropathy.

Materials and methods: In the HOME trial, 390 insulin-treated patients with type 2 diabetes were treated with 850 mg metformin or placebo up to three times daily for 52 months. We analyzed the association between metformin and changes in HbA1c, MMA and the Valk Score, a validated neuropathy score. We used structural equation modeling (SEM) analysis to estimate the mediation effects of MMA and HbA1c in the total effect of metformin on neuropathy score. We excluded patients with B12 deficiency at baseline or with B12 supplementation (n=15).

Results: In mixed model analysis, metformin, as compared to placebo, was associated with an increase of MMA at the end of the study (0.04 μmol/L, 95%CI 0.02 - 0.06, p=0.001). There was no significant difference in neuropathy score after 52 months between placebo (increase from 0.8±2.2 to 2.2±1.6) and metformin (1.1±2.1 to 3.7±1.3; ANCOVA: 0.03; 95% CI -0.03 - 0.08, p=0.41). However, SEM analysis showed that the effect of metformin on the neuropathy score consisted of a beneficial effect through lowering HbA1c (-0.02 per gram year of metformin) and an adverse effect through increasing MMA (0.04 per gram year). The model satisfied the goodness-of-fit test (χ^2=4.0; df=2, p=0.13; normated fit index=0.98). In addition, during the study, MMA did not differ significantly between treatment groups when stratified for B12 concentration, showing that metformin did not affect the biological relation between B12 and MMA.

Conclusion: Metformin not only reduces B12, but also progressively increases MMA. The increase of MMA in metformin users was associated with significant worsening of the neuropathy score. Furthermore, metformin did not affect the biological relation between B12 and MMA. These results indicate that metformin-related B12 deficiency is clinically relevant. Monitoring of B12 and, when available, MMA, in users of metformin should be considered.

Clinical Trial Registration Number: NCT00375388

OP 38 Bugs in the belly: microbiota and endotoxaemia

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Differential adaptation of human gut microbiota to weight loss and diabetes remission achieved by gastric bypass versus sleeve gastrectomy

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Background and aims: Bariatric surgery achieves weight loss and remission of type 2 diabetes through many mechanisms, potentially including modification of gut microbiota. By comparing human gut microbiota changes after two types of bariatric surgery: gastric bypass (GBP) and sleeve gastrectomy (SG), with similar metabolic outcomes and food intake at 1 year, we aim to examine the gut microbiota changes which differ by type of surgery and those that correlate with diabetes remission status achieved by both surgeries.

Materials and methods: Whole-metagenome shotgun (WMS) sequencing of genomic DNA fragments using Illumina HiSeq2000 was obtained from stool samples collected from 14 obese patients with type 2 diabetes pre-operatively and 1 year after either SG (n=7) or GBP (n=7) as part of a randomised, blinded clinical trial in which metabolic outcomes, including body composition, resting energy expenditure (REE), glycaemia, inflammatory markers, and food diaries were collected. Post-operative diabetes status was defined as partial remission (PR) or complete remission (CR) if HbA1c was ≤48 mmol/mol or ≤38 mmol/mol respectively and off glucose lowering therapy. Resulting shotgun reads were annotated with Kyoto Encyclopaedia of Genes and Genomes (KEGG).

Results: There were similar reductions in body weight (27.4 kg [SEM 5.3] v 23.5 kg [SEM 5.4], p=0.48), BMI (9.9 [SEM 1.8] v 7.6 [SEM 1.6], p=0.29) and REE (240 kcal [SEM 44] v 198 kcal [SEM 72], p=0.57) after GBP compared to SG. Estimated caloric intake and dietary components (fibre, fat, carbohydrate, protein) were similar between GBP and SG at baseline and 1 year. Diabetes remission occurred to a greater extent after GBP (4 CR, 1 PR) than after SG (1 CR, 4 PR). A greater change in taxonomy of gut microbiota was observed after GBP (3 major phyla differences) than after SG (no major phyla difference, only increase in *Bacteroidia* species). A significant increase in *Roseburia* species was found among those achieving diabetes remission after both GBP and SG. Functional analysis of gut microbiota metabolism by KEGG Orthology revealed a greater number of significant changes in KEGG gene function among those achieving diabetes remission after GBP (15 higher and 13 lower) than after SG (9 lower and 9 higher), and similarly for KEGG pathways (9 altered after GBP and 4 altered after SG). There were no common KEGG gene functions or pathways that were significantly altered among those achieving diabetes remission after both SG and GBP.

Conclusion: A greater proportion of gut microbiota are altered following GBP than SG surgery, likely reflecting the greater alteration to the gut ecological system achieved by GBP. The association of greater *Roseburia* species among those achieving diabetes remission after both GBP and SG supports a causal link with metabolic benefits.

Clinical Trial Registration Number: NCT01486680

Supported by: Maurice Wilkins Centre

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Does experimental metabolic endotoxaemia worsen high-fat diet-induced insulin resistance and obesity?

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Background and aims: Obesity and type 2 diabetes are metabolic diseases which have reached epidemic proportions worldwide. According to World Health Organization, about 347 million adults have diabetes and around 1.6 billion are overweight (400 million of them are obese). These metabolic disorders are associated with a low grade inflammation whose molecular origin is still unknown. Previous studies have highlighted the involvement of the gut microbiota and lipopolysaccharides (LPS), which are components of the cell wall of Gram (-) bacteria. It has been shown that a slight increase of LPS plasma levels, metabolic endotoxemia, could mimic the effect of a very high-fat diet and initiate insulin resistance and obesity. We have recently shown that LPS increase glucagon-like peptide 1 (GLP-1) plasma levels, a hormone which is known to stimulate insulin secretion. Our present work deals with i) the molecular mechanisms involved in this newly described pathway and ii) the physiological consequences of this LPS/GLP-1/Insulin cascade during an obesogenic diet.
Materials and methods: Molecular mechanisms are investigated through in vivo, ex vivo and in vitro experiments to study the effects of LPS on synthesis/secretion/degradation of GLP-1. The long-term outcomes of the induced metabolic endotoxemia are explored by a continuous infusion of LPS (300 μg/kg/day). Osmotic pumps are implanted intraperitoneally in high-fat fed mice for one or two months.
Results: In vivo, measurements of total and active GLP-1 plasma levels show that the increase of GLP-1 upon LPS stimulation is due to enhanced secretion (x3.91 compared to NaCl control group, $p<0.001$) rather than inhibition of its degradation. Stimulation of ileum explants or enteroendocrine cultured cells by LPS leads to an increased GLP-1 secretion suggesting a direct effect of LPS. This effect is mediated through toll-like receptor 4 (TLR4). Indeed, pharmacological (TLR4 antagonist) and genetical (TLR2/TLR4 KO mice) experiments suppress GLP-1 secretion. Very surprisingly, experimental metabolic endotoxemia induced by a continuous infusion of LPS for 30 days leads to improved metabolic consequences of an obesogenic diet. Compared to NaCl control mice, LPS-infused mice show a significant reduced fat mass measured by EchoMRI® (NaCl: 16.9%±1.3% ; LPS: 9.7%±0.8%, $p<0.001$). Hepatic lipid content estimated by Oil Red O staining indicate a significant decrease in LPS-treated mice ($p<0.001$). Finally, glucose metabolism and insulin sensitivity are also improved in LPS-treated mice as shown by an oral glucose tolerance test (aera under curve: NaCl: 819.7±22.2; LPS: 698.6±39.5, $p<0.01$) and an insulin tolerance test (aera under curve: NaCl: 216.5±10.1; LPS: 184±7.3, $p<0.05$).
Conclusion: We demonstrate for the first time that LPS modulate the secretion of GLP-1 through a direct regulation of enteroendocrine cells by a TLR4 pathway. In addition, our data suggest that 30 days of experimental metabolic endotoxemia does not worsen high-fat diet-induced insulin resistance and obesity but rather seem to improve it. Nevertheless further experiments are currently ongoing to test the effect of a longer infusion of LPS (60 days) and pharmacological approaches will define the specific involvement of GLP-1.
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Clc-5-deficient mice, a novel murine model for investigating the role of gut microbiota in obesity and metabolic syndrome

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Background and aims: Clc-5 (chloride channel 5) functions primarily as an electrogenic 2Cl-/1H+exchanger. Mice deficient in Clc-5 exhibit a phenotype strikingly similar to human Dent's disease. We have identified Clc-5 knockout (Clc-5KO) mice as a completely novel animal model of obesity and metabolic syndromes. The aim of this study is to characterize this new phenotype and identify the molecular mechanisms involved.
Materials and methods: We extensively characterized the obesity phenotype and molecular features of Clc-5KO mice using various technologies including QNMR, MRI, PCR, ELISA, and Western blot.
Results: We have identified a previously unrecognized and highly unique phenotype of Clc-5KO mice: they are all (100%) markedly obese on regular chow diet, with a body weight 25-35% higher than that of age-matched WT mice. QNMR analysis showed that Clc-5KO mice were constituted of 21% of total body fat compared to only 7% in WT controls. MRI analysis indicated a dramatic increase in both subcutaneous and visceral (abdominal) fats. All Clc-5KO exhibited glucose intolerance and 3-4-fold elevated levels of systemic proinflammatory IL-6 and MCP-1 (than WT), remarkably similar to those in human obesity. Moreover, the Clc-5KO mice exhibit a 3- to 10-fold increase in the expression of a panel of diabetes/obesity-related genes in the liver, including PPARγ, LXRα, RXRα, CD36 (a lipoprotein scavenger receptor), SRA (class A scavenger receptor), leptin-R, SREBP2, AMPKβ, and GHRP-R, all of which are the hallmarks of metabolic syndrome. More surprisingly, Clc-5KO mice had similar food assumption as the age-matched WT mice, suggesting that Clc-5KO mice acquire a more efficient dietary energy harvesting mechanism. We found a weakened bacterial killing capacity of the Clc-5KO macrophages compared to WT macrophages. Co-caging littermate WT with Clc-5KO mice led to a marked improvement of glucose intolerance and decreased body fats in Clc-5KO mice while impaired glucose intolerance in WT.
Conclusion: Our data clearly indicate that it is the altered intestinal flora that enable the Clc-5KO mice a higher energy-harvesting efficiency from dietary foods, while the WT microbiota can diminish this efficiency. We conclude that Clc-5 plays a critical and previously unrecognized role in lipid and glucose metabolism via modulating gut flora, and Clc-5KO mice can be used as a novel model of obesity, diabetes or metabolic syndromes.
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Diet interacts with host NOD2 genotype to control blood glucose and the gut microbiota

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Background and aims: Obesity is associated with inflammation, which is critical in causing insulin resistance. Sensing of bacterial components via Pattern Recognition Receptors (PPRs) contribute to this metabolic inflammation during obesity. The gut microbiome has also emerged as a mediator of metabolic inflammation and insulin resistance during obesity. However, familial transmission of microbiota during long term breeding can confound associations between immunity and taxonomy of the microbiota. Recently, our lab showed that deletion of Nucleotide Oligerization Domain 2 (NOD2) in mice promotes diet-induced inflammation, dysbiosis, and insulin resistance. The dysbiosis caused by NOD2 deletion was an independent factor that transmits insulin resistance. Here

we aim to circumvent issues associated with familial transmission of microbiota by generating WT and NOD2 KO littermates and tracking changes in metabolism, inflammation, and microbiome across the first 3 generations of breeding. We hypothesized that the dysbiosis phenotype of NOD2 deletion would first manifest in F2 generation NOD2 KO mice and that inflammation and insulin resistance would be exacerbated in these mice when comparted to F1 generation mice.

Materials and methods: See Fig. 1

Results: We confirmed that after 14 weeks of HFD Fx generation NOD2 KO mice (2556+/-105) were glucose intolerant relative to WT mice (2005+/-112) as measured by GTT AUC ($p<0.01$). In contrast, F1 generation WT (2265+/-113) and NOD2 KO (2150+/-145) littermates did not differ in GTT AUC ($p=0.55$). The role of NOD2 in regulating glucose during a HFD emerged in the F2 generation, with NOD2 KO mice (3168+/-115) being significantly more glucose intolerant than F2 WT mice (2596+/-115) on HFD ($p<0.01$). F2 NOD2 mice also trended towards having higher fasting blood glucose (FBG) ($p=0.069$, 12.7 vs 14.2 mM). F2 generation WT and NOD2 KO mice were then used as donors for microbiota transfer to germ-free WT mice. After 4 weeks of colonization while on HFD NOD2 KO recipient germ-free mice had significantly higher FBG than WT recipient germ-free counterparts ($p<0.01$, 11.6 vs 14.0 mM). Glucose intolerance (WT=1991+/-132 vs NOD2 KO=2688+/-142, $p<0.01$) and increased FBG (WT=10.8 vs NOD2 KO=12.3, $p=0.096$) characteristics persisted in F3 generation mice. Chow-fed WT versus NOD2 KO mice did not have altered glucose tolerance in any generation.

Conclusion: Our data shows an interaction between deletion of host NOD2 and lipid content in the diet that alters blood glucose. The gut microbes that thrive in the absence of NOD2 during a HFD promote hyperglycemia independent of host genetics. Data from F2 generation mice demonstrate that the divergence in microbiota between WT and NOD2 KO mice that drives glucose intolerance is not due to prolonged familial transmission during successive breeding of mice. Ongoing analysis will determine taxomic microbial markers and effects of the diet-microbiome interaction on inflammation in the gut and metabolic tissues.

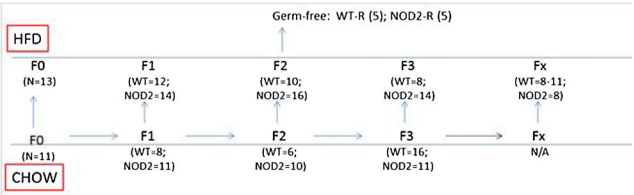

Figure 1: Materials and Methods. Mice from each generation were placed on a chow or 45% high fat diet (HFD) and glucose tolerance tests (GTT) were completed after 14 weeks. These data were compared with that of Fx generation WT and NOD2 KO mice – long term continuously bred colonies. Liver, adipose tissue, and cecums from each generation were harvested after 16 weeks HFD for measure of inflammatory markers and sequencing of the microbiome.

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OP 39 Diabetes in the elderly

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Frailty and the relationship between HbA_{1c} and mortality in elderly patients with type 2 diabetes

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Background and aims: In patients with type 2 diabetes mellitus, poor glycemic control is associated with an increased risk of all-cause and cardiovascular mortality. Frailty influences the relationship between blood pressure and mortality in old age. We hypothesized that the relationship between HbA_{1c} and mortality may also be influenced by the level of frailty.

Materials and methods: Patients with type 2 diabetes mellitus participating in a prospective observational cohort study in the Netherlands were included. Patients aged 60 years and older were selected from this cohort of primary care treated patients. Frailty was defined as a score less than 80 on the subscale 'physical functioning' of the RAND-36 questionnaire. After median follow-up for 14 years, multivariate Cox regression analyses were performed to evaluate the association between HbA_{1c} and mortality, adjusted for age, sex, BMI, duration of diabetes, systolic blood pressure, serum creatinine level, total cholesterol-HDL ratio, macrovascular complications, albuminuria, and the use of insulin. Analyses were performed in strata according to the frailty level ('physical functioning' score<80 and≥80) and diabetes duration.

Results: Approximately 60% of our study population was female. Median age (interquartile range) at baseline was 72 (67-77) years and median diabetes duration was 6 (3-12) years. Frailty was highly prevalent in our study population: 629 out of 858 patients (73%). Hazard ratios (95% confidence interval) of an increase in HbA_{1c} for all-cause and cardiovascular mortality in frail patients were 1.11 (1.02-1.21) and 1.19 (1.05-1.34), respectively (table 1). For the non-frail patients no significant associations were observed. When stratified according to diabetes duration, all confidence intervals were comparable between frail and non-frail patients, except for non-frail patients with a diabetes duration <5 years. Within this group no significant association was observed.

Conclusion: Higher HbA_{1c} was significantly related to increased all-cause and cardiovascular mortality in frail patients but outcomes were more or less comparable to the non-significant relationships in the non-frail population. Therefore, we conclude that frailty does not modify the relationship between HbA_{1c} and mortality in elderly patients with type 2 diabetes mellitus very much. Within non-frail patients with a diabetes duration less than 5 years, HbA_{1c} was not significantly related to mortality. Perhaps the positive relationships in frail patients with short diabetes duration reflect underlying morbidity.

Table 1. Hazard ratios and their 95% confidence intervals of HbA1c for all-cause and cardiovascular disease (CVD) mortality. Analyses were stratified according to frailty.

	Diabetes duration	All-cause mortality	Number of events All-cause mortality	CVD mortality	Number of events CVD Mortality
Total group		***1.12 (1.04-1.21)***		***1.16 (1.04-1.31)***	
	- < 5 years	1.09 (0.94-1.26)	199	1.13 (0.89-1.43)	76
	- ≥ 5 years	***1.15 (1.05-1.26)***	356	***1.19 (1.05-1.36)***	159
Non-frail subjects		1.18 (0.99-1.39)		1.06 (0.80-1.41)	
	- < 5 years	0.90 (0.63-1.29)	47	0.78 (0.41-1.46)	16
	- ≥ 5 years	***1.33 (1.10-1.62)***	61	1.24 (0.88-1.74)	27
Frail subjects		***1.11 (1.02-1.21)***		***1.19 (1.05-1.34)***	
	- < 5 years	***1.21 (1.02-1.44)***	152	1.26 (0.96-1.64)	60
	- ≥ 5 years	1.10 (0.99-1.22)	295	***1.19 (1.03-1.38)***	132

Adjusted for age, sex, BMI, duration of diabetes, systolic blood pressure, serum creatinine level, total cholesterol-HDL ratio, macrovascular complications, albuminuria, and the use of insulin were selected for their possible confounding effect.

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Optimum level of glycaemic control for the lowest mortality in very old adults with type 2 diabetes mellitus

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Background and aims: The complexity of care for diabetes patients increases substantially with age. Older adults with diabetes have higher rates of major diabetic complication and mortality. There are few studies conducted in the very old (age≥75 years) diabetic patients to investigate on optimal care for the very old adults, particularly glycemic target for this group of population. The aims of this study were to primarily explore which level of HbA_{1c} is associated with the lowest mortality and secondly for HbA_{1c} level with lowest diabetic-related complications in the very old diabetic patients.
Materials and methods: We conducted a retrospective cohort study of 1,000 participants with T2DM age≥75 years being treated in a university hospital during January 2007 to December 2013. Data collected were baseline characteristics including HbA_{1c} level, and geriatric syndromes such as dementia and frailty, a progressive decline in multiple organ systems and function which leads to vulnerability to complications and mortality. Primary outcome was analyzed in using Cox proportional hazard models and binary logistic regression for secondary outcomes.
Results: Included population had median age of 77(75-100) years with median HbA_{1c} of 7.0 (4.3-17.3) % and diabetic microvascular complications of 24.8%. Median follow-up time was 5.0(1.6-7.0) years. Overall mortality rate was 47.5/1000 person-year. HbA_{1c} had a U-shaped relationship with mortality; the lowest mortality was at HbA_{1c} of 6.0-6.9% and the highest at≥8.0% (hazard ratio [HR] 1.59; 95% confidence interval [CI] 1.04-2.45; P=0.034). Other factors influencing mortality were; GFR<30 mL/min/1.73 m2 (HR 3.52; 95% CI 2.05-6.04; P<0.001), age≥ 85 year (HR 2.91; 95% CI 1.91-4.43; P<0.001), frailty (HR 2.52; 95% CI 1.76-3.61; P<0.001), presence of coronary artery disease (CAD) (HR 2.02; 95% CI 1.39-2.93; P<0.001), male sex (HR 1.82; 95% CI 1.27-2.61; P=0.001), having both diabetic nephropathy and retinopathy (HR 1.95; 95% CI 1.10-3.43; P=0.021). While attending a special clinic (DM or geriatric) was a protective factor (HR 0.56; 95% CI 0.39-0.79; P= 0.001). Higher level of HbA_{1c} was related to more hypoglycemia especially in patients using insulin and having impaired renal function; and also more stroke events when HbA_{1c}≥8%. Moreover, frailty was significantly associated with stroke (odd ratio [OR] 2.92; 95% CI 1.58-5.38; P=0.001). Patients using beta blockers were more likely to develop CAD (OR 2.00; 95% CI 1.17-3.41; P=0.012) and heart failure (OR 2.28; 95% CI 1.20-4.31; P=0.011).
Conclusion: Optimum level of HbA_{1c} in the very old adults aimed for the lowest mortality should be less than 8%. However, attention should be drawn to other risk factors, such as reduced renal function, extreme age and frailty, which appeared to show more influence on mortality in older diabetic patients. In order to provide good care of diabetes for the very old, optimal goal should not focus only on glycemic level.
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Serum levels of advanced glycation end products and their receptor in the elderly diabetic patients with mild cognitive impairment

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Background and aims: Type 2 diabetes (T2DM) is a risk factor for Alzheimer's disease and mild cognitive impairment (MCI) in the elderly. The cause of cognitive impairment in diabetes is unknown, but it is most likely multi-factorial. Advanced glycation end products (AGEs) and their receptor (RAGE) play crucial roles in the pathogenesis of T2DM and associated complications, but RAGE is also an important cell-signaling receptor involved in cognitive impairment. The aim of the study was to evaluate serum levels of AGEs, RAGE and C-reactive protein (CRP) in elderly patients with T2DM with and without MCI and to determine the predictors (including AGEs, RAGE and CRP levels) of having MCI in elderly patients with T2DM.
Materials and methods: According to the criteria proposed by the MCI Working Group of the European Consortium on Alzheimer's Disease 276 elderly subjects with T2DM were screened for MCI (using the Montreal Cognitive Assessment: MoCA score) and selected into groups: 87 patients with MCI and 189 patients without MCI as a control. Data of biochemical parameters and biomarkers were collected. The serum levels of AGEs, RAGE and CRP were assessed using ELISA kit.
Results: In MCI patients serum levels of AGEs (2.19±1.12 ng/ml), RAGE (4.24±1.89 ng/ml) and CRP (7.6±2.7 mg/L) were significantly increased compared to controls (AGEs 1.04±0.82 ng/ml; RAGE 1.94± 0.91 ng/ml; CRP 3.9±2.0 mg/L; p<0.001). In group of subjects with MCI serum RAGE level was positively correlated with AGEs level (r=0.85; p <0.001) and with CRP level (r=0.54; p<0.001). RAGE, AGEs and CRP concentrations were positively correlated with HbA1c levels (RAGE: r= 0.78, p<0.001; AGEs: r=0.71, p<0.001; CRP: r=0.63, p<0.001) and negatively correlated with MoCA score (RAGE: r=-0.61, p<0.001; AGEs: r=-0.5, p<0.001; CRP: r=-0.37, p<0.001). The univariate logistic regression models revealed that variables which increased the likelihood of diagnosis of MCI in elderly patients with T2DM were: higher levels of HbA1c, RAGE, AGEs, CRP, triglicerydes, lower level of HDL cholesterol, previous cardiovascular disease (CVD), hypertension (HA) or use of antihypertensive drugs, hiperlipidaemia, retinopathy, nephropathy, increased number of co-morbidities, older age and less years of formal education. HA or use of anti-HA drugs (OR 3.48, 95% CI: 1.56-7.76, p=0.002), previous CVD (OR 3.34, 95% CI: 2.06-5.41, p<0.001), higher level of RAGE (OR 1.95, 95% CI: 1.24-3.06, p=0.004), and CRP (OR 1.55, 95% CI: 1.21-1.99, p=0.001), older age (OR 1.11, 95% CI: 1.0-1.23, p=0.039), and less years of formal education (OR 0.64, 95% CI: 0.5-0.82, p<0.001), are the factors increasing the likelihood of having MCI in elderly patients with T2DM in multivariable analysis.
Conclusion: In summary, serum levels of AGEs, RAGE and CRP are increased in MCI elderly diabetic patients compared to controls. A larger population-based prospective study needs to be performed to further confirm the relationship between AGEs, RAGE and the cognitive decline or progress to dementia. As an option, targeting the AGEs-RAGE system in diabetes especially with cognitive impairments through specific pharmacologic interventions might result in a clinical benefit for these patients.
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Protection from diabetic retinopathy, but not nephropathy

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Background and aims: Diabetic patients with nephropathy (DN) most often develop retinopathy (DR) as well, but those with DR frequently do not have DN suggesting that causal and protective factors may differ. Amongst the Joslin 50-Year Medalist we identified a cohort with DN, but not DR, providing the unique opportunity to characterize this unusual phenotype and possibly identify protective factors.
Materials and methods: This is a cross-sectional study of individuals with 50 or more years of type 1 diabetes, the Joslin 50-Year Medalists. The cohort was grouped into four categories by DN (eGFR lt 45 mL/min/ 1.73 m2) and proliferative DR (PDR, ETDRS gt 53) status. We compared groups of Medalists with DN and PDR (+DN/+PDR; n=63), DN and no PDR (+DN/ -PDR; n=30), no DN and with PDR (-DN/ + PDR; n=345), and no DN and no DR (-DN/-PDR; n=326).

Results: Of those with DN (+DN/-PDR and +DN/+PDR) the mean eGFR, serum creatinine and ACR in the groups were 37.5±8.5 v 33.9±8.8 mL/min/1.73 m2, 1.7±0.5 v 1.9±0.7 mg/dL,median [Q1, Q3] were 18.8 [8.0, 57.9] v 49.7 [14.0, 127.1] mcg/mg, respectively. Post-mortem pathology in +DN/-PDR (n=4) confirmed classic clinical DN with mesangial expansion and advanced glomerulosclerosis (DN class IIB and III). Those in the +DN/-PDR group were older at diagnosis (15.0±7.5, 10.4±5.5, 11.6±6.6, 10.5±5.9 years, $p<0.01$) and at study participation (72.1±7.2, 66.3±6.8, 66.1±7.3, 64.6±7.0 years, $p<0.01$). Surprisingly, those in the +DN/-PDR group had lower rates of cardiovascular disease (CVD) than expected for those with renal disease, 34.5% in the +DN/-PDR compared to 71.0% in the +DN/+PDR, 43.8% in -DN/+PDR, and 29.3% in -DN/ -PDR , $p<0.05$. Interestingly, the highest proportion of detectable c-peptide was in the +DN/ -PDR group (56.7% v. 30.7% +DN/+PDR, 37.4% -DN/-PDR, 32.2% -DN/+PDR, $p=0.03$). In addition, vascular endothelial growth factor (VEGF) response to stimulation (insulin and hypoxia) in fibroblasts was almost twice as high amongst those +DN/ -PDR (n=4) compared to those +DN/+PDR (n=6) (insulin 29% v 15%; hypoxia 40% v 29%). Those without DN or PDR (n=6) had the highest response to stimulation (insulin 94% and hypoxia 128%) over baseline. The VEGF response to stimulation mirrored the prevalence of CVD across the four DN/DR groups.

Conclusion: Among Medalists, the absence of PDR with DN is associated with a relative decreased prevalence of CVD, retention of beta cell function and higher VEGF response compared to the other +DN group indicating possible common protective factors against hyperglycemic toxicity for CVD and PDR.

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OP 40 The perfect cellular environment for beta cell differentiation

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Effects of quiescent and activated mesenchymal stromal cells on islet secretory functions

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Background and aims: Pre-culturing of islets in a direct co-culture with mesenchymal stromal cells (MSCs) has been shown to enhance islet function *in vitro* and to the outcome of islet transplantation *in vivo*. This study aimed to determine whether activation of MSCs by exposure to pro-inflammatory cytokines affects their ability to influence islet function.

Materials and methods: Adipose-derived MSCs and pancreatic islets were isolated from C57B1/6 and ICR mice. To determine the optimum time of co-culture required for MSCs to improve islet function, islets were co-cultured with quiescent MSCs in a direct-contact configuration and islets function was assessed by measuring glucose-stimulated insulin secretion (20 mmol/l; GSIS) at 24, 48 and 72 hours. Activation of MSCs was achieved by incubation (37C, 8 h) in the presence of the inflammatory cytokines TNF-α and IFN-γ (20 ng/ml each), and confirmed by measuring the relative expression of mRNAs for nitric oxide synthase-2 (NOS2) and chemokine C-X-C motif ligand 9 (CXCL9). Islets were cultured alone or in a direct-contact configuration with either quiescent or activated MSCs for 48 hrs before assessment of GSIS.

Results: *Co-culture incubation time:* GSIS measured from islets alone vs. islets co-cultured on MSCs at different time points revealed that a minimum of 48 hrs incubation time was needed for the MSCs to exert their beneficial effect on islets: islets-MSCs (2 mmol/l: 0.91±0.08 ng/islet/h; 20 mmol/l: 6.20±0.50) vs. islets-alone (2 mmol/l: 0.333±0.05 ng/islet/h; 20 mmol/l: 2.08±0.30, $p<0.001$, $n=3$ experiments, each of 10 observations). Activation of MSCs: Culturing MSCs for 8 h in the presence of TNF-α and IFN-γ was sufficient to activate MSCs as assessed by increased mRNA expression levels of NOS2 (quiescent MSCs 0.0001 vs. activated MSCs 0.1 relative expression) and CXCL9 (quiescent MSCs 3.4×10^{-5} vs. activated MSCs 0.6 relative expression). The activation of MSCs was maintained for 72 hours after removal of cytokines. Islets cultured alone (IA) for 48 hours responded to a stimulatory concentration of glucose with increased insulin secretion (2 mmol/l: 0.07±0.05 ng/islet/h; 20 mmol/l: 0.73±0.14). Glucose-induced insulin secretion was potentiated from islets that had been co-cultured with quiescent or activated MSCs compared to IA (quiescent-MSCs 20 mmol/l 4.99±1.1, activated MSCs 20 mmol/l 3.50±0.82 $p<0.05$). However, activated MSCs did not further enhance insulin secretion above that induced by co-culture of islets with quiescent MSCs.

Conclusion: Both quiescent and activated MSCs enhanced glucose-induced insulin secretion from islets when co-cultured in a direct co-culture configuration *in vitro*. MSCs activation by cytokine treatment did not further enhance insulin secretion above that induced by quiescent MSCs, but activated MSCs may also influence islet cell survival.

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Activation of pancreatic progenitors during isolation of porcine neonatal pancreatic cell clusters

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Background and aims: Generation of β cells *ex vivo* that are suitable for transplantation paves the way to correct beta-cell deficit in diabetic patients. While multiple step-wise protocols to differentiate human stem cells into functional β cells were proposed and early commitment of pluripotent cells into pancreatic progenitors is fairly efficient, the β cell maturation triggers are still largely unknown. Previous studies showed that *in vitro* cultivated porcine neonatal pancreatic cell clusters (NPCCs) exhibited primarily epithelial progenitor-like phenotype *in vitro* and could successfully ameliorate hyperglycemia in diabetic mice revealing a useful tool to explore islet maturation promoters.
Materials and methods: Current study sought to identify molecular profile of NPCCs upon isolation to better define their cellular properties. The NPCCs from 1-3 day new-born pigs were isolated using collagenase-based protocol and maintained *in vitro* for up to 4 days after dissociation. The mRNA and protein expression for insulin, glucagon, exocrine enzymes amylase and carboxypeptidase B, proliferation indicator Ki67 as well as the progenitor markers Pdx1 and Sox9 in time-course NPCC cultures were examined using semi-quantitative RT-PCR and immunofluorescence staining analysis, respectively.
Results: Histological analysis showed 3.4%, 7.6%, 8.6% and 11.4% of NPCCs are insulin+ in 1-,2-,3- and 4-day cultures, respectively, whereas semi-quantitative RT-PCR detected increased insulin and decreased exocrine enzyme carboxypeptidase B (CPB) mRNA expression over 4-day cultivation implying gradual enrichment of β cell population in culture. Strikingly, highly proliferating (30.1% Ki67+ cells in first 4-day cultures compared to 16.1% Ki67+ cells in 1-day old pig pancreas) pancreatic progenitors stained for Pdx1 (83.7%, 72.7%, 77.3% and 77.1% in 1-,2-,3- and 4-day cultures, respectively, compared to 51.9% in 1-day old pig pancreas) and Sox9 (respect 78.0%, 79.7%, 80.0% and 81.3% in 1-,2-,3- and 4-day cultures compared to 6.8% in 1-day old pig pancreas) were quickly induced after isolation indicating the pancreatic precursors could be propagated *in vitro*.
Conclusion: In summary, our results showed that islet precursors could be simply activated during NPCC isolation. NPCC-derived progenitors maintain their pluripotency in culture providing a useful platform to examine insulintropic and maturation-promoting effects of candidate molecules to direct human pluripotent cells into functional β cells.
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A small molecule that facilitates the differentiation into pancreatic endocrine cells from human ES/iPS cells

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Background and aims: Type 1 diabetes is an autoimmune disease characterized by the destruction of β-cells in the pancreas, absolute insulin deficiency and persistent high levels of blood glucose. Cases with difficult blood glucose control exist even using intensive insulin therapy. Although pancreas and pancreatic islet transplantation is established as the radical treatment of type 1 diabetes, problems of the transplantation therapies include shortage of donors and immune rejection. Generation of pancreatic β cells from embryonic stem (ES) cells or induced pluripotent stem (iPS) cells would lead to the development of a promising replacement therapy for insulin-dependent diabetes, such as type 1 diabetes. Researchers have aimed to differentiate pancreatic β cells from stem or progenitor cells for the goal. However, the induction efficiency of insulin-producing (INS(+)) cells from human ES/iPS cells has been low (less than 10%) in most reports. Aims: To establish an efficient differentiation method of INS(+) cells from human ES/iPS cells by identifying novel small molecule inducers.
Materials and methods: Based on the previously-reported differentiation protocol (Kunisada Y et al, 2012), human iPS cells were first induced into PDX1(+) pancreatic progenitors through SOX17(+) definitive endoderm, and then 4 factors (Nicotinamide, Forskolin, Dexamethasone and ALK5 inhibitor; 4Fs) and/or small molecules were added on the PDX1(+) cells. We used High-Throughput Screening (HTS) system to examine thousands of compounds from various libraries. Anti-insulin immunostaining was performed and the induction rate of INS(+) cells was quantitatively evaluated. Small molecules that more efficiently induce INS(+) cells than the previous protocol with 4Fs were defined as primary hits. Then, we evaluated the insulin secretory function of the INS(+) cells generated with the hit compounds. Finally, we established the INSULIN-GFP human iPSC reporter line to monitor and isolate the INS(+) cells and evaluated the function and gene expression of INS(+) cells differentiated from human iPSCs.
Results: Out of about 1,250 small molecules that we screened, one hit compound(compound SC)was identified. When the compound SC was combined with the previously-reported protocol with 4Fs, the induction rate of INS(+) cells was increased up to 15-20%. We found that compound SC facilitates the differentiation of endocrine cells including INS(+) cells both in vitro and ex vivo by expanding the NEUROG3(+) endocrine precursor cells. The INS(+) cells generated with compound SC showed the ability to secrete C-peptide in vitro. We then successfully established the INSULIN-GFP human iPSC reporter line, and are currently examining the mechanisms of action of compound SC to induce the pancreatic endocrine differentiation, based on the information of its target molecules and by gene expression analyses of purified INS(+) cells generated with compound SC.
Conclusion: We have identified one small molecule that facilitates the differentiation of human iPS/ES cells into pancreatic endocrine cells including insulin-producing β-like cells in vitro.

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Endocrine cell plasticity in human islets of Langerhans in type 2 diabetes

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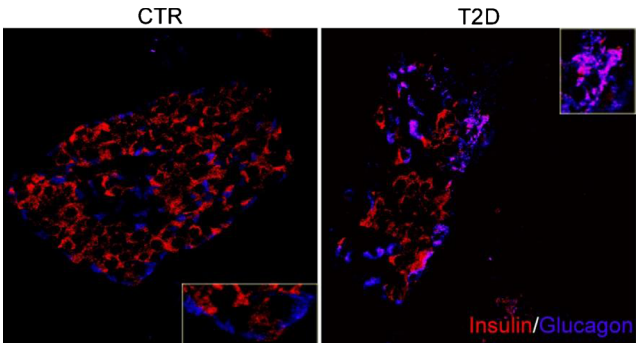
Background and aims: Studies in the last years indicate that the adult pancreas has an inherent regenerative capacity to produce new β-cells in response to increased metabolic demand or injury. Different mechanisms of beta cell regeneration in the adult pancreas have been proposed: 1)replication from endogenous β-cell, 2)neogenesis from non-β-cells precursors and 3)β-cell formation by transdifferentiation. The last mechanism consists in the conversion of one endocrine non-beta cell into beta by lineage reprogramming. It has been clearly identified in animal models; its existence and relevance in humans has never been demonstrated. Aim of this study was to examine the islet composition and architecture in control and diabetic subjects and to verify the existence of a transdifferentiation process in diabetic islets.

Materials and methods: We collected human pancreatic sections from 12 normal (7 M/5 F, mean age 69±7 years) and 14 diabetic donors (8 M/6 F; mean age 66.4±10.3 years). Sections were double/triple stained with antibodies directed against hormones (insulin,glucagon,somatostatin) and samples were analysed by confocal microscopy. Total islet area, β/α/δ-cell area, frequency of heterologous/homologous contacts among different endocrine populations, and the % of endocrine cells co-expressing insulin and glucagon (indicative of transdifferentiation) were quantitatively analyzed with the image-Pro 3D analyser software.
Results: In pancreatic sections derived from diabetic subjects there is a significant decrease of the total islet area (27±5% reduction; p=0.01) and cell density (15±7.9% reduction) compared to healthy controls, due to b-cell death and amyloid accumulation. In particular, we detected a 49±7% (p<0.0001) reduction in b-cell area of T2D subjects compared to healthy controls (particularly evident in the islet core). No significant modification of a-cell area was observed. The overlap coefficient between insulin and glucagon stainings (co-localization index) was significantly increased in diabetic subjects compared to controls (T2D 0.36±0.03; CTR 0.20±0.04; p<0.05), suggesting a transdifferentiation process. No correlation was found between the overlap coefficient and subjects' age, sex, diabetes duration, islet area. Interestingly, subjects with the highest overlap coefficient were under insulin treatment, suggesting that the phenomenon correlates with severe islet dysfunction.
Conclusion: The validation of transdifferentiation as an alternative mechanism occurring in adult diabetic pancreases and the understanding of how this event can be manipulated may lead to the design of new treatments aimed at promoting the survival of residual β-cell and preventing diabetes progression.



Supported by: PUR

OP 41 Eye on retinopathy

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Cumulative excess HbA_{1c} index may substantially predict retinopathy in type 1 diabetes patients, if we use HbA_{1c} data of total diabetes duration - a DCCT/EDIC subgroup analysis

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Background and aims: We found that an index, cumulative excess HbA1c value (CueA1C), may substantially predict retinopathy, if we use HbA1c data covering total diabetes duration, which may reflect the whole effect of metabolic memory in our type 1 diabetes mellitus (T1DM) patients who had only a short period (≤12 months) between diabetes onset and the beginning of observation. We also found that the longer the period without HbA1c data following onset, the lower the capacity for prediction in our cohort. To confirm these results, we examined the public data of DCCT/Epidemiology of Diabetes Interventions and Complications (EDIC).
Materials and methods: To examine only selected cases of DCCT/EDIC, those who had HbA1c data during nearly their entire period of hyperglycemia, we included 55 T1DM patients of primary prevention with the shortest diabetes duration (≤12 months) at DCCT baseline. We developed a window which we named 'half-yearly visit (HV)' to integrate HbA1c data and retinopathy steps of the final Early Treatment Diabetic Retinopathy Study (ETDRS) severity scale for persons. HV00 was DCCT baseline, and HV24 was at DCCT year 12. During DCCT, HbA1c values of an even number of quarterly visits (QVs) were applied to those of HVs: for example, the value of QV00 (DCCT baseline) was applied to HV00, QV02 to HV01, and so on. During EDIC, HbA1c values were applied to the corresponding HVs according to their data collection dates from DCCT baseline: for example, 11.75≤X<12.25 years' data to HV24. Missing values were estimated by time-weighted averaging from the HbA1c values of preceding and succeeding HVs. CueA1C was calculated by adding the values of (HbA1c -6.5)(%)x1/2(year) of all the HVs from onset to a given HV, supposing each case had one-year duration at HV00, keeping the same HbA1c value during that period. Simulated 3-, 6- and 9-year masked CueA1Cs were calculated in the same way but masking HbA1c data for 3, 6 and 9 years following onset respectively. Retinopathy was defined as DR+ at ETDRS step≥4, otherwise as DR- at a given HV. The prediction capacities of CueA1Cs for retinopathy between HV16 and HV24 (DCCT years 8 and 12, respectively) were examined by receiver operating characteristic (ROC) analysis and were compared by the method of DeLong. P<0.05 was considered significant.
Results: Of the 132 HVs which had retinopathy data between HV16 and HV24, 36 were DR+ and 96 were DR-. The area under the curve (AUC) for retinopathy was 0.885, 0.875, 0.777 and 0.653 in CueA1C, 3-, 6- and 9-year masked CueA1Cs respectively. The difference of AUC compared to CueA1C was not significant for 3-year masked CueA1C (P=0.39), but it was significant for 6-year (P=0.0002) and 9-year (P<0.0001) CueA1Cs. It seemed that CueA1C had a substantial capacity to predict retinopathy, and that the longer the period without A1C data, the lower the capacity for retinopathy prediction. The results in our previous study of Japanese patients seemed to be confirmed in the patients of the current DCCT/EDIC subgroup.
Conclusion: CueA1C index may have a substantial capacity to predict retinopathy in T1DM, if we use HbA1c data of total diabetes duration insofar as possible. We should consider information from the entire duration of diabetes in order to study the relation between hyperglycemia and retinopathy accurately.

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Cardiac autonomic function in relation to diabetic retinopathy development and progression in type 1 diabetic patients: a prospective observational study

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Background and aims: Denervation of the cardiovascular system is one of the earliest manifestations of diabetic autonomic neuropathy (DAN). Various studies have shown that it is strongly associated with microvascular complications of diabetes, microalbuminuria and diabetic retinopathy (DR). In this longitudinal observational study we aimed to determine whether cardiac autonomic fuction contributes to risk of DR development and progression in normoalbuminuric type 1 diabetic (T1DM) without syptomiatic DAN .

Materials and methods: One hundred fifty four normoalbuminuric T1DM patients were included in the study. Urine albumin excretion (UAE) was measured from two 24-h urine samples and determined as the mean of 24-h urine collections to minimize variability. Photodocumented retinopathy status was made according to EURODIAB protocol. Normoalbuminuria was defined as a UAE<30 mg/24 h. The battery of cardiovascular autonomic function (AFT) tests included: heart rate variation at rest (HRV-CV), HRV-CV during deep breathing (dbHRV-CV), Valsalva manoeuvre and active orthostatic test and blood pressure response to standing. In the frequency domain we measured high-frequency power (HF), and low frequency power (LF) using a fast Fourier transformation in order to calculate the power spectral density curve. Participants were reexamined after 18 months to update their glycaemic control, UAE and retinopathy status.

Results: The results of AFT in T1DM patients with NPDR and 107 free of DR are given in Table 1. Patients with NPDR showed significantly lower HRV at rest, HRV during deep breathing as well as deep breathing E/I ratio compared to group of patients without DR. There was a significant attenuation for both LF and HF Power in the NPDR group. In-between groups diabetes duration was significantly different although long-term glycaemic control assessed by glycated haemoglobin A1c (HbA1c) was similar. Six (3.89%) patients developed albuminuria during 18 months follow up period while 21 (13.36%) developed NPDR or progressed from NPDR to PDR. A Cox regression model adjusted for age, gender, disease duration, HbA1c, hypertension prevalence, ACE inhibitor use and smoking status showed that higher HRV-CV, dbHRV-CV as well as LF Power reduce the risk of DR incidence and progression (HR 0.664 (0.514 to 0.866), p=0.002; 0.873 (0.784 to 0.972), p=0.013 and 0.909 (0.892 to 0.964), p=0.014, resepectively).

Conclusion: In this prospective, longitudinal, observational cohort study, we demonstrated that AFT results might serve as an independent prognostic factor for the future development or progression of DR in T1DM patients even in the DAN absence.

Table 1. Clinical, laboratory data and cardiac autonomic function tests

Variable		Patients without retinopathy (N=107)	Patients with retinopathy (N=47)	p value
Age (yrs)		37(34-39)	39.5 (36-42.5)	0.279
Gender (N, %)	Female	52 (48.6)	28 (59.6)	
	Male	55 (51.4)	19 (40.4)	0.211
Diabetes duration (yrs)		11.5 (10.5-13.0)	20.0(17.5-22.5)	<0.001
HbA1c (%)		7.2(6.8-7.5)	7.4(6.9-7.8)	0.489
eGFR (mL/min/1.72m²)		108.1(105.6-110.5)	105.6(101.2-110.1)	0.301
UAE (mg/24h)		11.29(10.08-12.51)	11.82(9.86-13.77)	0.651
Autonomic neuropathy (N, %)		5 (4.7)	9 (19.15)	0.004
Spectral analysis of HRV				
HRV at REST – CV (%)		4.64(4.32-4.96)	3.68(3.21-4.15)	0.001
LF (EHz)		.0139(.0119-.0160)	.0135(.0106-.0165)	
LF Power (*10e–4 Hz²)		10.82(9.38-12.26)	7.06(5.64-8.48)	0.003
HF(EHz)		.2078(.1930-.2222)	.2379(.2140-.2620)	
HF Power (*10e–4 Hz²)		8.91(7.18-10.64)	5.91(3.56-8.28)	0.022
HRV during deep breathing-CV(%)		8.21(7.48-8.91)	6.48(5.35-7.62)	0.004
Deep breathing E/I		1,35(1.29-1.41)	1,31(1.19-1.43)	0.007
Valsalva Ratio		2,14(1.91-2.37)	2.23(1.79-2.68)	0.820
Max/min 30:15 ratio		1.61(1.45-1.75)	1.44(1.29-1.58)	0.171

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Effects of baseline haemoglobin A_{1c} and on-treatment blood pressure on outcomes in the VIVID-DME and VISTA-DME studies

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Background and aims: The VIVID-DME and VISTA-DME studies evaluated intravitreal aflibercept (IVT-AFL) vs. laser for diabetic macular edema (DME). The current subanalyses analyse associations between haemoglobin A1c (HbA1c) and blood pressure (BP) and visual and anatomic outcomes in these studies.

Materials and methods: In the VIVID-DME and VISTA-DME studies, 865 patients with diabetic macular edema (DME) were treated with IVT-AFL (2 mg every 4 weeks [2q4] or 2 mg every 8 weeks following 5 monthly doses [2q8]) or laser. In the current subanalyses, data from both studies have been integrated. The IVT-AFL treatment arms were pooled to increase the power of the analysis. HbA1c quartiles were defined using all available baseline data. On-treatment BP was calculated as ([mean systolic BP+mean diastolic BP]/2) using the average of measurements obtained every 4 weeks from baseline to week 52. Values were then divided into quartiles.

Results: Mean changes in best corrected visual acuity (BCVA) and central retinal thickness (CRT) by mean baseline HbA1c quartiles and by mean on-treatment BP quartiles are shown in the table. The most frequent ocular serious adverse event in a pooled analysis of VIVID-DME and VISTA-DME was cataract (2.4% and 1.0% vs. 0.3% for 2q4 and 2q8 vs. laser).

Conclusion: In the laser group but not the IVT-AFL group, visual and CRT improvements were less with increasing baseline HbA1c. There was no apparent correlation between increasing on-treatment BP and vision or CRT in either group. Visual benefits were consistently greater with IVT-AFL vs. laser, regardless of baseline HbA1c or on-treatment BP.

	Mean change in BCVA (letters)		Mean change in CRT (µm)	
Quartiles	IVT-AFL	Laser	IVT-AFL	Laser
Q1: HbA1c 4.5%-<6.7%	11.7	4.1	-200.9	-101.9
Q2: HbA1c 6.7%-<7.4%	11.9	1.9	-196.5	-83.1
Q3: HbA1c 7.4%-<8.6%	11.7	0.8	-195.5	-68.9
Q4: HbA1c 8.6%-14.7%	11.1	-0.3	-187.7	-43.3
Q1: BP 78.0-<99.7 mmHg	10.5	1.0	-137.8	-84.0
Q2: BP 99.7-<105.9 mmHg	11.0	1.6	-200.6	-62.1
Q3: BP 105.9-<111.2 mmHg	11.7	2.9	-208.0	-113.7
Q4: BP 111.2-<139.4 mmHg	11.3	-2.4	-207.1	-31.0

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Topical administration of GLP-1 receptor agonists prevents retinal neurodegeneration in experimental diabetes

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Background and aims: Retinal neurodegeneration is an early event in the pathogenesis of diabetic retinopathy (DR). Since glucagon-like peptide-1 (GLP-1) exerts neuroprotective effects in the central nervous system and the retina is ontogenically a brain-derived tissue. The aims of the present study were: 1) To examine the expression and content of GLP-1R in human and db/db mice retinas. 2) To determine the retinal neuroprotective effects of systemic and topical administration (eye drops) of GLP-1R agonists in db/db mice. 3) To examine the underlying neuroprotective mechanisms.
Materials and methods: Human study: retinas were obtained from the Tissue Bank of our Centre. A total of 8 diabetic donors and 8 non-diabetic donors matched by age and gender were included in the study. GLP-1R expression and content were analyzed by RT-PCR, Western-blot and immunohistochemistry. Animal studies: after 15 days of treatment by using subcutaneous or topical GLP-1R agonists the neurodegenerative features were examined. Functional abnormalities were assessed by electroretinography and neurodegeneration was assessed by measuring glial activation and the apoptotic rate. In addition, several representative mediators of apoptotic (Fas/FasL, caspase 8, Bax, p53), anti-apoptotic (BclxL), neuroinflammatory (iNOS), and insulin signaling (pAKT/AKT) pathways were also analyzed by Western blot. Glutamate (HPLC) and its main transporter GLAST (glutamate/aspartate transporter) were also determined.
Results: We have found abundant expression of GLP-1R in the human retina and retinas from db/db mice. Moreover, we have demonstrated that systemic administration of a GLP-1R agonist (liraglutide) prevents retinal neurodegeneration (glial activation, neural apoptosis and electroretinographical abnormalities). This effect can be attributed to a significant reduction of extracellular glutamate due to the prevention of the down-regulation of GLAST induced by diabetes. In addition, an increase of prosurvival signaling pathways was observed. We have found a similar neuroprotective effect using topical administration of native GLP-1 and several GLP-1R agonists (liraglutide, lixisenatide and exenatide). Notably, this neuroprotective action was observed without any reduction in blood glucose levels.
Conclusion: These results suggest that GLP-1R activation itself prevents retinal neurodegeneration. Our results should open up a new approach in the treatment of the early stages of DR.
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OP 42 Liver metabolism: from fat to facts

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Bioactive lipids dissociate steatosis and insulin resistance in the human liver in non-alcoholic fatty liver disease

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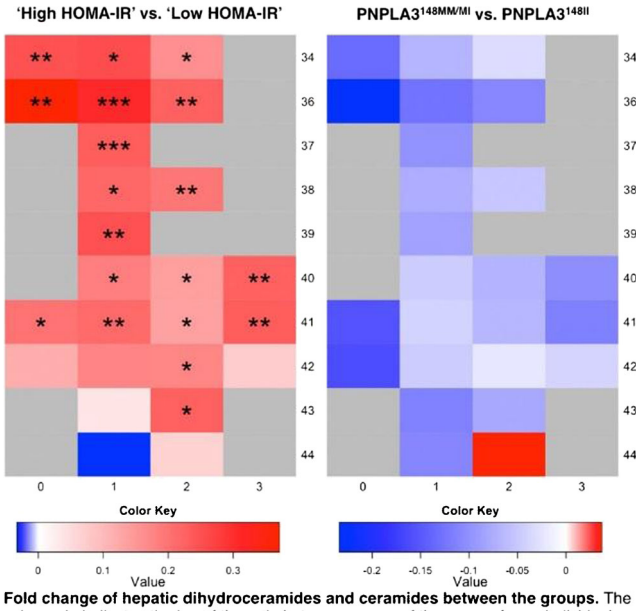
Background and aims: 'Metabolic NAFLD' is characterized by hepatic insulin resistance (IR) and the features of metabolic syndrome, while NAFLD due to the common (40% of Europeans) PNPLA3 I148M gene variant at rs738409 ('PNPLA3 NAFLD') is not. We used these two models to determine in the human liver whether i) NEFA or triglyceride (TG) composition; ii) bioactive lipids; iii) pathways of ceramide synthesis differ between 'Metabolic NAFLD' and 'PNPLA3 NAFLD'. The latter is of interest as recent studies in mice have identified 16:0-ceramide formed via the de novo ceramide synthetic pathway as the key mediator of hepatic insulin resistance.
Materials and methods: Liver biopsies from 125 subjects, characterized with respect to metabolic features and PNPLA3 genotype, were taken for profiling of molecular lipids using UHPLC-MS and liver histology. Subjects were divided into groups based on median HOMA-IR ('High HOMA-IR', n=62, vs. 'Low HOMA-IR', n=63) and the PNPLA3 genotype (PNPLA3148MM/MI, n=61 vs. PNPLA3148II, n=64). The groups were similar with respect to BMI and gender.
Results: Histologic liver fat was similarly 3-fold increased in 'High HOMA-IR' (15 [5 - 33]) vs. 'Low HOMA-IR' (5 [0 - 20], $p<0.002$) group, and in 'PNPLA3148MM/MI (15 [5 - 30]) vs. the 'PNPLA3148II' (5 [0 - 28], $p<0.04$) group. By definition, the 'High HOMA-IR' group was insulin-resistant vs. the 'Low HOMA-IR' group (HOMA-IR 4.8 [3.9-5.8] vs. 1.8 [1.3-2.7], $p<0.0001$), and also had decreased serum adiponectin (6.0 [4.0-8.7] vs. 9.5 [6.1-12.1] ng/ml, $p<0.0001$), while no changes were observed in PNPLA3148MM/MI vs. PNPLA3148II group (HOMA-IR 3.2 [1.7-5.1] vs. 3.2 [1.9-4.5], NS). The liver in 'High HOMA-IR' vs. 'Low HOMA-IR' was markedly enriched in saturated and monounsaturated TGs, dihydroceramides (34:0 and 36:0, reflecting de novo ceramide synthesis from 16:0 palmitate and 18:0 stearate, Figure), other ceramides (Figure) and in 16:0 and 18:0 but not 18:2 or 20:4 intrahepatic NEFA. Markers of other ceramide synthetic pathways were unchanged. In PNPLA3148MM/MI vs. PNPLA3148II, the increase in liver fat was due to polyunsaturated TGs while other TGs, diacylglycerols, ceramides (Figure) and intrahepatic NEFA were unchanged.
Conclusion: Equal increases in liver fat due to 'Metabolic NAFLD' and 'PNPLA3 NAFLD' are characterized by strikingly different hepatic lipidomes. Bioactive IR causing lipids, particularly ceramides from de novo synthesis from saturated fatty acids, are increased in 'Metabolic NAFLD' but not 'PNPLA3 NAFLD'. These differences imply measurement of liver fat allows diagnosis of NAFLD but not insulin resistance or its metabolic consequences.



Fold change of hepatic dihydroceramides and ceramides between the groups. The color code indicates the log of the ratio between means of the groups for an individual species. The y-axes denote the number of carbons and the x-axes the number of double bonds. *p<0.05, **p<0.01, ***p<0.001.

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Gene therapy with insulin receptor isoform a as an approach for the treatment of type 2 diabetes

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Background and aims: Type 2 diabetes mellitus is a complex metabolic disease and its pathogenesis involves abnormalities in both peripheral insulin action and insulin secretion by pancreatic beta cells. There are many proteins involved in insulin resistance and one of the most important is the insulin receptor (IR). Our laboratory has generated iLIRKO mice (inducible Liver Insulin Receptor KnockOut) that develops Type 2 diabetes in a very similar way to what happens in humans. Moreover, previous experiments in our laboratory found that IRA isoform, but not IRB, plays a direct role in the regulation of basal glucose uptake/transport through its specific association with endogenous glucose transporters (GLUT1 and GLUT2) in murine hepatocytes. With this background, we hypothesized that the expression of IRA in iLIRKO mice using AAVs (Adenoassociated virus) as a gene therapy approach could be a good option in order to reverse the diabetic phenotype. Thus, reconstitution of iLIRKO with IRA in the liver might increase glucose consumption, ameliorate hyperglycaemia and therefore impair the compensatory mechanisms through beta cell hyperplasia/hypertrophy that finally lead to beta cell failure.
Materials and methods: Recombinant hepatospecific AAV vectors (serotype 8) were constructed with a transgene cassette encoding either the IRA or IRB or the reporter gene GFP. Twenty-week-old mice were injected intravenously with the AAV viruses. Glucose and insulin tolerance tests were performed to evaluate insulin sensitivity and glucose clearance and follow the diabetic phenotype. In addition, insulin plasma levels were also measured by ELISA.
Results: The physiological effects of the disruption of insulin signaling in liver in iLIRKO mice were apparent 5 weeks after the induction of the IR deletion. By 3 months of age, male iLIRKO displayed pronounced glucose intolerance and insulin resistance. The expression of IRA in 20-week-old mice, but not IRB, was able to restore glucose tolerance and reverted insulin resistance about 8 weeks after the treatment. Moreover, plasma insulin levels were recovered in injected IRA iLIRKO mice as compared with IRB-injected or reporter gene-injected mice.
Conclusion: Our data demonstrate that the expression of IRA by gene therapy improves hyperglycemia by promoting an increased glucose uptake by the liver. The decrease on glucose plasma levels ameliorates the compensatory hyperinsulinemia that finally lead to beta cell failure and overt diabetes. Therefore, hepatic expression of IRA could be a therapeutic approach for Type 2 diabetes treatment.
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Metabolic reprogramming in male offspring in a non-dietary model of liver insulin resistance

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Background and aims: Several studies have focused on investigating the effects of early nutritional insults that increase the likelihood of developing type 2 diabetes in the offspring. Virtually none include the use non-dietary models manifesting hyperglycemia and hyperinsulinemia. We aimed to determine the effects of paternal versus maternal liver insulin resistance on the developmental programming in the offspring of the liver-specific insulin receptor knockout (LIRKO) mice.
Materials and methods: Male control offspring from 12 weeks of age LIRKO father (LF) and control mother, LIRKO mother (LM) and control father, and control mothers and fathers (C) were weaned at 3 weeks of age on a chow (21%) or high-fat diet (HFD) (60%) and followed for 12 weeks. Body weights and blood glucose levels were measure weekly. Insulin sensitivity, glucose tolerance, in vivo glucose-stimulated insulin secretion was assessed between 9 and 12 weeks of age in randomly selected mice from a least 3 different offsprings in each group. Mice were sacrificed at 15 weeks of age, blood, pancreases and liver were harvested for further analysis.
Results: As previously reported, 8 week-old LIRKO males and females exhibited insulin resistance and glucose intolerance compared to their respective controls. On a chow diet, LF (LF: 14.1±0.5; C: 17.5±0.5 g; LF vs C: $p<0.001$, 4 weeks of age): and LM (LM: 15.1±0.5; C: 17.5±0.5 g; LM vs C: $p<0.001$; 4 weeks of age) exhibited lower body weights until 9 weeks of age and compensated by gaining weight with age compared to controls. After 3 weeks on HFD, LM (LM: 27.7±1.0; C: 23.7±0.7 g; LM vs C: $p<0.01$; 6 weeks of age) but not LF gained more weight compared to C. LF and LM exhibited insulin resistance compared with C on both chow (LF vs C: p(area under the curve - AUC)<0.01 and LM vs C: p(AUC)<0.01; n=5-8) and HFD (LF vs C: p(AUC)<0.05 and LM vs C: p(AUC)<0.01, n=4-5) cohorts at 9 weeks of age. LF and LM maintained insulin resistance with age and after 8 weeks on HFD became hyperglycemic (LF, 110.2±6.2; LM, 107.2±4.1; C, 78.3±6.9 mg/dL; LF vs C and LM vs C: $p<0.01$; n=4) and LM hyperinsulinemic (LM, 2.63±0.66; LF, 1.62±0.67; C, 0.52±0.11 ng/ml; LM vs C: $p<0.05$; n=4) during fasting. LF and LM presented an augmented acute insulin secretion response to glucose (LF: 374.95±4.4; LM: 502.41±39.5; C: 223.65±36.4% of initial insulin levels; LF vs C: $p<0.001$; LM vs C: $p<0.001$; n=3; 2 minutes after stimulation) on chow. On HFD this response was abrogated in both groups. LF and LM had increased beta-cell

proliferation (LF: 0.42±0.06; LM: 0.44±0.02; C: 0.23±0.04% KI67+/Insulin+ cells; LF vs C: $p<0.05$; LM vs C: $p<0.01$; n=3-4) on chow comparing with C. After 12 weeks of HFD, LF and LM presented prominent hepatic steatosis. Analysis of genes involved in the regulation of liver glucose and fatty acid metabolism revealed LM and LF on chow diet exhibited downregulation of Glut2, Gpase, Cpt1, CD36, Elovl2, PPARα and PPARγ genes while the same groups on HFD showed upregulation of CD36, PPARα, PPARγ, Fas and Scd1.

Conclusion: Together these data suggest that prenatal hyperinsulinemia and hyperglycemia have detrimental effects on beta-cell adaptation and transcriptional regulation of hepatic metabolism in the offspring that may contribute to their impaired growth and metabolic response to dietary challenges.

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Liver insulin sensitivity is linked with resting sympathetic nervous activity in non-diabetic obese men

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Background and aims: Sympathetic nervous system activity may play a role in the aetiology of insulin resistance in humans. Obesity is associated with peripheral and hepatic insulin resistance and with basal sympathetic nervous hyperactivation. However, whether sympathetic nervous activation is more closely aligned with peripheral or hepatic insulin resistance in obesity remains unclear. We aimed to compare resting muscle sympathetic nervous activity (MSNA) between non-diabetic obese insulin-sensitive and insulin-resistant subjects stratified by liver and muscle insulin sensitivity.

Materials and methods: Forty-five non-diabetic obese subjects (22 men, 23 women) aged 51±11 years were studied. Two-step (low-dose [15] and high-dose [80] mU/m2/min) hyperinsulinaemic-euglycaemic clamps with deuterated glucose were performed. Subjects in the top tertile of endogenous glucose production (EGP) suppression were deemed Liversen; those in the bottom two tertiles were deemed Liverres. Subjects in the top tertile of glucose infusion rate (GIR) during the high-dose insulin clamp were deemed Musclesen; the bottom two tertiles were deemed Muscleres. Clinical and metabolic parameters were assessed. MSNA was measured by microneurography.

Results: MSNA did not differ between insulin-sensitive and insulin-resistant groups stratified by either liver or muscle insulin sensitivity. However, when men and women data were analysed separately, Liversen men had lower MSNA burst frequency compared to Liverres men (27±14 vs. 38±7 units; P=0.03). Musclesen men had lower MSNA burst frequency compared to Muscleres men (24±9±12.0 vs. 38±9 units; P=0.004). No differences in MSNA were observed in parallel analyses in women. MSNA burst incidence correlated positively with age (r=0.48, P<0.01) and visceral fat (r=0.3, P=0.05) in total cohort. In men, MSNA burst frequency correlated with the hepatokines high-sensitivity C-reactive protein (hsCRP) and fibroblast growth factor (FGF)19 (r=0.57, P=0.006; r=-0.47, P=0.03 respectively) and correlated negatively with liver insulin sensitivity (EGP suppression, r=-0.53, P=0.02).

Conclusion: MSNA burst frequency is lower in obese insulin-sensitive men compared to their insulin-resistant counterparts, when stratified by either muscle or liver insulin-sensitivity. Basal sympathetic nervous activity is related to liver insulin sensitivity and circulating hepatokines, suggesting a potential hepato-endocrine-sympathetic axis. Future studies are needed to clarify the influence of sympathetic nervous activity on liver insulin sensitivity in men.

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OP 43 From beta to alpha: shifting the diabetes paradigm

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Non-invasive in vivo monitoring of pancreatic beta cell insulin resistance

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Background and aims: Pancreatic β-cell dysfunction is central to the development of type 2 diabetes (T2DM) which has reached epidemic proportions. Because the pancreatic β-cell itself is a target for insulin action, β-cell insulin resistance can contribute to β-cell dysfunction. While *in vivo* tests are available to measure overall body or liver insulin resistance, there is currently no test to evaluate insulin resistance at the pancreatic islet/β-cell level in the living organism. In the present study we present a technique to report on insulin resistance in pancreatic β-cells *in vivo* .

Materials and methods: We transplant pancreatic islets into the anterior chamber of the eye (ACE) that express a fluorescent biosensor in their β-cells and monitor insulin resistance non-invasively and longitudinally by fluorescence microscopy at single-cell resolution in the living mouse. The biosensor is based on the different subcellular localization of FoxO1-GFP, i.e. cytosolic in insulin responsive cells and nuclear at insulin resistance.

Results: *In vitro* experiments in MIN6 cells and islets of Langerhans were performed to validate the function of the biosensor. In these experiments we monitored insulin resistance induced by pharmacological inhibitors as well as by the free fatty acid palmitate. The leptin deficient ob/ob mouse was used to validate the biosensor in vivo. At 3 and 10 months of age, lean control mice did not develop either whole-body insulin resistance (AUC (3 months)=845±56; AUC (10 months)=907±67) or β-cell insulin resistance (3 months of age: 1.1±0.6% insulin-resistant β-cells; 10 months of age: 1.8±0.9% insulin-resistant β-cells). In contrast, ob/ob mice were β-cell insulin resistant at 3 months of age (12.1±2.8% insulin-resistant β-cells, $p<0.001$ compared to ob-control (3 months) and $p<0.01$ compared to ob/ob (10 months)) and insulin responsive at old age (3.7±1.4% insulin-resistant β-cells). The β-cell insulin resistance was paralleling the whole-body insulin resistance (AUC (3 months)=1914±72; AUC (10 months)=827±89; $p<0.001$: 3 months compared to 10 months old ob/ob; $p<0.001$: 3 months old ob/ob compared to 3 months old ob-control). The *in vivo* imaging data were complemented with and confirmed by data from immunohistochemistry and Western blot analysis of *in situ* pancreatic islets.

Conclusion: Our technique will allow monitoring longitudinally and non-invasively at single cell resolution the dynamics of pancreatic β-cell insulin sensitivity/resistance in the context of overall insulin resistance, hepatic insulin resistance and glucose tolerance during progression and intervention of experimental T2DM in a 'personalized' medicine approach.

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Glucagon is a key factor in circadian glucose rhythm and clock gene regulation

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Background and aims: Metabolic regulation of glucose homeostasis is partly controlled by oscillations in the transcription of concurrent CLOCK genes. On the tissue level, these transcription patterns are strongly affected by nutritional intake and subsequent hormone release. Shift

work and/or an altered sleeping pattern are lifestyle factors that increase the risk of diabetes and other disturbances in glucose metabolism. Since dysregulation of glucagon accompanies disrupted insulin secretion in type 2 diabetes, we asked if glucagon signalling is involved in the circadian rhythm of clock genes and glucose homeostasis and whether glucagon secretion is regulated by circadian rhythm.

Materials and methods: To assess the effects of disrupted glucagon signalling on circadian glucose rhythm, we performed oral challenges using a mixed meal (60/20/20E% Glucose/Protein/Lipid) in wt and glucagon receptor knock-out mice (GCGR-/-) and measured glucose and insulin in plasma at *zeitgeber* times (ZT) 3 (3 h after start of 12 h/12 h light/dark cycle; light phase) and ZT15 (dark phase). To assess the effect of disrupted glucagon signalling on molecular clocks we analysed core clock gene expression in islets at ZT 3, 9, 15 and 21 using qPCR. To further examine circadian rhythm in glucagon dynamics, glucagon response following a meal challenge or a hyperinsulinaemic, hypoglycaemic clamp was measured in wt mice.

Results: During light phase (ZT3), we found a marked difference in basal (4.2±0.2 vs. 7.4±0.3 mmol/l, p<0.0001) and peak glucose (8.0±0.4 vs. 13.5±0.6 mmol/l, p<0.0001), and glucose-stimulated insulin peak (837± 174 vs. 2250±347 pmol/l, p<0.0001) in GCGR-/- compared to wt animals. In contrast, there was little difference in glucose levels and no difference in insulin levels between groups at the dark phase (ZT15). When applying the quantitative insulin sensitivity check index (QUICKI), we found that GCGR-/- differ greatly from wt in insulin sensitivity at ZT3 (0.90±0.07 vs. 0.51±0.02, p<0.0001) but not at ZT15. GCGR-/- mice displayed disrupted expression of clock genes *Bmal1, Clock, Rev-Erbα* and *Per1* at the mRNA level compared to wt. The glucagon response to a meal challenge did not differ between the ZT:s, however, the glucagon response to hypoglycaemia (4.8±1.2 vs. 1.9±0.5, p=0.035) and the insulin sensitivity measured using clamp (4.8±0.9 vs. 1.5±0.2, p=0.0034) was greater at ZT15 in wt animals.

Conclusion: In conclusion, this study shows that glucagon signalling is important for circadian glucose rhythm, as is highlighted by the fact that GCGR-/- mice differs in insulin dynamics from wt animals only during the light phase. This is further emphasized by the concurrent altered expression pattern of core clock genes in GCGR-/- mouse islets that suggests that altered glucagon signalling disrupts islet clock gene transcription.

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Hyperglucagonaemia after oral glucose and suppression of glucagon following i.v. glucose in totally pancreatectomised patients

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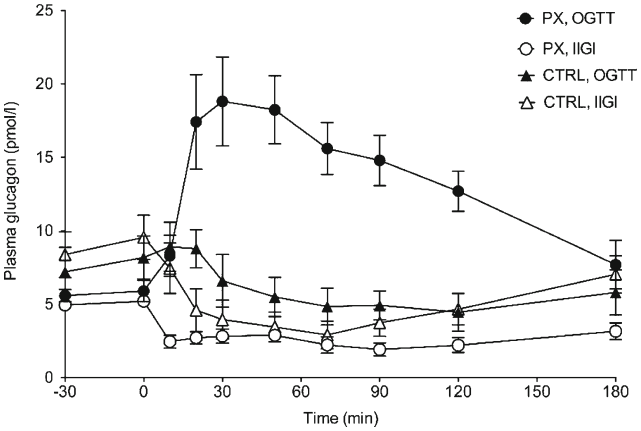
Background and aims: Glucagon is believed to be a pancreas-specific hormone and postabsorptive hyperglucagonaemia is thought to contribute to the hyperglycaemic state of patients with diabetes. This hyperglucagonaemia has been thought to arise as a consequence of alpha cell insensitivity to the glucagon suppressive effects of glucose and insulin combined with reduced insulin secretion. We hypothesised that postabsorptive hyperglucagonaemia represents a gut-dependent phenomenon occurring independently of plasma glucose and insulin concentrations. Therefore, we evaluated plasma levels of glucagon during OGTT and isoglycaemic i.v. glucose infusion (IIGI) - bypassing the gastrointestinal tract - in totally pancreatectomised patients.

Materials and methods: Ten totally pancreatectomised patients (PX) (age: 59.8±3.1 years [mean±SEM]; BMI: 21.5±1.3 kg/m^2; HbA_{1c}: 67.3±3.5 mmol/mol) and 10 healthy control subjects (CTRL) (age: 58.4±1.6 years; BMI: 22.9±0.8 kg/m^2; HbA_{1c}: 34.6±2.0 mmol/mol) underwent a 75 g-OGTT and a corresponding IIGI on two separate days. Analysis of plasma glucagon was carried out using a newly developed sandwich ELISA specific for fully processed human 29-amino acid glucagon.

Results: Significantly larger amounts of glucose were needed during the IIGI (in order to copy the 75 g-OGTT plasma glucose curve) in the PX group than in the CTRL group (83±3 g vs. 28±2 g, $p<0.001$). The PX group tended to have lower fasting levels of plasma glucagon compared to the CTRL group (5.4±0.2 pmol/l vs. 8.3±1.5 pmol/l, $p=0.064$), but exhibited a significant hyperglucagonaemic response during OGTT (Fig. 1). In contrast, IIGI suppressed plasma glucagon levels in both groups.

Conclusion: We demonstrate that fully processed 29-amino acid glucagon circulates in patients without a pancreas and that glucose stimulation of the gastrointestinal tract elicits significant hyperglucagonaemia whereas i.v. glucose administration results in suppression of plasma glucagon levels. These findings emphasise that gut-derived glucagon may play an important role in diabetic pathophysiology - also in diabetes secondary to total pancreatectomy. *Figure 1.* Time courses of plasma glucagon [mean± SEM] following OGTT and isoglycaemic i.v. glucose infusion (IIGI) in totally pancreatectomised patients (PX) and healthy control subjects (CTRL).



Clinical Trial Registration Number: NCT02006459
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A role for glucokinase in pancreatic alpha cell stimulus-secretion coupling

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Background and aims: One of the remaining controversial issues of glucose-regulated glucagon secretion is the identity of the glucose sensor linking glucose metabolism to hormone secretion. To strengthen the argument that glucokinase (GK) is this sensor in pancreatic alpha cells we decided on the following two aims: 1. Which hexokinases are expressed in pancreatic alpha cells? 2. Does manipulation of GK activity by inhibitors, activators or knockdown lead to a change in stimulus-secretion coupling in alpha cells?

Materials and methods: Primary pancreatic alpha cells were purified by FACS sorting from islets of 3 months old Wistar rats. Single cells RT-

PCR was performed for ca. 100 cells using primer sets for insulin, glucagon, somatostatin and pancreatic polypeptide to verify cell identity and to determine the expression patterns of glucose phosphorylating enzymes (GK and hexokinase I to III). 5'race combined with cloning and sequencing was performed to identify the isoforms of glucokinase mRNAs present in pancreatic alpha cells. For glucagon secretion studies the cells were seeded onto 25 mm cover glasses and transduced with an adenovirus expressing glucagon-pHluorin under the control of the glucagon promoter. 72 h after transduction the cover glasses were moved to a ZEISS Axiovert TIRF microscope and glucagon release events were imaged, analyzed and quantified. GK inhibitors and a GK activator were used acutely while knockdown of GK expression was achieved by treatment with siRNA. To verify the glucagon release measurements performed by TIRF microscopy, glucagon secretion from isolated primary alpha cells was measured by glucagon RIA.

Results: Single cell RT-PCR showed that >90% of analyzed pancreatic alpha cells were positive for glucokinase mRNA (>90 analyzed cells). All analyzed cells were negative for hexokinase I-III. 5'Race and sequence analysis revealed that 63% of the analyzed mRNAs (45/71) were the full length neuroendocrine GK form, resulting in a functional protein while 37% had deletions resulting in non-functional proteins. Primary alpha cells show a twofold difference in glucagon release at 3 mM vs 4 mM glucose (22.4±6 release events/minute and 100 μm2 (RE) vs. 11,2±2 RE; $p<0.001$). The same difference in release at these two glucose concentrations was observable using RIA (3%±0.5 of content released at 3 mM glucose vs 1.5%±0.1 at 4 mM; $p<0.05$). Treatment with glucokinase inhibitors 5-thioglucose and deoxyglucose led to an increased release at 4 mM glucose concentration (22±3 RE or 24±4 RE respectively; $P<0.001$ vs 4 mM glucose) while treatment with the activator RO0281675 led to a decreased release at 3 mM glucose (9,68±2RE; $p<0.001$ vs 3 mM glucose). 80% knockdown of glucokinase mRNA, which was verified by RT-PCR, led to a loss of glucose sensitivity resulting an increased release at 4 mM glucose (14.4±0.9 RE) and a decreased release at 3 mM glucose (16.2±1 RE).

Conclusion: Rat alpha cells express the functional neuroendocrine form of glucokinase. Single rat alpha cells respond to an increase of glucose concentration with a decrease in glucagon release with a set point between 3 and 4 mM glucose. Activation of glucokinase lowers this set point while inhibition of Glucokinase raises it. Knockdown of glucokinase leads to a loss of glucose responsiveness. Taken together these data indicate that glucokinase is indeed the glucose sensor linking glucose concentration to glucagon secretion in pancreatic alpha cells.

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OP 44 Logistics of diabetes care

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A systematic review of educational, psychosocial, behavioural and health service organisational interventions to improve outcomes for young adults with type 1 diabetes

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Background and aims: Many young adults (YA) with type 1 diabetes (T1D) experience poor outcomes as a result of their condition. Reasons may include poor knowledge and self management skills, poor clinic attendance, poor adherence with treatment recommendations and engaging in high risk behaviours. The aim of this systematic review was to identify, describe and synthesise the evidence regarding the effectiveness of any educational, psychosocial, behavioural and health service organisational interventions to improve outcomes for YA with T1D.

Materials and methods: Five electronic databases were searched between June and September 2014. Expert groups were contacted to identify relevant grey literature. Any studies that addressed interventions related to patient education and/ or support, behaviour, lifestyle change, or health service organisational change for YA between 15-30 years old with T1D were included. Interventions which aimed to improve outcomes by purely pharmacological or physiological means were excluded. A narrative synthesis of all relevant studies was undertaken.

Results: Papers (n=1,700) were screened by 2 authors (a 3rd author screened if there were discrepancies), 19 quantitative studies published between 1991 and 2013 met the inclusion criteria. Four major categories were identified; Health Services Organisation (n=4), Technology (n=9), Support (n=2) and Education (n=4). Quality of the studies was variable, 1 randomised controlled trial (RCT), 3 retrospective, 7 feasibility/ acceptability studies and 8 studies with a pre/ post design were identified. All the studies were of relatively short duration (3 days - 12 months, 1 study was 36 months), 12 had convenience/ opportunistic samples (often with small sample sizes) and 10 had no/ inappropriate controls. Outcome measures included HbA_{1c} (n=14), frequency of episodes of hypoglycaemia/ diabetic ketoacidosis, diabetes knowledge, clinic attendance, telephone contact time with diabetes nurse, insulin administration, treatment satisfaction, self care activities, attitudes and behaviours towards intervention, number of blood glucose (BG) tests, BG cue awareness and compliance. The majority reported weak evidence for the intervention improving the outcomes of interest and where significance was reported, it often deteriorated over time. The use of E-health (text messaging and internet), newer insulin administration devices and smarter BG self monitoring devices in the review highlighted the importance of Technology. Papers in the Health Services Organisation category mainly aimed at impacting on diabetes management/ engagement by the introduction of a structured/ coordinated approach to transition, some incorporated educational elements (n=2), and technology (n=1). Papers in both the Education and Support categories aimed to improve management of diabetes, specifically glycemic control and 2 aimed to enhance awareness of BG cues.

Conclusion: This systematic review and narrative synthesis has highlighted a lack of well designed and well described interventions, in particular RCTs, aimed at improving health outcomes for YA with T1D. Further high quality research is warranted in this area.

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Patients education focusing on physical activity - impact on habitual activity levels, exercise tolerance, metabolic control and life quality

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Background and aims: The aim of study was to evaluate physical activity changes in T2DM patients after standard education and educational program with specific target of its increase and influence on metabolic control and patients life quality.

Materials and methods: Prospective randomized unblinded study was performed with 79 T2DM patients randomized to standard educational program consisting of 5 sessions and similar program with addition of 15 minute simple physical exercises during each session, use of pedometer ("standard" and "active" education groups correspondingly). Physical activity was evaluated by IPAQ questionnaire and average number of steps per day by pedometer. 6-minute walk test (6-MWT) distance was used for assessment of exercise tolerance, SF-12 v.2 questionnaire for life quality. HbA1c and body weight changes characterized metabolic control. Patients were observed for 6 months after finishing education program. Data are presented as M(SD) format, Mann-Whitney U-test was used for statistical analysis.

Results: 39 patients were randomized to standard and 38 to active group. At baseline groups were comparable for age, diabetes duration, HbA1c, BMI, IPAQ score, 6-MWT distance, number of steps per day and physical component score of SF-12 (see Table). For mental component score of SF-12 there was statistically significant difference in favor of standard group. As it is shown in data table, in 6 months patients of active group had increase in physical activity and 6-MWT distance, increase in number of steps per day compared to standard education. Also they had significantly larger decrease of HbA1c, improvement of PCS and trend for improvement of MCS scores of SF-12. Then both standard and active group were evaluated together higher self-reported activity (METs/wk >1500) was associated with significantly more weight loss compared to baseline (-1.9(2.4) vs. -0.7(2.7) kg p=0.04), larger 6-MWT distance (460(62) vs. 415(81) m p=0.01), better life quality on physical component score (42(7.2) vs. 37(7.9) p=0.006).

Conclusion: In T2DM patients education can lead to significant increase in physical activity together with improvement of metabolic control and life quality. Its effectiveness was significantly enhanced by introducing practical physical exercises and patients' participation in physical activities together with use of pedometers with minimal additional time and costs. Not only daily patients activities but also their tolerance to physical exercise was increased which can improve patients' attitude and capacity for habitual exercise. Higher habitual exercise levels were associated with better weight control and life quality thus improving health care for diabetic patients.

Standard and active groups characteristics at baseline and 6 months after therapeutic education.

	Standard	Active	p-level
	Baseline		
Age, y.	63(6.0)	64(7.0)	0.9
Diabetes duration, y.	5.2(6.4)	5.2(6.0)	0.9
HbA1c, %	7.1(1.4)	7.3(1.5)	0.8
BMI, kg/m^2	30.5(6.4)	32.2(6.9)	0.2
IPAQ score, METs/wk	1200(787)	1227(709)	0.7
6-MWT distance	412(71)	399(88)	0.5
Steps per day	6195(2705)	6166(2391)	0.9
SF-12 PCS	39(9.4)	37(7.3)	0.4
SF-12 MCS	45(8.3)	41(7.9)	0.03
	6-months visit (compared to baseline)		
dHbA1c	-0.3(0.85)	-0.8(1.1)	0.006
dMETs/wk	+363(1077)	+1361(1559)	0.005
d 6-MWT distance	+16(33)	+49(71)	0.01
d Steps per day	-286(2105)	+1609(2096)	0.02
dPCS	+1(7.1)	+4(6.0)	0.04
dMCS	0(7.9)	+3(8.4)	0.07

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Study on efficacy of long-term education for achieving compensation of type 1 diabetes mellitus in children and adolescents

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Background and aims: Today education is a basic component in therapy of patients with diabetes mellitus in most countries. It was interesting to assess achievement of compensation in children and adolescents with type 1 diabetes mellitus within a 5-year period after training. The work was initiated to assess level of knowledge and compensation degree in children and adolescents with type 1 diabetes mellitus in "Type 1 Diabetes School".

Materials and methods: A five-day training course was conducted in "Type 1 Diabetes School" at our Center. The training was conducted by means of a structured program containing all appropriate sections. Before and after training course all participants were tested with a questionnaire containing 30 key questions for self-control. On the basis of the findings children and adolescents with type 1 diabetes mellitus were divided into groups, trained and untrained. 54 of 80 children and 38 of 57 adolescents were preliminary trained, 26 children and 19 adolescents got no training. DS5 Glycomat (USA) was used to measure glycated hemoglobin (HbA1c) by means of high pH anion-exchange liquid chromatography. Certified by the National Glycohemoglobin Standardization Program this method became the reference one. It helps demonstrate the predicting role of HbA1c level as a criterion for assessment of compensation.

Results: Before training children gave right answers to 20% of questions only. In 5 years they could give right answers to 85% of questions, mean HbA1c in trained and untrained children was 7.8% and 10.8%, respectively. Children who got no training, those with low level of knowledge and motivation both in them and their parents were hospitalized at the intensive care units more frequently both before and after training. Within follow-up period they gave right answers to 15% of questions only. The trained adolescents with type 1 diabetes mellitus initially could give right answers to 30% of questions, in 5 years there were 70% of right answers. In the untrained adolescents, level of knowledge remained as low as it was before and after 5-year follow-up, mean HbA1c level was 8.1% and 10.1% in the trained and untrained adolescents, respectively.

Conclusion: In the group of children trained for 5 years HbA1c mean level was 7.8%, in those untrained it was 10.8%. In the groups of trained and untrained adolescents mean HbA1c was 8.1% and 10.1%, respectively. Better compensation and level of knowledge in children as compared with those among adolescents confirm the role of family in the type 1 diabetes mellitus control.

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Effectiveness of nurse-led telecoaching in optimising diabetes control: results of a randomised controlled trial in patients with type 2 diabetes

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Background and aims: About a half of people with type 2 diabetes do not reach their treatment targets, increasing the risk of future complications. Patient education has been shown to contribute to a better glycaemic control. Diabetes education by phone may improve patient inclusion and increase efficiency, however the effectiveness of telephone

education in type 2 diabetes is uncertain. The objective was to study the effect of "The COACH Program", a well-established Australian telecoaching program for patients with chronic conditions, on glycohemoglobin and other modifiable risk factors in patients with type 2 diabetes in primary care.
Materials and methods: Randomized controlled trial in Belgium wherein people between 18 and 75 years were selected on hypoglycaemic agent consumption, recruited by their sickness fund and randomized to telecoaching or usual care. The intervention was delivered by diabetes nurse educators and consisted of five telephone sessions spread over 6 months, aimed at achievement of guideline-recommended diabetes treatment targets. The primary outcome was change in HbA1c at 6 months in the entire study group and the subgroup with HbA1c≥7% at baseline (further "subgroup"). The secondary outcomes were: total cholesterol (TC), LDL, HDL, triglycerides, BP and BMI at 6 and 18 months follow-up. The measurements were performed by trial nurses and the blood samples analyzed in a single lab. Linear mixed-effect model was used to compare the change in outcomes within and between groups.
Results: 574 eligible patients were randomized to the telecoaching (N=287) or the control group (N=287). Their median age was 64 years, 62% were men, 86% on oral antidiabetics therapy. The mean (SD) baseline HbA1c,% was 7.0 (1.0) and 7.9 (0.9) in the subgroup, TC: 176 (38) mg/dl, BP: 133 (17)/75 (10) mmHg, BMI: 30 (5) kg/m2. 85% of patients in the telecoaching group attended the intervention as planned. The attrition rate was 11% and 16% in the intervention group and 9% and 14% in the control group at 6 months and 18 months respectively. At 6 months, the reduction in HbA1c,% (95% CI) in the intervention group was -0.2 (-0.3 to -0.1, p=.000) and in the subgroup -0.5 (-0.7 to -0.3, p=.000); in the control group 0.0 (-0.1 to 0.1, p=.703) and -0.1 (-0.3 to 0.1, p=.411) in the respective subgroup. The between-group difference in HbA1c was 0.2 (0.1 to 0.3, p=.003) and 0.4 (0.1 to 0.7, p=.002) in the subgroup. Other between group differences at 6 months were observed only in BMI (kg/m2): 0.4 (0.1 to 0.7, p=.003) and TC (mg/dl): 6 (2 to 11, p=.011), in favor of the intervention. At 18 months, the sustained between-group difference in HbA1c was 0.1 (0.0 to 0.3, p=.065) and 0.3 (0.0 to 0.6, p=.033) in the subgroup. No between-group difference in other outcomes was observed at 18 months.
Conclusion: The COACH Program improved the glycaemic control, total cholesterol and body mass index at 6 months in people with type 2 diabetes in Belgian primary care. Overall, the differences between groups did not sustain at 18 months. In patients with HbA1c≥7% at baseline, the clinically relevant difference in glycaemic control slightly declined but stayed significant at 18 months follow-up. Nurse-led telecoaching in type 2 diabetes is an effective alternative to face-to-face patient education.
Clinical Trial Registration Number: NCT01612520
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OP 45 Burning fat: oxidation in muscle

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The effect of a short-term high-fat overfeeding on plasma levels of acylcarnitines in young, healthy men with low or normal birth weight
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Background and aims: Low birth weight (LBW) subjects have an increased risk of developing type 2 diabetes later in life compared with normal birth weight (NBW) subjects. Also, an impaired oxidation of fatty acids and a subsequent accumulation of lipid species have been implicated in the pathogenesis of type 2 diabetes. Acylcarnitines are markers of an incomplete beta-oxidation in mitochondria. Here, we investigated whether LBW and NBW subjects had different plasma levels of acylcarnitines after a control diet and a high-fat, high-calorie diet and whether they changed these levels in response to overfeeding. Also, we examined whether the levels were related to insulin secretion and/or sensitivity.
Materials and methods: Twenty LBW (0-10th percentile) and twenty-six NBW (50-90th percentile) young, healthy men were in a random order given a three-day control diet and a five-day high-fat, high-calorie diet. After these challenges, we measured fasting plasma levels of forty-five acylcarnitine species by tandem mass spectrometry and assessed differences in these levels between birth weight groups or diets by unpaired or paired t-tests, respectively, or Wilcoxon signed rank tests. Also, we performed intravenous glucose tolerance tests and hyperinsulinaemic euglycaemic clamps to assess insulin secretion and sensitivity and evaluated associations between levels of acylcarnitines and these measures by regression analyses adjusted for age, body mass index, and birth weight.
Results: LBW subjects had higher plasma levels of eight acylcarnitine species, of these five hydroxyl- (OH)/dicarboxyl- (DC) species, as well as of total acylcarnitines and total OH-/DC-acylcarnitines after the control diet compared to NBW subjects, but no differences were seen after the high-fat, high-calorie diet. Furthermore, LBW and NBW subjects decreased levels of twelve species and increased levels of three or four species, respectively, and additionally decreased the level of total acylcarnitines in response to overfeeding. Also, the level of total OH-/DC-acylcarnitines was inversely associated to the serum level of insulin and hepatic insulin resistance after the control diet.
Conclusion: LBW subjects had a higher plasma level of total acylcarnitines after the control diet compared to NBW subjects, which indicates that they have an incomplete beta-oxidation of fatty acids in mitochondria. Also, their higher level of total OH-/DC-acylcarnitines suggests that they have a higher omega-oxidation of fatty acids in endoplasmic reticulum and/or an incomplete beta-oxidation of OH-/DC-fatty acids. LBW and NBW subjects decreased the level of total acylcarnitines in response to overfeeding, which may be a normal response to an acute overfeeding followed by an overnight fast with a minor release and oxidation of fatty acids in this less fasted state. Acylcarnitines do not seem to adversely affect insulin secretion or action. On the contrary, the level of total OH-/DC-acylcarnitines was inversely associated to insulin resistance, which suggests that omega-oxidation may be a scavenger pathway for oxidation of fatty acids that could accumulate as lipid species that impair insulin signalling.
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Suppression of plasma free fatty acids similarly reduces myocardial lipid content and left ventricular systolic function in type 2 diabetic patients as in controls

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Background and aims: Type 2 diabetes (T2DM) is closely associated with the development of heart failure, which could in part be due to impaired substrate metabolism and accumulation of myocardial lipids (MYCL). To better understand substarte metabolism of the diabetic heart,this study investigated the impact of an acute pharmacological inhibition of adipose tissue lipolysis leading to reduced availability of circulating FFA on MYCL and heart function in T2DM.
Materials and methods: 8 T2DM (Age:56±11a;BMI:28±3.5 kg/m2;HbA1c:7.29±0.88%) were investigated on two study days in random order, following administration of Acipimox /Placebo and compared to previously assessed data in healthy controls. MYCL and heart function was measured by 1H-magnetic-resonance-spectroscopy and tomography at baseline, at 2 and at 6 hours.
Results: Acipimox reduced MYCL by -39±41% as well as systolic heart function significantly (Ejection Fraction (EF): -13±8 and cardiac index: -16±15% compared to baseline) to the same extent than in healthy controls., FFA strongly correlated with changes in MYCL ΔMYCLMR1vsMR3: r=0.707; p=0.002) and EF (ΔEFMR1vsMR3: r=0.651; p=0.006). Diastolic heart function remained unchanged.
Conclusion: Inhibition of adipose tissue lipolysis is associated with a rapid depletion of MYCL-stores and reduced systolic heart function in T2DM. These changes were comparable to those previously found in insulin sensitive controls. MYCL thus likely serves as a readily available energy source to cope with short-time changes in FFA availability in the diabetic state.
Clinical Trial Registration Number: NCT01980524

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Saturated fatty acids increase intramuscular lipid by activation of lipogenic pathways dependent on SREBP-1c with no modification of fatty acid transport or lipid oxidation

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Background and aims: Dietary saturated fatty acids induce insulin resistance in muscle with the increment in intramuscular lipid accumulation (IMLA). The mechanism by which dietary fatty acids modulate IMLA are not clear. The aim of this study is evaluate the mechanism that could be regulating the IMLA under different dietary fatty acid composition.
Materials and methods: A group of rats was assigned to an isoenergetic and normocaloric diet, each with a significantly different composition of saturated (SD), monounsaturated (MD) and polyunsaturated (PD) fatty acids and they were feed for a month. Samples of muscle tissue were taken to determine tissue lipid accumulation and to study the gene expression levels of fatty acids transports FATP1, FATP4, FAT/CD36 and FABPpm, muscle lipid oxidation marker CPT1 and the lipogenic markers SREBP-1c, ACC1 and SCD1. Additionally, L6 cells were differentiated and then treated with Palmitic, Oleic, and Linoleic fatty acid for 24 h. Fatty acid cytotoxicity was evaluated by the gene expression levels of BCL2, P53 and Casp3. After treatment lipid accumulation and the same genetic markers as in muscle tissue samples were studied. Analysis of differences between groups was performed using Kruskal-Wallis or U of Mann-Witney test. Associations between variables were evaluated by Spearman corretation test. A P value <0.05 was considered statistically significant.
Results: Animal weights did not vary according to diets. Muscle lipid accumulation were significantly higher in animals feed with CD (p=0.01). The expression levels of CPT1 and fatty acid transport proteins FATP1, FATP4, FAT/CD36 were no different between diets, however FABPpm gene expression levels were significantly lower in animals fed with SD (p<0.01), with an inverse correlation with the IMLA (r=-0.59, p<0.01). SD raised muscle lipogenic markes with the increment of SREBP-1c , ACC1 and SCD1gene expression levels (p<0.01, p=0.05 and p=0.03 respectively); a direct association between these gene expression levels and the IMLA was found (r=0.54, p=0.02; r=0.59; p<0.01 and r=0.52, p=0.03 respectively). Fatty acids did not induce apoptosis. L6 lipid content (CLC) was higher in cells treated with any of the fatty acids than in control group, with no differences between the fatty acid treatment. None of the measured gene expression levels were different according to the control group nor to fatty acids treatment. However, FATP1 and FABPpm gene expression were negative associated with the lipid content (r=-0.64, p<0.01 and r=-0.62, p<0.01 respectively), while lipogenic markers SREBP-1c, ACC1 and SCD1 gene expression levels were directly correlated with CLC (r=0.44, p=0.04; r=0.46, p=0.03 and p=0.54, r=0.01 respectively).
Conclusion: The elevated levels of IMLA in the presence of saturated fatty acids are associated with the induction of SREBP-1c gene expression and its regulated lipogenic pathways with the increment of ACC1 and SCD1 gene expression levels, rather than the modification in the fatty acid transport or the muscle lipid oxidation.
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Role of Rab-GTPases in the regulation of fatty acid metabolism in skeletal muscle

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Background and aims: Besides glucose, fatty acids play a central role in the energy metabolism of skeletal muscle. Deficiency of the Rab GTPase-activating protein (Rab-GAP) TBC1D1 leads to an increase of fatty acid uptake and fatty acid oxidation accompanied by a shift in skeletal muscle energy substrate preference from glucose towards fatty acids. It is the aim of this project to find the underlying mechanisms that drive the shift towards fatty acid metabolism under conditions of Tbc1d1-deficiency.
Materials and methods: Cell culture experiments were performed using differentiated C2C12 myoblasts. Selected Rab GTPases (Rab8a, 10, 14, 28)that function as potential substrates of TBC1D1 were silenced via siRNA technology. Subsequently, fatty acid uptake was determined measuring the incorporation of ^{3}H-Palmitic acid. In addition, siRNA oligos for Rab-GTPase knock-down (KD) were transfected into Extensor digitorum longus (EDL) and Soleus muscle of 12 - 15 week old C57BL/6 males via in vivo Electrotransfection (IVE). Seven days after electrotransfection intact EDL and Soleus muscle were dissected and ^{3}H-Palmitic Acid oxidation was measured.
Results: In cultured C2C12 myotubes the Knock-down (KD) of Tbc1d1 led to a significant increase in fatty acid uptake (+41%, SEM±21%). KD of Rab8a, Rab10 and Rab14 reduced fatty acid uptake (Rab8a: -43%, SEM±13%; Rab10: -41%, SEM±9%;

Rab14: - 23%, SEM±20%). In contrast to this Rab28 KD did not affect fatty acid uptake in these cells. In both, glycolytic EDL and oxidative Soleus muscle the siRNA-mediated KD of Rab8a caused a significant reduction of fatty acid oxidation (EDL: -35%, SEM±9%; Soleus: -42%, SEM±8%). KD of Rab10, however, did not alter fatty acid oxidation in EDL or Soleus muscle.

Conclusion: We showed that Rab8a, Rab10 and Rab14 influence fatty acid uptake in cultured C2C12 myotubes in a similar fashion, while knock-down of Rab28 did not lead to changes in fatty acid uptake in these cells. Ex vivo experiments revealed that the siRNA-mediated KD of Rab8a led to a significant reduction of the ex vivo fatty acid oxidation of intact isolated EDL and Soleus muscle. In contrast to the cell culture experiments, KD of Rab10 did not affect ex vivo fatty acid oxidation in both, EDL and Soleus muscle.

OP 46 Watching the granule: novel regulators of beta cell exocytosis

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Rabs and RIM are differentially involved in docking and tethering of insulin granules

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Background and aims: Glucose dependent insulin release is markedly reduced in the islets obtained from type 2 diabetic subjects. Insulin is released by regulated exocytosis and docking of insulin granules at the plasma membrane, followed by the assembly of the secretory machinery is a prerequisite for insulin release. We have recently shown that central elements of the secretory machinery assemble after granule docking, but it remains unclear whether granule docking sites can exist before arrival of a granule. Current models propose that docking of the granules occurs through binding to either raft-like clusters of SNARE proteins or to structural proteins such as RIM1, in all cases implying at least partial assembly of the secretory machinery prior to docking. However, direct evidence for this is lacking.

Materials and methods: Dual-color TIRF microscopy of INS1 cells 832/13 combined with quantitative image analysis was used to study the association of GFP-labeled proteins with granules labelled with NPY-mCherry at the plasma membrane.

Results: We show that the insulin granule transits to stably docked state from a loosely tethered state a few seconds after the arrival of the granule. This transition is induced by recruitment of syntaxin and munc18-1, and that recruitment of munc13 and SNAP25 was delayed further. Here we extend on this work and present quantification of several exocytosis proteins including rab3 and 27, rabphilin, granuphilin, RIM1, tagged with EGFP at the insulin granule release site during insulin granule docking, priming and exocytosis. We find that the Rab3 interacting protein RIM1 and Rabphilin were enriched at docking sites prior to granule tethering and docking. A slow but significant increase of RIM1 fluorescence was seen during granule maturatrion into the releasable pool (priming), suggesting roles for RIM1 in both docking and priming. None of the other proteins were present before granule arrival, but they were instead recruited during docking or even later during priming. Granules that docked successfully carried Rab3 and Rabphilin, whereas those that only temporarily tethered did not. In contrast, Rab27 and its effector Granuphilin were present on both types of granules.

Conclusion: We conclude that sites enriched with RIM1 at the plasma membrane act as tethers for incoming granules. The likely binding partners on the granule for this weak interaction are rab27/granuphilin. Conversion into the stably docked state that is required for exocytosis occurs only when rab3/rabphilin are also present on the granule. Successful docking coincides with acute clustering of syntaxin/munc18, and we propose that this cluster then nucleates assembly of the exocytosis machinery. It may thus underlie the docking defect observed with reduced expression of SNARE and accessory proteins during type 2 diabetes.

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Clustering of L-type CA^{2+}-channels promotes exocytosis of individual secretory granules

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Background and aims: Ca2+ entry through voltage gated L-type calcium channels triggers release of insulin-containing secretory granules, and is required for 1st phase insulin secretion. Indirect electrophysiological and biochemical evidence suggest that L-type channels functionally couple to the exocytosis machinery by binding of the α1 subunit of L-type channels to SNARE proteins through its synaptic protein interaction site (synprint). Clustering of L-type channels near docked granules is thought to facilitate exocytosis by generating local domains where Ca2+ is sufficiently elevated. However, direct evidence for channel clustering and localized Ca2+ influx near granules is lacking.
Materials and methods: EGFP-labeled Ca2+-channel subunits (Cav1.2) were imaged together with a granule marker in human islet cells and Ins1 cells using TIRF-microscopy. Local Ca2+ domains were visualized using Fluo 5 F-am or membrane-targeted lyn-R-GECO. Ca2+-currents were recorded using whole cell patch clamp.
Results: Cav1.2-EGFP formed clusters on a hazy background that partially colocalized with a subset of the docked granules. Image analysis indicated specific binding of Cav1.2-EGFP to granules, which was confirmed by single molecule imaging demonstrating reduced mobility of CaV1.2-EGFP near docked granules. This binding was not detectable when the synprint fragment was coexpressed, suggesting that the peptide competes with and uncouples Cav1.2 from the exocytosis machinery. Ca2+-channel clusters formed at granules at least 60 s after their arrival at the plasma membrane, and rapidly dispersed after exocytosis. In depolarized cells, docked granules that exocytosed (responders) carried significantly more CaV1.2-EGFP than those that failed to do so (failures). Endogenous Ca2+-channels were visualized functionally as Ca2+ microdomains; these measurements indicated localized and more rapid Ca2+-entry at responder granules compared with failures or random sites. Modeling of Ca2+-influx confirmed that the observed rates of Ca2+-entry and distribution are consistent with localized Ca2+-influx near granules. Statistical analysis indicates that the probability of exocytosis is at least 1000-fold higher when a channel is bound to the granule than when it is 500 nm away.
Conclusion: Using high resolution microscopy we identify in human and rat insulin secreting cells a granule pool where localized Ca2+ influx leads to exocytosis with high release probability and minimal latency, corresponding to the immediately releasable pool (IRP). Prior to exocytosis these granules slowly recruit on average L-type Ca2+-channels through an interaction that involves the channels' synprint site. When this interaction is interrupted by expression of exogenous synprint or when Ca2+ is raised by release from intracellular stores, rapid exocytosis is absent. This arrangement leads focal Ca2+ influx that supports rapid and selective exocytosis of the associated granules with minimal requirements for Ca2+-influx. Thus, IRP granules are docked at the plasma membrane and associated with L-type Ca2+-channels.
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MiR-335 regulates exocytotic proteins and affects glucose-stimulated insulin secretion through decreased Ca^{2+}-dependent exocytosis in beta cells

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Background and aims: Ca^{2+}-induced exocytosis is essential for insulin to be secreted from beta-cells, and in islets from type-2 diabetic (T2D) donors the expression of several genes coding for exocytotic proteins is reduced. Largely this phenomenon cannot be explained by polymorphism; rather it is likely due to epigenetic factors like microRNAs (miRNAs). Indeed, previous studies have identified a number of miRNAs with differential expression in the islets from T2D donors and the Goto-Kakizaki (GK) rat. One of the upregulated miRNAs in the GK rat is miR-335, predicted to target several exocytotic genes amongst those *Stxbp1* is a validated target. Here we aim to investigate whether miR-335 regulates the expression of exocytotic genes and affects insulin secretion and exocytosis in beta-cells.
Materials and methods: Insulin secretion was measured by radio immuno assay. Exocytosis and docking of insulin granules was studied by capacitance measurements using the patch-clamp technique and by TIRF microscopy. Rat miR-335 was overexpressed using chemically-modified mature microRNA mimic in INS-1 832/13 beta-cells by transfection. Gene knockdown was performed with RNAi. Protein and mRNA levels were analysed with Western Blot and RT-qPCR, respectively.
Results: Overexpression of miR-335 (OE335) in INS-1 832/13 cells reduced insulin secretion at 16.7 mM glucose compared to control cells (SCR) ($n=3$; $p<0.01$). This was not due to reduced insulin content ($n=3$) or voltage-dependent Ca^{2+} currents ($n=15$-17) but rather a direct effect on second phase exocytosis; the measured increase in membrane capacitance was extensively decreased in OE335 cells (42±23 f. *vs* 128±18 fF; $n=11$-14; $p<0.01$). In agreement, exocytotic events measured by TIRF microscopy were reduced in OE335 cells (0.032 ± 0.001 min^{-1} μm^{-2}; $n=8$) compared to SCR cells (0.049 ± 0.003 min^{-1} μm^{-2}; $n=8$; $p<0.001$). However, TIRF microscopy revealed similar density of docked granules in both OE335 and SCR cells ($n=51$-53). The exocytotic genes coding for Snap25, Stxbp1 and Synaptotagmin 11 (Syt11), known to have reduced expression in T2D islets, are putative targets of miR-335. In accordance, protein levels of Snap25, Stxbp1 and Syt11 were decreased in OE335 cells compared to SCR cells ($p\leq0.05$; $n=4$ for each protein). Snap25 and Stxbp1 have previously been proven essential in insulin exocytosis, but the role of Syt11 is still uncertain. Interestingly, Syt11 silencing in INS-1 832/13 cells resulted in decreased first phase exocytosis measured as increase in membrane capacitance (161±22 f. *vs* 251±34 f. in control cells; $n=9$-13; $p<0.05$).
Conclusion: We suggest that miR-335 is crucial in the regulation of glucose-stimulated insulin secretion. It functions by reducing the exocytotic proteins Snap25, Stxbp1 and Syt11 which modulates Ca^{2+} dependent exocytosis in beta-cells. The progression of T2D is facilitated by failure of beta-cell adaptations to secret more insulin. We believe our study contributes with novel insights as to how single dysregulated miRNA may affect pancreatic beta-cell function.
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Effects of high glucose and K^+ depolarisation on secretion and insulin granule mobility in MIN6 cells and mouse beta cells

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Background and aims: To further characterize the differences between nutrient- and depolarization-induced insulin secretion, we compared insulin secretion and parameters of insulin granule mobility in MIN6 cells and MIN6 pseudo-islets and in normal mouse beta-cells and islets.

Materials and methods: Insulin granules were labeled by transient transfection with insulin-EGFP in MIN6 cells and by adenoviral transduction in primary mouse beta-cells. The granules present in the submembrane space of the cell footprint were imaged by TIRF microscopy. The cells were continuously perifused at 37°C with oxygenated medium containing the respective stimuli. The image files (1 sequence=200 images=25 seconds) were evaluated by an in-house written program to achieve an observer-independent quantification. The insulin secretion of MIN6 pseudo-islets and mouse islets was measured by perifusion with ELISA of the fractionated efflux.

Results: The perifused islets and pseudo-islets were stimulated by 40 mM K^+ for 10 min and, after a wash-out period, by 30 mM glucose for 10 min. In MIN6 pseudo-islets 30 mM glucose was much less effective than 40 mM K^+. When the K^+ depolarization preceded the glucose stimulus, glucose was entirely ineffective. In mouse islets glucose was about equi-effective with K^+ depolarization. When the K^+ depolarization preceded the glucose stimulus, the glucose-induced secretion had a sluggish onset, but still reached the same maximal level. Under control condition, the number of granules in the first image of the sequences was 337 ±34 per MIN6 cell but only 68±15 per beta cell. In both cell types this parameter remained essentially stable during imaging. Due to continuous arrivals at and departures from the evanescent field the total number of granules under this condition were 6972±873 per sequence in MIN6 cells and 1439±281 in beta-cells. In MIN6 cells about 5720 granules per sequence were classified as short-term resident granules (residence time≤1 s) and about 118 granules were long-term residents (residence time≥25 s). The corresponding values for beta-cells were 1334±247 and 15±5, respectively. Apparently, the proportion of long-term resident granules is lower in beta cells than in MIN6 cells. The stimulation pattern in beta cells differed from that of MIN6 cells, in that only K^+, but not glucose increased the number of arriving granules and diminished the number of long-term resident granules.

Conclusion: The insulinotropic efficacy of nutrient stimulation is much higher in beta-cells than in MIN6 cells, while both have comparable responses to K^+ depolarization. This may be related to the proportionally lower number of tethered granules in beta-cells. Unexpectedly, the granule mobility in beta cells was less influenced by insulinotropic stimulation than that in MIN6 cells.

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OP 47 Biomarking of atherosclerosis

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Lack of association between plasma PCSK9 and LDL-cholesterol in patients with controlled type 1 diabetes

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Background and aims: Pro-protein convertase subtilisin/kexin type 9 (PCSK9) is a post-transcriptional inhibitor of LDL-receptor (LDLR). PCSK9 impairs LDL clearance by promoting the LDLR degradation. PCSK9 is secreted by hepatocytes and binds to the extracellular domain of the LDLR, causing degradation in the lysosomes. Previous studies have shown that, in non-diabetic population, PCSK9 was positively correlated to the circulating plasma concentrations of total cholesterol and especially LDL cholesterol (LDL-C). In case of good glycemic control, lipid profile of patients with type 1 diabetes mellitus (T1DM) is different from what is observed in case of poor glycemic control; it can be observed an increase in plasma triglycerides and higher LDL-C levels in case of poor glycemic control. A previous study has shown that PCSK9-LDL association was lost in patients with type 2 diabetes mellitus with poor glycemic control. Here, we aimed to determine the relationship between circulating levels of PCSK9 and LDL-C in subjects with T1DM depending on glycemic control.

Materials and methods: A multicentric study of PCSK9 plasma levels was performed in 202 patients with T1DM whose diagnosis was made before age 40 and insulin treatment initiated within 12 months after diagnosis. They were free of any hypolipidemic agent. Fasting blood samples were obtained from a biological collection. Plasma concentrations of PCSK9 were mesured using a sandwich ELISA assay (CycLex).

Results: The mean age was 38 years and diabetes duration 18 years. The mean HbA1c was 8.3%. Plasma PCSK9 concentrations ranged from 85 to 868 ng/ml (mean 314 ng/ml). A positive correlation was found between PCSK9 and age and systolic blood pressure. Plasma PCSK9 was positively correlated with total cholesterol, triglycerides, LDL-C, apoB (Table 1). There was a tendency for a positive association between PCSK9 and HbA1C. In multivariate analysis, PCSK9 (log) was independently associated with LDL-C (p=0,04) and HbA1c (p=0,03). However, the association between PCSK9 and LDLC seemed to be limited to the patients with "uncontrolled" diabetes: PCSK9 was still correlated with LDL-C (p=0.0004) in “uncontrolled” diabetics (HbA1c≥8%) while this correlation was lost (p=0.52) in “controlled” diabetics. Plasma PCSK9 levels were significantly higher in “uncontrolled” diabetes subgroup than in “controlled” diabetics (p=0.001).

Conclusion: This first study evaluating PCSK9 in patients with T1DM shows a positive correlation between PCSK9 and total cholesterol, LDL and triglycerides; this correlation is lost in patients with good glycemic control in contrast to patients with poor glycemic control.

Variable	Univariable linear regression		Multivariable linear regression
	R^2	p-value	p-value
Age	0,023	**0,03**	0,16
BMI	0,02	**0,04**	0,31
Systolic BP	0,036	**0,007**	0,12
Estimated GFR	0,0003	0,82	0,55
HbA1c	0,015	0,08	**0,03**
Total cholesterol	0,039	**0,005**	-
Triglycerides	0,03	**0,01**	-
LDL-C	0,05	**0,001**	**0,04**
ApoB	0,039	**0,006**	-

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Dysfunctional endothelial progenitor cells in diabetes: establishing a link between epigenetics and metabolic memory

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Background and aims: Endothelial progenitor cells (EPCs) have regenerative capacities and play an important role in vessel wall homeostasis by promoting angiogenesis and maintaining endothelial integrity. The EPCs dysfunction, observed in type I and II diabetes mellitus, contributes to the insurgence of vascular complications in diabetic patients. These complications persist and progress even after good long-term glycemic control, a phenomenon termed "metabolic memory", suggesting that EPC dysfunction might also last even after glycemia normalization. We investigated whether the exposure to pathologic glucose concentrations affected human cord blood-derived CD34+ cell functions and whether these defects persisted after glucose normalization.
Materials and methods: CD34+ cells were purified from cord blood of healthy donors and expanded in normal-glucose (NG; with 30 mM mannitol for osmotic control) or high-glucose (HG; 30 mM) serum-free medium plus cytokines (FLT3 50 ng/ml, SCF 50 ng/ml, IL3 20 ng/ml and IL6 20 ng/ml) for up to 30 days. The proliferative capacity of CD34+ cells was assessed by producing a growth curve over 30 days and performing doubling time analyses. Telomere length was evaluated by quantitative real-time PCR. Migration ability toward stromal cell-derived factor-1 (SDF-1) was determined using a modified Boyden chamber. CXCR4 protein and mRNA expression were assessed by flow cytometry and quantitative real-time PCR, respectively. The methylation status of CXCR4 gene was assessed by methylation-sensitive restriction enzyme digestion followed by quantitative real-time PCR.
Results: We previously showed that after 20 days hyperglycemia dramatically impaired CD34+ cell proliferation, migration and differentiation into pro-angiogenic cells. Here we demonstrated that high glucose affected CD34+ proliferation by inducing cell senescence. Indeed, after 20 days HG-CD34+ cells presented a significant telomere shortening when compared to the NG-CD34+ cells ($p \leq 0.05$ vs NG). In addition, we also showed that the reduced migration toward SDF-1 in HG-CD34+ cells was associated to a down-regulation of CXCR4 receptor, both in terms of protein ($p \leq 0.001$ vs NG) and mRNA ($p \leq 0.05$ vs NG). Importantly, this defect was maintained even after 72 hours of relief from hyperglycemia ($p \leq 0.05$ HG and exHG vs NG). To address whether epigenetic regulation contributed to defective CXCR4 expression in HG-CD34+ cells, we investigated the methylation status of the CXCR4 gene using methylation-specific restriction enzymes. We found that after 20 days HG-CD34+ cells presented a significant ($p \leq 0.05$ vs NG) hypermethylation of CpG island I, IV and V of CXCR4 gene when compared to NG-CD34+ cells.
Conclusion: These data suggest that hyperglycemia negatively affects CD34+ stem cell function by promoting premature senescence and reducing migration ability towards SDF-1 via CXCR4 gene methylation. Interestingly, this latter defect, which persists after glucose normalization, provides the first evidence of an epigenetic contribution to the establishment of "metabolic memory" in CD34+ cells, paving the way to new pharmacological targets.
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FDG-PET-CT assessed subclinical arterial inflammation is associated with glycaemic control, central obesity, and systolic blood pressure in type 2 diabetes patients

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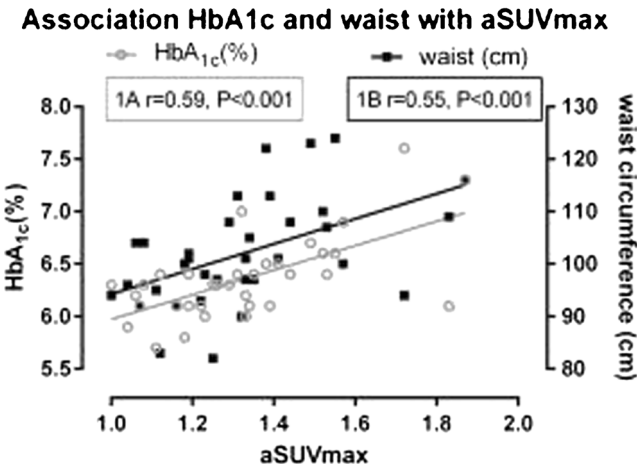
Background and aims: In type 2 diabetes mellitus (T2DM), subclinical atherosclerotic changes occur early in its course. We investigated the association of arterial inflammation and calcification assessed with 18-Fluorodeoxyglucose positron emission tomography-computed tomography (FDG-PET-CT) with atherosclerotic risk factors, skin advanced glycation end products (AGEs)), and microalbuminuria in early T2DM.
Materials and methods: 34 patients with treatment naïve T2DM, without cardiovascular disease , were studied (age 63 [IQR, 55-66] yrs, 65% male, BMI 31.5 [28.6-36.8] kg/m2, HbA1c 6.4±0.4% (46±4.3 mmol/mol), 17% microalbuminuria. A whole body FDG-PET-CT scan was performed to assess arterial inflammation. Standardized uptake value (SUV)max was calculated according to EARL guidelines and CT was used to score arterial calcification (AC: visual score: 0(no calcification) to 4(>50% calcified plaque)). SUVmax and AC were measured in 4 segments (carotid arteries, ascending aorta and aortic arch, descending and abdominal aorta, and iliac and femoral arteries) and calculated for the total aortic tree (aSUVmax, mean of 4 segments). Clinic and 24-hour ambulatory blood pressure (ABPM) was assessed. Skin AGEs were measured as skin autofluorescence.
Results: aSUVmax and AC were not associated. aSUVmax was associated with BMI ($r=0.64$, $P<0.001$), HbA1c (figure 1A), waist circumference (1B), systolic BP(1C) and pulse pressure ($r=0.39$,$P=0.025$; comparable results for ABPM), urinary albumin/creatinine ratio ($r=0.38$, $P=0.031$), and not with SAF, age, gender, smoking, and lipids. After multivariate adjustment ($r2=0.73$, $P<0.001$), only HbA1c, ($\beta=0.50$, $P<0.001$), waist circumference ($\beta=0.45$, $P<0.001$), and SBP ($\beta=0.40$, $P<0.001$) remained significant. AC was associated with male gender ($r=0.48$, $P=0.004$), age ($r=0.51$, $P=0.002$), BMI ($r=-0.43$, $P=0.011$), and SAF ($r=0.37$, $P=0.036$) and not with other mentioned risk factors. After multivariate adjustment ($r2=0.39$, $P=0.001$) only male gender ($\beta=0.49$, $P=0.003$) and SAF ($\beta=0.34$, $P=0.033$) remained significant.
Conclusion: These results show that in treatment naïve T2DM, glycemic control, central obesity, and SBP are independent determinants of FDG-PET-CT assessed subclinical arterial inflammation. Interessingly, while the extent of arterial calcification did not show any relation with arterial inflammation, it was independently associated with skin AGEs, suggesting discordant pathways at different stages of atherosclerotic disease.



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RVX-208 acts via an epigenetic mechanism to lower Major Adverse Cardiovascular Events (MACE) in patients with atherosclerosis and especially in those with diabetes mellitus

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Background and aims: RVX-208 binds selectively to the second ligand domain of Bromodomain and Extra-Terminal (BET) proteins and inhibits their activity. Each BET protein has two bromodomains that bind acetylated lysines in histones. When a BET protein binds ligand, it recruits transcriptional machinery to DNA and thereby modifies gene activity. Analysis of pooled data from two phase 2b trials; SUSTAIN and ASSURE revealed a 55% relative risk reduction (RRR) of MACE in RVX-208 treated patients (n=331) vs. placebo (n=168). But in those with diabetes mellitus (DM) RVX-208 treatment lead to a 77% RRR of MACE vs. placebo. RVX-208 increased production of ApoA-I yielding more high-density lipoprotein (HDL) particles. While these effects should lower MACE, the magnitude was more than expected, prompting studies to identify properties of RVX-208 beyond its effects on ApoA-I/HDL.
Materials and methods: Plasma biomarkers from SUSTAIN and ASSURE trials were analyzed. Microarray data from RVX-208 treated primary human hepatocytes (PHH) or human whole blood (HWB) were used to identify differentially expressed genes, and guide measurements of specific proteins in clinical samples to confirm key findings.
Results: Biomarkers from the trials showed significant increases ($p<0.05$, unless specified) between RVX-208 vs. placebo in: HDL-c (+3 mg/dL), ApoA-I (+7.5 mg/dL), large HDL (+0.7 umol/L), HDL size (+0.1 nm), and total HDL particles (+1.8 umol/L, $p<0.07$). Glucose in all patients (n=499) or in those with DM (n=192) given RVX-208 or placebo was unchanged vs baseline. In patients with DM (n=119) and low HDL (<40 mg/dL), RVX-208 reduced glucose by -0.3 mmol/L but in placebo it increased +0.9 mmol/L. These modest changes do not predict the MACE reductions. Thus microarrays were used to survey PHH and HWB exposed to RVX-208. In PHH, RVX-208 decreased expression of genes in pathways for cholesterol & fatty acid synthesis, innate immunity and glucose processing. Most profound were effects on complement and coagulation pathways, where RVX-208 downregulated expression of 19/26 and 20/33 genes respectively. These data were confirmed by RT-PCR of key mRNAs. Furthermore, specific complement and coagulation proteins were found to be decreased in plasma from the trials (range 7-12% vs. baseline). Microarrays from HWB exposed ex-vivo to RVX-208 identified pathways with known roles in atherogenesis including: pro-inflammatory signaling, cell-cell interactions and extracellular matrix organization. RVX-208 significantly downregulated several pro-atherogenic genes (43/56) but upregulated anti-atherogenic genes (9/17), that control monocyte recruitment, migration and activation, macrophage function, inflammatory signaling and plaque stability to suggest an overall anti-atherosclerotic benefit.
Conclusion: RVX-208 treatment is associated with marked MACE reductions in SUSTAIN and ASSURE patients and especially in those with DM. RVX-208 modifies cellular epigenetics to impact multiple biological processes that underlie CVD. Combined effects of RVX-208 on reverse cholesterol transport, vascular inflammation, innate immunity, atherosclerosis and thrombosis may explain its efficacy in reducing MACE.
Clinical Trial Registration Number: NCT01423188, NCT01067820

OP 48 Not child play: paediatric metabolism

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A trans-ethnic meta-analysis of genome wide association studies reveals new loci associated with childhood obesity

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Background and aims: Childhood obesity has more than doubled in the last 30 years. Children who are obese are more likely to be obese as adults and are more at risk for several adult health problems such as type 2 diabetes and heart disease. To search for genetic variants that are associated with childhood obesity, we performed a trans-ethnic meta-analysis of 22 genome wide association studies with European, African, South American, and East Asian ancestry.
Materials and methods: The discovery stage of the meta-analysis has 10,668 cases, defined as greater than the 95th percentile of BMI between 2 and 18 years old, and 12,647 controls, defined as less than the 50th percentile all throughout childhood.
Results: Using the MANTRA algorithm, we meta-analyzed approximately 19 million SNPs, and found 14 loci that attained genome wide significance ($P<5\times10\text{-}8$). 10 of these loci (FTO, TMEM18, FAIM2, MC4R, SEC16B, ADCY3, TNNI3K, GNPDA2, FHIT, and TFAP2B) have been previously discovered, while 4 (PHLDA1, METTL15, RBMS3, and COTL1) are novel. More than 100 loci were found to be suggestive of association ($P<5\times10\text{-}6$), 7 of which (HOXB3, ADCY9, NEGR1, PTPB2, OLFM4, LINGO2 and SULT1A2) have previously been found to be associated with BMI or Obesity. All of these loci will be carried over into our replication effort, which will narrow down the list even more.
Conclusion: In summary, should these loci survive our replication effort, we will have implicated new pediatric obesity loci.
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CReP loss-of-function causes beta cell failure and diabetes

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Background and aims: A well balanced endoplasmic reticulum (ER) function is crucial for insulin release and survival of pancreatic β-cells. Deficient as well as excessive/prolonged ER stress can cause β-cell dysfunction/death and diabetes. Phosphorylation of the α subunit of eukaryotic translation initiation factor 2 (eIF2α) is key for protein folding homeostasis in the ER. CReP is a cofactor for protein phosphatase 1 (PP1), which is required for eIF2α de-phosphorylation. Here we report on the first mutation in PPP1R15B, encoding CReP, in two siblings with young-onset diabetes.
Materials and methods: Exome sequencing was performed in the index case. PP1 binding was examined in HEK293T cells expressing wild type or mutant CReP. CReP, PUMA, DP5 and Bim were silenced by RNA interference in INS-1E and FACS-purified primary rat β-cells. Insulin secretion was measured by ELISA. eIF2α phosphorylation was examined by Western blot and gene expression by qPCR. Apoptosis was assessed by Hoechst 33342/propidium iodide staining and by Western blot for caspases and cytochrome c release.
Results: Two siblings, born to unaffected parents, had early-onset insulin treated diabetes (autoantibody negative, with low but detectable C-peptide levels) diagnosed at 15 and 28 years. This was associated with short stature and microcephaly. Exome sequencing identified a homozygous R658C mutation in PPP1R15B in the index case, which was confirmed by Sanger sequencing in the two siblings. Introducing the R658C mutation in a CReP plasmid diminished the ability of CReP to bind PP1 in vitro and de-phosphorylate eIF2α. CReP silencing in INS-1E cells increased eIF2α phosphorylation. CReP-deficient β-cells had reduced insulin content and reduced glucose-induced insulin secretion. CReP silencing induced apoptosis in INS-1E cells (14±3% apoptosis with siCReP vs 4±0% with control siCT, $p<0.05$, n=4-5) and primary β-cells (siCReP 22±3% vs siCT 15±2%, $p<0.05$, n=5). CReP silencing induced caspase-9 cleavage and cytochrome c release from the mitochondria, indicating activation of the intrinsic pathway of apoptosis. CReP deficiency induced a 2-3-fold increase in mRNA expression of the pro-apoptotic BH3-only proteins PUMA and DP5 ($p<0.05$, n=6). Silencing of PUMA, DP5 and Bim partially protected CReP-deficient β-cells against apoptosis (11-13% apoptosis vs 16%, $p<0.05$, n=3-4).
Conclusion: We have identified a homozygous missense mutation in PPP1R15B leading to a syndrome that includes young-onset diabetes and microcephaly. The mutation diminishes the ability of CReP to bind PP1 and de-phosphorylate eIF2α, leading to β-cell dysfunction and apoptosis. Our findings support the concept that dysregulated eIF2α phosphorylation, decreased in Wolcott-Rallison syndrome or increased by the PPP1R15B mutation, is deleterious to β-cells and leads to diabetes.
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Characterisation of rapid progressors to type 1 diabetes among children with HLA-conferred disease susceptibility

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Background and aims: To assess the characteristics of rapid progressors to type 1 diabetes (T1D) among children with HLA-conferred disease risk.
Materials and methods: We followed from birth 7410 children (52.6% males) with HLA-conferred disease risk for development of islet autoimmunity and progression to T1D over a median follow-up time of 13.2 years (range 0.9-18.2). Islet cell autoantibodies (ICA), autoantibodies to insulin (IAA), to glutamic acid decarboxylase (GADA), and islet antigen 2 (IA-2A) were analyzed for screening for β-cell autoimmunity. Disease progression was considered rapid when T1D was diagnosed within 1.5 years after the seroconversion to autoantibody positivity. We analyzed the association between rapid progression and 25 non-HLA single nucleotide polymorphisms (SNPs) predisposing to T1D.
Results: Among the 7410 children, 1563 (21.1%) tested positive for diabetic autoantibodies, and 221 (14.1%) of the seroconverted children progressed to T1D by the end of 2012. The median time from seroconversion to diagnosis was 0.35 (0.02-1.46) years in rapid progressors (n=

46, 20.8%) and 4.3 (1.5-15.8) years in slower progressors. Compared to slower progressors, rapid progressors had higher frequency of multipositivity (76 vs. 54%; P=0.008), higher titers of ICA (10.0 vs. 5.0 JDFU; P=0.003) and IAA (9.1 vs. 6.6 RU; P=0.02) at seroconversion, and higher prevalence of a single nucleotide polymorphism (SNP) in the FUT2 gene (major allele G homozygotes, 74 vs. 25%; P<0.001) among individuals carrying the high risk HLA genotype. Furthermore, rapid progressors carried more frequently a combination of ≥3 of these risk SNPs in comparison to slower progressors (74 vs. 57%; P=0.04). Compared to autoantibody-positive non-progressors, rapid progressors were younger at seroconversion (1.6 vs. 5.5 years; P<0.001), carried more often the high risk HLA-DQB1*02/*0302 genotype (48 vs. 26%; P=0.001), had higher frequency of IAA (80 vs. 13%; P<0.001), GADA (58 vs. 13%; P<0.001), and IA-2A positivity (31 vs. 3%; P<0.001), and higher titers of ICA (10.0 vs. 4.0 JDFU; P<0.001), IAA (9.1 vs. 0.3 RU; P<0.001), GADA (6.9 vs. 0.1 RU; P<0.001), and IA-2A (0.11 vs. 0.08 RU; P<0.001) at seroconversion. In comparison to autoantibody-positive non-progressors, rapid progressors with a high risk HLA genotype carried more often the predisposing SNP in the FUT2 gene (74 vs. 32%; P=0.001).

Conclusion: At seroconversion, individuals with rapid progression to T1D are characterized by young age, high-risk HLA genotype, higher autoantibody titers, positivity for multiple autoantibodies, higher prevalence of the predisposing SNP in the FUT2 gene and a higher number of risk SNPs per individual. Such children should be considered as a target for careful monitoring and aggressive intervention.

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Prevalence of monogenic diabetes in the Norwegian childhood diabetes registry estimated by targeted deep sequencing

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Background and aims: 10-15% of children with diabetes do not have auto-antibodies at diagnosis, and it is unknown how many of these who have an underlying monogenic form of diabetes. We therefore screened all auto-antibody negative patients in the Norwegian Childhood Diabetes Registry for five monogenic diabetes genes. Our aim was to estimate the prevalence of common forms of monogenic diabetes in childhood diabetes in Norway.

Materials and methods: The Norwegian Childhood Diabetes Registry includes 95% of all children with newly diagnosed diabetes from 2002. By 17.03.2015, the registry contained clinical data (onset of diabetes, family history and treatment) for 3882 children, as well as serum and DNA samples. 469 negative as well as 469 GAD and IA-2 antibody positive individuals were screened for HNF1A, HNF4A, GCK, HNF1B, and INS sequence variants using TruSeq Custom Amplicon sequencing. Classification of DNA variant pathogenicity was performed blindly to auto-antibody status.

Results: Mean coverage was 488X with 97% of the target covered at >20X. We found 58 rare exonic and splice variants and classified them according to clinical criteria for pathogenicity (class 1-5). 6.7% of auto-antibody negative patients had a sequence variant classified from unknown to pathogenic (class 3-5) compared to 2.8% of the auto-antibody positive patients (P=0.005, OR=2.51). For the stricter classification (class 4-5), the corresponding numbers were 3.9% and 0.2%, respectively. Interestingly, 4.1% of the auto-antibody negative patients showed an enrichment of HNF1A variants of pathogenicity class 3-5; compared to 0.8% of the auto-antibody positive patients (P=0.001, OR=4.99).

Conclusion: This is a population-based study screening all the auto-antibody negative patients for five monogenic diabetes genes. Our results suggest that the prevalence of monogenic diabetes in the Norwegian Childhood Diabetes Registry is in the range of 3.9 - 6.7% among GAD- and IA-2 antibody negative patients. Molecular screening in all auto-antibody negative patients should now be considered for routine diagnostics.

Supported by: The Norwegian Diabetes Association

PS 001 Cardiovascular complications in type 2 diabetes

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Gender difference in diabetes related excess risk of cardiovascular events: When does the 'risk window' open?

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Background and aims: Diabetic women have a significantly higher excess risk of cardiovascular events than diabetic men, when compared with their respective non diabetic counterparts. However, whether this is evident along the entire period of life or whether this gender driven 'risk window' opens only in some life's periods, remains still uncertain. This study has retrospectively followed up, along a period of eight years (from 2005 to 2012) a cohort of diabetic patients living in Tuscany, a region of centre of Italy, comparing between genders the effect of age on diabetes related excess risk of hospitalization for acute myocardial infarction (AMI), ischemic stroke (IS), and congestive heart failure (CHF).
Materials and methods: The database used for this investigation was obtained from linking three datasets: the first concerning hospital discharges with main diagnosis of AMI (ICD-9-CM:410.xx), IS (ICD-9-CM:430.xx, 431.xx, 432.xx, 434.xx or 436.xx) or CHF (ICD-9-CM: 402 to 428), from all Tuscan hospitals over the period 2005 to 2012, the second was the general population registry of all inhabitants of Tuscany and the third a dataset containing the registry of all known diabetic patients from Tuscany. The effect of diabetes, identified by hospital discharge with ICD-9-CM code: 250.xx, and/or by the presence in the regional diabetes registry, on hospitalization for AMI, IS, CHF, was separately measured in men and in women according to a Cox regression analysis model, along the entire period 1st January 2005 to 31st December 2012.
Results: On a total of 3,192,203 inhabitants aged more than 16 yr (47% males), we counted 24,605 hospitalizations for AMI (16,251 in men and 8,354 in women), 26,953 for IS (14,848 in men and 12,105 in women), 17,628 for CHF (8,403 in men and 9,225 in women). After adjusting for age, the diabetes related excess risk, expressed as hazard ratio (HR:95%CI), was, overall, significantly higher in women than in men hospitalized for AMI [2.629(2.484-2.781) vs. 1.963 (1.877-2.053); $p< 0.05$], but similar between genders for those hospitalized for IS [2.103(2.000-2.209) vs. 2.131(2.041-2.224); p=ns] and CHF [2.631(2.499-2.769) vs. 2.550(2.418-2.688); p=ns]. After stratifying the population by age decades, however, diabetic women hospitalized for AMI had a significantly higher excess risk than diabetic men, along the entire age interval between decade 45-54 yr up to age class 75-84 yr, with the highest difference found in age class 45-54 yr [5.827(4.299-7.720) in women vs. 2.879(2.493-3.304) in men; $p<0.05$]. In patients hospitalized for IS and CHF diabetic women had an excess risk higher than men from age class 55-64 yr up to class 75-84 yr, with the highest difference in age decade 55-64 yr in both [4.139(3.594-4.745) vs. 3.044(2.769-3.339) and 6.828(5.683-8.156) vs. 4.109(3.611-4.663) respectively; $p<0.05$ for both].
Conclusion: In this cohort of Tuscan population the excess risk of cardiovascular events linked with diabetes, is significantly different between genders. With respect to AMI, diabetic women are more disadvantaged, compared to diabetic men, with a gender driven 'risk window' which mostly opens in the perimenopausal age. As to IS and CHF, it opens later, in the postmenopausal age, and at a lesser extent. All this prompts attention to a timely, gender oriented, prevention of cardiovascular events in people with diabetes.

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N-terminal prohormone of brain natriuretic peptide is a predictor of heart failure complicating type 2 diabetes: the Fremantle Diabetes Study Phase II

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Background and aims: N-terminal prohormone of brain natriuretic peptide (NT-proBNP) predicts incident heart failure in the general population but can be influenced by factors such as renal disease and obesity that are common in type 2 diabetes. The aim of this study was to determine the prognostic utility of NT-proBNP for hospitalisation or death due to heart failure in type 2 diabetes.
Materials and methods: We used baseline data and incident outcomes from the Fremantle Diabetes Study Phase II, a community-based longitudinal observational study. Patients were recruited between 2008 and 2011 and heart failure events were obtained from validated linked data up to end-June 2013. Plasma NT-pro BNP was measured by commercial assay with inter-day imprecision ≤4.0% at concentrations up to 406 pg/mL and limit of detection 5 pg/mL. The optimum plasma NT-pro BNP cut-points for prevalent and incident heart failure were identified from the area under the receiver operating characteristic curve (AUC) and Youden's Index. Cox regression with age as time-scale identified independent predictors of heart failure.
Results: Of 1,551 type 2 diabetes participants (mean age 66 years, 52% male, median diabetes duration 9 years), 101 (6.5%) had a prior hospitalisation for heart failure. The geometric mean (SD range) NT-proBNP was greater in those with vs without prior heart failure (456 (99-2098) vs 75 (19-291) pg/mL, $P<0.001$). In those <75 years, the AUC for NT-proBNP for identifying prevalent heart failure was 0.83 (0.77-0.89) with an optimum cut-off of 122 pg/mL, and 0.74 (0.65-0.83), with an optimum cut-off of 275 pg/mL, in those ≥75 years. During a mean ± SD 3.6±1.1 years' follow-up (5,153 patient-years), 80 (5.5%) of the remaining 1,450 patients were hospitalised with/died from heart failure, an unadjusted incidence of 15.5 (95% CI 12.3-19.3) per 1000 patient-years. NT-proBNP was greater in those with vs without incident heart failure (424 (107-1685) vs 68 (19-246) pg/mL, $P<0.001$). The NT-proBNP AUC for incident heart failure was 0.79 (0.71-0.88) with an optimum cut-point of 114 pg/mL in those aged <75 years (sensitivity, specificity, PPV and NPV: 72.4%, 76.4%, 8.3% and 98.9%), and 0.78 (0.71-0.85) and 282 pg/mL for those aged ≥75 years (sensitivity, specificity, PPV and NPV: 76.3%, 74.0%, 30.2% and 95.5%). Significant independent predictors of incident heart failure were longer diabetes duration, insulin use and ln(NT-proBNP).
Conclusion: NT-proBNP cut-offs for predicting incident hospitalisation with/death from heart failure in our contemporary type 2 diabetes cohort were similar to those for defining prevalent heart failure. For those ≥75 years, NT-proBNP cut-offs for both prevalent and incident heart failure were lower than for diagnosis of heart failure in the general population (>450 pg/mL).
Supported by: NHMRC Project Grant APP1042231; Novo Nordisk Diabetes Support Scheme 2015

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Fructosamine is a risk factor for myocardial infarction and all-cause mortality: longitudinal experience from the AMORIS cohort

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Background and aims: Glycation is linked to microvascular complications of diabetes and also to macrovascular events. Fructosamine is a biomarker of glycation but its associations to macrovascular complications are not well documented. The aim of this study was to evaluate fructosamine as a risk factor of myocardial infarction and all-cause mortality in a large population based cohort.
Materials and methods: Information on glucose and fructosamine was obtained from subjects of the large AMORIS cohort between 1985 and 1996 (n=338,443) who were followed for 19 years on average. None of the subjects were hospitalized at the time of blood sampling and samples were collected either at routine health check-ups or in primary care. Incident cases of myocardial infarction and death from any cause were identified from national patient and cause of death registers respectively with no loss to follow-up. Cox regression with censored data was used to estimate hazard ratios between relevant clinical groups of glycemic status.
Results: The incidence of myocardial infarction (n=21,526 cases) and all-cause mortality (n=73,458 deaths) increased at fructosamine levels above 2.30 mmol/L indicating prediabetes. For myocardial infarction, the sex- age- fasting- and entry period adjusted hazard ratio in subjects above 2.70 mmol/L vs reference subjects with normal glucose tolerance was 2.9 (95% CI: 2.7-3.1) (Figure 1). The corresponding hazard ratio for all-cause mortality was 2.3 (95% CI: 2.2-2.4). These associations remained basically unchanged after adjustment for total cholesterol, triglycerides, albumin and social class. When additional adjustment for glucose was performed the associations were attenuated but remained. Furthermore, the associations remained stable when smoking and hypertension were accounted for in a subset of the cohort (n=3,178). In a proportion of the subjects with simultaneous measurements of fructosamine, HbA1c and fasting glucose respectively (n=9,746), similar associations across the biomarkers were observed with somewhat lower hazard ratios for fasting glucose.
Conclusion: There is a strong association between increased fructosamine levels and myocardial infarction and death from any cause when major cardiovascular risk factors are accounted for. This association could only partly be explained by glucose levels and fructosamine may add or complement to risk assessment in this setting.

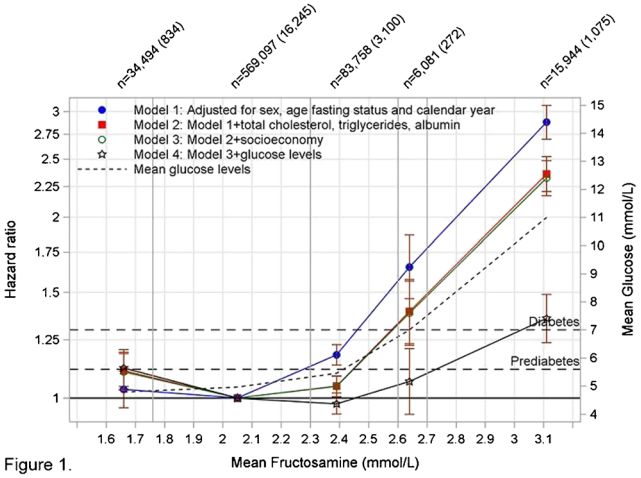

Figure 1.

Supported by: Jungner Foundation for laboratory medicine

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Single nucleotide polymorphisms at the Niemann-Pick C1-Like 1 gene locus significantly predict cardiovascular events in coronary patients with type 2 diabetes

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Background and aims: Niemann-PickC1-like1 (NPC1L1) protein is critically involved in intestinal cholesterol absorption. Recently, genetic variation at NPC1L1 locus has been linked with cardiovascular event risk. The association of NPC1L1 variants with cardiovascular events in patients with type 2 diabetes (T2DM) is unknown and is addressed in the present study.
Materials and methods: We prospectively investigated the impact of NPC1L1 tagging single nucleotide polymorphisms (SNPs) rs3187907, rs10264715, rs4720470, rs217420, rs17655652, and rs17725246 on the incidence of vascular events in 943 patients undergoing coronary angiography for the evaluation of stable coronary artery disease. Furthermore, variants rs217434, rs2072183 and rs41279633 were included based on previously published associations.
Results: As is shown in the table, variants rs3187907, rs10264715, rs217420, rs17725246 as well as rs217434, rs2072183 and rs41279633 significantly predicted cardiovascular events both in the total study population and specifically in patients with T2DM after multivariate adjustment including statin use. Tagging variants rs4720470 and rs17655652 neither in the total study population nor among T2DM patients predicted cardiovascular events.
Conclusion: We conclude that common NPC1L1 variants significantly predict cardiovascular events, particularly in patients with T2DM.

Table: NPC1L1 SNPs as predictors of future cardiovascular events

SNP	HR [95%CI] - all patients	p-value– all patients	HR [95%CI] - T2DM patients	p-value - T2DM patients
rs3187907	1.32 [1.02-1.69]	0.032	1.63 [1.05-2.55]	0.031
rs217434	1.31 [1.02-1.68]	0.033	1.54 [1.00-2.36]	0.049
rs10264715	1.40 [1.10-1.78]	0.006	1.75 [1.16-2.63]	0.007
rs217420	1.41 [1.11-1.79]	0.005	1.71 [1.17-2.51]	0.006
rs2072183	1.34 [1.05-1.70]	0.018	1.53 [1.03-2.26]	0.036
rs41279633	1.45 [1.12-1.88]	0.005	1.67 [1.11-2.52]	0.015
rs17725246	1.42 [1.10-1.83]	0.007	1.73 [1.15-2.59]	0.009

Supported by: Land Vorarlberg

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Diabetes as a risk factor for acute coronary syndrome in women compared with men: a systematic review and meta-analysis

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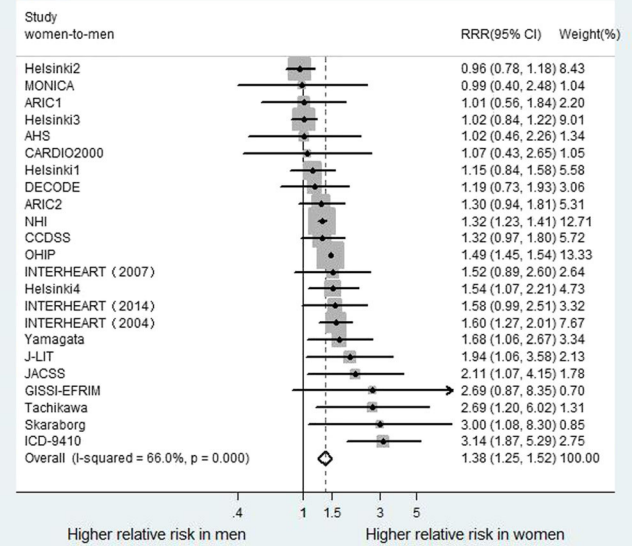
Background and aims: Diabetes mellitus is a significant cause of death and disability worldwide and is a strong risk factor for acute coronary syndrome. Whether diabetes confers the same excess risk of acute coronary syndrome between the sexes is unknown. Therefore, we undertook a systematic review and meta-analysis to estimate the relative risk for acute coronary syndrome associated with diabetes in men and women.
Materials and methods: We systematically searched PubMed, Embase, and Cochrane Library databases for both case-control and cohort studies published between Jan 1, 1966, and Dec 3, 2014. Studies were included if they reported sex-specific estimates of the relative risk (RR), hazard ratio (HR), or odds ratio (OR) for the association between diabetes and acute coronary syndrome, and its associated variability. We pooled the sex-specific RR and their ratio (RRR) between women and men, using a random-effects model with inverse-variance weighting.
Results: We included 9 case-control and 10 cohort studies with data for 10856279 individuals and at least 106703 fatal and non-fatal acute coronary syndrome events. The pooled maximum-adjusted RR of acute coronary syndrome associated with diabetes was 2.46(95%CI 1.92-3.17) in women and 1.68(95%CI 1.39-2.04) in men. In patients with diabetes compared with no diabetes, women therefore had a significantly greater risk of acute coronary syndrome_the pooled women-to-men RRR was 1.38(95%CI 1.25-1.52, p<0.001), with no evidence of publication bias.
Conclusion: Women with diabetes have a roughly 40% greater excess risk of acute coronary syndrome, compared with men with diabetes.



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Concordance of glucose based and of HbA_{1c} based diagnoses of diabetes in patients with established coronary atherosclerosis: a comparison between men and women

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Background and aims: Concordance between glucose based and HbA1c based diagnoses of diabetes differ between populations. Here, we aimed at investigating their concordance in men and in women with stable coronary artery disease (CAD).
Materials and methods: We measured fasting glucose as well as HbA1c and performed standard 75 g oral glucose tolerance tests in a consecutive series of 711 patients, 513 men and 198 women, who had angiographically proven coronary artery disease (CAD) but not previously diagnosed diabetes. Based on glucose values, diabetes was diagnosed with a fasting plasma glucose (FPG) ≥126 mg/dl or a postchallenge glucose ≥200 mg/dl 2 hours after the oral glucose load; based on HbA1c values diabetes was diagnosed with an HbA1c ≥6.5%.
Results: Among men, 33 had diabetes based on fasting or postchallenge glucose values, of whom 26 also had diabetes according to the HbA1c criterion. Of the 480 men who did not have diabetes based on glucose values, 446 also did not have diabetes according to HbA1c criteria; among women, 3 had diabetes based on glucose values, of whom 2 also had diabetes according to the HbA1c criterion. Of the 195 women who did not have diabetes based on glucose values, 185 also did not have diabetes according to HbA1c criteria. Concordance of Glucose and HbA1c criteria was similar in men and women (92% and 94%; p= 0.335). Applying glucose criteria as a standard, sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of the HbA1c criterion for men were 78.8%, 92.9%, 43.3%, and 98.5%, respectively. For women, sensitivity, specificity, PPV and NPV of the HbA1c criterion were 66.7%, 94.9%, 16.7%, and 99.5%, respectively.
Conclusion: We conclude that concordance of glucose and HbA1c criteria among patients with stable CAD is high and is similar in men

and women with CAD. However, for both sexes the sensitivity of the HbA1c criterion is poor in this patient population.

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Glycaemic control and excess mortality in patients with type 2 diabetes

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Background and aims: The excess risk of all-cause and cardiovascular mortality in individuals with type 2 diabetes and various levels of glycemic control and renal complications has not been evaluated.
Materials and methods: Individuals with type 2 diabetes registered in the Swedish National Diabetes Registry after 1/1/1998 were included in this study. For each patient, 5 controls were randomly selected from the general population and matched by age, sex, and county. All were followed until 31/12/2011 through the Swedish cause-specific death registry.
Results: Over a mean follow-up of 4.6 and 4.8 years respectively, 77,117 (17.7%) of 435,369 patients with diabetes and 306,097 (14.5%) of 2,117,483 controls died; corresponding rates for cardiovascular mortality were 7.9% vs 6.1% (adjusted hazard ratio (HR) 1.15 (95% confidence interval (CI): 1.14-1.16) and 1.14 (1.13-1.15), respectively. The excess risk of all-cause and cardiovascular mortality increased with younger age, worse glycemic control, and severity of renal complications. In patients younger than 55 years having hemoglobin A1c (HbA1c), ≤6.9% (≤52 mmol/mol), the HR for all-cause mortality was 1.92 (1.75-2.11), which decreased to 0.95 (0.94-0.96) in those older than 75 years. If normoalbuminuria coexisted the corresponding hazard ratios were 1.60 (1.40-1.82) and 0.76 (0.75-0.78) respectively, with a significant risk reduction also in those 65-75 years old, hazard ratio 0.87 (0.84-0.91).
Conclusion: Although there is evidence suggesting that the overall excess risk of mortality among individuals with type 2 diabetes has dropped to historically low levels of around 15%, our study shows that remains high in certain patient groups, and is still substantially increased in individuals younger than 55 years even among those with glycemic control within target ranges and normoalbuminuria. Our study highlights the important need to continue efforts to improve life expectancy in people with diabetes type 2. This appears to be particularly important for younger middle-aged people.
Supported by: SIMSAM

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Evaluation of aldehyde dehydrogenase from human erythrocytes of patients with type 2 diabetes mellitus

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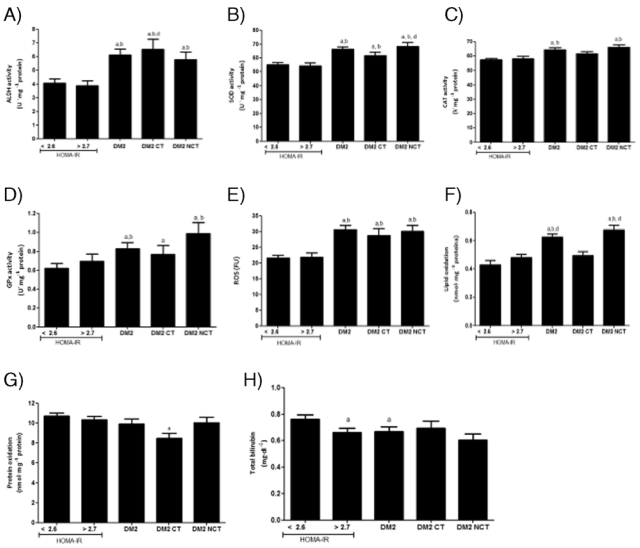
Background and aims: Type 2 diabetes (DM2) is a complex disease that is typically diagnosed in midlife and is characterized by progressive defects due to insulin secretion decreased or insulin action accompanied by changes in the metabolism of carbohydrates, lipids and proteins. There is evidence that oxidative stress (OS) plays a major role in the insulin resistance and in the pathogenesis of DM2, then the oxidative stress association has been described for several years. As one of the consequences of oxidative stress is lipid peroxidation (LP) levels increase where the aldehyde dehydrogenase (ALDH) enzymes are involved in the detoxification of aldehydes formed during this LP increase.
Materials and methods: In this study one of the proposals was to evaluate, how the activity ALDH is changed in patients suffering from insulin resistance and DM2 as well, a full evaluation of anti-oxidant and oxidant systems in human erythrocytes was performed with the aim of analyzing their activity and association with DM2, via absorbance and fluorescence techniques.The study subjects consited of a total of 542 patients, aged 35-88 years. Groups were classified based on HOMA-IR, the cut off was 2.6 being 232 subjects showed no insulin resistance, 133 subjects showed insulin resistance and 177 patients with DM2. The average time the patients were diabetes diagnosis was 9.2±7.2 years. Also, the DM2 patients were categorized by glycemic control DM2 CT (DM2-HbA1c 6.9, n=82) and without control DM2 NCT (DM2-HbA1c 7, n=95).
Results: Interestingly, increased ALDH activity was observed in the diabetic group, it seems to be associated with the severity of the disease and might be a compensatory effect against oxidative stress (noticed in controlled and uncontrolled patients).The antioxidant enzymes activity (SOD,CAT,GPx), bilirrubin and stress oxidative markers levels (ROS, lipid and protein oxidation) are modified during the development of DM2. Previous studies have shown that this could probably occur during the beginning of a certain pathology causing the increase in the antioxidant capability to diminish the oxidative stress and finally the oxidative damage. Additionally, associations between phenotypic characteristics with the antioxidant and oxidant systems were noticed between age, gender, glucose, HbA1c and HOMA-IR, probably for the oxidative stress severity of the diabetes.
Conclusion: Our findings suggest that glycemic control and insulin resistance have an influence on the oxidative stress in DM2 . DM2 presented more oxidative stress than HOMA-IR>2.6 and HOMA-IR<2.7. Therefore, our obtained data suggest that oxidative stress play a role in the DM2 pathology and the evaluation of these antioxidants enzymes as ALDH and oxidative markers could help in the severity of the type 2 diabetes and improving redox status.



Antioxidant enzymes activities and Oxidative stress markers and bilirubin levels in erythrocytes lysates.

A) ALDH, B) SOD, C) CAT and D) GPx, E) ROS, F) Lipid oxidation and G) Protein Oxidation H) Bilirubin. HOMA-IR < 2.6, HOMA-IR > 2.7, DM2: diabetes mellitus, DM2 CT: controlled diabetes mellitus HbA1c < 6.9, DM2 NCT: uncontrolled diabetes mellitus HbA1c >7. Each bar represents mean ± SD. Statistical significance [a] P < 0.05 vs HOMA-IR < 2.6, [b] P < 0.05 vs HOMA-IR > 2.7, [c] P < vs DM2 and [d] P < 0.05 vs DM CT with a Kruskal-Wallis test followed by the multiple comparison test proposed by Dunn's Method.

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PS 002 Complications in type 1 diabetes

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The epidemiology of diabetes, diabetes related mortality, complications, obesity and target HbA_{1c} in Russian Federation: the national diabetes register

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Background and aims: Due to the progressive growth of diabetes mellitus (DM) and diabetic complications and the rising costs of their treatment the data of diabetes registers are important for planning of healthcare system. The aim of our study was to evaluate the trends in epidemiology of DM, DM-related mortality, complications, obesity and target HbA1c in Russian Federation (RF).
Materials and methods: The study summarized the data of National diabetes register based on medical records of DM patients in more than 2000 clinics of primary care in 31 regions of RF. Primary care is provided by DM specialists (mostly) or general practitioners, the data entered manually in online diabetes register, powered by Microsoft Dynamics CRM platform let data export/import to/from MS Excel. Statistics was performed by IBM SPSS Statistics version 19.
Results: In 31 regions of RF in 2014 have been registered 1984791 DM patients compared with 1882483 in 2013 (+5.4%): type 2 (T2DM) 92.2%, type 1 (T1DM) 5.6%, other types 2.2%; 99.4% adults (≥18 yr.), 0.4% children, 0.2% adolescents; gender M/F T1DM 52.5/47.5%, T2DM 28.1/71.9%. The average prevalence of T1DM was 1.56 cases/1000 population, T2DM 25.72 cases/per 1000 population with the wide range of variability among regions from 0.19 to 2.66 and 6.47 to 34.97/1000 population, respectively. The incidence of DM: in 2014 registered 103277 new DM cases, 30.9% less compared to 2013 (T2DM 92.1%, T1DM 3.3%, other types 4.6%). In 2014 registered 26 450 DM-related deaths, the rate of mortality compared to 2013 also decreased by 27.7%. The leading cause was cardiovascular disease: cumulatively heart failure, stroke and myocardial infarction - 50.7% of deaths in T2DM and 34.5% in T1DM, the third leading cause in T1DM - kidney failure (7.9%), in T2DM - oncology (7.5%). Mortality rate due to diabetic coma remains fairly high in T1DM (2.1%). The most frequent complications in T1DM/T2DM: diabetic neuropathy 38.4/19.0%, retinopathy 34.7%/15.3%, hypertension 18.8/37.6%, nephropathy 20.2/4.9%, CKD as GFR<60 9/17%, macroangiopathy 16.7/8.3%. Only 9% of T2DM patients had normal body weight, 33.7% were overweight and 57% had obesity; in T1DM 51%, 26% and 11%, respectively. HbA1c in 2014 T2DM: 35.8% of patients <7.0% and another 35.7% <8.0%, that in some T2DM cases can be considered as suboptimal target achieved; in T1DM the situation was somewhat poorer: 26.1% had HbA1c <7.0% and 30.3% of patients <8.0% (2013: 38.0/37.4%; 24.2/33.7% for T1/T2DM, respectively).
Conclusion: The data of National diabetes register have shown increased DM prevalence in RF over 2013-14 years that is clearly in concordance with the global worldwide trends, but decrease in DM incidence. Apparently, we are dealing not with true reduction but rather with inappropriate registration of new DM cases in primary care. With the general trend of DM-related mortality decrease the cardiovascular pathology remain the main cause of death both in T1 and T2DM. The registered in primary care frequency of complications looks rather underestimated, especially compared with the data of epidemiology studies with active screening. The data demonstrated a very high level of overweight and obesity in DM population that raises the issue of crucial importance for preventive measures. Based on HbA1c level, we consider the situation as fairly stable but need the further efforts for the better DM control.

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Glycaemic control and prevalence of complications in adolescents and young adults with type 1 diabetes: longitudinal data from to Norwegian diabetes quality registers

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Background and aims: Good glycemic control delays the onset and progression of microvascular complications, but this can be difficult to achieve, particularly in young people with type 1 diabetes.The aim of this study was to assess glycemic control and the prevalence of complications in a contemporary cohort of adolescents and young adults in Norway.
Materials and methods: Data was obtained by linking the Norwegian Diabetes Register for Adults and the Norwegian Childhood Diabetes Registry. We identified 874 patients with type 1 diabetes (diagnosed before age 14) who were aged between 18-30 in 2013. All available HbA_{1c} data from the age of 14 until the age of 30 were obtained from the two registers. We calculated updated median HbA_{1c} for each patient, together with median HbA_{1c} for each year (14-30). The patients were grouped according to glycemic control. Good control was defined as HbA_{1c}<7.5% (58 mmol/mol) for patients<18 years of age and<7.0% (53 mmol/mol) for patients aged 18-30, and poor control as HbA_{1c}> 9.0% (75 mmol/mol). The last available data on retinopathy, nefropathy, blood pressure, lipid profile, smoking habits, weight and insulin device was obtained from the adult register.
Results: Median age of the cohort was 23, median age at diabetes onset 9 years and diabetes duration was 15 years. 51% were males, 18% were ex-smokers or current smokers and 40% were treated with continuous subcutaneous insulin infusion. Median HbA_{1c} rose during adolescence to peak at 9.3% (78 mmol/mol) at age 17 in females and 9.1% (75 mmol/mol) at age 19 in males thereafter decreasing steadily in both genders. Females had a higher HbA_{1c} result compared to males at ages 16-18 years ($p<0.01$). The proportion of patients achieving good glycemic control was worst at 18 years of age (3%) and only 18% of patients achieved good glycemic control in their late twenties. 26% of the patients had retinopathy and/or nephropathy with no gender differences. 72% of patients with poor glycemic control both as teenagers and young adults had complications. In a binary logistic GEE model updated median HbA_{1c}, smoking habits, diabetes duration and age at diagnosis predicted complications ($p<0.05$).
Conclusion: Longitudinal data from two Norwegian diabetes quality registers show that glycemic control is still far from satisfactory in adolescents and young adults, females have higher HbA_{1c} than males in their middle teens. The prevalence of microvascular complications was 26% for both genders and 72% in the group with sustained poor glycemic control.

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Long-term trends in cardiovascular risk factors in type 1 diabetes: nationwide monitoring of 38,169 patients from 1996 to 2014

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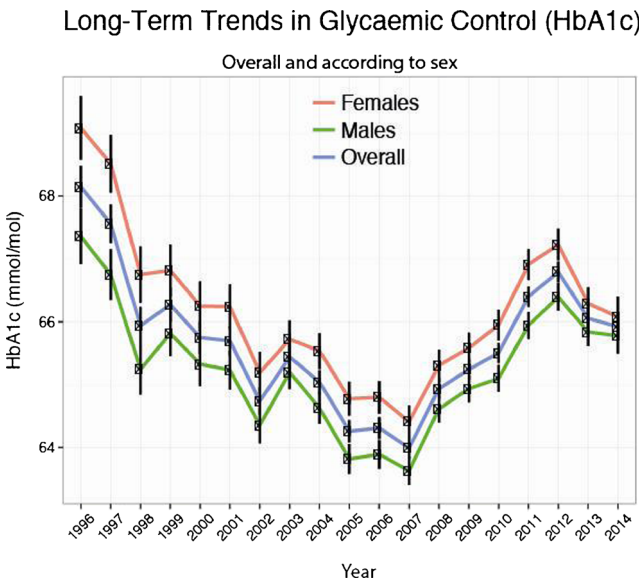
Background and aims: The last decades have witnessed remarkable advances in the management of type 1 diabetes. The risk of complications has presumably been reduced by intensive insulin therapy, improved insulin delivery and glucose monitoring, blood pressure control and lipid lowering therapy. Yet long-term trends in risk factor control has not been examined. We aimed to examine trends in six cardiovascular risk factors from 1996 to 2014, in the overall cohort and in relation to socioeconomic categories.

Materials and methods: We included all patients with type 1 diabetes entered in the Swedish National Diabetes Register from 1996 to 2014 (n= 38,169 contributing 457,577 appointments). We calculated adjusted estimates of glycated haemoglobin (HbA1c), systolic blood pressure (SBP), LDL cholesterol (LDL-C), body mass index (BMI), physical activity and smoking.
Results: HbA1c declined from 68.1 mmol/mol to 64.0 mmol/mol from 1996 to 2007 and then reversed to 66.8 in 2012, declining slightly in the remaining two years. By the end of the study period there was no improvement in HbA1c since the turn of the millennium (Figure 1). Individuals with a college/university degree had 4 mmol/mol lower HbA1c than individuals with 9 years or less education and there was no trend towards reduced differences. Overall LDL-C declined from 2.85 in 2002 to 2.59 in 2014, which was, however, not significantly lower than LDL-C in 2006. Higher education, but not higher income, was associated with lower LDL-C. BMI increased linearly, from 24.7 kg/m2 to 26.1 kg/m2 from 1996 to 2014. SBP decreased from 131.2 mmHg in 1996 to 125.9 mmHg in 2014. Odds ratio for being a smoker in 2014, compared with 1999, was 0.75 (95% CI 0.69 to 0.82); there were staggering differences in relation socioeconomic status, with no tendencies towards reduced gaps. Smoking rates declined more in the background population than among persons with type 1 diabetes. Physical activity doubled between 2004 and 2007 and then remained unchanged for the remaining seven years. Odds ratio for being physically active in 2014, compared with 2001, was 5.25 (95% CI 5.05 to 5.45).
Conclusion: Blood pressure control has been successful. Smoking rates have declined but smoking cessation has been less less successful among persons with type 1 diabetes, as compared with persons without diabetes. Promotion of physical activity has been successful. Less stable improvements are noted for HbA1c and LDL-C. BMI is increasing steadily. Socioeconomic differences are pronounced.



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Gender aspects in type 1 diabetes patients undergoing angiography: a registry report

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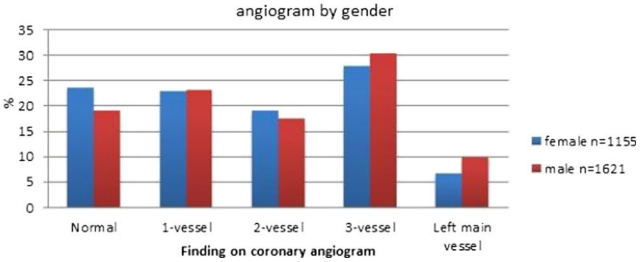
Background and aims: In patients without diabetes, widespread coronary artery disease (CAD) is more common among men compared to women. This difference is often attenuated if diabetes is present. Few studies have addressed type 1 diabetes (T1D) in this context. The aim of this study was to describe angiographic findings and assess long term mortality also by affected coronary vessels in women and men with T1D.
Materials and methods: All patients undergoing coronary angiography in Sweden 2001-2012, included in the Swedish Coronary Angiography and Angioplasty Registry (SCAAR) and registered in the Swedish National Diabetes Registry (NDR) with T1D were included. T1D was defined both with an epidemiological definition and also using the clinicians' diagnosis and onset age before 50 years. Coronary angiogram was visually judged by the local coronary interventionist and divided into normal (including atheromatosis/stenosis <50%), one-, two-, three-, and left main-vessel disease. Patients were followed for mortality until 31 December 2012.
Results: We included 1180 women and 1664 men undergoing coronary angiography. Mean age 57.5±11 years in both women and men. Mean diabetes duration 37.0±14.1 years in women, vs. 33.5±14.0 years in men ($p<0.0001$). Mean HbA1c 67±14 vs. 66±14 mmol/mol ($p=0.26$), LDL 2.7±0.9 vs. 2.6±0.9 mmol/L ($p=0.83$), HDL 1.65±0.49 vs. 1.43±0.47 mmol/L ($p<0.0001$), systolic blood pressure 136±18 vs. 136±17 mmHg ($p=0.33$) in women and men respectively. Men had more albuminuria 51% vs. 42% ($p<0.0001$) and women more retinopathy 68% vs. 64% ($p<0.05$). Main indications for coronary angiography were stable coronary artery disease (CAD) 36 vs. 34%, unstable acute CAD 39 vs. 37% and ST-elevation myocardial infarction (STEMI) 9 vs.12%, other indications 18 vs. 16% in women and men respectively. CAD findings by gender are presented in Figure 1. Normal angiography was more common in women (24% vs. 19%). The mean follow-up period was 7.2 (SD 2.2) years and total mortality did not differ (unadjusted) in women compared to men. In women diagnosed with 2- and 3-vessel disease crude mortality seemed higher compared to men.
Conclusion: In patients with T1D admitted for coronary angiography women more often had a normal angiogram whereas men more often had 3- vessel and left main vessel disease. Thus the advantage related to female gender seen in the general population is not completely attenuated by the presence of T1D. Despite less widespread angiographic findings in women, total mortality did not differ, which might be explained by longer diabetes duration in women.

Figure 1: Percentage of coronary artery disease findings on coronary angiogram by gender



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What makes a survivor? Description of patients with very long duration of type 1 diabetes with and without a history of micro- or macrovascular complications

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Background and aims: Type 1 diabetes (T1D) is associated with an increased risk of micro- and macrovascular disease. Some individuals with T1D develop such complications very early, while others may never experience them, despite long diabetes duration. The aim of this study was to describe the risk factor distribution in subjects with very long duration of T1D, with and without micro- and macrovascular complications.

Materials and methods: The study included individuals with T1D registered in the Swedish National Diabetes Registry (NDR) between 2002 and 2004. The distribution of biological, lifestyle- and socioeconomic factors were compared between subjects with and without a history of cardiovascular disease (CVD), and also between subjects with and without a history of renal impairment or severe retinopathy.

Results: Of those with a diabetes duration >50 years (n=583), 290 subjects had a history of CVD and 240 subjects had a history of kidney disease or severe retinopathy, while 180 individuals were free of these diagnoses. Detailed risk factor distributions in these groups are given in Table 1. Factors that differed significantly between subjects with and without CVD were age, HbA1c, body mass index (BMI), HDL-cholesterol, LDL-cholesterol, albuminuria, antihypertensive- as well as lipid-lowering medication, and income. The same factors differed significantly between subjects with and without history of renal impairment or severe retinopathy, except for BMI, HDL-cholesterol and income.

Conclusion: Individuals with T1D who have survived >50 years without either micro- or macrovascular complications have significantly lower age, HbA1c, and triglyceride levels compared to patients with a history of such complications. They also have higher LDL-cholesterol levels, less albuminuria and less antihypertensive and lipid-lowering medication. Although these differences in risk factor burden may contribute to explain the presence of diabetic complications, unknown protective factors may be present.

Table 1. Risk factor distribution for subjects with a diabetes duration >50 years

	History of cardiovascular disease			History of renal impairment or severe retinopathy		
	Yes	No	P-value	Yes	No	P-value
N	240	343		290	293	
Age, years	66.7 ± 7	63.9 ± 7	0.001	66.4 ± 8	63.7 ± 7	<0.001
Male sex (%)	54.2	51.0	0.45	53.1	51.5	0.70
HbA1c, mmol/mol	64 ± 12	62 ± 12	<0.001	64 ± 12	61 ± 11	0.02
BMI, kg/m²	25.6 ± 4.0	24.8 ± 3.6	0.02	25.6 ± 4.2	25.0 ± 3.4	0.06
SBP, mmHg	142 ± 18	143 ± 18	0.52	144 ± 19	142 ± 17	0.32
DBP, mmHg	71 ± 9	71 ± 10	0.43	72 ± 9	71 ± 10	0.16
Total cholesterol, mmol/l	4.8 ± 0.9	5.1 ± 0.9	0.92	4.9 ± 0.9	5.1 ± 0.9	0.10
HDL, mmol/l	1.6 ± 0.5	1.8 ± 0.5	<0.001	1.7 ± 0.5	1.8 ± 0.5	0.08
LDL, mmol/l	2.6 ± 0.8	2.8 ± 0.8	0.001	2.7 ± 0.8	2.8 ± 0.8	0.03
Triglycerides, mmol/l	1.3 ± 0.7	1.1 ± 0.5	<0.001	1.3 ± 0.7	1.1 ± 0.5	<0.001
Antihypertensive medication (%)	81.9	63.9	<0.001	78.7	63.9	<0.001
Lipid medication (%)	61.8	41.7	<0.001	61.0	39.4	<0.001
Albuminuria (%)	51.7	35.6	<0.001	54.1	30.4	<0.001
Microalbuminuria (%)	27.1	21.3	0.11	26.6	20.8	0.10
Macroalbuminuria (%)	24.6	14.3	0.002	27.6	9.6	<0.001
Severe retinopathy (%)	22.9	14.0	0.005	69.7	13.0	<0.001
Smoker (%)	7.6	5.8	0.41	5.9	7.8	0.34
Married (%)	65.8	61.2	0.26	64.5	61.8	0.50
High education level (%)	14.6	19.5	0.12	14.5	20.5	0.06
Income (% in top quintile)	9.2	15.5	0.03	11.0	14.7	0.19

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Determinants of HbA_{1c} in patients with type 1 diabetes in seven Swedish county councils

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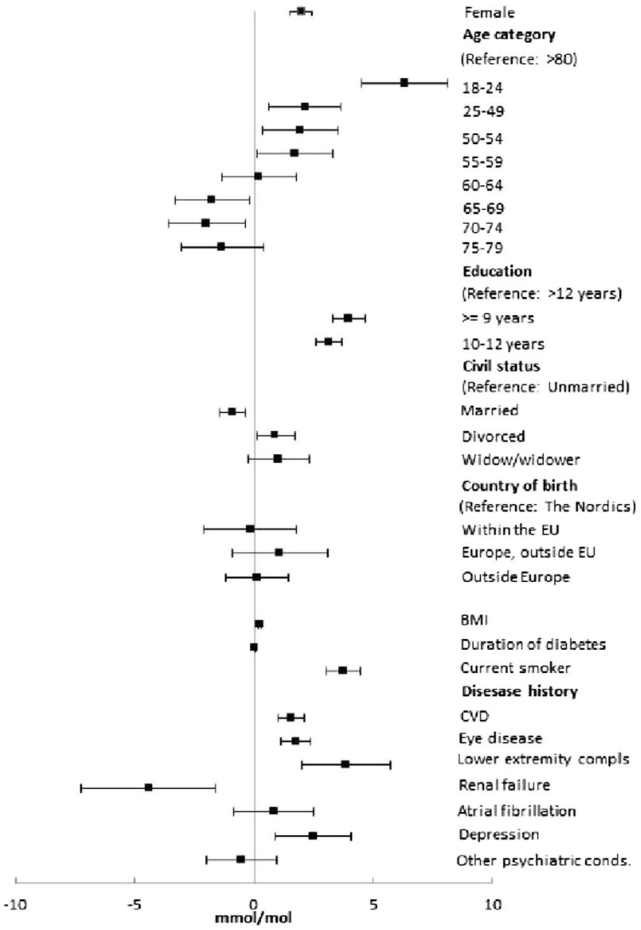
Background and aims: In order to make fair comparisons between the results of different health care providers, proper consideration of the case-mix of the populations they serve is important. HbA1c is often used as a metric to indicate the quality of diabetes care, it is therefore of value to determine what patient characteristics affect this outcome. As part of the ongoing National Collaboration for Value Based Reimbursement and Monitoring Systems, we therefore set out to investigate what factors are associated with HbA1c in a large retrospective cohort of persons with type 1 diabetes.

Materials and methods: This was a retrospective register study where we analyzed persons 18 years or older, with a health care contact and a diagnosis of diabetes during 2010-11 in the administrative systems of seven Swedish county councils (Dalarna, Jämtland Härjedalen, Skåne, Stockholm, Uppsala, Västra Götaland and Östergötland), covering ~70% of the Swedish population and linked this data to data from the National Diabetes Register, socioeconomic data from Statistics Sweden and data on filled prescriptions from the Prescribed Drug Register. We estimated a random effect model on HbA1c after one year of follow-up, including socioeconomic, demographic and clinical factors.

Results: Based on a complete case approach, 13 396 patients were analyzed. Women had on average higher HbA1c than men. Blood sugar control seemed to be better with higher age. Of the socioeconomic factors, higher education was associated with lower levels of HbA1c, as was being married. By contrast, we found no association between HbA1c and being born outside the EU. A history (previous 2 years) of diabetes related complications were associated with higher levels of HbA1c, which is likely due to high levels of HbA1c being an indicator of what is causing the complications in the first place. The exception to this pattern was patients with renal failure.
Conclusion: Apart from obvious demographic factors such as age and gender as well as disease history, educational and civil status are important factors to take into consideration when comparing obtained HbA1c levels between health care providers. This also raises the question of the need for additional focus on education directed towards these groups to facilitate improved diabetes management.



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HbA_{1c} and incidence of micro- and macrovascular disease in patients with very long duration of type 1 diabetes

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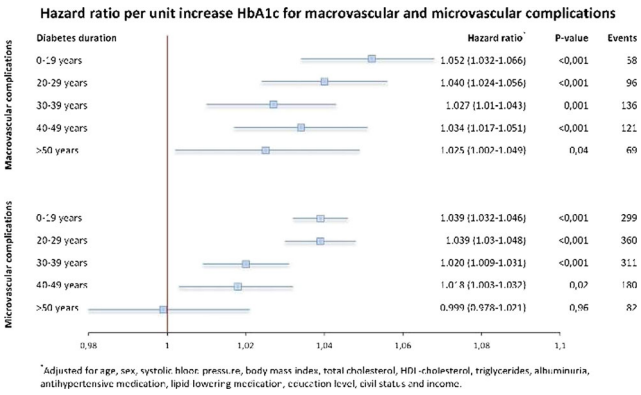
Background and aims: Type 1 diabetes (T1D) is associated with an increased risk of micro- and macrovascular disease. Glycemic control is a strong predictor of these complications. The aim of this study was to explore whether HbA1c is associated with incidence of micro- and macrovascular disease in subjects with very long duration of T1D.
Materials and methods: Individuals with T1D included in the Swedish National Diabetes Registry (NDR) between 2002 and 2004 without a history of cardiovascular disease (CVD) and albuminuria (n=7081) were followed until the first event of CVD, death or until December 31, 2012. Furthermore, subjects without a history of renal impairment, severe retinopathy and albuminuria (n=6577) were followed until the first event of renal impairment, severe retinopathy, death or until December 31, 2012. Cox proportional hazards regression was used to study the relation between HbA1c and incidence of CVD, renal impairment and severe retinopathy. The models were adjusted for age, sex, systolic blood pressure, body mass index, total cholesterol, HDL-cholesterol, triglycerides, albuminuria, antihypertensive medication, lipid-lowering medication, education level, civil status and income. The analyses were stratified into five levels of diabetes duration: 0-19 years, 20-29 years, 30-39 years, 40-49 years, and >50 years.
Results: During a mean follow-up time of 9 years 480 individuals were diagnosed with CVD (7.3 per 1000 person-years). In addition, 1232 subjects were diagnosed with renal impairment or severe retinopathy (21.7 per 1000 person-years). After adjusting for potential confounding factors, incidence of CVD was significantly associated with HbA1c levels in all duration strata (Figure 1). Hazard ratios decreased with longer diabetes duration. A similar relationship was observed with incidence of microvascular disease. HbA1c levels were not associated with incidence of microvascular disease in patients with >50 years duration.
Conclusion: HbA1c is a predictor of micro- and macrovascular disease independent of duration of T1D. The results suggest that HbA1c is a weaker risk factor in persons with very long diabetes duration, indicating the importance of other known and unknown protective factors in this very selected group of patients.



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Application of a validated prognostic model to identify type 1 diabetic patients at high risk for major outcomes

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Background and aims: A prognostic model for assessing individual risk for major outcomes (CHD, stroke, ESRD, amputation, blindness and all-cause death) in type 1 diabetes (T1DM) has been developed from the EURODIAB PCS and tested in three other prospective cohorts. The model uses routinely available features: age, HbA1c, waist-hip ratio, urinary ACR and HDL. It identifies three groups of T1DM at low- (LR, score lower than 16), intermediate- (IR, score 16-20) and high- (HR, score

higher than 20) absolute risk. This model has been applied to a cohort of 774 T1DM (367 F, 47%; 407 M, 53%) attending our outpatients clinic.
Materials and methods: Mean age and diabetes duration (DD) were 40.2±11.7 years and 19.3±12.2 years; HbA1c 7.8±1.2% (range 5.2-15.1%); urinary ACR (median) 0.49 mg/mmol (0.03-142.6); HDL 62.5 ±15.2 mg/dl (8-103 mg/dl), WHR 0.92±0.06 (0.74-1.14). Risk distribution was evaluated in the entire cohort and after exclusion of 41 T1DM subjects (5.3%) with major outcomes.
Results: Risk distribution was LR n. 466 (60.2%), IR n. 205 (26.5%) and HR n. 103 (13.3%) in the whole cohort, and LR n. 461 (62.9%), IR n. 195 (26.6%) and HR n. 77 (10.5%) after exclusion of T1DM with major outcomes. Major outcomes were more frequent in HR (26/103; 25.2%) and IR (10/205; 4.9%) than in LR (5/466; 1.1%; $p<0.0001$). Mean absolute risk at 3, 5, 7 and 10 years in the 733 subjects with no previous outcomes were, respectively, 1.4, 2.4, 3.4 and 4.9% for LR; 3.8, 6.5, 9.2 and 13.1% for IR; 11.7, 18.9, 25.4 and 33.9% for HR. Distribution of risk was similar in M and F: LR 58.7 vs. 61.9%, IR 25.5 vs. 27.5% and HR 15.7 vs 10.6% ($p=0.113$), but HR was more common in M ($p=0.037$). IR and HR increased with quartiles of DD: DD lower than 9 years 16.6 and 1.6%; 10-18 years 24.8 and 4.9%; 19-28 years 24.7 and 13.9%; higher than 28 years 40.9 and 34.8% ($p<0.0001$). By multiple regression, main factors associated with IR&HR were age ($r2=0.801$), logACR ($\Delta r2=0.097$) and HbA1c ($\Delta r2=0.039$; $p<0.0001$ for all). Other recognized prognostic factors (not included in the model because of weak additional effects) were strictly correlated with those included in the model; indeed, these risk factors increased from LR to HR: sBP 120±14, 132 ±17 and 145±20 mmHg; dBP 72±8, 75±9 and 75±10 mmHg; LDL cholesterol 113±29, 122±30 and 120±29 mg/dl; non-HDL cholesterol 120±32, 131±35 and 132±33 mg/dl; triglycerides 69 (55-91 IQR range), 82 (63-106) and 101 (69-137) mg/dl; fibrinogen 320±64, 353±63 and 376±67 mg/dl; uric acid 3.6±1.0, 3.8±1.9 and 4.8±4.7 mg/dl; on the other hand, eGFR (CKD-EPI) decreased progressively: 108±15, 99±13 and 83±20 ml/min/1.73 m2 ($p<0.001$ for all).
Conclusion: In our cohort of T1DM, 30-40% of subjects had high (about 10%) or intermediate risk (about 25%) for major outcomes. Subjects at IR and some at HR are also present among T1DM with short DD. This model allows to trace for each T1DM an exact risk profile to be used in the clinical practice with the aim of improving treatment for all risk factors.
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PS 003 Genetics of metabolism

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SNP x SNP interactions in the VPS13C/C2CD4A/C2CD4B locus modulate diabetes risk

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Background and aims: Single nucleotide polymorphisms (SNPs) in the VPS13C/C2CD4A/C2CD4B locus have been found to be associated with type 2 diabetes, fasting plasma glucose, 2-hour plasma glucose and insulin secretion in genome-wide association studies (GWAS). Some of the weakly linked SNPs demonstrate opposing effect directions. The functional impact of these genetic variants is unknown. Our objective was to examine a broad panel of intermediary traits in our extensively phenotyped cohort.
Materials and methods: We genotyped 21 tag SNPs in the locus. Metabolic traits from oral glucose tolerance tests conducted in 2273 participants and MR-based body fat distribution parameters in a subgroup were jointly examined using region-based analyses. Key variants were identified by iterative leave-one-out tests and single SNP association tests. Haplotype-association tests and linear regression tests with interaction models were conducted. Insulin secretion and insulin sensitivity were additionally examined in subgroups with hyperglycemic and hyperinsulinemic-euglycemic clamps, respectively.
Results: Joint tests confirmed the previously established association with post-challenge glucose and insulin secretion. After adjustment for sex, age and BMI, region-wide analysis indicated an association with insulin clearance ($p=0.01$), fasting non-esterified fatty acids (NEFA, $p=0.009$), glycerol ($p=4\cdot10^{-4}$), and subcutaneous abdominal adipose tissue volume ($p=10^{-5}$). For rs11629596 and rs1425270, a near genome-wide level of significance was detected for fasting glycerol and insulin clearance ($p=4\cdot10^{-7}$ and $p=7\cdot10^{-7}$, respectively). We demonstrated SNP×SNP interactions on metabolic traits centered around rs72752005. This SNP also augmented the association of the GWAS-SNP rs7172432 with diabetes (adjusted per-A-allele OR of rs7172432 on diabetes: 1.03 [CI 0.48-2.19], 1.44 [CI 0.98-2.11] and 2.63 [1.07-6.55] for strata of the A/A, A/G and G/G genotypes of rs72752005, respectively).
Conclusion: In the VPS13C/C2CD4A/C2CD4B locus, we demonstrate pleiotropic metabolic effects with strong SNP x SNP interactions affecting multiple metabolic traits and association with diabetes. Specific SNP combinations in this locus could bear a much higher diabetes risk than SNP effect estimates from GWAS that originally identified this genetic region.

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Genome-wide multi-phenotype rare variant association analysis detects effect of *ZNF259* on fasting insulin and triglyceride levels

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Background and aims: Individuals with type 2 diabetes (T2D) have increased risk of coronary heart disease (CHD), suggesting shared pathogenesis. While adverse fasting insulin (FI) levels have a central role in T2D development, CHD is causally related to dyslipidaemia and triglycerides (TG), in particular. For common variants (minor allele frequency, MAF>5%), single-trait analyses have shown an enrichment of FI associations among SNPs preselected on Metabochip for TG and waist phenotypes. However, novel associations could be detected via i) multi-phenotype analysis by accounting for the correlation between traits, and ii) analysis of low-frequency and rare variants (MAF≤5%, both denoted by RVs).
Materials and methods: We have developed a method and software tool for Multi-phenotype Analysis of RVs (MARV), and have applied it to jointly analyse FI, TG and WHR using data from 4788 individuals from the Northern Finland Birth Cohort 1966. Individuals were genotyped on the Illumina370CNV array and imputed to the 1000 Genomes Project all ancestries reference panel (March 2012). FI/TG/WHR were adjusted for sex, BMI and three principal components to control for population structure. The following transformations were applied: natural logarithm for FI and inverse normal for the residuals of TG and WHR.
Results: We identified RV associations, at genome-wide significance ($p<1.7\times10^{-6}$, Bonferroni correction for 30,000 genes) in *ZNF259*, which maps to a common variant GWAS locus for TG and CHD. Based on BIC, the model with TG and FI provided the best fit ($p_{model}=2.2\times10^{-9}$), and stronger associations than in univariate analyses ($p_{TG}=1.1\times10^{-7}$; $p_{FI}=0.22$). *ZNF259* included 18 rare variants with MAF ranging from 0.01% to 4.9%, including three missense SNPs.
Conclusion: Common variants at *ZNF259* have previously been associated with lipid levels and CHD but not FI. Using the MARV method and tool, we demonstrate for the first time a role of *ZNF259* RVs in T2D/CHD-related trait variability, suggesting shared pathophysiology.
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SNPS in FGF5 and ZNF652 show parent-of-origin specific effects on blood pressure in families from the Botnia study

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Background and aims: Hypertension affects ~1 billion people and causes ~9.4 million deaths worldwide each year. There is a strong heritable component; however the genetic variants identified so far explain only ~1% of the heritability. There are differences in disease expression between the genders that cannot be fully explained by hormonal differences alone, and some genetic basis for the sex-specific effects has been suggested in previous studies. Further, Lascaux-Lefebvre et al (Diabetes Metab. 2001) and others have suggested the impact of parental histories and specifically, a stronger maternal influence on blood pressure. Parent-of-origin effects (POE) could explain some of these biases, but they would not be detected in a case-control setting. Our study aims to explore these effects in two of the largest family based cohorts.
Materials and methods: Families from the Botnia cohort (n=4189, 1083 families) were genotyped on the Sequenom mass array for 13 SNPs that has previously shown an association with blood pressure in GWAS studies and Systolic Blood Pressure (SBP), Diastolic Blood Pressure (DBP) and Mean Arterial Pressure (MAP) were measured. Correlations between the parent and offspring phenotypes were analysed, and tests for association and parent-of-origin effects were performed using a family based linear regression model (qTDT). SNPs showing parent-of-origin effects will be replicated in families from the Hungarian Transdanubian Biobank (n=9279, 1022 families).
Results: The correlation between SBP, DBP and MAP were stronger between mothers and offspring (r_{MAT}) than between fathers and offspring (r_{PAT}) (SBP: $r_{MAT}=0.222$ $r_{PAT}=0.140$ $p_{DIFF}=0.002$, DBP: $r_{MAT}=0.215$ $r_{PAT}=0.120$ $p_{DIFF}=0.001$, MAP: $r_{MAT}=0.245$ $r_{PAT}=0.137$ $p_{DIFF}=<0.001$). The SNP rs16998073 in fibroblast growth factor 5 (*FGF5*), showed the strongest POE.. The A-allele, previously associated with lower DBP, showed a trend for lower DBP when inherited from the father (Pat_effect=-0.021, $p_{PAT}=0.07$), but higher DBP when inherited from the mother (Mat_effect=+0.020, $p_{MAT}=0.08$), with the total test for POE reaching significance ($p_{POE}=0.005$). The POE test also reached significance for MAP (Pat_effect=-0.019, Mat_effect=+0.020, $p_{POE}=0.006$) and SBP (Pat_effect=-0.019, Mat_effect=+0.015, $p_{POE}=0.04$). The SNP rs16948048 near zinc finger protein 652 (*ZNF652*) reached significance for POE ($p_{POE}=0.03$) on DBP, with the A allele, previously associated with lower DBP, associated with lower DBP when inherited maternally (Mat_effect=-0.029, $p_{MAT}=0.03$) but neutral when inherited paternally (Pat_effect=+0.003, $p_{PAT}=0.79$) in the current study.
Conclusion: We show here for the first time parent-of-origin specific effects of SNPs previously associated with blood pressure. This could explain some of the gender specific effects in traits and the correlation between individual parents and offspring observed and highlights the need to consider parent-of-origin effects while investigating genetic associations for blood pressure traits. Studies to replicate this parent-of-origin effect in families from Hungary are underway.
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Heritability of human serum lysophospholipids and ether phospholipids

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Background and aims: Although lipidomics, the study of lipids, have a great potential as clinical tool for monitoring metabolic changes in health and disease, hardly anything is known about the heritability of lipids. We therefore use the cohort of our intervention study in twins to investigate similarities and differences in the lipid profile of mono- and dizygotic twins.
Materials and methods: In the NUGAT (NUtriGenomic Analysis in Twins) study, 46 healty twin pairs (34 monozygotic, 12 dizygotic) were standardized for their nutritional behavior due to a period of six weeks on a high-carbohydrate, low-fat diet. This standardization was followed by an interventional low-carbohydrate, high-fat diet for another six weeks. Extensive characterization of the metabolic responses was performed after the low fat (LF) and after 1 (HF1) and 6 (HF6) weeks of high fat diet (HFD) to evaluate rapid and long-term effects. A lipid profile of 307 lipids out of 18 classes were part of the examination including 2 classes of lysophospholipids (n=27) and 2 classes of ether phopholipids (n=39). For the study of heritability, the ACE model was applied. This model is based on a correlation analysis of the mono- and dizygotic twins, in which the proportion of variance is determined that can be traced back to

additive genetics (A), common environment (C) and individual environment (E) of the twins. The ACE model was also checked against the AE model.

Results: Both classes of lysophospholipids showed stable and high proportions of heritability (A≥0.5) for the screening (Scr) and during the study. Although both classes didn't differ very much and showed the same reaction pattern, the lysophosphatidylcholines (LPC) had slightly higher inheritance (Scr: 0.72, LF: 0.60, HF1: 0.80, HF6: 0.66) than the lysophosphatidylethanolamines (LPE; Scr: 0.70, LF: 0.55, HF1: 0.75, HF6: 0.51). The single members of LPC (n=18) and LPE also showed these high A-values with the solitary exception of the LPCs with 19 C-atoms and different number of double bonds (0≤A≤0.46). In contrast the concentrations of the ether phospholipid classes seemed much more environmentally determined (0≤A≤0.46). Moreover the reaction pattern differed between the ethers of phosphatidylcholine (PC-O; Sc: 0.00, LF: 0.00, HF1: 0.46, HF6: 0.00) and the ethers of phosphatidylethanolamine (PE-O; Sc: 0.24, LF: 0.40, HF1: 0.41, HF6: 0.00). Their single members also varied in their heritability. For the PC-Os (n=26) the length of their fatty acids seemed to have the strongest impact. The longer the fatty acids, the less heritable they were.

Conclusion: Although we understand more and more, how lipids act and interact under different environmental conditions, it is necessary to clarify how and how much we can affect these progresses in individuals (e.g by nutrition). There is evidence that especially lipids with odd fatty acids were able to reduce the diabetes risk. Our data showed strong differences in the heritability of lipid classes and also that classes like lysophospholipids, which can be associated with inflammation, seemed to be genetically determined. These findings may be part of an explanation for phenomena like metabolically healthy obesity and the ability to compensate the unfavorable effects of HFDs.

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Use of Danish National Registers to identify parental history of type 2 diabetes for gene-environment interaction and association studies

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Background and aims: Family history is one of the major independent risk factors for type 2 diabetes, reflecting both a shared underlying genetic susceptibility and environment. The study of interactions between genetic variants and family history of diabetes is a powerful way of examining gene-environment interactions as it investigates whether the over-all level of known and unknown genetic and environmental factors which cluster in families, modifies the genetic associations. We aimed (1) to assess whether it is feasible to obtain systematic data concerning parental history of diabetes (PHD) through linkage between several Danish National Registers (DNR) and (2) to enrich a composite genetic study of diabetes (The LuCAMP study) with register-derived PHD data in order to examine the modifying effect of PHD on known genetic markers of diabetes risk.

Materials and methods: We obtained records of diabetes diagnosis for the parents of the 5447 LuCAMP participants (968 with T2DM) by record linkage in the DNR. Logistic regression was used to assess the interaction between PHD and 30 known genetic variants associated with diabetes risk, adjusting for age and sex.

Results: Among participants with diabetes, 42% (n=409) had PHD, while only 28% of participants without diabetes had PHD (n=1236); OR for diabetes by PHD: 1.92 (95%CI: 1.66;2.22). Four SNPs (rs5945326, rs7957197, rs340874 and rs7578597) showed nominally statistically significant ($p<0.05$) interaction with PHD (either parent). For three of these (rs5945326, rs7957197, and rs7578597) the diabetes risk associated with the risk allele was lower among people with PHD (OR_{int}:0.77, 0.69 and 0.63 respectively), while for rs340874 the risk was higher (OR_{int}:1.24). Odds ratios were broadly consistent when examining maternal PHD, paternal PHD and PHD from both parents separately for these fours SNPs, although not all sub-analyses were statistically significant. Five further SNPs (rs8042680, rs13266634, rs896854, rs7961581 and rs10010131) showed nominally statistically significant interactions with maternal PHD, paternal PHD or PHD from both parents without showing a significant interaction for PHD from either parent. None of the interaction p-values remained statistically significant after Bonferroni correction for multiple comparisons.

Conclusion: We showed that it is possible to use the Danish National Registers to enrich extant genetic studies in Denmark with parental history of diabetes data. Our approach can be used to discover signals of modification of some genetic determinants of diabetes by parental history of diabetes.

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Familial clustering of Japanese patients with adult-onset type 1 diabetes mellitus from the database of "Tokai Clinical Study Group of Diabetes"

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Background and aims: The prevalence of Type 1 diabetes mellitus (T1DM) in Japanese is known to be much lower than in Caucasians. Meanwhile, previous studies reported that the λsibling(λs), which represents the ratio of the risk of T1DM in siblings of patient with T1DM to that of general population, was much higher in Japanese than in Caucasians(15 in Caucasians and 65-271 in Japanese, respectively). However, those studies were based on the data of subjects whose onset of T1DM was under the age of 20 years old, and showed the risk only in siblings. The aim of this study is to investigate familial clustering of T1DM in Japanese subjects with adult-onset T1DM.

Materials and methods: This cross sectional study was performed with 737 subjects with T1DM from the database of "The Tokai Clinical Study Group of Diabetes" enrolled from 28 hospitals. Assuming the subjects as probands, the familial clustering was investigated using questionnaires regarding the family history, including parents, siblings, and children, of T1DM. In addition, we classified the probands into the different groups according to the subtypes of T1DM development; slowly progressive, acute, and fulminant-onset, or their onset-age; 0-12 , 13-19 , 20-39 , and 40≦ years old.

Results: The mean age of probands was 53.6 years old, and the onset of T1DM in 85.3% of them was above the age of 20 years old. 6.2% of the probands had at least one T1DM family member. The prevalence of T1DM was 2.3% in siblings, 1.2% in parents and 0.8% in children. The risk of T1DM in the siblings, parents, and children of proband was respectively 164, 89, and 59 times higher than the general population in which the prevalence is 0.014% in Japanese. Among 3 subtypes of T1DM, the acute-onset group showed the highest familial risk (acute-onset; 2.4%, slow progressive-onset; 1.0%, fulminant-onset; 1.1%). Meanwhile, the one-way Cochran-Armitage trend test demonstrated that,

among 4 groups of their onset-age, the earlier-onset group showed the significantly higher risk of T1DM in their siblings and fathers, but not mothers. Furthermore, among 17 families in which both parents and their children had T1DM, the onset-age of T1DM of children in 16 families was earlier than that in their parents'.
Conclusion: In this study, the risk of T1DM in the family of Japanese patients with adult-onset T1DM was much higher than that of Caucasians, even though the prevalence of T1DM is much lower in Japan. Furthermore, families of acute-onset and early-onset probands showed the highest risk of developing T1DM, suggesting that the high familial clustering could be attributed to genetic factors. This study also demonstrated that the parental risk in early-onset proband was associated to father, but not mother, and this finding might support EURODIAB study that showed the high risk in children of fathers with T1DM. The children of probands in 16 out of 17 families were observed to develop T1DM earlier than their parents, supporting that the incidence of T1DM especially in young children is worldwide increasing. However, further study is needed to elucidate the genetic susceptibility contributing the most to the high familial clustering in Japanese.

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Family history of diabetes and cardiovascular disease is associated with cardiometabolic markers in offspring at age 12: the PIAMA study

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Background and aims: The associations of family history of type 2 diabetes and cardiovascular disease (CVD) with cardiometabolic markers in offspring have been well documented. Type 2 diabetes and CVD share risk factors and often co-occur. We investigated cardiometabolic risk factors in children with a (combined) family history of diabetes and CVD (myocardial infarction (MI) and stroke).
Materials and methods: We used data of 1379 children of the Dutch Prevention and Incidence of Asthma and Mite Allergy (PIAMA) birth cohort, for whom family history of diabetes, MI and stroke were parent-reported, and cardiometabolic markers (waist circumference (WC), cholesterol, blood pressure (BP), glycated haemoglobin (HbA1c)) were measured at age 12. The following categories were created for each child: 'no family history'; 'moderate family history' (1-2 grandparent(s) with late disease onset); or 'strong family history' (parent(s) with disease; grandparent(s) with early disease onset; or 3-4 grandparents with late disease onset). Differences in cardiometabolic markers between children with no family history and children with a moderate or strong family history were assessed by multiple linear regression analyses.
Results: Children with a strong family history of diabetes, (N=146, 11%) had 2.02 cm higher WC (95%CI 0.91-3.13), 0.19 mmol/l higher total cholesterol (TC) (95%CI 0.06-0.32), 0.18 higher total-to-high-density-lipoprotein cholesterol ratio (TC/HDLC ratio) (95%CI 0.004-0.36) and 0.68 mmol/mol higher HbA1c (95%CI 0.17-1.20) than children with no family history of diabetes. Children with a strong combined family history of diabetes and CVD (N=414, 30%) had 0.13 mmol/l (95%CI 0.03-0-23) higher TC and 0.17(95%CI 0.03-0.31) higher TC/HDLC ratio than children with no family history. Moderate family history of diabetes or moderate combined family history were not associated with any of the cardiometabolic markers.
Conclusion: At age 12, children with a strong family history of diabetes and a strong combined family history of diabetes and CVD had significantly higher levels of cardiometabolic markers compared to children without family history. Family history of these diseases, in particular family history of diabetes, is a useful tool to identify children at high risk for the development of future cardiometabolic diseases in order to offer preventive interventions.

PS 004 Body fat distribution and metabolism

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The identification of wrist circumference cut off for insulin resistance prediction in a population of children/adolescents

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Background and aims: Insulin resistance plays a central role in the pathogenesis of the metabolic syndrome and its prevalence in the paediatric population is increasing, particularly among obese children/adolescents. In a previous study we observed a close relationship among wrist circumference, its bone component, and insulin resistance in overweight/obese children/adolescents. The aim of this study was to identify the wrist circumference cut off for the prediction of insulin resistance in a population of children/adolescents.
Materials and methods: We recruited n=1134 (M=583, F=551; mean age: 10.3±2.9) overweigh/obese children/adolescents in the Department of Pediatrics of our University and n=114 (M=58, F=56; mean age: 11.8 ±2.9) normal weight controls in a hospital. We evaluated the following anthropometric and biochemical parameters: body weight (kg), height (cm), wrist circumference (cm), BMI-z score, fasting glucose (ml/dl), fasting insulin levels (μU/ml). The wrist circumference was measured using a tension-gated tape measure positioned over the Lister tubercle of the distal radius and over the distal ulna. The subjects were divided into two groups according to Tanner stage (TS): prepubertal (TS 1), pubertal (TS from 2 to 5). Insulin resistance was estimated according to the homeostasis model assessment of insulin resistance (HOMA-IR). HOMA-IR cut off values for insulin resistance in the pre-pubertal period were: 2.22 in females and 2.67 in males; in the pubertal period were: 3.82 in females and 5.22 in males. The data were analyzed through logistic regression and ROC curves, with SAS software 9.3.
Results: We observed that the cut-off of wrist circumference to predict insulin resistance in the pre-pubertal subjects were: ≥15.8 cm in males (sensitivity 0.70, specificity 0.73), ≥15.4 cm in females (sensitivity: 0.36, specificity: 0.82); in the pubertal subjects were ≥17.7 cm in males (sensitivity: 0.52, specificity: 0.84) and ≥15.2 cm in females (sensitivity: 0.85, specificity: 0.40). The linear regression demonstrated a positive correlation between wrist circumference and HOMA-IR (R2=0.09, p<0.0001).
Conclusion: The wrist circumference, an easy method to detect anthropometric parameter, could be utilized to identify children/adolescents with increased risk for insulin resistance, thus avoiding testing the entire population of overweight/obese children.

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Ratio of waist-to-calf circumference is associated with NAFLD and hepatic fibrosis in type 2 diabetes independently of obesity and insulin resistance

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Background and aims: Although the term sarcopenic obesity refers to the loss of skeletal muscle mass with increased body fat that occurs with ageing, assessment of sarcopenic obesity is not feasible due to the requirement of specific imaging modalities such as dual-energy X-ray absorptiometry. For a clinical practice, we previously validated waist-to-calf circumference ratio (WCR) as a simple index to assess the disproportion between abdominal obesity and leg muscle lean mass, which were significantly associated with carotid atherosclerosis in patients with type 2 diabetes (Diabetes Care. 2011;34:2067-71). Sarcopenic obesity is closely linked to various cardiometabolic disorders, while its influence on non-alcoholic fatty liver disease (NAFLD) or steatohepatitis has not been fully determined in type 2 diabetes. Therefore, we aimed to investigate whether WCR is associated with NAFLD and hepatic fibrosis in patients with type 2 diabetes.
Materials and methods: This was an observational study performed in 5,806 consecutively enrolled patients with type 2 diabetes. Hepatic steatosis was diagnosed using ultrasonography. Insulin resistance was assessed using the insulin tolerance test (ITT). NAFLD fibrosis scores (NFS) and FIB-4 scores were calculated to define advanced fibrosis in subjects with NAFLD.
Results: The prevalence of NAFLD and obesity (BMI≥25 g/m2, Asian definition) were 44.6% and 38.9%, respectively, in the whole study population of type 2 diabetes. The proportion of NAFLD was higher in subjects with increasing WCR tertiles regardless of gender (the lowest WCR tertile vs. the highest WCR tertile, 32.6% vs. 52% in men, 24.1% vs. 58.0% in women, both Ps<0.0001). This relationship between WCR tertiles and NAFLD appeared significant even after stratification with obesity or insulin resistance status assessed by ITT (Ki values). Multiple logistic regression analysis also demonstrated this independent association between WCR tertiles and NAFLD after adjusting for confounding factors related to obesity or insulin resistance (ORs=1.32 to 1.36 in men, Ps<0.01 and ORs=1.14 to 1.24 in women, Ps<0.05). Serum levels of liver enzymes (AST and ALT) and an inflammatory marker, CRP were all elevated with increasing WCR tertiles in both men and women (All Ps< 0.001). Furthermore, among the NAFLD population, subjects with the highest WCR tertile were likely to have advanced fibrosis compared with other individuals regardless of obesity (hepatic fibrosis assessed by NFS and FIB-4: ORs=2.27 and 2.15, respectively; both Ps<0.001).
Conclusion: WCR is associated with increased risks of NAFLD and advanced hepatic fibrosis in type 2 diabetes independently of obesity or insulin resistance. This suggests that WCR which indirectly reflects sarcopenic obesity may be a useful and practical anthropometric index to predict fatty liver and its fibrosis in type 2 diabetes.
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Associations between type 2 diabetes-related genetic scores and OGTT derived traits, in obese children and normal weight controls

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Background and aims: A total of 62 single nucleotide polymorphisms (SNPs) have been associated with type 2 diabetes (T2D) and pooled together into an additive T2D genetic risk score (GRS-T2D) in European adults. Of these SNPs, 21 have been recognized to affect β-cell function and 10 to affect insulin resistance (IR), and have been pooled into a β-cell function GRS (GRS-β) and an IR GRS (GRS-IR) respectively. Here, we aim to investigate the associations between these GRSs and OGTT derived traits in obese and normal-weight adolescents and young adults.
Materials and methods: In a sample of 1,076 obese children/adolescents (11.4±2.8 years old) and 1,265 normal weight adolescents and young adults (21.1±4.4 years old) of European ancestry, we performed a

standard OGTT to assess fasting glucose homeostasis traits, i.e. fasting plasma glucose [FPG] (mmol/l), fasting insulin [FI] (mU/l), HOMA-B (20*FI/[FPG-3.5]) and HOMA-IR (FPG*FI/22.5), post-load glucose homeostasis traits, i.e. insulinogenic index [II] (ΔInsulin30'/ΔGlucose30'), insulin sensitivity index [ISI] (k/sqrt[Glucose0'*Insulin0'*Average-Glucose*Average-Insulin]), disposition index [DI] (ISI*100*Insulin30'/[glucose30'*(glucose30'-3.89)]) and 2-hours plasma glucose [2-hG] (mmol/l), and the presence of pre-diabetic conditions, i.e. impaired glucose tolerance [IGT], and impaired fasting glucose [IFG]. The genotyping of SNPs was performed with Metabochip arrays (Illumina). We assessed the associations of T2D-related traits with GRS-T2D, GRS-β and GRS-IR using linear or logistic regression models adjusted for gender, age and BMI. Here, we only report associations that were significant after Bonferroni multiple test correction (i.e. with a $p<0.0015$).

Results: The GRS-IR was not associated with any variable either in obese children or in normal weight controls, while the GRS-T2D was associated with only II (-0.02 per allele) and DI (-0.02 per allele) in normal weight young adults but with no variable in obese children. GRS-β was associated with HOMA-B (-0.02 per allele), II (-0.04 per allele) and DI (-0.03 per allele) in normal weight children, with FPG (+0.02 per allele) and DI (-0.03 per allele) in obese children, and with FPG (+0.01 per allele), HOMA-B (-0.02 per allele), II (-0.03 per allele) and DI (-0.03 per allele) in meta-analyzed data. None of the scores was associated with any binary trait.

Conclusion: Among T2D related genetic scores, only GRS-β shows significant associations with glucose homeostasis traits in both obese children/adolescents and in normal weight controls. These associations highlight that GRS-β influences both fasting and post-load glucose homeostasis. The fact that GRS-T2D is associated with II and DI only in normal weight participants, may be explained by a higher influence of non genetic factors in obese compared to non obese subjects.

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Age associated DNA methylation changes in adipose tissue is linked to type 2 diabetes

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Background and aims: Ageing is a risk factor for several common diseases, including type 2 diabetes, and has been suggested to contribute to increased epigenetic variability. Epigenetic factors, e.g. DNA methylation, provide a link between genetic and non-genetic factors, and could be involved in phenotype transmission and disease development. The current aim was to investigate the effect of age on DNA methylation in human adipose tissue.

Materials and methods: The Infinium HumanMethylation450 BeadChip was used to analyse the genome-wide DNA methylation pattern in adipose tissue from 96 healthy men with an age span between 23 and 80 years. The association between age and DNA methylation of individual CpG sites was tested using a random effect mixed model, including cohort as the random effect variable and age, BMI, and HbA1c as fixed factors.

Results: DNA methylation data was obtained for 456,800 CpG sites in the 96 men. Age was positively correlated with the average level of DNA methylation in adipose tissue ($p=1.1\times10\text{-}5$). After correction for multiple testing, we found 31,567 individual CpG sites significantly associated with age. Among these sites, 90.6% showed a positive whereas only 9.4% showed a negative relation between age and the level of DNA methylation, in agreement with the result of average DNA methylation. Of the CpG sites significantly associated with age, 24,514 are annotated to 11,036 unique genes, whereas 7,053 of the CpG sites are intergenic. We proceeded to test if the age-associated methylation differences also can be identified in diseased subjects, by comparing our results with a recently published case-control study identifying 15,627 CpG sites with differential DNA methylation in adipose tissue from subjects with type 2 diabetes compared with non-diabetic age-matched controls. We found an overlap of 1,278 differentially methylated CpG sites, 90% of which changed in the same direction due to age and type 2 diabetes. These CpG sites include many novel loci, but also established diabetes susceptibility genes such as JAZF1. To obtain further biological insight into the significant associations between DNA methylation and age, we used WebGestalt to perform a KEGG pathway analysis on the 11,036 genes with one or more CpG sites significantly associated with age. Significant enrichment was found for 31 pathways, including e.g. the MAPK and Wnt signaling pathways, pathways in cancer and type 2 diabetes mellitus.

Conclusion: We have shown extensive epigenetic alterations in adipose tissue due to ageing, some which previously also have been shown to be present in type 2 diabetic patients. Our study supports a role for epigenetic mechanisms in the development of age related diseases such as type 2 diabetes.

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Lower risk for obesity among adolescents living at higher altitudes: a cross-sectional analysis in the Peruvian population

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Background and aims: A significant inverse association between obesity and altitude has been reported in the adult population of the United States, adjusting for multiple covariates. However, whether this relationship extends to the pediatric populations remains unknown. The aim of the present study was to determine the association between altitude and obesity among adolescents, adjusting for several factors.

Materials and methods: We explored the association between altitude and obesity in the adolescent population of Peru, a country with topographic, socio-economic, cultural, and ethnic features differing from those of the United States and Europe. We utilized data publicly available online from the Food and Nutrition National Center of Peru, CENAN, for 2009-2010, the last period for which data relevant for a model analysis are available. The CENAN used a nationally representative sample selected by the National Home Survey. An important feature of this survey is the on-site measurements of body weight and height using standardized methods. Final analysis included 7,618 adolescent subjects (14-19 years old). Obesity was defined as the BMI-for-age in months at or above 95th percentile for sex. Percentiles were estimated using the Centers for Disease Control and Prevention 2000 growth charts. Multilevel mixed-effects Poisson regression was used to estimate the relative risk for obesity, adjusting for several factors including age, sex, physical activity, out-migration rate, urbanization, and latitude, accounting for nested data (region level) and the random effects between subjects.

Results: The crude prevalence of obesity among adolescents was 3.7% between 0−1,499 m and 0.9% at high altitude (≥1,500 m). Boys had substantially less crude prevalence of obesity at high altitude as compared with girls living at high altitude (0.86% and 0.93%, respectively). Overall, the relative risk for obesity, adjusted for age, sex, physical activity, out-migration rate, urbanization, and latitude was as follows:

1.00 between 0−499 meters (reference category), 1.63 (95% confidence interval 1.05 to 2.53) between 500−1,499 m, 0.59 (0.33 to 1.07) between 1,500−2,999 m, and 0.28 (0.14 to 0.56) at ≥3,000 m.
Conclusion: Among Peruvian adolescents, the risk for obesity was lower at altitudes above 3,000 m. These findings suggest that geographical elevation is an important factor linked to obesity not only among adults but also in the pediatric population. Exploring the underlying mechanisms of this association could provide new insights in the pathogenesis of obesity.
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Metabolic syndrome in elderly women: Is waist circumference the correct 'entrance criteria'?
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Background and aims: More than 20% of the world's adult population is considered having metabolic syndrome (MetS). MetS is used to identify patients at greater risk of developing cardiovascular diseases (CVD) and type II diabetes (T2DM). The consensus definition set out by the International Diabetes Federation (IDF) defines large waist circumference as the 'entrance criteria' for identifying MetS and thereby high risk patients. This study aimed to investigate the risk of developing CVD based on number of MetS risk factors rather than waist circumference. Subjects were further stratified based on body weight and body composition in order to determine rate ratios (RR) and incidence within the specified groups.
Materials and methods: Demographics, DEXA scans and serum samples were collected from 1999-2001 on 5855 women aged 73.1±6.3. CVD diagnoses were collected from Danish National Disease Registries primo 2015. Subjects with a preexisting CVD event at baseline were excluded from analysis. Presence of MetS and number of abnormalities were assessed by the IDF criteria for triglycerides, HDL-cholesterol, blood pressure, and fasting glucose. Initially, RR for a CVD event was determined in subjects with ≥2 MetS risk factors (corrected for age and BMI) by Poisson regression analysis. Thereafter, RR for development of CVD was determined based on BMI (corrected for age and number of MetS risk factors). Lastly, stratification was made based on BMI (underweight (UW): <18.5, normal weight (NW): 18.5-24.9, overweight (OW): 25.0-29.9, fat: ≥30) and body composition defined as central/peripheral (C/P) fat mass ratio stratified into quartiles (Q1-Q4) in order to determine RR and incidence in the specific groups.
Results: An increase in CVD risk of 27% was found throughout the cohort solely by number of MetS risk factors (≥2 vs. <2), RR=1.27 (1.14-1.42). Moreover, the risk for developing CVD was increased with increasing BMI (UW: RR=0.88 (95% CI: 0.52-1.47), NW: RR=1 (ref), OW: 1.18 (1.04-1.32), FAT: RR=1.29 (1.11-1.50)). This indicates that both number of MetS risk factors and BMI is associated with CVD events. However, within all groups, regardless of stratification (BMI or body composition), a significant increase in risk of CVD event was found for subjects having ≥2 MetS risk factors (Table 1). This indicates that number of risk factors, rather than BMI or body composition, are the underlying denominator for CVD outcome. The CVD incidence increased between groups highlighting that increasing BMI and central fat mass increase the risk of a CVD event.
Conclusion: Number of risk factors and BMI are both of importance in defining subjects at risk of developing CVD. However, the risk of developing a CVD event was increased with ≥2 MetS risk factors regardless of BMI or body composition in elderly women. It is proposed that number of risk factors should be used for optimal identification of patients at increased risk of CVD. Large waist circumference or BMI >30 should be employed as an individual risk factor rather than the 'entrance criteria' for MetS in elderly women.

				CVD RISK ≥2 vs. <2 MS risk factors		CVD INCIDENCE	
		Person years at risk	Number of cases	RR	95% CI	Incidence pr 1000 person years	95% CI
BMI	UW	598	28	2,28	[0,81-6,41]	46,8	[32,3-67,8]
	NW	17808	917	1,27	[1,06-1,51]	51,5	[48,3-54,9]
	OW	16392	982	1,28	[1,09-1,50]	59,9	[56,3-63,8]
	FAT	6162	418	1,22	[0,95-1,58]	67,8	[61,6-74,7]
C/P RATIO	Q1	9963	504	1,28	[0,98-1,67]	50,6	[46,4-55,2]
	Q2	9944	558	1,14	[0,92-1,41]	56,1	[51,6-61,0]
	Q3	9918	536	1,38	[1,11-1,70]	54,0	[49,7-58,8]
	Q4	8997	600	1,33	[1,09-1,62]	66,7	[61,6-72,2]

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Different relationship between body fat distribution and diabetes mellitus: analysis of the 2008-2010 Korean national health and nutrition examination surveys
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Background and aims: The aim of this study was to investigate the association between the regional body fat distribution, especially leg fat mass and the presence of diabetes mellitus (DM) in older populations.
Materials and methods: A total of 3,027 men and 3,548 postmenopausal women aged 50 years or older were analyzed from Korea National Health and Nutrition Examination Surveys (2008-2010). Body compositions including muscle mass and regional fat mass were measured using dual-energy X-ray absorptiometry.
Results: Obesity indices such as body weight, body mass index (BMI), waist circumference, total body fat (%) and truncal/arm fat mass were higher in diabetes patients compared with those non-diabetes patients. However, leg fat mass was lower in postmenopausal women with diabetes but not in men (Table 1). In multiple logistic regression analysis, a 1-kilogram increase in trunk fat mass was associated with 15% increase in the presence of DM in men and 19% increase in women after adjustment for confounding factors. Conversely, each kilogram increase in leg fat mass was significantly associated with 51% reduction of DM in men and 44% reduction in women. In partial correlation analysis adjusted for age, leg fat mass was positively correlated with appendicular skeletal muscle mass and HOMA-β. There was significant negative association between leg fat mass and glycated hemoglobin in both genders and fasting glucose in women (Table 2). When the participants were classified four groups according to sex-specific leg fat mass quartiles, the odds ratios (OR) for the presence of DM significantly decreased gradually as leg fat mass increased in both sexes (men: Quartile 1 vs. Quartile 2 vs. Quartile 3 vs. Quartile 4 : OR [95% CI]=1 vs. 0.43 [0.31-0.60] vs. 0.32 [0.23-0.46] vs. 0.13 [0.09-0.21]; women: Quartile 1vs. Quartile 2 vs. Quartile 3 vs. Quartile 4 : OR [95% CI]=1 vs. 0.41 [0.30-0.57] vs. 0.32 [0.22-0.46] vs. 0.13 [0.08-0.21], respectively) after adjustment for potential confounding factors (Table 3). To define the relative importance of leg fat mass and leg muscle mass on DM, we categorized subjects into four groups: HF-LM, HF-HM, LF-LM, and LF-HM based on the median values of leg fat mass and leg muscle mass. As a result, higher leg fat mass significantly lowered the risk of DM regardless of leg muscle mass status in both genders ($p < 0.001$).
Conclusion: In conclusion, our study demonstrated that, in contrast to trunk and arm adiposity, there is a favorable association of leg adiposity

with DM in Korean adults aged 50 years or older. We also found the contributory effects of lower extremity fat on DM are more dominant than those of lower extremity muscle. Our findings support the notion that subcutaneous fat and glucose metabolism are intimately interlinked.

Table 1. Characteristics of the study population according to the presence of DM

	Men			Women		
	Non-DM (N=2514)	DM (N=513)	P-value	Non-DM (N=3079)	DM (N=469)	P-value
Age (year)	63.24±8.85	64.29±8.38	0.011	63.68±8.88	67.04±7.97	<0.001
Weight (Kg)	65.08±9.60	67.84±9.66	<0.001	56.06±8.56	58.91±9.07	<0.001
BMI (kg/m^2)	23.44±2.91	24.43±2.90	<0.001	23.92±3.16	25.24±3.36	<0.001
Waist circumference (cm)	84.38±8.50	88.32±8.67	<0.001	81.49±9.15	87.04±9.23	<0.001
Current smoking (%)	847 (33.8%)	164 (32.0%)	0.424	140 (4.6%)	16 (23.4%)	0.272
Regular exercise (%)	471 (18.8%)	83 (16.2%)	0.169	392 (12.8%)	43 (9.2%)	0.031
SBP (mmHg)	124.13±17.16	125.67±16.33	0.061	125.53±17.87	131.44±18.24	<0.001
DBP (mmHg)	77.03±10.45	75.35±10.26	<0.001	76.43±10.05	75.70±10.15	0.144
Fasting glucose (mmol/l)	5.4±0.6	8.1±2.6	<0.001	5.84±1.54	5.84±1.28	<0.001
HbA1c (mmol/mol)	44.0±5.85	56.1±13.28	<0.001	43.9±2.9	57.4±12.95	<0.001
HOMA-β	104.24±52.73	61.42±55.92	<0.001	117.37±53.01	75.72±66.83	<0.001
Total cholesterol (mmol/l)	4.85±0.92	4.69±0.99	0.025	5.28±0.91	5.17±1.01	0.025
LDL cholesterol (mmol/l)	2.80±0.89	2.57±0.96	<0.001	3.22±0.83	3.05±0.91	<0.001
HDL cholesterol (mmol/l)	1.18±0.29	1.08±0.26	<0.001	1.25±0.28	1.16±0.27	<0.001

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Predictors of severe obesity and associated morbidity in the Malmö Preventive Project: a population-based cohort study

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Background and aims: Severe obesity represents an increasing medical problem of relevance for type 2 diabetes. This study aimed to evaluate risk markers for developing severe obesity as well as the prospective morbidity risk associated with severe obesity in a population-based screening and intervention programme.
Materials and methods: In total, 18,200 individuals from a population-based cohort underwent a baseline examination in 1972-1991 and were re-examined in 2002-2006 in Malmö, Sweden. In total, 809 (4.4%) patients with severe obesity (BMI ≥35 kg/m2) were found at re-examination, and predictive risk factors from baseline were identified. Total and cause-specific morbidity were followed in national registers in all severely obese patients, as well as in age- and sex-matched normal weight controls (n=1618).
Results: Men and women developing severe obesity differed significantly from matched controls in baseline variables associated with the metabolic syndrome (BMI, triceps skinfold, blood pressure, fasting-glucose, triglycerides), as well as in fasting-insulin, liver enzymes, cholesterol, and uric acid. Both male and female obese subjects were on average shorter than controls ($p<0.001$). Female but not male severe obesity was associated with family history of hypertension. Abstaining from alcohol was more common in female severely obese subjects ($p<0.001$), but not among male subjects ($p=0.324$), compared with normal-weight controls. Incident cancer ($p=0.002$), coronary events ($p=0.022$), stroke ($p=0.002$), heart failure ($p<0.001$), atrial fibrillation ($0<0.001$), and diabetes ($p<0.001$) during follow-up was higher among severely obese persons compared to normal-weight controls.
Conclusion: Variables associated with the metabolic syndrome are significant predictors for development of severe obesity after a long-term follow-up. Severe obesity is associated with cardiovascular morbidity, cancer and incident diabetes. This calls for improved weight control in persons with certain predictors of this condition, as well as better risk factor control in patients with severe obesity.
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Maternal exposure of autoantibodies protects the child from islet autoimmunity in early childhood if the mother does not have type 1 diabetes (TEDDY)

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Background and aims: Maternal islet autoantibodies transferred via placenta indicate presence of type 1 diabetes (T1D) or an increased risk of T1D in the mother. However, little is known concerning the child´s risk of islet autoimmunity (IA) or T1D. The aim was to explore whether exposure of maternal autoantibodies in absence of maternal T1D affects the child´s risk to develop IA in early childhood.
Materials and methods: Children with an increased genetic risk for T1D were followed from birth in The Environmental Determinants of Diabetes in the Young (TEDDY-study). Maternal venous blood sample was collected at the child´s age of 9 months if: the mother had T1D (n=297), of a child of a mother without T1D that was positive for any islet autoantibodies at age 3-6 months or if the mother had any other type of diabetes (type 2, gestational or maturity onset of diabetes in the young (MODY) (n=538).
Results: During the follow-up for a median of 65 months (IQR 49-86) IA developed in 14.1% (42/297) of children born to a mother with T1D and in 12.8% (69/538) of children born to a mother without T1D. Children exposed to maternal islet autoantibodies from non-T1D mothers were less likely to develop IA (HR=0.26; 95%CI 0.08-0.84; p=0.0236) as compared with un-exposed children. In contrast, children exposed to maternal autoantibodies and born to mothers with T1D had similar risk of IA as compared with un-exposed children. (HR=1.08; 95%CI 0.58-2.0; p=0.8137).
Conclusion: Exposure of maternal autoantibodies indicated decreased risk for IA in early childhood in children without a mother with T1D, but not for children to mothers with T1D as compared with un-exposed children.
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Identification of potential circulating beta cell biomarkers

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Background and aims: Therapies to promote the preservation and restoration of functional pancreatic β-cell mass are essential for the treatment of type 2 diabetes (T2D). A major challenge for the development of such therapies is the lack of adequate biomarkers to assess changes in β-cell health with theraputic interveniton and disease stage. Monitoring β-cell mass and function during the disease progression could lead to a better

understanding of pathogenesis and an improved treatment. In this study, proteomic analysis of human islets of Langerhans was carried out in order to search for such biomarkers.

Materials and methods: Human islets and exocrine pancreatic tissue were provided by the Nordic Network for Clinical Islet Transplantation. One and two dimensional (2D) SDS-PAGE was used for separation of islet and exocrine tissue extracts and the entire 1D gel lanes or the 2D gel spots with two fold increased expression in islets, were subjected to in-gel digestion. The resulting peptides were analyzed by liquid chromatography FTICR mass spectrometry. Proteins were identified by searching against the Swiss-Prot database using Mascot, requiring at least two unique peptides, at 95% level of confidence for a valid protein identification. Following in silico filltering for islet enriched expression using the Human Protein Atlas, candidates were further investigated using Western blotting (WB) and immunohistochemistry (IHC). Quantification of Secretagogin (SCGN) levels in human plasma from age, body mass index, and gender matched T2D patients and healthy control subjects, provided by Uppsala University Hospital,was enabled by ELISA (BioVendor).

Results: In the islet protein extracts, 1700 proteins were identified and following in silico filltering for islet enriched expression, one of the candidate proteins, SCGN, were highly expressed in human islets both on mRNA and protein level. Furthermore, WB also showed that the protein was secreted into the culturing media with an indication that high glucose (16.5 mM) exposure for 1 h resulted in higher intracellular levels and reduced SCGN levels in the cell media. Double staining of human pancreatic tissue with SCGN and insulin showed co-localisation of the two proteins, indicating that SCGN was predominantly expressed in β-cells. Finally, ELISA allowed for the quantification of SCGN in plasma of T2D patients (n=15) as well as in healthy subjects (n=16). Significantly increased SCGN levels (p=0.03) could be observed in the diabetic patients.

Conclusion: To identify a circulating biomarker of β-cell mass offers a challenge since the dispersed islets constitutes only 1-2% of the total pancreatic mass, and the insulin producing β-cells even a smaller fraction within the islets. This demands that a suitable biomarker must be highly expressed and secreted mainly from the β-cells in order to reflect their health, functional status or mass. SCGN met these criterias and could also be quantified in human plasma, suggesting a potential of SCGN as a β-cell biomarker. However further studies will be required to determine whether the increase of plasma SCGN reflects the mass of β-cells present in the islets of T2D patients.

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Altered lipid metabolism preceding onset of islet autoimmunity and type 1 diabetes

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Background and aims: Dysregulation of lipid and amino acid metabolism has been shown to take place already prior to the first seroconversion to islet autoimmunity in progression to Type 1 diabetes (T1D). Previously we also observed by lipidomic analysis of cord blood that lipid dysregulation is found in newborn infants who developed T1D early in life while it is not associated with development of beta-cell autoimmunity in general. In order to gain a global view of metabolome in progression to islet autoimmunity and T1D, here we studied serum metabolome in children from the DIABIMMUNE cohort, who seroconverted to one or more autoantibodies and matched controls.

Materials and methods: Children were divided into four groups according to the number of autoantibodies they developed during the follow-up: 12 children progressed to two or more islet autoantibodies (≥2 Aab) group, 8 children developed one Aab persistently (1pAb), 21 children developed one Aab transiently (1tAb). 81 children were controls matched for gender, HLA genotype, country (Finland, Estonia) and date of birth. Among the 12 children from ≥2 Aab group, four progressed to T1D. The study included a longitudinal series of samples (cord blood, 3, 6, 12, 18, 24, 36 months of age) from three case groups and controls. Metabolomic analysis of serum samples was performed by applying two global analytical platforms to study molecular lipids and polar metabolites, respectively. The two metabolomics datasets (876 molecular lipids and 355 polar metabolites) were clustered using the model-based clustering, which resulted in 18 and 13 clusters of molecular lipids and polar metabolites, respectively. Linear mixed effect models were applied at cluster level to further analyze the metabolomics data. The fixed effects accounted for in the models were group effects at different time points, effect of age as well as the effect of age of seroconversion to islet autoantibodies.

Results: The significant differences were mainly observed for the ≥2 Aab and 1pAb groups, while 1tAb group tended to have very similar metabolite profiles as the controls. Specifically, in ≥2 Aab group, triglycerides were downregulated at early age and lysophosphatidylcholines were upregulated, as compared to controls. In contrast, in the 1pAb group triglycerides were upregulated while lysophosphatidylcholines were downregulated. No significant changes were observed for major membrane lipids such as phosphatidylcholines (PC). Regarding metabolites, in ≥2 Aab and 1 Aab groups the cluster containing TCA cycle metabolites and metabolites of branched chain amino acid (BCAA) metabolism (excluding BCAAs themselves) was downregulated in cord blood. In ≥2 Aab group this cluster remained downregulated while in the 1Aab group became upregulated at about 2 years of age.

Conclusion: The children who seroconvert to two or more autoantibodies (as well as to T1D) have markedly different metabolic profile from those children who progress to a single islet autoantibody. Identification of the underlying pathways may lead to improved disease detection and to novel clues for disease prevention.

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Antigen-based immunotherapies do not prevent progression of autoimmune diabetes: a systematic review and meta-analysis

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Background and aims: Antigen-based immunotherapies aim to attenuate the destructive autoimmune process against pancreatic β-cells in patients with recent-onset autoimmune diabetes. We performed a systematic review and meta-analysis to assess their efficacy and safety in tertiary prevention of autoimmune diabetes.

Materials and methods: We searched in major bibliographic databases (MEDLINE, COCHRANE, EMBASE), trial registries, conference proceedings and reference lists of included studies and relevant reviews, for randomized controlled trials of antigen-based immunotherapies (GAD-alum, Diapep277, oral insulin, nasal insulin, NBI-6024, insulin B-chain fragment) in patients with recent-onset autoimmune diabetes (less than 60 months after diagnosis or C-peptide levels after mixed meal stimulation more than 0.2 nmol/L). Our primary outcome was residual β-cell function by means of fasting and stimulated (both glucagon and mixed meal) C-peptide. We also synthesised data for change in HbA_{1c} and daily insulin dose, incidence of any and severe hypoglycaemic events, and incidence of severe adverse events. Finally, we assessed risk of bias and graded the quality of the body of evidence.

Results: We identified 22 eligible studies, 15 of which were included in the meta-analysis. Risk of bias was deemed high in 14 of them, while in the remaining two it was unclear or low. Overall, there were no differences in fasting [weighted mean difference (WMD) 0.01 nmol/L; 95% CI -0.09 to 0.11; I^2=73%] or mixed meal stimulated (WMD 0.02 nmol/L/min; 95% CI -0.08 to 0.12; I^2=50%) C-peptide compared with placebo. Glucagon stimulated C-peptide was maintained higher (WMD 0.13 nmol/L/min; 95% CI 0.05 to 0.21; I^2=0%) in patients treated with Diapep277. Moreover, there was no change in daily insulin dose (WMD 0.02 IU/kg; 95% CI -0.04 to 0.09; I^2=51%) or HbA_{1c} (WMD -0.06%; 95% CI -0.35 to 0.23; I^2=42%) versus placebo. Finally, antigen-based immunotherapies were not effective in reducing the incidence of severe hypoglycaemic events. Nonetheless, there was no increase in the incidence of severe adverse events (risk ratio 0.87; 95% CI 0.53 to 1.44; I^2=0%). The overall quality of the body of evidence was very low.
Conclusion: Antigen-based immunotherapies are not effective in preventing the progression of autoimmune diabetes in newly-diagnosed patients.

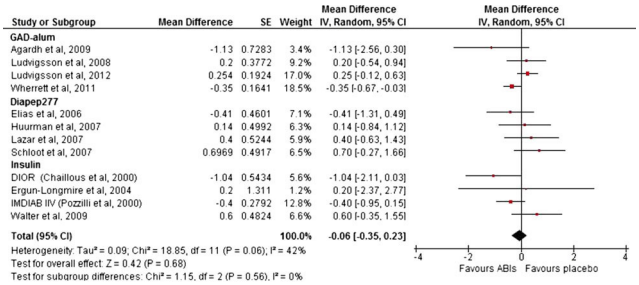

Figure. Forest plot depicting weighted mean difference for change in HbA_{1c}. SE: standard error, IV: inverse variance, ABIs: antigen-based immunotherapies.

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Residual beta cell function in diabetes children followed and diagnosed in the TEDDY study compared to controls

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Background and aims: Participation in prospective follow-up studies is associated with a reduction in symptoms and ketoacidosis at diabetes onset, but it is not known whether this close monitoring also leads to better outcomes beyond diagnosis. We hypothesize that children diagnosed with type 1 diabetes through The Environmental Determinants of Diabetes in the Young (TEDDY) study are diagnosed earlier in the time course of diabetes and that they will have a higher level of C-peptide at diagnosis compared with control children diagnosed through the community.
Materials and methods: TEDDY children were enrolled at 3-4.5 months of age and followed every 3 months for autoantibodies against insulin, GAD65 and IA-2 and for the development of clinical diabetes. Control subjects are matched to diabetic TEDDY subjects by age of diagnosis within one year and have at least one islet autoantibody at diagnosis. After diagnosis of type 1 diabetes, all participants undergo visits at diabetes onset and then at 3, 6, 12, 18 and 24 months and annually thereafter. The primary outcome measure is the AUC of C-peptide in response to a Mixed Meal Tolerance Test (MMTT) over time. The goal is to follow all subjects until the loss of detectable endogenous C-peptide.
Results: A preliminary analysis of 46 TEDDY and 43 control children revealed that TEDDY children diagnosed with diabetes often had no symptoms (58%) at onset and none had ketoacidosis (DKA) compared to 100% with diabetes symptoms and 18% DKA in the controls. Mean HbA1c was lower in TEDDY (6.4%) than control (7.6%) children ($p<0.0001$). The TEDDY children had significantly higher residual C-peptide AUC than the controls analyzed by a generalized linear model with repeated measures ($p=0.0062$). The decrease in residual C-peptide during follow up did not differ between TEDDY and control children ($p=0.1976$).
Conclusion: The earlier diagnosis of type 1 diabetes in TEDDY children studied so far appears to be associated with higher MMTT levels of residual C-peptide. Although the loss of C-peptide may be parallel, it remains to be determined whether early symptom-free diagnosis of diabetes has a long term benefit.
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Serum microRNAs as novel biomarkers for diagnosis of type 1 diabetes mellitus

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Background and aims: New Strategies and biomarkers for early detection of type 1 diabetes mellitus (T1DM) are sorely needed. We aimed to explore the diagnostic value of serum microRNAs (miRNAs) in newly diagnosed patients with T1DM.
Materials and methods: Serum miRNA expression was investigated in patients with T1DM (duration of diabetes≤1 year) and age-, gender-matched healthy individuals recruited between August 2011 and June 2014. Firstly, microarray was used to screen 2006 miRNAs in 6 T1DM patients and 6 healthy controls. Quantitative reverse-transcriptase polymerase chain reaction assay (qRT-PCR) was then applied to evaluate the expression of differentially altered miRNAs. A logistic regression model was developed using a training cohort (characteristics as shown in table). Area under the receiver operating characteristic curve (AUC) was used to assess diagnostic accuracy. Finally, the model was validated by leave-one-out cross-validation and another independent validation cohort (characteristics as shown in table).
Results: Microarray analysis discovered that thirty-one serum miRNAs were significantly different between T1DM patients and healthy controls (fold expression change≥2, $P<0.05$). Six miRNAs (miR-642a-3p, miR-320c, miR-1225-5p, let-7b-5p, miR-26b-5p, miR-144-3p) were verified by qRT-PCR in the training cohort ($P<0.05$ for each). A logistic regression model combining miR-320c, miR-1225-5p, age and body mass index (BMI) was developed, which provided a high diagnostic accuracy of T1DM (AUC=0.817 for training data set, 95% confidence interval 0.732-0.904, P=0.000). The cross-validation error rate based on logistic regression models was 18.3%. The satisfactory diagnostic performance of the model persisted in the validation cohort (AUC=0.804, 95% confidence interval 0.688-0.921, P=0.000).
Conclusion: Our results identified a panel based on the expression of serum miRNAs with considerable diagnostic value for T1DM. Further research is necessary to explore whether these have clinical implications for early detection of disease.

Table. Characteristics of participants in the training and validation cohorts

Characteristics	T1DM cases	Healthy controls	*P* value
Training cohort	**n = 40**	**n = 56**	**----**
Age (years)	22.31 ± 1.90	20.00 ±1.34	0.308
Female (n, %)	26 (65%)	35 (62.5%)	0.802
BMI‡ (kg/m^2)	18.75 ± 0.46	19.73 ± 0.38	0.104
Disease duration (months)	8.54 ± 0.74	----	----
HbA_{1c}§ (%)	7.4 (6.4, 9.8)	5.1 (4.9, 5.3)	0.000
FCP¶ (ng/ml)	0.25 (0.09, 0.34)	----	----
PCP2h‖ (ng/ml)	0.57 (0.27, 0.80)	----	----
Validation cohort	**n = 33**	**n = 29**	**----**
Age (years)	21.18 ± 1.76	20.90 ±2.14	0.919
Female (n, %)	18 (54.5%)	14 (48.3%)	0.622
BMI (kg/m^2)	18.42 ± 0.63	21.28± 0.50	0.001
Disease duration (months)	6.93 ± 1.28	----	----
HbA_{1c} (%)	8.9 (7.5, 12.6)	5.1 (4.9, 5.2)	0.000
FCP (ng/ml)	0.28 (0.10, 0.55)	----	----
PCP2h(ng/ml)	0.54 (0.29, 0.75)	----	----

Data are presented as mean ± SE or median (interquartile range) for continuous variables and n (%) for categorical variables; ‡ BMI: body mass index (kg/m^2); § HbA_{1c}: glycated hemoglobin A_{1c}; ¶ FCP, fasting serum C-peptide; ‖ PCP2h, 2-hour postprandial serum C-peptide.

Supported by: Sun Yat-sen University Clinical Research 5010 Program(2007030)

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Predicting islet cell death using miRNAs: small molecules giving big insights

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Background and aims: Death of insulin-producing beta cells is a common feature of type 1 diabetes (T1D), including after islet transplantation as well as Latent Autoimmune Diabetes of Adults (LADA) and type 2 diabetes (T2D). The death of these islet beta cells is a slow process that commences several months-years prior to clinical onset of T1D. We currently lack tools to quantitatively detect islet cell loss prior to the clinical onset of diabetes, or to predict the progression of established T1D. MicroRNAs (miRNAs), a subset of small non-coding (nc)RNAs, are promising biomarkers as i) some of these are significantly altered in circulation between individuals with or without diabetes, ii) show high stability in blood, iii) offer ease of detection and iv) are resistant to freeze-thaw degradation. We identified a set of ncRNAs that are either enriched in islet beta cells or differentially expressed in individuals with diabetes. This set of ncRNAs, which we refer to as the RAPID signature (RNA-based Analysis for Predicting Islet Death) can potentially predict islet beta cell death during diabetes progression.
Materials and methods: Next Generation Sequencing (NGS) and quantitative (q)PCR profiling studies were conducted on developing human pancreases and adult islets. MicroRNAs in plasma from individuals with newly diagnosed or established T1D, compared to age and gender matched controls, were profiled on the OpenArray™ (OA) platform followed by validation on ViiA7 platform.
Results: To determine the most reproducible high-throughput qPCR platform on which to assess the RAPID signature, we undertook a robust comparison of multiple nano- and micro-fluidics platforms (OA, Dynamic Array® (DA), TaqMan Low Density Arrays/TLDAs and the standard 96-well (low throughput) platform on the ViiA7 system). Data from OA was more reproducible (median assay CV 2.1%) than DA and best correlated with the 96-well platform (R2=0.88). We therefore used OA platform for future assays. NGS and qPCR studies identified 20 miRNAs that are enriched during pancreas development and remain highly expressed in adult islets. Twenty four other miRNAs were identified to be differentially expressed (fold change >2, P-value <0.05) in individuals with T1D, compared to age and gender matched controls (see Fig. 1). Validation of the above selected miRNAs in multiple T1D individuals (vs controls) will lead to refining the RAPID signature into a minimal set of miRNAs that can reliably measure the death of islet beta cells and diabetes progression.
Conclusion: Our studies will inform medical researchers and clinicians as to how to predict the development of T1D, and monitor response to interventions such as islet transplantation, vaccines and drugs that aim to retard beta cell loss or promote beta cell regeneration, and also guide the development of new treatments to lessen the burden of diabetes.

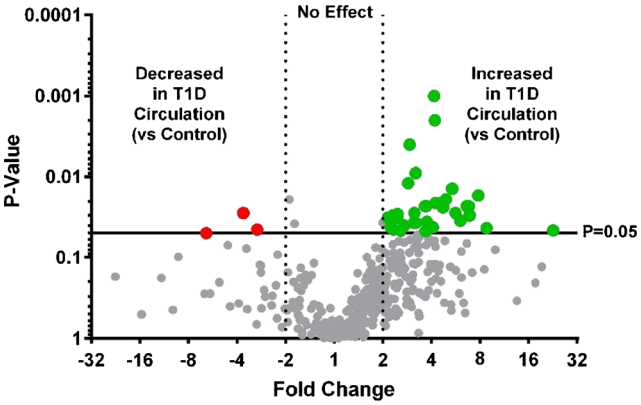

Figure 1. Volcano plot of differentially expressed circulating microRNAs in T1D individuals compared to age and gender matched controls.

Supported by: Funding to AAH from NHMRC and JDRF Clinical Trials Network, Australia.

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A toddler with type 2 diabetes

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Background and aims: Type 2 Diabetes Mellitus continue to be a major health problem related to pediatric obesity. Early identification of pediatric patients at risk, prompt diagnosis and early therapy are major factors to partial reversal of the disease.
Materials and methods: We present a case report of early onset of Type 2 Diabetes in a 3 year-old child with description of the clinical course.
Results: A 3 ½year-old Hispanic female presented to the pediatric endocrinology clinic for evaluation of obesity. She had symptoms of polyuria and polydipsia. Her past medical history was unremarkable. She was born full term. Birth weight was 3.2 kg. Her family history is significant for obesity in parents with no history of diabetes. Diet review revealed poor family nutritional habits with uncontrolled counting of calories and fat. On physical examination, weight was 35 kg (>95 percentile) and height was 95% percentile. Her BMI was also 95% for age. The patient had no cushinoid features, no thyromegaly and her Tanner Stage was I.

Laboratory tests revealed: Fasting plasma glucose 230 mg/dl (12.6 mmol/l) hemoglobin A1C 7.2% insulin 48.8 ulu/ml (339 pmol/L)(normal range 0-29.1uiu/ml, 0- 202 pmol/L) C-peptide 6.9 ng/nl 2.2 nmol/L (normal range 0-3.3, 0- 1.09 nmol/L). Lipid and liver panels were normal. Immune markers for Type 1 Diabetes were negative. Based on symptoms, physical findings of obesity and laboratory results the diagnosis of Type 2 Diabetes was made. The patient was started on liquid Metformin 500 mg daily. Diabetes and nutritional education were performed. We asked the family to implement lifestyle modification by controlling their food portions, total caloric intake and increased physical activities. The patient made significant improvement in weight reduction that lead to normalization of blood glucose levels. The Metformin therapy was decreased by 50% each month, and then stopped. Six months after diagnosis, the patient was at 75% of weight, had normal blood glucose levels, hemoglobin A1C of 5.3% and was off therapy.

Conclusion: Reversal of type 2 diabetes in children is possible by:

1. Early screening of obese children
2. Early diagnosis
3. Appropriate therapy
4. Lifestyle modification

PS 006 Physical activity and other life style factors affecting glycaemia

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Physical activity in a large cohort of insulin-treated type 2 diabetes patients: PROGENS ARENA study

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Background and aims: Data from multiple studies present a consistent picture indicating that regular physical activity substantially reduces the risk of type 2 diabetes mellitus (T2DM). Additionally, in subjects with a diagnosis of T2DM, exercise helps to increase insulin sensitivity, control weight, and improve cardiovascular risk profiles. Unfortunately, the level of physical activity seems to be low among T2DM individuals. Moreover, it was suggested that starting insulin therapy may be associated with an even further decrease in physical activity. We aimed to estimate the level of physical activity in a large cohort of insulin-treated T2DM patients. All of them were participants of the PROGENS ARENA study which was designed to assess the role of behavior-oriented educational intervention in T2DM.

Materials and methods: There were 2500 insulin treated T2DM patients (55.41% women) included into the study. The mean age of the study participants was 64.92±9.31 years, mean BMI 31.44 kg/m2±4.49, mean diabetes duration 12,43±6.86 years, mean baseline HbA1c 8.46% ±1.21. All the patients used both prandial and basal insulins. The proportion of subjects using prandial short acting human insulin was 50.27%, others used rapid acting insulin analogues (in the model of multiple daily injections or as a component of a pre-mixed regimen). At the study onset all the patients filled out a questionnaire concerning health-oriented behaviors including physical activity.

Results: Only 57.4% of examined T2DM patients (51.79% women and 64.36% men) declared any form of deliberate physical activity. Most of the group (82.33%) reported a minimal level of exercise, such as walk or seasonal garden activities. Only 17.4% of patients declared moderate physical activity (swimming, jogging, biking); it is of note that this level of activity was declared more often by men than women (24.88% vs 11.37%). Intense exercise (including participation in sport competitions) was reported only by 0.27% of the study participants. The majority of T2DM patients declared undertaking any form of deliberate physical activity only 1-2 times per week, exercise was performed on daily basis only by 22.26% of T2DM patients.

Conclusion: Insulin-treated T2DM patients are characterized by a very low level of physical activity. The subject of exercise should be considered a very important target in diabetes education.

Supported by: Bioton company

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Physical activity in patients with type 2 diabetes and hypertension: insights into motivations and barriers from the MOBILE study

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Background and aims: While Physical Activity (PA) is key in the management of type 2 diabetes (T2DM) and hypertension, it is difficult to implement in practice and many patients do not achieve recommended levels of PA.
Materials and methods: Cross sectional, observational study conducted in 2014 in France. Participating physicians (cardiologists and diabetologists) were asked to recruit two physically-active and four inactive patients, screened with the Ricci-Gagnon self-questionnaire (active if score ≥16). Patients subsequently completed the validated IPAQ-SF self-questionnaire, as well as all the physicians to assess their own level of PA. The objective was to evaluate the achievement of an individualized HbA1c target and blood pressure goal (<140/90 mmHg) in the active vs. inactive cohort, to explore the correlates for meeting both targets by multivariate analysis, and to examine the barriers and motivations to engage in PA as expressed by active and inactive patients.
Results: 1766 patients were analyzed. Active (n=628) vs. inactive (n=1138) patients were more often male, younger, less obese, had shorter durations of diabetes, fewer complications and other health issues, such as osteo-articular disorders ($p < 0.001$ for all). Their diabetes and hypertension control was better and obtained despite a lower treatment burden. The biggest difference in PA between the active vs. inactive patients was the percentage of patients who declared engaging in regular leisure-type PA (97.9% vs. 9.6%), also reflected in the percentage with vigorous activities in IPAQ-SF (59.5% vs. 9.6%). Target glycemic and blood pressure control was achieved by 33% of active and 19% of inactive patients ($p<0.001$). In multivariate analysis, active patients, those with fewer barriers to PA, with lower treatment burden, and with an active physician, were significantly more likely to reach the combined glycemic and blood pressure goal. Having an active physician was the strongest correlate: participants were 4 times more likely to be at targets vs. those with an inactive physician. The physician's role also emerged in the motivations (reassurance on health issues, training on hypoglycemia risk, and direct prescription and monitoring of the PA by the physician). A negative self-image was the highest ranked barrier for the inactive patients, followed by lack of support and encouragement, and by medical concerns and fear of injury.
Conclusion: Physicians should consider PA prescription as seriously as any drug prescription, with the same need to monitor compliance and the response-effect, and take into account motivations and barriers to PA to tailor advice to patients' specific needs and reduce their perceived constraints.
Supported by: Novartis

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Physical activity and the incidence of diabetes: evidence from the China Kadoorie Biobank study

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Background and aims: The estimated prevalence of diabetes in China is high and increasing. Higher physical activity is associated with decreased risk of diabetes in Western populations, but evidence from Chinese populations is limited. Moreover, most previous studies have assessed mainly recreation- related physical activity rather than total activity from different domains.
Materials and methods: We analysed prospective data of over 0.5 million people aged 30-79 years who were recruited into the China Kadoorie Biobank Study from 10 areas in China. Information about the frequency, duration and type of activity was collected via a questionnaire and an estimate of intensity in metabolic equivalent of task (MET) was assigned to each activity based on the literature. Physical activity was calculated as MET hours per day (MET-h/d) spent on work, commuting, housework, and non-sedentary recreational activities. Data on diabetes incidence was collected via linkage with mortality and morbidity registries and the national health insurance system. After excluding individuals with a prior history of cardiovascular disease or diabetes (including screen detected) at baseline, Cox regression models were used to yield hazard ratios (HRs) relating physical activity to diabetes risk adjusting for age, sex, study area, body mass index and other potential confounding factors. The HRs were corrected for 'regression dilution bias' using a self-correlation (~0.52) for MET-h/d ascertained from a re-survey of about 20,000 individuals conducted on average 3 years later to ascertain the association with "usual" physical activity.
Results: The study population included 463,347 participants (59% female; mean age 51 years; mean physical activity 21.8 MET-h/d). During 7-years of follow-up there were 8866 incident diabetes events recorded. There was a strong inverse association of "usual" physical activity with risk of incident diabetes with a log-linear dose-response relationship. Comparing the highest (≥40 MET-h/d) with the lowest (<8 MET-h/d) physical activity group, there was a 27% lower (95% CI: 18%, 36%) risk of diabetes. Each usual 4 MET-h/d increase, (approximately an hour of brisk walking per day), was associated with 2.7% (95%CI: 1.4%, 4.1%) risk reduction. The associations did not change materially after exclusions of individuals with other prior chronic diseases, exclusion of the first two years of follow-up, and varied little across different subgroups of participants.
Conclusion: In Chinese adults, higher usual physical activity was associated with a lower risk of diabetes. These findings suggest that targeted strategies to increase levels of physical activity in China could have a major health impact.
Supported by: MRC, BHF

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Evolution of physical activity patterns and subsequent type 2 diabetes risk: results from the E3N-EPIC cohort study

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Background and aims: Physical activity (PA) is the main modifiable risk factor to prevent or postpone type 2 diabetes mellitus (T2DM). However, the association between the evolution of PA and subsequent risk of T2DM is not well known. The aim of this study was to evaluate the

association between 5-year changes in patterns of PA and T2DM incidence in the large E3N-EPIC cohort study.
Materials and methods: The study population was constituted of 60,992 women followed from 1997 to 2008. During the follow-up, 1,982 cases if T2DM were observed. Mutually exclusive patterns of PA were constructed using latent class analysis (LCA) and latent transition analysis (LTA). The associations between the 5-year evolutions (from 1993 to 1997) of PA patterns and incident T2DM were assessed by multivariate Cox models. Results were expressed as risk ratios (RR) and 95% confidence intervals.
Results: Four PA patterns were identified: «high PA level», «moderate PA level», «gardening/do it yourself» and «low PA level». Between 1993 and 1997, a total of 10,952 women (18%) increased somehow their levels of PA. After adjustment for established risk factors of T2DM and stratification on body mass index (BMI), maintaining a high level of PA was associated with a decrease of T2DM incidence when compared to women who stayed at a low level of PA (RR=0.77 (0.60-0.99) and 0.71 (0.58-0.87) for women with a BMI lower than or higher than 25 kg/m^2 respectively). Increasing the level of PA between 1993 and 1997 was associated with a borderline decreased risk in both strata of BMI (RR=0.89 (0.73-1.10) and 0.89 (0.77-1.03) for women with a BMI lower than or higher than 25 kg/m^2 respectively).
Conclusion: Using an innovative method in T2DM epidemiology, associations between evolutions of PA patterns and T2DM were quantified for the first time in such a large population. We have found that maintaining a high level of PA over time was associated with a decreased risk of T2DM, independently of BMI.
Supported by: Fondation Coeur et Artères, VITADIAB Project

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Lower mortality with physical activity in type 1 diabetes of extreme duration: Joslin 50-Year Medalist study
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Background and aims: Physical activity (PA) is often discussed as a crucial aspect of type 1 diabetes management, however, very little is understood about the effect of PA on mortality in those with the disease. This analysis examines the association in a unique group of individuals who have had type 1 diabetes for 50 or more years (the Joslin 50-Year Medalists).
Materials and methods: All Medalists (n=955, 54.1% female) provided documentation of 50 years of insulin dependence, resided in the US, and were examined at the Joslin Diabetes Center. The median HbA1c was 7.1%, age 65.0y, duration of diabetes 53.0y, age at onset 11.0y, with a median BMI of 25.6 kg/m2 and daily insulin dose of 0.4 U/kg. PA was assessed by self-report and validated by the Paffenbarger Questionnaire.
Results: Medalists who reported being physically active (77.5%) had a significantly lower HbA1c (7.1 v 7.2%, p<0.01), BMI (25.3 v 27.4 kg/m2, p<0.0001),) and triglycerides (64.0 v 73.5 mg/dL, p<0.001). Additionally, those reporting PA had higher eGFR (72.7 v 66.4 mL/min/1.73 m2, p<0.01) and HDL-C (64.0 v 57.0 mg/dL, p<0.0001) levels. The rates of cardiovascular disease (CVD) (37.1 v 48.9%) and nephropathy (10.3 v 21.0%) were significantly different in the active group compared to the inactive group (p<0.01 and p<0.0001, respectively). Proliferative diabetic retinopathy and neuropathy were not significantly different (p>0.05). Ten percent (n=99, 42.4% female) of Medalists have passed away, 61 (61%) of whom reported being physically active. Among these individuals median age (74.0 v 64.0y, p<0.0001), age at diagnosis (12.0 v 11.0y, p<0.01), duration (57.0 v 52.0y, p<0.0001), HbA1c (7.2 v 7.1%, p=0.05), and eGFR (53.5 v 73.7 mL/min/1.73 m2, p<0.0001), were significantly different than the rest of the cohort. Prevalence of neuropathy, nephropathy, and CVD were higher in the deceased group (p<0.05). The most frequent cause of death was CVD (n=42), followed by cancer (n=9), end-stage renal disease (n=5), and accidents (n=5). We investigated the influence of PA on mortality by Cox proportional hazards regression. The group crude hazard ratio (HR)=0.47, (95%CI 0.31, 0.72) for PA compared to non-PA indicates a substantial protective effect. After adjusting for potential covariates including, age, sex, HbA1c, history of CVD, nephropathy, and use of antihypertensives, PA remained independently associated with protection from all-cause mortality (HR=0.50, 95%CI 0.31, 0.78). To counter the possibility of reverse causality we duplicated analyses without subjects who passed away within 1 or 3 years of enrollment into the study and found similar results.
Conclusion: This longitudinal analysis confirms that PA is associated with increased survival probability independent of other factors in an aging population of older type 1 diabetic individuals, providing evidence of PA as an important means of reducing the burden of mortality in this population.
Supported by: NIH JDRF

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Skin autofluorescence is related to tobacco smoke and nicotine exposure
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Background and aims: Tobacco smoke has been found to be an exogenous source of reactive glycation products. Skin autofluorescence (SAF), reflecting advanced glycation endproducts (AGE) accumulation in the skin, has been shown to predict diabetes-related cardiovascular complications and is higher among smokers compared to non-smokers. In the present study, we examined the association of different smoking behaviors and nicotine exposure with SAF using both epidemiological and metabolomics data.
Materials and methods: We used a large population-based dataset from the LifeLines Cohort Study and metabolomics data from the Qatar Metabolics Study of Diabetes (QMDiab). Smoking behavior and exposure to tobacco smoke from others (secondhand smoking (SHS)) were assessed in a questionnaire in 9,009 individuals including 314 individuals (3.5%) with type 2 diabetes (T2DM) participating in the LifeLines Cohort Study. In QMDiab nicotine exposure was measured in saliva, plasma and urine in 374 individuals of whom 190 individuals (51%) with type 2 diabetes. SAF was measured non-invasively in all individuals using the AGE Reader.
Results: Urinary cotinine N-oxide, a marker of nicotine exposure, was found to be positively associated with SAF in QMDiab (p=0.03). HbA1c (p=0.010) and type 2 diabetes (p<0.0001) were most strongly associated with higher SAF levels among former and current smokers participating in the LifeLines Cohort Study. We observed a gradual increase in SAF with the number of hours being exposed to SHS. SAF levels of former smokers decreased to levels of never smokers after 15 years of smoking cessation (Figure 1).
Conclusion: We have demonstrated that tobacco smoke exposure, assessed by questionnaire as well as by cotinine N-oxide in urine, is significantly associated with SAF. Both active smoking and exposure to

secondhand smoking are associated with higher SAF levels. Smoking cessation has beneficial effects on SAF levels.

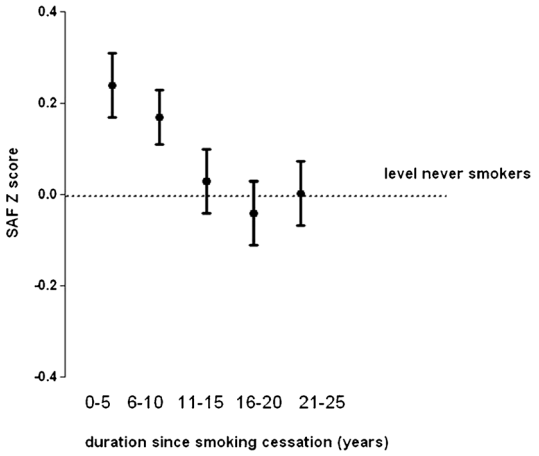

Figure 1. Effect of smoking cessation on skin autofluorescence in former smokers (LineLines data)

Dots show mean SAF Z-scores adjusted for age, GFR and diabetes. Bars reflect standard error of the mean. SAF, Skin autofluorescence; GFR, glomerular filtration rate

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Association between diabetes and exposure to pesticides: a systematic review and meta-analysis

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Background and aims: Diabetes mellitus is a worldwide epidemic, currently affecting more than 350 million people and expected to reach 550 million by 2050. It represents a major global public health challenge. Diabetes aetiology is considered to be an interplay between genetic and environmental factors. Emerging evidence suggests that environmental contaminants, including pesticides play an important role in the pathogenesis of diabetes. We performed a systematic review of observational studies that assessed the association between exposure to pesticides and diabetes.
Materials and methods: A comprehensive literature search of peer-reviewed original research pertaining to pesticide exposure and any health outcome, published until December 2014, with no language restriction, was conducted. The association between exposure to any pesticide and all types of diabetes was examined. Separate analyses for studies that recruited only Type 2 diabetes (T2D) participants were performed. Odds ratios (OR) using fixed and random-effects models were calculated. Random effects are reported in view of the presence of heterogeneity. Risk estimates were transformed in order to be comparable across studies. Heterogeneity was assessed using the I^2 (0-100%).
Results: We identified 21 studies assessing the association between pesticides and diabetes, in a total of 66,714 individuals (5,066 cases/ 61,648 controls). Most studies did not report the specific diabetes type. The summary OR for the association of the exposure to any type of pesticide and diabetes was 1.61 (95% CI: 1.33-1.95, $p=9\times10^{-7}$), with large heterogeneity ($I^2=68.2\%$). Studies evaluating T2D only (n=12 studies), showed a similar summary effect 1.64 (95% CI 1.39-1.92, $p=2\times10^{-9}$) however heterogeneity disappeared ($I^2=0\%$). Analysis by type of pesticide showed an increased risk with less heterogeneity ($I^2=0$-87%). The summary effects ranged from 1.19 (95% CI: 1.05-1.35, p=0.007, $I^2=0\%$) for the exposure to chlordane, up to 2.34 (95% CI: 1.53-3.59, $p=9\times10^{-5}$, $I^2=43.7\%$) for exposure to trans-nonanchlor. The study type and the measurement of the exposure did not affect the effect estimate. Egger's test was significant suggesting the presence of small-study effects. High heterogeneity was attributed mainly to the synthesis of observational studies.
Conclusion: This systematic review supports the hypothesis that exposure to various types of pesticides increases the risk of diabetes. Subgroup analyses did not reveal any differences in the risk estimates based on the type of studies or the measurement of the exposure. Analysing each pesticide separately reduces heterogeneity and the different effect sizes suggest that some pesticides are more likely to contribute to the development of diabetes than others.
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PS 007 Patient centered studies in diabetes

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Diabetes mellitus as a risk factor for community-acquired pneumonia in hospital admissions

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Background and aims: Respiratory infections are frequently associated with diabetes mellitus (DM). Health Authorities and Medical Societies recommend several general measures (e.g., smoking cessation and control of chronic illnesses), as well as flu and pneumococcal vaccination of people with DM. Portugal has one of the highest prevalence rates of DM within the European context. We have therefore, carried out a retrospective, nation-wide register analysis of hospitalized patients that aimed to estimate the prevalence of DM among community-acquired pneumonia (CAP) patients and evaluate the impact of DM in hospital length of stay and in-hospital mortality.
Materials and methods: Anonymized data from 157,291 CAP adult patients referring to the period 2009-2012 were extracted from the National Hospital Discharge Database of DRGs (Diagnostic-Related Groups). Patients included in the study had CAP as main diagnosis on admission within the age range 20-79 that matched the PREVADIAB - National Prevalence Study. Data for analysis were clean from double entries, day cases and hospitalizations >90 days. We also excluded individuals with HIV and with iatrogenic immunosuppression. Nation-wide DM statistics from 2009 to 2012 were collected from the PREVADIAB study.
Results: Within the 74,175 CAP episodes that matched the inclusion criteria we found a high burden of DM that tended to increase over time, from 23.7% in 2009 to 28.1% in 2012. Importantly, the DM prevalence in CAP patients was significantly higher when compared to the national DM prevalence ($P<0.0002$), particularly in women. We found that episodes of CAP in DM patients had in average 0.8 days longer hospital stay as compared to patients without DM ($P<0.0001$) totalling an estimated 15,370 days of stay attributable to DM in 19,212 CAP admissions. In-hospital mortality was also significantly higher in CAP patients that have DM (15.2%) vs without DM (13.5%) ($P=0.002$).
Conclusion: Taken together, our data show a significant increase in the prevalence of DM in subjects hospitalized with CAP reinforcing the relevance of DM as a risk factor for CAP. This longitudinal study provides indications that DM patients acquiring CAP are older, have longer hospitalization time and have higher mortality rates than CAP patients without DM. Importantly, our study also highlights that the relative impact of diabetes was greatest in younger adults (20-39 years) and in women. Thus, this nation-wide study identified people with DM as a priority group for adoption of general measures to prevent CAP, and more specifically for flu and prophylactic pneumococcal vaccination. These results have informative value for strategies of patient awareness and future healthcare guidance, particularly for people with diabetes under 40 years and for DM women.
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Influenza in patients with diabetes and obesity: vaccine effectiveness against hospitalised influenza and complications after hospitalised influenza-like illness

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Background and aims: Diabetic subjects are at risk for influenza complications, and vaccination has long been recommended in this population in France. Recent data show that the same applies to obese subjects, and recent French recommendations are to vaccinate subjects with a BMI≥ 40. Since 2012-13, we have conducted multicentre test negative case

control studies to estimate influenza vaccine effectiveness (IVE) against hospitalized laboratory confirmed influenza in Europe. A sub-analysis performed on the data from 6 hospitals in France considered the results among subjects with diabetes (DM) and obesity (Ob).

Materials and methods: During the 2012-13 and 2013-14 seasons, we swabbed all patients hospitalized for influenza related illness, aged≥18, and reporting an influenza-like illness (ILI) within 7 days before admission (cases: PCR positive for influenza; controls: negative for any influenza virus). Influenza vaccination was recorded. By logistic regression we calculated IVE by influenza subtype (adjusted for age, sex and number of children) and risk factors for complications (adjusted for age, sex, influenza infection, vaccination, DM and Ob).

Results: Among the 848 patients included, influenza was confirmed in 253. IVE was 69% (95% IC: 38-84, P<0.001) and 40% (95% IC: 01-64, P<0.05) in the 2012-13 and 2013-14 seasons, resp. DM or Ob was present in 286 subjects (DM: 197; Ob: 152; both: 63), influenza was confirmed in 85/286, and vaccine coverage was 54%, 45% and 44% in DM, Ob and other subjects, resp. (P=0.05). Subjects with DM were older (DM: 71±17; Ob: 61±18; both: 69±12; other: 65±20, P=0.001). Overall adjusted IVE in subjects with DM or Ob was 55% (95%CI: 19-75, P< 0.01). Table 1 shows IVE according to virus subtype, metabolic disease and season. Complications during hospitalizations for ILI were respiratory failure (N=160), heart failure (N=100), renal failure (N=79) and death (N=27). In multivariate analysis, renal failure was associated with DM (OR 2.57, 95%CI: 1.55-2.48, P <0.001) and Ob (OR 1.73, 95%CI: 0.99-3.03, P=0.05). Heart failure was associated with age (65-74: OR 2.57, 95%CI: 1.28-5.16, P=0.008); ≥75: OR 4.15, 95%CI: 2.26-7.64, P= 0.0001), Ob (OR 1.76, 95%CI: 1.04-3.00, P=0.03) and DM (OR 1.55, 95%CI: 0.99-2.51, P=0.07). Respiratory failure, death and length of inpatient stay were not associated with DM or Ob.

Conclusion: In patients with DM or Ob, IVE is similar to that reported in general population. However vaccine coverage is insufficient, especially in Ob. DM and Ob increase the risk of cardiac and renal complications in subjects hospitalized with ILI.

	Obesity (N = 152)				Diabetes (N = 197)				Obesity or diabetes N=286)			
	cases/total		Adjusted IVE	P	cases/total		Adjusted IVE	P	cases/total		Adjusted IVE	P
	V	nV	(IC 95 %)		V	nV	(IC 95 %)		V	nV	(IC 95 %)	
Overall	10/68	30/84	63 (8; 85)	0.03	24/106	32/91	47 (-4; 73)	0.06	29/140	56/146	55 (19; 75)	< 0.01
Season 2012-2013	5/36	21/45	82 (31; 95)	0.01	15/55	18/45	51 (-26; 81)	0.13	18/74	36/77	64 (19; 84)	0.01
Season 2013-2014	5/32	9/39	21 (-200; 79)	0.72	9/51	14/46	49 (-40; 81)	0.19	11/66	20/69	46 (-32; 77)	0.17
Against AH3N2*	5/63	7/61	29 (-198; 83)	0.64	14/96	11/70	21 (-93; 68)	0.59	15/126	16/106	29 (-63; 69)	0.41
Against AH1N1*	3/61	12/66	52 (-113; 89)	0.33	1/83	10/69	93 (39; 99)	0.01	4/115	22/112	81 (38; 94)	< 0.01
Against B *	2/60	10/64	88 (35; 98)	0.01	8/90	8/67	22 (-136; 74)	0.65	9/120	15/105	55 (-18; 83)	0.10

Table 1: IVE according to metabolic disease, season and virus type. Cases: PCR positive for influenza; V: vaccinated; nV: non vaccinated; *: subjects with another influenza virus type were excluded from control group.

Clinical Trial Registration Number: NCT01764152 & NCT02027233
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Direct medical costs of severe hypoglycaemic events amongst type 2 diabetes patients in the UK: a retrospective database study

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Background and aims: Hypoglycaemia is a common complication in type 2 diabetes mellitus (T2DM) patients. Treatments raising insulin levels independently of blood glucose, such as oral insulin secretagogues (sulfonylureas and glinides) and exogenous insulin, can increase the risk of hypoglycaemia. This study aims to provide an up to date estimate of the direct medical costs of severe hypoglycemic events amongst T2DM patients to the UK healthcare system.

Materials and methods: A retrospective cohort design was undertaken with linked UK Clinical Practice Research Datalink (CPRD) and Hospital Episodes Statistics (HES) databases, selecting patients initiated on any mono, dual, or triple combination therapies for treatment of T2DM between 2008 and 2012, and observing severe hypoglycemic events and costs estimated up to end of regimen +90 days, or end of recorded data (minimum of 1 day, maximum 6 years). All patient characteristics were described at treatment initiation for mono, dual, or triple combination therapies. Severe events were defined as events requiring hospital treatment with hypoglycaemia-specific diagnosis or procedure code. Costs were estimated using Healthcare Resource Groups (HRGs version 4) and tariffs derived from the National Tariff 2013-14, and primary care follow-up appointment costs using Personal Social Services Research Unit (PSSRU) 2014, and drug prescription costs using Prescription Cost Analysis for England 2008-13. Results from the linked sample were extrapolated using the full HES census dataset to provide national cost estimates.

Results: There were 857 severe events observed, with a mean observation period of 510; 522; 503 days (mono, dual, triple therapy respectively, regardless of regimen), among 60,335; 31,610; 10,298 patients identified in the linked sample. 56; 58; 61% of admitted patients were male, with a mean age of 63.8; 62.4; 61.2 years in three groups. Mean diabetes duration at admission was 3.0; 5.6; 7.3 years in the three groups, with a mean HbA1c of 7.7%; 8.3%; 8.8%. The event rate was 5.3; 7.0; 6.6 per 1000 patient-years in the three groups with a mean per-event secondary care cost of £3,558; £3,477; £2,989, and primary care follow-up cost of £56 per event in each group. Extrapolating to national data (17,622 episodes observed in full 2013-14 HES data), estimated national cost to the NHS per year was £61,193,413.

Conclusion: Severe hypoglycemic events impose a substantial burden upon the NHS. Further research is required to quantify the extent to which events could be prevented, and the associated potential cost savings to NHS budgets.

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Towards a better understanding of acute post-prandial hyperglycaemic episodes: a patient perspective

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Background and aims: Prolonged or severe episodes of hyperglycaemia have been associated with the development of micro- and macrovascular long term complications such as neuropathy and atherosclerosis. Previous studies have demonstrated comparable effects of acute hyperglycaemia on cognitive function and mood to hypoglycaemia. This study aimed to explore the impacts of acute post-prandial hyperglycaemia (PPHG) on health-related quality of life in people with insulin-dependent diabetes mellitus.

Materials and methods: Twenty-four adults (≥18 yrs) with Type 1 (n= 14) and Type 2 (n=10) insulin-dependent diabetes took part in a qualitative study. Data were collected via one-to-one telephone interviews in the US (n=10) and two focus groups in the UK (n=14). The study was reviewed and approved by an independent ethics board and participants provided written informed consent. Interviews and focus groups were audio-recorded and transcribed verbatim. Anonymised transcripts were analysed using a thematic analysis approach, supported by qualitative software (MAXQDA).

Results: A number of common themes were identified associated with participants' awareness and experience of acute PPHG (such as variability, frequency and duration). Some participants felt that the food eaten and activities undertaken had an effect on the speed with which glucose rose and the time taken for glucose levels to return within normal range.

Increased thirst and frequency/urgency of urination, tiredness, feeling lethargic and suddenly hot and sweaty were consistent symptoms when discussing PPHG. Other symptoms included dizzy spells and "fogginess", impaired concentration and difficulty thinking quickly. Some participants worried about these symptoms and found them "unnerving". Participants' perceived physical and cognitive impacts of acute PPHG could affect their work and perceived ability to drive. Experiences of acute PPHG episodes also prompted changes in behaviour including avoiding social events, leaving early or avoiding them altogether, arranging with employers to take breaks in the working day and constant worry that resulted in restrictive diets. Anxieties about PPHG episodes were most frequently related to concerns that repeated episodes could have on long term health. Some participants expressed particular fears that severe and long lasting episodes (compared to short lived "routine" periods of PPHG) would lead to diabetic complications.

Conclusion: This study advances our understanding of the every-day impact of acute PPHG on individuals with diabetes, which to the authors' knowledge has not been previously explored using qualitative methods. Analysis of the interview transcripts provided rich insight into participants' experiences of PPHG. The data will be used to guide development of a self-reported outcome measure of the impact of PPHG, to allow a more systematic and quantitative study involving a larger sample.

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Hospital incidental diagnosis of diabetes: a population study

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Background and aims: Uncertainty remains on how the diagnosis is being made of new, not previously identified, incident diabetes. Newly diagnosed diabetes, has been, indeed, mainly identified after admission to intensive care units or after hospitalizations due to acute cardiovascular events and seems to bring about, in such conditions, an excess risk of fatal events. Purpose of this study was to present a new method apt to identify incidentally diagnosed diabetes (IDD) among a large population of hospital discharged patients, determining, moreover, their risk of mortality, as compared with that of subjects with known diabetes (KD).

Materials and methods: Among 214,991 individuals discharged in year 2011 from both medical and surgical wards of all hospitals of Tuscany, Italy we identified IDD when all these conditions were satisfied: a.: absence in the regional diabetes registry, b.: no previous hospital discharge with main or secondary diagnosis of diabetes (ICD-9-CM 250.xx), c.: no past prescriptions of glucose lowering drugs (GLD) (ATC class A10A or A10B), d.: no evidence of legal certifications including those enabling patients to free prescriptions of consumables, clinical chemistry tests, specialists' consultations related to the diabetic condition. Diagnosis of incidental diabetes also required e.: record for at least two GLD prescriptions, with the first prescription occurring within 30 days after the index discharge and the second over the next six months. In addition, two-year (2012-2013) adjusted mortality risk, expressed as hazard ratio (HR), was tested through a Cox regression analysis, comparing IDD subjects and KD patients having had at least one hospital admission in 2011 and alive at 1st January 2012 (n=865 and 31,715 respectively).

Results: IDD was found in 974 patients in one year, corresponding to 375.6 per 100,000 hospitalized people. The great majority of IDD patients were discharged by medical wards (88%). The adjusted relative risk [RR(95%CI)] of IDD, as evaluated in hospitalized population, exponentially increased with age and was three-fold higher in migrants of non-Italian ancestry; RR: 3.18 (1.87-5.42). Furthermore IDD risk was associated with male gender; RR: 1.24 (1.09-1.41) and with greater burden of existing co-morbidities, as evaluated by Charlson Co-morbidity index in previous hospitalizations; [(RR:63.51 (49.78-81.03) in those with Charlson Co-morbidity index≥2], while was reduced among patients of general practitioners adhering to shared guidelines resulting in a proactive model of care delivery RR: 0.75(0.63-0.91). In the cohort of IDD patients alive at 1st January 2012, (n=865) the hazard ratio(HR) of two-year-mortality, adjusted for main confounders, was not different from that of matched KD subjects (HR=1.08; 95%CI: 0.92-1.26; p=NS).

Conclusion: IDD is relatively common among hospitalized individuals and, in our population, occurs more commonly in older male subjects, migrants of non-Italian ancestry, and in patients of physicians non-adhering to a shared care model of diabetes care. Finally, people with IDD have similar mortality risk compared with matched KD individuals.

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Normoalbuminuric male but not female patients with type 2 diabetes have a normal life expectancy after 14 years of follow-up (ZODIAC-50)

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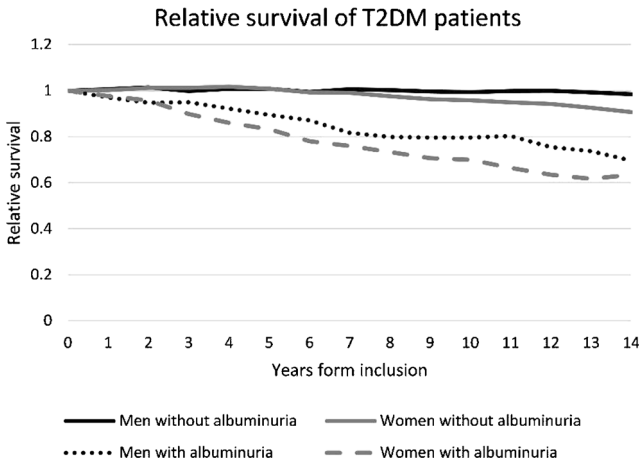
Background and aims: When compared to the general population, the relative risk for cardiovascular mortality in patients with type 2 diabetes (T2DM) has been described to be substantially higher in women compared to men. To what extend gender influences mortality risk in the Netherlands is unclear as it is known from previous studies that overall mortality in patients with diabetes in the Netherlands is low compared to many other countries. The aim of the present study was to investigate gender differences in life expectancy in primary care treated patients with type 2 diabetes in the Netherlands.

Materials and methods: Patients with T2DM participating in a prospective observational cohort study (ZODIAC) in the Netherlands were included. Inclusion in this cohort took place in 1998, 1999 and 2001. Vital status was assessed in 2013 and life expectancy was calculated using relative survival analysis. Using the Hakulinen method, the relative survival was expressed as the ratio of observed survival of patients observed divided by the survival of the general population in the Netherlands with the same age (data available from Statistics Netherlands). Analyses were performed for men and women separately and for subgroups of patients with and without albuminuria.

Results: A total of 1791 patients was included of which 56% was female. Mean age was 67 (SD 12) years and the median diabetes duration was 4 (ICR: 2-9) years. Microalbuminuria was present in 29% and macroalbuminuria in 9% of patients at baseline. After 14 years 49% (874) patients died, of which 43% from cardiovascular causes. The relative survival was 0.87 (95%CI: 0.80 - 0.93) for men and 0.81 (95%CI: 0.75 - 0.87) for women with T2DM. In the subgroup of patients without microalbuminuria the relative survival was 0.99 (95%CI: 0.90 - 1.05) for men and 0.91 (95%CI: 0.84 - 0.98) for women. In the subgroup of patients with albuminuria the relative survival was 0.70 (95%CI: 0.58 - 0.81) for men and 0.63 (95%CI: 0.54 - 0.74) for women (figure 1).

Conclusion: In primary care treated T2DM patients, the life expectancy of men and women was 13% and 19% lower after 14 years of follow-up compared to men and women in the general population, respectively. In the subgroup of patients without albuminuria, male patients had the same life expectancy compared to the general population, while the life expectancy in women still was lower. These results confirm that overall, patients with T2DM, have a lower life expectancy compared to the general population, but that the absence of albuminuria increases this expectancy considerably, even to normal in men.



PS 008 Diet and hyperglycaemia

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Fruit and vegetable consumption and risk of prediabetes and type 2 diabetes in a Swedish prospective study

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Background and aims: Fruit and vegetable intake has been proposed for the prevention of type 2 diabetes (T2D) but the evidence from observational studies is inconclusive or even scarce when it comes to other glucose tolerance abnormalities, like prediabetes. Therefore, we investigated the association between fruit, vegetable or combined fruit and vegetable intake and the risk of deterioration in glucose tolerance (to prediabetes or T2D), in a prospective population-based cohort study.
Materials and methods: Subjects were 2296 men and 3180 women aged 35-56 years old at baseline, without previously diagnosed diabetes, who participated in the Stockholm Diabetes Prevention Program and were followed for 8-10 years. For all participants, anthropometric and clinical measurements, an OGTT for the assessment of prediabetes (impaired fasting glucose and/or impaired glucose tolerance) or type 2 diabetes, an extensive questionnaire on lifestyle factors and a food frequency questionnaire were carried out at both baseline and follow-up. Men and women differed in fruit and vegetable consumption, so we used sex-specific tertiles of intake for our analyses.
Results: Median intake of fruit and vegetables at baseline was higher among women compared to men: 185 vs 103 g/day for fruit ($p<0.001$) and 163 vs 117 g/day for vegetables ($p<0.001$). Higher intake of fruit (>148 compared to <64 g/day) was associated with a 27% lower risk of deteriorating glucose tolerance in men, after adjusting for age, family history of diabetes, BMI, physical activity, smoking, education and blood pressure (OR: 0.73, 95% CI: 0.55-0.98); more specifically the risk of developing prediabetes from normal glucose tolerance (NGT) decreased by 26% (OR: 0.74, 95% CI: 0.52-1.05). Vegetable consumption in men was inversely but not significantly associated with the risk of prediabetes or T2D. In women, vegetable consumption appeared protective, and especially the middle tertile of total vegetable intake was significantly associated with a lower risk of developing T2D from NGT (OR: 0.42, 95% CI: 0.20-0.90). Intake of a specific vegetable subgroup (green leafy vegetables) reduced also the risk of developing T2D in women. The multivariate adjusted OR for the highest (>42 g/day) compared to the lowest (<12 g/day) intake of green leafy vegetables was 0.33 (95% CI: 0.16-0.68). The consumption of fruit and vegetables combined showed no significant associations.
Conclusion: Higher consumption of fruit was associated with a reduced risk of deteriorating glucose tolerance in men, especially for the progression from NGT to prediabetes, whereas in women vegetable consumption and specifically higher intakes of green leafy vegetables, reduced the risk of developing T2D from NGT. Overall, the findings from this Swedish study population add to the evidence of a beneficial effect of fruit and vegetables on T2D risk, and provide new insight for the prevention of prediabetes.
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Exposure to persistent organic pollutants in early pregnancy and risk of gestational diabetes mellitus

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Background and aims: Persistent Organic Pollutants (POPs) are a group of diverse substances, including polychlorinated biphenyls (PCBs) and organochlorine pesticides that are resistant to biodegradation and ubiquitously present in our environment. Exposure to endocrine disrupting chemicals such as POPs has been linked to type 2 diabetes and metabolic disturbances in epidemiological and animal studies, but little is known about POPs exposure during pregnancy and the development of gestational diabetes mellitus (GDM). The purpose of this study was to determine the extent to which, exposure to current low levels of different POPs in the first trimester of pregnancy is associated with GDM risk in 639 women from the RHEA pregnancy cohort in Crete, Greece.
Materials and methods: Concentrations of several PCBs, dichlorodiphenyldichloroethene (DDE), and hexachlorobenzene (HCB) were determined in first trimester maternal serum by triple quadrupole mass spectrometry. POPs were treated as continuous variables on a log10 scale. We defined total PCBs as the sum of all congeners, non dioxin-like PCBs as the sum of PCB 153, 138, 170 and 180, and dioxin-like PCBs as the sum of PCB 118 and 156. Pregnant women were screened for gestational diabetes mellitus (GDM) between 24 and 28 weeks of gestation, and GDM was defined by the criteria proposed by Carpenter and Coustan. Adjusted odds ratios (ORs) and 95% confidence intervals (CIs) were estimated using multivariable logistic regression models.
Results: Geometric mean HCB, DDE and PCBs serum concentrations in pregnant women were 88, 2043 and 295 pg/ml respectively. 68 (7%) women had GDM. On multivariable regression analyses, a 10-fold increase in total PCBs was associated with increased risk of GDM (OR= 4.44; 95% CI: 1.17, 16.79) after adjusting for pre-pregnancy BMI and several other confounders. The association was more pronounced for non dioxin-like PCBs (OR=4.42; 95% CI: 1.19, 16.43). Prenatal DDE and HCB exposure were not significantly associated GDM risk.
Conclusion: These findings suggest that women with high PCBs levels in early pregnancy had higher risk for GDM. Further studies are needed to replicate these results and to evaluate potential biological mechanisms underlying the observed associations.
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Comparison of glycaemic outcome and relation to changes in liver fat content in low-carb vs low-fat dietary regimes for prediabetic subjects

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Background and aims: Very low carb diets (LCD) were reported to rapidly reduce liver fat and to improve glucose metabolism more effectively than low fat diets (LFD) in metabolic syndrome subjects. However, this observation is confounded by small sample sizes and could not be replicated by others. The aim of this study was to investigate the effect of a sequential short-term very low carb hypocaloric vs. low fat hypocaloric diet followed by an isocaloric moderately low carb or low fat dietary intervention on glycemic parameters, intrahepatic lipids (IHL) and risk factors for cardiovascular disease in prediabetic subjects.
Materials and methods: In a subcohort of the Prediabetes Lifestyle Intervention Study (PLIS; n=99) we conducted a two-step dietary intervention with two different regimes. The initial 3 wk-phase was hypocaloric (ca. 1500 kcal/day), the second phase (11 months) represented the maintenance period. We compared a 3 week very low-carbohydrate diet followed by moderate carb diet (short term: < 40 g carbohydrates/day, long term: < 40% of daily energy intake) and a low-fat diet (fat intake<30% of daily energy intake). Metabolic assessment took place after 3 weeks, 6 and 12 months and was based on 2-hour oral glucose tolerance tests and magnetic resonance spectroscopy (for IHL).
Results: While both interventions showed similar significant short-term reduction and long-term maintenance of reduced glucose levels and liver fat content (LCD vs LFD; relative change in IHL after 6 months: -42,2± 29,2% vs -24,6±65,8%, p=0,382), short-term reductions in mean (± SD) fasting glucose levels were significantly greater in the LCD-group (-11,1 ±12,1 mg/dl vs -3,7±10,9 mg/dl, p=0,002). After 3 weeks, reduction of blood pressure was greater in LCD (systolic: -12±13 mmHg vs -4± 15 mmHg, p=0,002; diastolic: -7±8 mmHg vs -4±9 mmHg, p=0,019), whereas in triglycerides the LCD-improvement reached only a trend effect (p=0,073). In the LFD-group, reductions in C-reactive protein (CRP: 0,1±3,6 mg/dl vs -0,7±2,2 mg/dl, p=0,017) and high density lipoprotein (HDL: -0,8±10,6 mg/dl vs -6,2±6,2 mg/dl, p=0,001) could be observed, but did not sustain in the maintenance period. The same long-term attenuation applied to blood pressure and triglycerides. No short-term diet-specific effect on low density lipoprotein (LDL) was observed (p= 0,263) after 3 weeks, whereas a significant reducing LCD-effect was assessed after 6 months (-19,1±20,8 mg/dl vs -9,9±18,3 mg/dl, p= 0,003). Correlation analysis showed, that despite similar magnitude of metabolic changes, liver fat reduction under low-fat diet is tightly linked to weight loss (p=0,003), but not in the low-carb group (p=0,170).
Conclusion: Low-carb and low-fat diets are similarly effective to reduce glycemic parameters and liver fat content, whereas the improvement in other cardiovascular risk parameters was more pronounced under the low-carbohydrate diet. The underlying mechanisms appear to differ regarding dietary composition suggesting an individualization of dietary strategies.
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Association of dairy foods with impaired glucose metabolism and diabetes mellitus in The Maastricht Study

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Background and aims: Observational studies suggest an inverse association between total dairy intake and diabetes risk. However, there is a lack of information on the relation of specific dairy products with impaired glucose metabolism (IGM) and type 2 diabetes (T2DM).
Materials and methods: Individuals aged 40-75 y were recruited for The Maastricht Study. All participants filled out a 254-food item food frequency questionnaire (FFQ), covering 41 specific dairy items that captured differences between full fat, semi-skimmed, and skimmed products, as well as fermented and non-fermented products. Glucose metabolism status was assessed by an oral glucose tolerance test and participants were informed on their glucose metabolism status after returning the FFQ. 2,508 Individuals were available to estimate odds ratios (ORs (95%CI)) for IGM (n=501) and newly diagnosed (ND) T2DM (n=129), with

adjustment for BMI, physical activity, smoking, education, energy intake, and intake of vegetables, fruits, meat, and fish.
Results: For IGM, fully adjusted analyses revealed inverse associations, with ORs (95% CI) comparing the highest with the lowest tertile of intake of 0.73 (0.55 - 0.96) for skimmed products and 0.74 (0.54 - 0.99) for fermented products. These dairy products were not associated with ND T2DM. In contrast, full fat products were positively associated with ND T2DM (OR (95%CI): 2.01 (1.16-3.47)), whereas total dairy intake was inversely associated with ND T2DM (OR (95%CI): 0.46 (0.24-0.86)).
Conclusion: Individuals with a high consumption of skimmed and fermented products had a lower odds of having IGM, and individuals with a high consumption of total dairy products had a lower odds of having ND T2DM. High intake of full fat products was not related to IGM, but increased the odds of ND T2DM.
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Adherence to Mediterranean diet reduces ten-year diabetes risk. The role of TNF-a and homocystein as potential mediators
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Background and aims: Beyond its cardioprotective effects, Mediterranean diet has been reported to offer an anti-diabetic protection. The aim of this study was to investigate the effect of long term adherence to Mediterranean diet on ten-year diabetes incidence, and examine inflammatory and oxidative stress biomarkers as candidate mediators of this relationship.
Materials and methods: At baseline (2001-2), a random sample of 1514 men and 1528 women (>18 years) without any clinical evidence of cardiovascular disease, were enrolled in the study. Several socio-demographic, clinical, biochemical and other variables were studied in relation to diabetes development (i.e., fasting blood glucose >125 mg/dL or the use of antidiabetic medication, WHO, ICD-10 criteria). Adherence to Mediterranean diet was evaluated using MedDietScore (range 0-55) and score tertiles (low, moderate and high adherence to the diet) were calculated. In 2011-2012 the ten-year follow up was performed.
Results: 191 new diabetes cases were recorded. The ten-year incidence of diabetes was calculated 13.4% and 12.4% among men and women respectively. Moderate and high adherence to Mediterranean diet were found to reduce the risk of diabetes by 49% (95%CI: 0.30, 0.88) and 62% (95% CI: 0.16, 0.88) respectively, compared with low adherence, taking into consideration age, sex, years of education, family history of diabetes, hypercholesterolemia, hypertention, smoking habits, physical activity and central obesity (waist circumference≥94/80 for men and women respectively). Trend analysis revealed a logarithmic relationship (p=0.042). Men with waist circumference >94 cm and women >80 cm were found to benefit the most. Whole grains, fruits and legumes were found to exert the greatest predictive ability. When various biomarkers were entered in the fully adjusted model, the antidiabetic effect of Mediterranean diet was found to be mediated by TNF-a and homocysteine levels.
Conclusion: The present work demonstrates the beneficial role of Mediterranean dietary pattern against type 2 diabetes development, which extends Mediterranean diet's therapeutic potential to other cardiometabolic disorders. The possible participation of TNF-a and homocystein in this pathway is proposed. The components of Mediterranean which are responsible for this protection require more investigation.
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A high diet quality based on dietary recommendations does not reduce the incidence of type 2 diabetes in the Malmo Diet and Cancer cohort
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Background and aims: A high diet quality index based on the Nordic nutrition recommendations and Swedish dietary guidelines has previously been associated with reduced risk of cardiovascular disease and mortality in the Malmö Diet and Cancer cohort. The aim was to investigate if this diet quality index was associated with risk of type 2 diabetes.
Materials and methods: Out of 26,172 participants (44 to 74 years) from the Malmö Diet and Cancer cohort study, 2,829 type 2 diabetes incident cases were identified from registers during 18 years of follow-up. A modified diet history method was used to estimate dietary intakes. A diet quality index was constructed based on adherence to the recommended intakes of saturated fat, polyunsaturated fat, fish and shellfish, dietary fiber, fruit and vegetables, and sucrose.
Results: After adjustments for potential confounders, we observe no significant association between diet quality index and type 2 diabetes risk. Hazard ratio for highest vs. lowest index category were among men 1.05 (95% CI: 0.88, 1.25; P-trend=0.94) and among women 1.03 (95% CI: 0.87, 1.22; P-trend=0.34). Exclusion of individuals reporting a substantial diet change in the past and energy mis-reporters did only marginally affect the results.
Conclusion: This study found no significant association between a high diet quality index based on the current dietary recommendations and reduced incidence of type 2 diabetes. Further investigation is needed to better understand how overall diet quality and individual dietary components are associated with the development of type 2 diabetes.
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Paternal chronic high-fat diet consumption reprogrammes the gametic epigenome and induces transgenerational inheritance of metabolic disorder
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Background and aims: Genetic and non-genetic (environmental) factors are strongly implicated in obesity. Increasing evidence indicate that paternal obesity is associated with epigenetic inheritance of metabolic disorders in the next generations. However, a direct evidence of the molecular carriers remains elusive. We hypothesized that paternal chronic high-fat diet (HFD) consumption transgenerationally alters the gametic epigenome, affecting the metabolism of the offspring.
Materials and methods: F0-male rats fed either HFD or chow diet for 12 weeks were mated with chow-fed dams to generate F1 and F2 offspring. Motile spermatozoa were isolated from F0 and F1 breeders to determine DNA methylation and small non-coding RNA (sncRNA) expression pattern by deep sequencing.
Results: Newborn offspring of HFD-fed founders had reduced body weight and decreased beta-cell mass when compared to offspring from chow-fed breeders. F1 and F2 adult females from F0 founders fed a HFD

were glucose intolerant and resistant to HFD-induced weight gain. No major phenotype was observed in male offspring, suggesting sex-specific responses. HFD-fed F0 and their F1 male offspring presented similar changes in the epigenome of spermatozoa, showing altered DNA methylation and sncRNA expression profile when compared to respective controls. Altered expression of the miRNA let-7c in sperm of F0 and F1 breeders was also observed in the white adipose tissue of female offspring, leading to a transcriptomic shift in let-7c putative target genes.
Conclusion: Our results indicate that chronic HFD consumption transgenerationally reprograms the gametic epigenome, which may be the molecular mechanism involved in the transmission of environmentally-induced metabolic dysfunction to the next generations.
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The effect of haptoglobin polymorphism and PPAR-γ agonists on carotid artery intima-media thickness in type 2 diabetic patients: seven years follow-up

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Background and aims: Patients with Type 2 diabetes mellitus are predisposed to accelerated atherosclerosis. Subclinical atherosclerosis can be measured by carotid artery intima-media thickness (cIMT), which predict cardiovascular and all-cause mortality independently. Lately the role of non traditional risk factors such as haptoglobin polymorphism, elevated homocysteine levels, inflammatory factors, etc. have emerged in the pathogenesis of diabetic vascular complications. Haptoglobin (Hp) plays an important role in iron metabolism and additionally modulates atherosclerosis given its antioxidant effect. Hp (1-1) provides a stronger antioxidant effect as compared to Hp (1-2) and Hp (2-2) polymorphisms. A strong association between haptoglobin polymorphism and coronary sclerosis, as well as carotid artery intima-media thickness (cIMT) has been reported previously. Peroxisome proliferator-activated receptor-γ (PPAR-γ) agonists (rosiglitazone and pioglitazone) are oral antidiabetic agents with insulin sensitizer properties. These bind to nuclear PPAR-γ receptor and have pleiotropic effects, some of which are independent of their glucose lowering action. There are no data available in the literature regarding the long term effect of these agents on cIMT and their association with haptoglobin polymorphism. Our aim was to investigate the effect of haptoglobin polymorphism on cIMT in Type 2 diabetic patients during a 7 years follow-up with respect to the applied treatment (antidiabetic agents, aspirin) as well as metabolic and conventional cardiovascular risk factors.
Materials and methods: We recruited 114 patients in 2005 and 2006, and Hp polymorphism, CRP level, HbA1c and lipid parameters were determined. Seven years later control examinations were performed and we tried to find any association between Hp polymorphism and changes in cIMT. During the follow-up period 29 patients were treated with rosiglitazone for 46±23 months on average. Following rosiglitazone restriction, treatment of 11 patients was continued with pioglitazone for 12 ±5 months.
Results: Our results showed a decrease in cIMT of patients treated with PPAR-γ agonists compared to those having been on other medications. In the latter group, an increase in cIMT was noticed and the difference between the two groups was significant ($p=0.003$). Regarding PPAR-γ treatment and Hp polymorphism significant improvement of cIMT in Hp(1-1 1-2) patients ($p=0.016$), and moderate, non-significant increase of cIMT in Hp(2-2) group was found, respectively. There was no significant difference in lipid parameters and HbA1c between the groups, nor aspirin treatment and smoking influenced significantly the changes in cIMT.
Conclusion: In patients with Hp(1-1 1-2) polymorphism PPAR-γ agonists significantly decreased the subclinical atherosclerosis in Type2 diabetics during the seven years follow-up.
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Effect of exercise on Pharmacokinetics (PK) of Basal Insulin peglispro (BIL) and insulin Glargine (GL) in type 1 diabetes mellitus patients

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Background and aims: BIL is a novel basal insulin that has a prolonged duration of action related to a delay in insulin absorption and a reduction in clearance. As exercise may potentially affect insulin absorption, this study investigated the effect of exercise on PK of BIL and GL.
Materials and methods: Forty patients with T1DM received 0.5-U/kg subcutaneous doses of either BIL or GL once daily in the thigh for 20 days. In each treatment arm, patients were randomised to 1 of 2 sequences, providing an intra-subject comparison: a 45-minute exercise challenge at 50%-70% VO_2 max (17 hours postdose; ~6 hours after a meal) on Day 17 and no exercise on Day 20, or vice versa. On both days, PK samples were collected for 24 hours after insulin dosing. Frequent blood sampling for PK and blood glucose (BG) was conducted from 17 to 20 hours postdose (0-3 hours after start of the exercise).
Results: Mean BIL concentrations increased with exercise, reaching a peak at 1 hour and returning to pre-exercise levels within 3 hours after starting exercise. BIL AUC during a dosing interval at steady state ($AUC_{0\text{-}24,ss}$) was increased by 13% with exercise compared to no exercise. BIL AUC for 3 hours after the start of exercise ($AUC_{0\text{-}3}$) and maximum observed concentration at steady state ($C_{max,ss}$) were increased by 63% and 64%, respectively, with exercise compared to no exercise. These differences were statistically significant. In contrast, for GL, these PK parameters were not affected by exercise. Mean baseline (pre-exercise) BG levels were 138 mg/dL (7.7 mmol/L) with exercise vs 122 mg/dL (6.8 mmol/L) without exercise in the BIL treatment arm, and 145 mg/dL (8.0 mmol/L) with exercise vs 148 mg/dL (8.2 mmol/L) without exercise in the GL arm. Over the 3-hour period after exercise start, mean (±SD) BG levels were similar with or without exercise following BIL or GL dosing (BIL: 86.0±26.5 mg/dL [4.8±1.5 mmol/L] with exercise vs 90.7± 30.2 mg/dL [5.0±1.7 mmol/L] without exercise; GL: 113±31.8 mg/dL [6.3±1.8 mmol/L] with exercise vs 125±48.9 mg/dL [6.9±2.7 mmol/L] without exercise).
Conclusion: While exercise transiently increased systemic exposure following BIL but not GL dosing, the mean glucose concentration appeared comparable. With the reduced receptor binding affinity and attenuated peripheral glucose uptake of the hepato-preferential drug BIL, further research is warranted to delineate the hypoglycaemia risk with exercise.
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Improvement in hepatic metabolism is associated with reduced conversion to diabetes in IGT subjects treated with pioglitazone (ACT NOW study)

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Background and aims: Increased liver enzymes are markers of fatty liver disease (FLD) and of progression to type 2 diabetes (T2DM). Pioglitazone has been shown not only to delay onset of T2DM, but also to ameliorate fatty liver and improve hepatic function. We recently have shown that subjects with FLD have increased plasma amino acid concentrations and that the ratio of glutamate/(serine+glycine) (GSG index) was associated with degree of hepatic fibrosis measured by liver biopsy. The aim of this study was to evaluate the changes observed in parameters of hepatic function after 2.4 year of pioglitazone treatment in subjects with impaired glucose tolerance (IGT) and the relationship with onset of T2DM.
Materials and methods: 441 IGT subjects who participated in the ACT NOW Study and had complete end-of-study metabolic measurements were randomized to receive pioglitazone (45 mg/day) or placebo and were observed for a median of 2.4 years. Indices of insulin sensitivity (Matsuda index [MI]), lipid profile (triglycerides, HDL, LDL), liver enzymes, plasma amino acid concentrations, adipose tissue IR (FFA x Ins), hepatic IR index (Glu 0-30 min x Ins 0-30 min), and index of hepatic damages, ie GSG index and AST/ALT score were measured.
Results: Pioglitazone reduced IGT conversion to diabetes by 72% ($P<0.0001$) and decreased AST (from 26 to 22 U/l), ALT (from 30 to 24 U/l) and TG (from 123 to 106 mg/dl) concentrations compared to placebo ($p<0.001$). Pioglitazone, compared to placebo, markedly decreased hepatic IR (-2637 vs -259 mMxpM), AST/ALT (+0.08 vs -0.08) and the GSG index (-0.6 vs +0.9), all $p<0.0001$ documenting improved hepatic metabolism. Changes in GSG index correlated with changes in hepatic IR ($r=0.26$), adipose tissue IR ($r=0.29$) and matsuda index ($r=-0.28$, all $p<0.0001$). In the pioglitazone group, after 2.4 year, fewer subjects converted to T2DM (n=15 vs 45). New T2DM subjects showed increased or no change (rather than decrease) in GSG index, hepatic IR, AST, ALT and TG concentrations.
Conclusion: Reduced conversion of IGT to T2DM after pioglitazone treatment is strongly related to the improvement in hepatic function.
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Effectiveness of shared decision making using a support decision tool to achieve treatment targets in type 2 diabetes mellitus patients. A randomised trial (OPTIMAL)

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Background and aims: No more than 15% of type 2 diabetes mellitus (T2DM) patients achieve all treatment goals regarding glycaemic control, lipids and blood pressure. Shared decision making (SDM) may increase that percentage; however, not all support decision tools are appropriate. Because the ADDITION-Europe study demonstrated two (almost) equally effective treatments but with slightly different intensities, it may be a good starting point to discuss with the patients their diabetes treatment, taking into account both the intensity of treatment, clinical factors and patients' preferences. We aimed to evaluate whether such an approach increased the proportion of patients that achieve all three treatment goals.
Materials and methods: Cluster-randomised trial in 35 general practices that participated from 2002-2009 in the ADDITION study. Both ADDITION and non-ADDITION T2DM patients, 60-80 years, known with T2DM for 8-12 years, were included. GPs from the intervention group were trained in SDM with the decision support tool. During the first visit the intensity of treatment, personalised targets and treatment priorities were established. Participants underwent 3-monthly check-ups as usual and a yearly rearrangement of priorities and discussion of treatment targets with the repeated use of the decision tool. The control group continued treatment as before. Follow-up: 24 months. Primary outcome: the proportion of patients who achieve all three treatment goals (intensive treatment: HbA1c 45-53 mmol/mol (6.5-7%), BP <135/85 mmHg, total cholesterol<3.5 mmol/mol; less intensive treatment: Hba1c <53 mmol/mol, BP<140 mmHg, total cholesterol <4.5 mmol/mol). Intention-to-treat analyses (ITT). To analyse the proportion of achieved treatment goals for blood pressure, lipids and HbA1 relative risks will be calculated, with the control group as reference. $P<0.05$ is considered statistically significant.
Results: In total 157 participants. In the intervention group 25 patients decided to continue less intensive treatment and 10 patients continued

intensive treatment. 34 patients (61.1%) switched from less intensive to intensive treatment and 3 the other way round. After one year, mean values of HbA1c, BP and total cholesterol had not changed in both groups. Compared to the control group, the proportion of patients achieving treatment targets in the intervention group did not improve (table 1). Two-years results will be available in Mai 2015 and presented during EASD Annual meeting 2015.

Conclusion: After shared decision making more than 60% of type 2 diabetes patients decided to choose stricter treatment targets. This led to a non-significant decrease in achieved treatment targets after 1 year, without a change in mean values for HbA1c, blood pressure and cholesterol levels.

Table 1: Patients who achieved separate and all treatment targets. Baseline and 1 year follow-up (n, %), with relative risk and p-value between groups.

	Intervention group, Patients on target		Control group, Patients on target			
	Baseline n=72	Follow up n=71	Baseline n=85	Follow up n=85	Relative risk	p-value
Hba1c (mmol/mol)	49 (68.1)	54 (76.1)	44 (55.0)	49 (62.0)	1.23 (0.99-1.52)	0.079
Blood pressure (mmHg)	36 (50.0)	32 (45.1)	39 (50.0)	43 (54.4)	0.83 (0.60-1.15	0.327
Total cholesterol (mmol/l)	33 (45.8)	23 (32.4)	40 (54.1)	35 (49.3)	0.66 (0.44-1.00)	0.060
All three treatment targets	12 (16.7)	10 (14.1)	13 (15.3)	14 (16.5)	0.86 (0.40-1.81)	0.824

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The effect of a vegetarian vs conventional hypocaloric diet on serum concentrations of persistent organic pollutants in patients with type 2 diabetes

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Background and aims: Emerging evidence suggests that environmental factors described as persistent organic pollutants (POPs) are involved in the development of type 2 diabetes (T2D). POPs may be present especially in fatty fish, fish and seafood, meat and dairy products. The aim of this study was to explore the effect of a vegetarian vs. conventional diet on serum levels of POPs in T2D patients after 12 weeks of a dietary intervention.

Materials and methods: 74 subjects with T2D were randomly assigned to either follow a vegetarian diet without fish or meat (n=37) or a control group who followed an isocaloric conventional diabetic diet (n=37). Both diets were calorie restricted (-500 kcal/day). To measure insulin sensitivity, the hyperinsulinemic (1 mU.kg-1.min-1) isoglycemic clamp was conducted. β-cell function was assessed using a mathematical model after a test meal. Magnetic resonance imaging of the abdomen was performed to measure the amount of visceral fat. We measured serum levels of 44 POPs. Dioxins and dioxin-like POPs were analyzed by isotope dilution high resolution gas chromatography and mass spectrometry after clean up on silica and carbon columns. Non-dioxin-like POPs were analyzed by gas chromatography with electron capture detector. All measurements were performed at 0 and 12 weeks. For statistical analysis we used repeated measures ANOVA and a multivariate regression model. Correlations were calculated using Pearson's correlations.

Results: We did not observe any difference between the groups in serum levels of most POPs in response to both hypocaloric diets. In the groups combined, changes in serum concentrations of the POPs were correlated to changes in HbA1c ($r=+0.34$; $p<0.01$), fasting plasma glucose ($r=+0.41$; $p<0.01$) and β-cell function measured as insulin secretion at a reference glucose level ($r=-0.37$; $p<0.01$), independent of changes in body weight and volume of visceral fat.

Conclusion: Our findings support the relationship between POPs and diabetes, especially β-cell function.

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Differential effects of a two-year insoluble cereal fiber intervention in prediabetic subjects (the OPTImal FIber Trial - OPTIFIT)

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Background and aims: The development of type 2 diabetes is believed to be associated with some dietary factors including potential beneficial effects of dietary fibre. Epidemiological studies have highlighted their putative preventive role and especially insoluble fibre seems to improve insulin sensitivity and therefore reduce the risk of type 2 diabetes. On this behalf a prospective 2-year-study was performed to investigate whether a fibre supplementation can delay or inhibit the increase in blood glucose in subjects with prediabetes. Aim of the program was an increased intake of dietary fibre through change of diet (lifestyle intervention course) additionally to fibre supplementation.

Materials and methods: We performed a 24-months randomized, placebo-controlled, double-blind study involving 180 adults with impaired glucose tolerance. They were randomized into two groups receiving different types of fibre supplement. All subjects started with a one-year lifestyle program including group-based consultations and 4-day food records at regular intervals. During the second year of study they only received their study supplement and were asked to continue their regular intake twice daily. Blinded fibre supplement was either enriched with insoluble oat fibre adding 15 g of fibre per day to the normal nutrition or served as a placebo. Every 6 months anthropometric data and serum markers were assessed. In total 136 participants completed the first study year and 108 subjects completed the whole trial.

Results: The 2-h-OGTT glucose levels improved significantly in both intervention groups after the first study year (placebo-group: Δ -10,5 mg/dL, $p=0.020$ vs. fiber-group: Δ - 17,3 mg/dL, $p<0.001$) as well as markers of insulin sensitivity (HOMAIR, OGIS180, ISIFFA), weight ($p<0.001$), waist ($p<0.001$) and hip circumference ($p<0.001$). In the fiber-group visceral and subcutaneous adipose tissue were significantly reduced (p 19 g fiber/day, Δ -17,0 mg/dL, non-adjusted $p=0.002$) compared to the lowest fiber consumption (<15 g fiber/day, Δ -10,6 mg/dL, non-adjusted $p=0.102$). Several features of metabolic syndrome were significantly improved in both groups at the end of the study. Due to the supplementation the subjects in the fibre group effectively enhanced their fibre intake by approx. 13,4 g per day (placebo-group: 1,5 g per day), revealing good and continuous compliance of the study participants

Conclusion: The OPTIFIT-study showed that also in Germany a lifestyle intervention can significantly improve risk factors for type 2 diabetes. An increased intake of dietary fibre can significantly improve parameters of glucose levels and insulin sensitivity. Fat metabolism, anthropometric data and the extent of fatty liver disease were positively affected by the intervention.

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Identifying people at high risk for type 2 diabetes: preliminary results from the Kerala Diabetes Prevention Programme

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Background and aims: The majority of people with type 2 diabetes mellitus (T2DM) live in low- and middle- income countries. Limited longitudinal studies among Asian Indians have assessed the performance of diabetes risk scores in identifying those at high risk for T2DM. We evaluated the performance of a diabetes risk score to recruit participants into a diabetes prevention program and determined the one-year change in glucose metabolism.
Materials and methods: The Kerala Diabetes Prevention Program is a cluster randomised controlled trial of a peer-led, group-based lifestyle intervention on reducing the incidence of T2DM in rural Kerala, India. The present analyses were limited to those in the control arm, with preliminary data available. At baseline, the Indian Diabetes Risk Score (IDRS), with a cut-point ≥60 was used to identify those at high risk for T2DM among 1306 participants. Participants with an IDRS cut-point ≥60 and without known T2DM attended a mobile clinic and underwent an oral glucose tolerance test (OGTT). Pre-diabetes and diabetes were defined using the World Health Organisation criteria.
Results: The mean (standard deviation) age of participants in the control arm was 47.1 (7.4) years and 47% were females. The IDRS risk factors were age ≥35 years (97.9%), family history of diabetes (51.9%), physical inactivity (79.4%) and central obesity (72.4%). At baseline, 47.1% (n=615) of participants screened were identified as being at high risk for T2DM, using the IDRS and of those 54.5% (n=335) had normal glucose tolerance (NGT), 29.1% (n=179) had pre-diabetes and 16.4% (n=101) had diabetes, based on OGTT. Of those with NGT at baseline, 26% progressed to pre-diabetes and 3.2% progressed to T2DM, by one year. Among those with pre-diabetes at baseline, 27.6% reverted to NGT and 19% progressed to T2DM by one year.
Conclusion: This is one of the first diabetes prevention programs in a developing country to use a diabetes risk score to identify people at high risk for T2DM. This study identified a large proportion of people with NGT who progressed to pre-diabetes or diabetes within one year, these participants would normally be excluded from diabetes prevention programs. These findings provide evidence to support the usefulness of a diabetes risk score to identify people at high risk for T2DM, among Asian Indians.
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Implementation and preliminary outcomes of the Madrid city council diabetes prevention program (ALAS, High Risk Strategy) applied to workers

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Background and aims: In people with prediabetes, lifestyle interventions directed at weigth lossand increasing physical activity are effective in preventing the progression to type 2 diabetes. The Madrid City Council Diabetes Prevention Program (ALAS, High Risk Strategy), has beeb implemented among its employees. Aims: 1 -To achieve 5%-7% weight reduction ; 2- To improve glycemic control.
Materials and methods: During a medical examitation, Madrid City Council workers are encouraged to complete the Findrisc test. Those with positive results, that means, puntuation≥15 are asked to undergo Oral glucose tolerance test (OGTT). When they fulfill criteria for prediabetes, they are invited to participate in the ALAS program. It is delivered over 6 months and consits of 10 group sessions in a Workshop format. There are 6 weekly core sessions the main content of which are: healthy diet, physical activity and emotional management, and 4 more sessions directed at maintenance.
Results: 852 workers fulfilled Findrisc test. 76 people got positive results, from these, 53 (69,73%) underwent (OGTT). Up to February 2015, 43 participants have completed the Madrid City Council Diabetes Prevention Program (ALAS, High Risk Strategy), 30 had Prediabetes (70%) and 13 Normoglycemic at high risk (30%). Participants ages ranged from 37 to 68 (mean 53.6 SD=7,6), 20 males (46,5%) and 23 females (53,5%). At program completion, participants lost a mean of 4,73 Kg or 5,03% of body weight and 23% have normalized the glycemic control; 35% achieved 5% or more weight loss; 56% people attended 6 or more sessions. Weight loss was negatively associated with age, and positively with male gender and attendance. Factors contributing to program success are their implementation in the setting of work health screening, given the employees busy schedules and family responsabilities.
Conclusion: In people with prediabetes, lifestyle interventions are effective to achieve weight loss and to improve glycemic control. It should be encouraged to pass the Findrisc Test during health examinations in the workplace. Such a program could be introduce as part of the health screening examination that companies provide. It could be relevant to the health of employees to implement Diabetes Prevention Program in the workplace.

PS 010 Prevalence of type 1 diabetes and gestational diabetes around the world

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The prevalence of gestational diabetes largely increases if the WHO-1999 cut-off values of the 75-gram oral glucose tolerance test are replaced with the WHO-2013 criteria

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Background and aims: The prevalence of gestational diabetes (GDM) is rising and contributes to a growing burden to global obstetric health care. In 2013, the WHO adopted the stricter cut-off glucose values for diagnosis of GDM of the IADPSG-2010 criteria. In the Netherlands, we have used the WHO 1999 criteria for GDM so far. In this study, we evaluated the prevalence of GDM applying the currently used WHO-1999 criteria compared to the new WHO-2013 criteria.
Materials and methods: Retrospective evaluation of blinded laboratory results of all 75-gram OGTTs performed between January 2011 and August 2014 by Certe, a regional primary- and secondary healthcare laboratory which covers a large rural area and urban area in northern Netherlands of approximately 550,000 inhabitants. Women could be referred by their midwife or gynaecologist to perform an outpatient OGTT if they had risk factors for GDM or symptoms suggestive of GDM (e.g. polyhydramnion). From this database, GDM prevalence was determined using the current national guidline based on the diagnostic WHO-1999 criteria: positive if fasting plasma glucose is ≥7.0 mmol/l and/or 2-h glucose value ≥7.8 mmol/l after a 75-gram OGTT. GDM prevalence was also determined using the WHO-2013 cut-off values: fasting plasma glucose ≥5.1 mmol/l and/or 2-h glucose value ≥8.5 mmol/l after a 75-gram OGTT.
Results: A total of 6130 OGTTs were carried out in the study period, 193 (3%) were incomplete (0.6% failed because of intolerance caused by nausea/vomiting, for the remaining 2.4% there was no registration of the cause of failure). Mean (±SD) age of the women was 30.3±5 years. The prevalence of GDM in this cohort was 19% using the WHO-1999 cut-off values and 30% using the WHO-2013 cut-off values (+58%). GDM was diagnosed solely on fasting glucose in 64% by WHO-2013 criteria contrasting to 1% by WHO-1999 cut-off values where the 2-h glucose value was the major determinant of GDM-diagnosis. Prevalence of GDM increased with maternal age category (WHO1999/WHO2013): 18-25 years 14%/23%; 25-30 years 14%/24%; 30-35 years 21%/33%; 35-40 years 24%/35%; >40 years 30%/48%.
Conclusion: Adjusting our national guideline to the new WHO-2013 criteria will have a major impact on the prevalence of GDM, a 1.5 fold increase mainly because a larger proportion of GDM based on abnormal fasting glucose levels. This will lead to a higher referral rate to secondary obstetric care, necessitating a shared-care model for obstetric and diabetes care between primary and secondary obstetric care facilities. Furthermore it remains to be seen whether the WHO-1999 GDM-negative but WHO-2013 GDM-positive screeners have better maternal and neonatal outcomes.

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Prevalence and risk factors for thyroid pathology in patients with diabetes mellitus

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Background and aims: The aim of this study was to assess the prevalence and risk factors for thyroid pathology among patients with type 1 and type 2 diabetes mellitus
Materials and methods: The study was conducted in our clinic during the period of January 2012 - December 2013. It was a retrospective study. The total study population included 829 patients with type 1 (14.8%) and type 2 diabetes (85.2%). The study group consisted of 532 women (64.2%) and 297 men (35.8%).
Results: Thyroid pathology was found in 78 (63.4%) of patients with type 1 diabetes and 507 (71.8%) of patients with type 2 DM. In type 1 diabetes group with established thyroid pathology the most common disorder was autoimmune thyroid disease - 45 (36.5%), while nodules were diagnosed in 31 patients (25.2%). In type 2 DM group the most common disorder were nodules - 332 (47.0%), while patients with auto-immune thyroid disease were 152 (21.5%). In type 2 DM group the risk of developing thyroid pathology increased by 1,074 times in comparison to type 1 diabetes. The risk of developing nodules increased by 1,142 times accordingly, and the risk of auto-immune thyroid disease decreased. The comparative analysis of patients with single and multiple nodules showed a statistically significant difference in terms of age - patients with multiple nodules were older - 61,35±10,78 years and those with single nodules 57,7±12,45 years. In type 1 diabetes mellitus a statistically significant correlation between age and presence of nodular formations was found. This correlation was not established in terms of auto-immune thyroid disease. In patients with type 2 diabetes mellitus statistically significant correlation was established between age and thyroid pathology in general as well as between age and the presence of nodular formations. In terms of AITD such correlation was not found.
Conclusion: Data comparison in type 1 and type 2 diabetes mellitus showed that aging increases the risk of nodular changes in both type 1 and type 2 diabetes, i.e., age can be perceived as an absolute risk factor for developing thyroid pathology, namely nodular pathology. According to our data in type 1 and type 2 diabetes, female sex is a risk factor for the development of nodular pathology and AITD.

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Plasma lipids at type 1 diabetes onset predicts residual beta cell function after 6 and 12 months

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Background and aims: The exact mechanisms triggering and regulating progression towards beta-cell failure in type 1 diabetes (T1D) are poorly understood. It is generally acknowledged, however, that both genetic and environmental components are involved. A detailed understanding of the pathogenesis of T1D is a criterion for the development of preventive strategies. In particular, the identification of early metabolic modifications is promising in the study of etiological pathways. Recent research indicates that modulations of multiple plasma phospholipids precedes the presence of autoantigens in the development of T1D. These observations suggests the potential of metabolomic biomarker panels in monitoring health and potential decline in beta-cell function before T1D symptoms appear. Thus, we hypothesize that new biomarkers in the form of metabolite panels, and in particular lipids, in plasma from children with recent onset T1D will reflect the remaining beta cell function. This could lead to the development of new biomarker panels for decline in beta-cell function before T1D manifests itself clinically.

Materials and methods: For this study we used a total of 129 patients <15 years of age followed in the Danish Remission Phase Study. The patients were followed for 12 months (n=40 additionally returned at 60 months) from the diagnosis of T1D. Demographic and anthropometric data, insulin therapy as well as blood samples for centralized measurement of Hemoglobin A1c and meal-stimulated C-peptide were collected prospectively 1, 3, 6, 12 and 60 months after diagnosis. Multiple reaction monitoring lipidomics were used to characterize the lipid profiles of the participants of the cohort at each time point.
Results: Seven diacylglycerols (DG) and total amount of DG all of them carrying either 16:1 or 18:1 fatty acids, or both, were significantly associated, after Benjamini-Hochberg correction, to a decrease in stimulated C-peptide after six months, and three of these: DG 16:1/16:1, DG 18:1/18:1 and DG 16:1/18:1 and total DG were also associated to a decrease in stimulated C-peptide after 12 months. Six triacylglycerols (TG) and total TG were also associated to a decrease in stimulated C-peptide after six months, and three of these and total TG were also associated to a decrease in stimulated C-peptide after 12 month. All of the TGs contained at least one 16:1 or 18:1 fatty acid. Six cholesterol ester (CE) and total CE were associated to a decrease in stimulated C-peptide at six months, of these three and total CE were associated to stimulated C-peptide decrease at 12 months. CE 24:0 were only associated to a decrease in stimulated C-peptide after 12 months. Oxidated PC (OxPC) and PC 36:4a were significantly associated to an increase in stimulated C- peptide after 6 and 12 month. Another phospholipid, sphingomyelin 34:2 were also associated to a decrease in stimulated C-peptide after 12 months.
Conclusion: These observations provide evidence that lipid disturbances predicts decline in beta cell function, and could by closer examinations lead to better classification of patients and the progression of the disease as well as to the identification of new pharmaceutical drug targets.
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A comparison of diabetes type 1 in South Asian and Caucasian patients in the UK: the Type 1 in Minority Ethnic populations (TIME) study

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Background and aims: Diabetes type 2 (T2D) is up to six times more common in people of South Asian origin than in white Caucasians in the UK. Furthermore, onset is earlier with higher morbidity and mortality. Diabetes type 1 (T1D) has not previously been characterised in this group, despite South Asians comprising over 7% of the UK population. Recent reports suggest the prevalence of T1D is similar across South Asian and white Caucasian ethnicities. The aim of the TIME study was to characterise and compare T1D between South Asian and background white Caucasian populations.
Materials and methods: We performed a case controlled analysis of 417 patients across two centres in the West Midlands, UK (a region in the UK with one of the highest South Asian populations) matching for age and gender (one South Asian patient matched to two white Caucasian). Data for 177 patients was analysed from the Queen Elizabeth Hospital (QEH) and 240 patients from New Cross Hospital (NCH).
Results: The results are shown in Table 1. Values are median (interquartile range) with * indicating a p value <0.05.
Conclusion: In conclusion, we found that South Asians with T1D presented at a later age, and had more significant macrovascular risk factors than white Caucasians. Interestingly, there was no difference in glycaemic control or prevalence of microvascular complications. As for T2D, these findings have important implications for service delivery and personalised healthcare.

Table 1: A comparison of diabetes type 1 in South Asian and Caucasian patients in the UK

Characteristic (number of pts)	NCH South Asian (80)	NCH Caucasian (160)	QE South Asian (59)	QE Caucasian (118)
Male	43 (54%)	86 (54%)	34 (58%)	68 (58%)
Female	37 (46%)	74 (46%)	25 (42%)	50 (42%)
Age (yrs)	33.5 (23.75-45)	34 (23.75-45)	36 (28-44.5)	36 (28 – 44.75)
Age at diagnosis (yrs)	16 (9.75-24)*	12 (8-18)*	17.5 (10.75-26)	15 (10-22)
Duration of disease (yrs)	17 (9-28.25)*	21.5 (13.75-32)*	17 (8-23)	19 (7-28.5)
HbA1c (mmol/mol)	75 (61.5-88.5)	76 (63-91)	66.1 (55.25-81.75)	70.5 (61-83.6)
Systolic BP (mmHg)	121 (113-132)	125 (115-132)	130 (120.5-141.5)	131.5 (120.3-144)
Diastolic BP (mmHg)	-	-	86 (80.5-90)*	82 (77.25-88.75)*
Weight (kg)	72.4 (58.9-82.35)	73.6 (64-90)	74 (58.65-85.75)	76.5 (66.2-88)
Height (cm)	168 (157-175.5)*	170.5 (161-178)*	168 (165-173)	176 (169.5-182.5)
BMI (kg/m²)	25.6 (22.55-28.4)	25.7 (22.5-30.4)	30.9 (22.8-37)	25 (22.6-28)
Total cholesterol (mmol/L)	4.7 (3.9 – 5.45)	4.6 (4-5.3)	4.45 (3.8-5.45)	4.1 (3.7-4.95)
HDL (mmol/L)	1.3 (1.0 – 1.6)*	1.4 (1.2-1.65)*	-	-
Cholesterol/HDL	3.6 (2.9-4.5)*	3.2 (2.7-4.0)*	-	-
Creatinine level (µmol/L)	75 (66-87)	78 (69-87)	-	-
eGFR (ml/min/1.73m²)	97.3 (82.2-109.9)	91.2 (79.3-103.9)	-	-
Albumin/ Creatinine ratio (mg/mmol)	2.4 (0.7-3.6)	2.5 (0.75-3.5)	-	-

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National childhood diabetes register: disease onset, diabetes control and therapeutic data in the Czech Republic

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Background and aims: The Czech National Childhood Diabetes Database (ČENDA) is a centralized web-based national database that stores treatment and outcome data in children and adolescents with diabetes, allowing for anonymous comparison among diabetes clinics. The goals of the ČENDA database are to provide longitudinal data of childhood diabetes control and to identify strengths and weaknesses in the system of care. Here we present the first two years of the database.
Materials and methods: Since 2013, the database collects data on every patient treated by 48 participating pediatric diabetes outpatient clinics. Importantly, a prerequisite for participation is to report every patient cared for. Data include characteristics of the disease onset and annual summaries of clinical care, including every tested HbA1c value. Protocols have been implemented ensuring complete anonymization and patient data protection.
Results: The database contains data of 2192 children and young people with diabetes; this is estimated to be 70% of all Czech pediatric patients according to the highly accurate national incidence register. In 2013, complete annual data were available for 1972 patients (1025 boys and 947 girls) up to the age of 18. Of these, 95% had type 1 diabetes, 3% had genetically proven monogenic diabetes, 1% had type 2 diabetes and 1% were stated as other type of diabetes. Median HbA1c was 66.3 (average 69.3; IQR 57-78) mmol/mol. The proportion of children reaching target therapeutic goal of 59 mmol/mol declines from 34% in 0-4 years old children to 25% in 15-18

the years old. Out of children treated by insulin, 75% were on multiple daily injections (MDI), 24% were on continuous subcutaneous insulin infusion (CSII) and 1% were on conventional therapy. The proportion of children treated by CSII rises significantly with age, being 6.8% in 0-4 years old children and 33% in the 15-18 years old. HbA1c values do not differ between children on MDI and CSII therapy. The total daily insulin dose varied by age and diabetes duration; the lowest dose was observed in children younger than 4 years (0.65 U/kg/day), the highest dose was in adolescents 15-18 years old (0.84 U/kg/day). Celiac disease was reported in 11.5%, and 18.3% of children were treated for autoimmune thyroid disease. Significant differences have been noted between clinics in almost all of the observed parameters.
Conclusion: With a coverage over 70%, the ČENDA database offers a representative view of childhood diabetes in the Czech Republic. The comparison of pediatric diabetes clinics shows large differences in treatment processes, complications and outcomes, notably in the proportion of patients reaching therapeutics targets. Detailed data analyses will help identify key areas for further improvement of the system of pediatric diabetes care.
Supported by: Czech Diabetes Society, MZČR National action plans and concepts

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Rising incidence of childhood type 1 diabetes in Wielkopolska province, Poland

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Background and aims: Over the past decades, a rapidly increasing incidence of childhood type 1 diabetes has been reported in many parts of the world. In Poland, the first epidemiological register was conducted in Wielkopolska between 1970-1985, where the estimated average incidence rate was 4.4/105 for the age group 0-16 years. In 80s and early 90s of the 20th century according to data from epidemiological programs EURODIAB and DiaMond, Poland was included to a very low incidence of type 1 diabetes countries with an incidence rate estimated around 6.6 / 105. This study aims to assess the current incidence of type 1 diabetes among children in Wielkopolska.
Materials and methods: The analysis involved cases of new onset type 1 diabetes that were recorded in Wielkopolska Register of Childhood Diabetes from 2008 to 2013. The degree of completeness of the registry overall has been estimated at 95.0% The denominator for the analysis were children≤14 years with residency in the study area, which was defined geographically to correspond with administrative and census boundaries of Wielkopolska. Diabetes was diagnosed based on WHO criteria and first day of insulin administration was assumed the time of diagnosis. Total, sex-, and age-specific incidence rates per 100,000 person-years were calculated for each calendar year. A direct standardization method was used to estimate age and sex standardized rates, assuming a reference population comprising equal numbers in each of the sex- and age-specific groups (0-4, 5-9, and 10-14 years). The 95% CI was calculated using the Gaussian approximation to the Poisson log-likelihood. The demographic date was obtained from the Statistical Office in Poznan.
Results: 571 new cases of type 1 diabetes: 259 girls and 312 boys were identified during the period January 2008 - December 2013. The mean age at diagnosis was 9.0±4.3 years. The trend for increased incidence of type 1 diabetes has been observed in children aged 0-14 (2008: 15.6/105, 95% CI: 8.6-22.4; 2013: 24.3/105, 95% CI: 20.2-28.4). The highest annual incidence was reported among those aged 5-9 years (2008 - 22.8/105- 95% CI: 15.6-30.0; 2013 - 27.2/105- 95% CI: 19.7-34.6). The fastest incidence increase was found in the youngest age group (2008 - 7.1/105, 95% CI: 3.2-11.0; 2013 - 17.4/105 , 95% CI: 11.5-23.2). Diabetes has occurred more often in autumn and winter months comparing to spring/summer months, except for 2012 when the seasonality was not observed
Conclusion: Incidence rate of type 1 diabetes is rising in Wielkopolska. Such rapid increase in very short period rather is associated with environmental factors than changing in genetic background. Seasonality of the disease is not a regular occurrence, which suggests increasing role of non-seasonal factors in the growing incidence. The fastest increase of type 1 diabetes incidence was noticed among the youngest children. The peak incidence is steadily moving towards younger age groups of children which indicates that paediatric diabetes health care will be extended and there is a higher risk of developing chronic complications in younger patients. The accelerating trend needs to be well monitored due to the search for its causes, prevention and the possibility of customizing medical care.

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Effect of ethnicity on clinical presentation with type 1 diabetes and on humoral autoimmunity, within ADDRESS-2 (After Diagnosis Diabetes Research Support System)

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Background and aims: In type 1 diabetes (T1D), most comparisons across the literature are within and between countries, but not by ethnicity per se. That is, in part, due to the fact that many of the countries are relatively homogenous with regards to ethnicity. Here, we examined the effect of ethnicity on the clinical presentation and humoral autoimmunity, within people with incident T1D recruited to ADDRESS-2.
Materials and methods: ADDRESS-2 comprises of a multi-ethnic cohort of people with incident T1D, aged 5-60 years old, characterized on the basis of clinical and demographic features and, in many, islet-specific antibodies (Ab) (GAD65, IA-2, ZnT8). From 01/09/2011 to 28/02/2014, patients with T1D were recruited to ADDRESS-2, within 6 months of diagnosis, from 134 recruiting sites in the UK.
Results: 1,580 patients were recruited (1,453 White-Europeans (WE); 909 male; 867 children). Of the non-WE patients, 52 were Asian (A), 20 Black (B), 55 Mixed/Other (MO). Osmotic symptoms (95%), weight loss (84%), fatigue (84%) were the main presenting symptoms and occurred in comparable frequency in all ethnic groups ($p>0.05$). Symptom duration was greater in WE people ($p=0.002$). 42% presented with diabetic ketoacidosis. Ketoacidosis rates were similar in WE (43%) and non-WE (38%) ($p>0.05$). Ethnicity did not independently predict symptom duration or mode of clinical presentation. Family history of diabetes (FHDM) was reported at comparable frequencies between ethnic groups ($p>0.05$). A ethnicity was a strong, independent predictor of FHDM (OR=1.9; $p=0.03$). Ethnicity did not predict HbA1c or insulin dose requirements at the time of presentation. Of all patients, 926 (59%) provided a blood sample for Ab testing. 84% (774/926) had at least one Ab present. Ab positive status was associated with WE (85%) more than non-WE (67%) ethnicity ($p=0.001$). WE ethnicity was a strong, independent predictor of Ab presence in children (5-16 years; OR=4.1; $p=0.01$) and adults (17-60 years: OR=2.3; $p=0.02$). A (OR<5; $p=0.01$) and B (OR<5; $p=0.04$) ethnicities strongly and independently predicted Ab absence. MO ethnicities less strongly predicted that (OR<0.5; $p=0.08$). None of the potential confounding variables that may explain the effect of ethnicity increased the OR above 0.5 for non-WE ethnicities, on multiple regression analyses. WE patients were significantly more likely to have multiple

(versus single) Ab present compared to non-WE patients (RR=1.9; p=0.01).

Conclusion: ADDRESS-2 is a registry-based recruitment study of representative patients with a clinical diagnosis of T1D, over a wide age range and multiple ethnicities. It offers important information on the clinical and anthropometric characteristics of patients presenting with T1D. Although T1D is a condition that mostly affects people of WE ethnicity, there were no major differences in presentation across different ethnicities. Presence of islet cell Ab was much higher in WE patients compared with non-WE. Ethnicity was a strong, independent predictor of Ab status and frequency in children and adults presenting with T1D.

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Characteristics of newly diagnosed adults with type 1 diabetes in the UK and their evolution over the first 5 years

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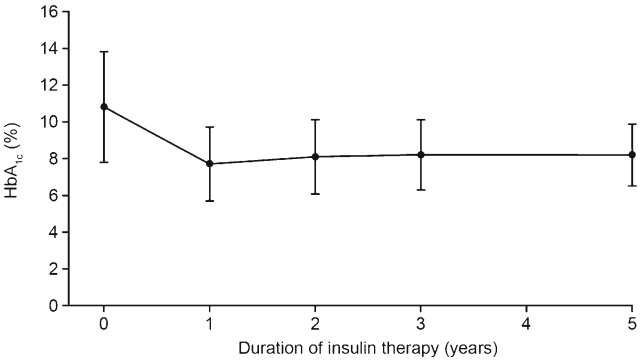
Background and aims: The aim of this study was to ascertain the weight, BMI characteristics and comorbidities of adults with newly diagnosed type 1 diabetes (T1D) in the UK and assess the impact of initiating insulin therapy on their glycaemic control and weight profile over the first 5 years after diagnosis.

Materials and methods: This study was a non-interventional, retrospective, cohort study utilising data from the UK Clinical Practice Research Datalink (CPRD) database between 2001 and 2013. Patients were selected based on a recorded diagnosis (C10* read codes) of T1D or fulfilling the NHS criteria for T1D, in whom onset of continuous insulin treatment was within 12 months of diagnosis. Patients included were aged 18-85 years, with a body mass index (BMI) of 11-50 kg/m^2 and a full medical history of at least 12 months prior to diagnosis (n=2430). Using this dataset, HbA1c and BMI levels were compared at baseline and 12, 24, 36 and 60 months after insulin therapy was initiated.

Results: At baseline, patients (63.3% male, 36.7% female) had a mean ± SD age of 40.8±16.1 years, HbA1c of 10.8%±3.0, and a BMI of 25.3±5.5. Mean BMI and BMI distribution of study patients were comparable to that of the UK general population. Following initiation of insulin therapy, mean HbA1c statistically significantly (only taking inter-subject variation into consideration) declined from 10.8%±3.0 to 7.7%±2.0 at 12 months (P<0.01), but subsequently statistically significantly increased to 8.1%±2.0 at 24 months, remaining stable thereafter at 8.2%±1.9 and 8.2%±1.7 at 3 and 5 years, respectively (Figure 1). Mean BMI consistently increased from 25.3 ±5.5 at baseline, to 27.2±5.8, 27.4±5.7, 27.5±5.6, and 27.9±5.5 kg/m^2 at 1, 2, 3 and 5 years, however, only the increase in the first 12 months was statistically significant (only taking inter-subject variation into consideration).

Conclusion: Whilst insulin therapy exerted an initial reduction in HbA1c at 12 months, mean HbA1c at 12 months was still above the NICE targets, and further deteriorated soon after, stabilizing between 2 and 5 years after initiation of treatment. After beginning insulin therapy, T1D patients were susceptible to long-term cumulative weight gain and increase in BMI, with deeper entrenchment into pathophysiological BMI weight categories. This study supports the concept of "glycaemic streaming" and suggests that further intensification of efforts is required right from diagnosis and initiation of insulin therapy to support patients with T1D to achieve and maintain glycaemic control targets. Currently weight control is a side issue in UK T1D patient education and treatment strategies which focus primarily if not almost exclusively on the titration of insulin to achieve HbA1c targets. This study illustrates the need for greater emphasis on weight management in national T1D education programmes.



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PS 011 Genetics of metabolism: insights from animal studies

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Obesity associated variants within FTO are functionally connected to multiple genes in the locus

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Background and aims: Several studies directly implicated FTO as the obesity gene. Genome-wide association studies have linked non-coding variants within FTO introns with increased risk for obesity and type 2-diabetes. Recently, our group has discovered that the obesity-associated elements within fto interact with Irx3, a distant gene on the genome that appeared to be the functional obesity gene. Our data showed that FTO obesity-associated region contain enhancers that drive Irx3- like expression, whereas Fto expression is primarily regulated by regions proximal to its promoter. eQTL mapping showed altered Irx3 expression (not Fto) in the human brain and null mice demonstrated that Irx3 is also a regulator of body mass and composition. However, involvement of Fto gene and/or other genes in the phenotype couldn't be rejected.

Materials and methods: To identify all possible genomic interactions between the 47 kb obesity-associated interval and promoters of genes located within a megabase window downstream FTO locus, we performed circular chromosome conformation capture followed by high throughput sequencing (4C-seq) in adult mouse brain (8 weeks). In order to determine a potential role for Irx5 expression (and exclude IRX6) in the regulation of body mass index and metabolism, we used the CRISPR-Cas9 system to create Irx5 and Irx6 Knock-out mice.

Results: Using 4C-seq, we observed strong long -range interaction between the obesity-associated FTO intron not only with Irx3, but also Irx5. No interaction was observed with IRX6 promoter. Mice homozygous for an Irx5 and Irx6 null allele are viable and fertile, with no evidence of embryonic lethality. Irx5KO mice showed a significant reduction in body weight. Importantly, the percentage of fat mass was significantly reduced without marked change of the lean mass ratio, due to an increase in browning of white adipose tissue. Similar phenotype was observed for Irx3KO mice. After the mice were subjected to 45% high fat diet (HFD) for 10 weeks, Irx5KO showed improved glucose intolerance and insulin resistance compared to control littermates. Irx6KO mice did not show any weight loss phenotype and HFD lead to glucose intolerance similar to observed in WT controls. Compound animals Irx3/Irx6 and Irx3/Irx5 are being generated and heterozygote animals are being evaluated for metabolic phenotypes.

Conclusion: These results strongly suggest that Irx5, together with Fto and Irx3, is also responsible for controlling body mass, body composition and energy metabolism. Our data shows that obesity-associated SNPs within FTO are functionally connected with multiple genes and that misregulation of Irx3 and Irx5 expression, but not with Irx6, that is indeed located in another transcription activation domain (TAD), might lead to obesity in mice.

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Identification of novel susceptibility genes for diabetes- and obesity-related traits in two backcross populations of obese and lean mice

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Background and aims: Type 2 diabetes (T2D) in humans is influenced by a combination of numerous adipogenic and diabetogenic alleles. In outcross experiments of obese and lean mouse strains, several QTL (Quantitative Trait Loci) for obesity and hyperglycemia were separated. Mouse models could therefore help to identify several key obesity and diabetes modifier genes. In order to find further disease genes we performed two new crossbreeding approaches and subsequent QTL analysis. The obese New Zealand Obese (NZO) mouse which represents a polygenetic model for the human metabolic syndrome and T2D, was crossed with the two lean mouse strains C3HeB/FeJ and 129/OlaHsd.

Materials and methods: We measured phenotypic traits associated with obesity and T2D in two backcross populations of 600 individuals each (300 males, 300 females). Animals were fed a high-fat-diet (45% fat/cal.) throughout the experiment. Body weight, body composition, plasma glucose and insulin concentrations during fasted and fed states were measured at different time points. At 21 weeks of life mice were sacrificed and tissues were harvested for the analysis of metabolic regulators. We genotyped both N2 populations with the help of a genome-wide high-density SNP panel. Linkage analysis was performed by in silico calculation of phenotype-genotype associations. Moreover, differential gene expression between the parental strains in liver, skeletal muscle, WAT, BAT and pancreatic islets was analysed by Affymetrix microarrays.

Results: Genetic linkage analysis revealed several highly significant QTL for metabolic traits in both backcross populations. In the NZOxC3HeB/FeJ backcross, major QTL for blood glucose were identified on chromosomes 4 (32 cM, logarithm of odds (LOD) 8.4), 7 (12 cM, LOD 14.0) and 15 (13 cM, LOD 7.5). Interestingly, a QTL for blood glucose at 32 cM on Chr. 4 was detected in both backcrosses (NZOx 129/OlaHsd and NZOxC3HeB/FeJ), indicating the contribution of an NZO allele. However, the largest effects were contributed by Chromosome 7, where maximum LOD scores overlapped for blood glucose, body weight, lean mass, final plasma insulin and BMI. Moreover, this locus appeared to be responsible for almost 80% of the total prevalence of hyperglycaemia in the male NZOxC3HeB/FeJ backcross population. Using microarray data we were able to detect several candidate genes within the loci with tissue-specific differential expression between the strains.

Conclusion: We identified novel QTL associated with obesity and T2D through a mouse genetics approach. Moreover, we were able to determine promising candidate genes for the diabetogenic effect of the most striking QTL on chromosome 7. Sequencing and functional assays of these candidates will help to identify the causal genes. Furthermore, interval-specific congenic introgression of 129, C3H and NZO into diabetes-resistant C57BL/6J will be performed to narrow down the candidate region and therefore serve as a second approach to identify novel diabetes genes.

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Obesity modulates Ankrd26 gene expression by inducing epigenetic changes of its promoter

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Background and aims: Ankyrin repeat domain 26 (Ankrd26) gene is a novel actor in the pathogenesis of obesity and T2D. This gene indeed plays an important role in the control of food intake, fat mass and glucose tolerance in vivo in mice. Based on our MeDIPseq data, Ankrd26 gene seems to be a target sensitive to environmental exposure and to epigenetic modifications. Here, we aimed to investigate whether obesity in vivo impacts on Ankrd26 gene transcription by causing epigenetic modifications
Materials and methods: Obesity was induced by feeding 8 weeks (w) old male C57BL/6 mice with a high fat diet (HFD; ~60% energy from fat) up to 22 w. Gene expression was evaluated by Real-time qPCR in tissues from obese mice and lean mice (STD). DNA methylation of Ankrd26 promoter was investigated by MeDIP-qPCR and bisulfite sequencing analysis in both lean and obese mice
Results: HFD-fed mice are heavier than STD-fed mice already upon 8 w of obesogenic treatment reaching a 50% increase of body weight compared with lean mice at the end of the treatment. Obese mice exhibit also increased fasting blood glucose, impaired glucose tolerance and reduced insulin sensitivity at 8 and 22 w compared with controls (Table 1). Upon 8 w of treatment Ankrd26 mRNA expression is slightly decreased in the epididymal white adipose tissue (WAT) from obese mice compared with lean mice (STD 0.544±0.036 AU vs. HFD 0.521±0.021 AU). By contrast, 22 w of treatment leads to a significant decrease of Ankrd26 mRNA in WAT from obese mice (STD 0.509±0.011 AU vs. HFD 0.483± 0.008 AU, $p<0.001$). Similar to WAT, Ankrd26 mRNA levels are decreased in the tibialis skeletal muscle (STD 0.578±0.019 AU vs. HFD 0.558±0.011 AU, $p<0.01$), but not in the liver (STD 0.492±0.023 AU vs. HFD 0.500±0.028) from obese mice upon 22 w of HFD compared to controls. Consistent with the lower Ankrd26 expression in obese mice, MeDIP-qPCR analysis shows a 2-fold increase in DNA methylation enrichment of a region covering the Ankrd26 promoter (-462 bp to -193 bp) in pooled WAT genomic DNA from 22 w HFD-fed obese mice compared with lean mice (STD IP=0.407±0.171% vs. HFD IP 0.814±0.047%, $p< 0.05$). Bisulfite sequencing also reveals in obese mice a consistent and specific hyper-methylation of 2 cytosine residues at -436 bp (% of methylation: STD 62% vs. HFD 90%, $p<0.001$), and -431 bp (% of methylation: STD 38% vs. HFD 83, $p<0.001$) from Ankrd26 Transcription Starting Site. By contrast, no differences in the methylation state of these two specific CpG sites were observed between lean and obese mice upon 8 w of treatment (% of methylation -436 bp: STD 71% vs. HFD 83%; % of methylation -431 bp: STD 65% vs. HFD 58%)
Conclusion: Obesity regulates in a time dependent manner the expression of the Ankrd26 gene by inducing methylation of specific CpG sites on Ankrd26 promoter

Variable	8 weeks		22 weeks	
	STD (n=11)	HFD (n=12)	STD (n=9)	HFD (n=12)
Body weight (g)	24.7 ± 0.6	34.4 ± 0.9[a]	26.4 ± 0.9	38.8 ± 1.0[b]
Fasting glucose (mmol/l)	5.9 ± 0.3	7.7 ± 0.9[a]	5.8 ± 0.5	9.1 ± 0.5[b]
GTT AUC (mmol/l 120 min-1)	725.7 ± 42.2	1290.7 ± 59.1[a]	653.6 ± 57.8	1344.2 ± 76.1[b]
ITT AUCi (mmol/l 120 min^{-1})	741.6 ± 4.8	355.7 ± 44.0[a]	681.0 ± 68.5	312.9 ± 52.2[b]

Data are means ± SE of determinations in at least 10 mice per group. [a]$p<0.001$, 8-weeks HFD-fed mice vs 8-weeks STD-fed mice; [b]$p<0.001$, 22-weeks HFD-fed mice vs 22-weeks STD-fed mice

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New animal models reveal that two genes are candidates for the onset of type 2 diabetes associated with obesity in rats

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Background and aims: Obesity is a major factor for the onset of type 2 diabetes. However, little is known how obesity induces the onset of type 2 diabetes. So far, many genes responsible for obesity and type diabetes were identified by genome-wide association studies, but only few genes are common between obesity and type 2 diabetes. Since it is difficult to directly search for diabetogenic genes associated with obesity, the use of double congenic rats introgressing both fa/fa obese and diabetogenic gene is an essential component of genetic investigation. In this study, we investigate to identify diabetogenic gens associated with obesity using new obese animal models.
Materials and methods: We generated several congenic strains: (1) single lean congenic introgressed Nidd2 QTL derived from OLETF rat into F344 (F.O-Nidd2/of), (2) single obese congenic introgressed Lepr-/- locus into F344 (F.ZF-Lepr) and (3) double congenic rats having obese and Nidd2 QTL region (F.ZF-Lepr&Nidd2/of). Each strain was phenotyped for plasma glucose, insulin levels and insulin resistance by insulin tolerance test (ITT). In 25 weeks of age, they were sacrificed and then liver, skeletal muscle and abdominal fat tissues were dessected and stored at -80°C until use. RNA and protein levels were determined using reverse transcription-quantitative PCR and western blotting analyses, respectively.
Results: The double congenic strain, F.ZF-Lepr&Nidd2/of (Lepr−/− and Nidd2/of) showed significantly higher glucose levels (60, 90, 120 min after glucose loading : $p<0.001$ vs F.ZF-Lepr), and significantly lower hypoglycemic response to insulin than those of the obese control strain, F.ZF-Lepr (Lepr−/−) (30, 60, 90, 120 min after insulin injection : $p<0.05$ vs F.ZF-Lepr). These phenotypes were observed specifically in the obese strains, but not in the lean strains. These results indicate that the Nidd2/of locus harbours a diabetogenic gene associated with obesity. We measured the expression of 60 genes in the Nidd2/of QTL region between the strains and found that the levels of the mRNA expression of five genes were significantly different between the strains under condition of obesity. Three genes among the five genes were differentially expressed both in obese and lean rats, while the other two genes, Coq2 and Plac8 were differentially expressed specifically only in the obese rats, suggesting that these genes are candidates for the onset of type 2 diabetes associated with obesity in rats (Coq2 : $P=0.00387$ and Plac8 : $p=0.00122$)
Conclusion: Our data show that Coq2 and Plac8 were candidate genes for the onset of type 2 diabetes associated with obesity in rats.

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Nicotinamide nucleotide transhydrogenase and the glucose regulation of mitochondrial glutathione oxidation, NADPH concentration and insulin secretion in mouse islets

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Background and aims: Changes in glutathione redox state (E_{GSH}) were recently measured with the redox-sensitive GFP2 probe fused to glutaredoxin 1 (GRX1-roGFP2) and its mitochondrial form (mt-GRX1-roGFP2) in rat islet cell clusters and human islets. We found an inverse correlation between 1) the acute glucose-mediated reduction in E_{GSH} and 2) the acute rise in NAD(P)H autofluorescence and glucose stimulation of insulin secretion (GSIS). Nicotinamide nucleotide transhydrogenase (NNT) is one of several NADPH-producing enzymes in mitochondria that could contribute to these acute glucose effects. Its spontaneous inactivating mutation in C57BL6/J mice was shown to reduce the GSIS

due to reductions in ATP production, β-cell depolarization and Ca^{2+} influx. Here, we tested the impact of a lack of NNT on the acute glucose regulation of mitochondrial E_{GSH} and its role in GSIS and key stimulus-secretion coupling events by comparing islets from C57BL6/J mice with islets from closely-related C57BL6/N mice that express wild-type NNT (J vs. N islets).

Materials and methods: Islets were isolated from 8-16 week-old female C57BL6/J and /N mice. After isolation, the islets were (co)infected with adenoviruses coding (mt-)GRX1-roGFP2, NNT+mCherry (to express wild-type NNT in J islets) or mCherry alone as control, and cultured for 2-4 days. The mitochondrial and cytosolic E_{GSH}, the reduced and oxidized forms of NADP and NAD, ATP and the sum ATP+ADP, the intracellular Ca^{2+} concentration, and insulin secretion were measured after 30-60 min incubation or during perifusion at increasing glucose concentrations from 0.5 (G0.5) to 30 mmol/L (G30) in the presence of 4.8 or 30 mmol/L extracellular K^+ (K4.8 or K30).

Results: In N islets, glucose acutely and significantly reduced mitochondrial E_{GSH} as in rats and humans while increasing NADH/(NADH+NAD^+) (G30 0.22±0.03 vs. G0.5 0.05±0.01, $p<0.01$), NADPH/(NADPH+$NADP^+$) (G30 0.72±0.08 vs. G0.5 0.48±0.06, $p<0.05$) and ATP/(ATP+ADP) ratios (G30 0.83±0.003 vs. G0.5 0.69±0.003), $p<0.001$), Ca^{2+} and insulin secretion in K4.8 or K30 ($p<0.05$). In J islets, the glucose-induced rises in NADH/(NADH+NAD^+) (0.21±0.04 vs. 0.04±0.01, $p<0.01$), ATP/(ATP+ADP) (0.85±0.01 vs. 0.71±0.02, $p<0.001$) and [Ca^{2+}]i were similar to those in N-islets. There were also no significant differences between mice for their lack of cytosolic E_{GSH} response to glucose. In contrast to N islets, mitochondrial E_{GSH} was low at all glucose concentrations and NADPH/(NADPH+$NADP^+$) was not increased in G30 compared to G0.5 (0.83±0.03 vs. 0.78±0.03) and not decreased by 10 μM of mitochondrial uncoupler FCCP in G30 (0.83±0.02). The GSIS in K4.8 and K30 was 66% lower ($p<0.05$) in J vs. N islets, but the relative amplifying action of glucose in K30 was preserved (fold-increase vs. G0.5). Importantly, adenovirus-mediated expression of NNT in J islets restored the glucose regulation of mitochondrial E_{GSH} and the glucose (G30 0.84±0.01 vs. G0.5 0.52±0.04, $p<0.001$) and FCCP (0.64±0.05, $p<0.05$ vs. G30) regulation of the NADPH/(NADPH+$NADP^+$) ratio as observed in N islets, and significantly increased GSIS in both K4.8 and K30 conditions.

Conclusion: NNT is responsible for the glucose-induced rise in NADPH/(NADPH+$NADP^+$) ratio and reduction in mitochondrial E_{GSH} in pancreatic islets, and increases the GSIS by acting at a site distal to Ca^{2+} influx. The results also suggest that NNT works in the reverse mode of operation in islets exposed to a low glucose concentration or to FCCP.

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Mitochondrial mutations affect cellular calcium homeostasis in the aging organism and could support development of type 2 diabetes mellitus

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Background and aims: It was postulated that mutations in the mitochondrial genom (mtDNA) which accumulate in age can contribute to the pathogenesis of type 2 diabetes mellitus (T2DM). In mice there is a maximum value in ROS generation in liver at the age of 9 months. At an advanced age there is a decreased value in ROS generation and a reduced membrane potential in C57BL/6NTac-mice (control). However, in conplastic C57BL/6NTac-mtNOD/LtJ-mice (mtNOD) carrying a point mutation in complex IV of the respiratory chain ROS generation remains constantly high and membrane potential increased in age. The aim of this study was to examine if an altered calcium homeostasis contributes to this adaption in the mtNOD mouse strain.

Materials and methods: Hepatocytes of 3, 6, 9 and 12 months old control- and mtNOD mice were isolated and transfected with the cyto(plasmatic)-CAR-GECO and the mito(chondrial)-GEM-GECO vector. Fluorescence intensity of the resulting proteins depends on calcium binding. Cells were analysed after starvation and subsequent glucose supply (25 mmol/l) by means of fluorescence microscopy. In addition, RNA was isolated from hepatocytes and gene expression of uncoupling protein 2 (UCP2) and the mitochondrial calcium uniporter (MCU) were investigated by quantitative Real-Time PCR.

Results: Cytosolic Calcium influx after glucose supply was comparable at the age of 3 months in both strains. At the age of 6 months this influx was reduced in controls and mtNOD mice. The cytosolic calcium influx was highest in 12 months old controls with a significant increase compared to 6 months old mice. In contrast, in mtNOD mice cytosolic calcium influx in 9 and 12 months old animals was comparable to 3 months old animals. Interestingly, the age dependent pattern of glucose-induced cytosolic calcium influx was in line with the gene expression of UCP2 suggesting a relationship of both parameters. At the age of 3 months mitochondrial calcium influx was significantly higher in mtNOD mice compared to controls. A slight increase of the mitochondrial calcium influx was observed in controls during aging, whereas there is a strong and significant decrease in mtNOD mice. Notably, the age dependent decline of the glucose-induced mitochondrial calcium influx after fasting occurred inversely to the increase in the mitochondrial membrane potential in hepatocytes. Gene expression of the MCU was comparable in 3 month old animals. It remained unchanged during aging in controls, whereas a significant increase was observed in 9 and 12 versus 3 months old mtNOD mice.

Conclusion: The mitochondrial calcium influx declined at the age of 9 months in both strains compared to the cytosolic calcium influx in hepatocytes. However, control and mtNOD mice adapted in a different manner. Control mice increased the cytosolic calcium influx, whereas mtNOD up-regulated MCU gene expression. Our data suggest reduced sensitivity to external triggers including insulin in mtNOD mice in age. Thus, the C57BL/6NTac-mtNOD/AtJ mouse strain represents an interesting model to study trigger effects of age dependent mitochondrial regulation in the pathogenesis of T2DM.

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Deletion of the type 2 diabetes-associated gene product STARD10 in mice impairs insulin secretion and action

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Background and aims: Genome-wide association studies have identified more than 90 loci associated with type 2 diabetes risk. Recently-identified common genetic variants in the ARAP1/STARD10 locus affect fasting proinsulin levels (rs11603334) and glucose-induced insulin secretion (rs1552224) in man. The implicated single nucleotide polymorphisms (SNPs) are located in the 5'UTR of the ARAP1 gene and ~40 kb downstream of the STARD10 gene. The product of the latter gene, StAR-related lipid transfer (START) domain containing 10, is involved in the intracellular transport of phospholipids. Here, we show that global deletion of the StarD10 gene in mice leads to marked defects in both insulin secretion and insulin sensitivity.

Materials and methods: Null alleles of StarD10 (Tm1a), bearing a LacZ-neo-STOP cassette downstream of exon 2, were provided through the International Mouse Phenotyping Consortium (IMPC) and maintained on a C57BL6 background. Loss of StarD10 mRNA from liver and islets was confirmed by qRT-PCR. Transgenic mice were generated by pronuclear injection of DNA encoding FLAG-tagged StarD10 under tetracycline-regulated promoter control. Crossing with RIP7-rtTA mice, expressing the reverse tetracycline transactivator under the control of the rat insulin promoter, allowed ß-cell-specific, tetracycline-inducible expression in adult mice, and was confirmed by qRT-PCR analysis and

Western (immuno-) blotting. Measurement of glycemic parameters in vivo, islet isolation, intracellular Ca2+ imaging and insulin secretion, were performed using standard protocols.

Results: Compared to wild type (WT) littermates, mice deleted globally for StarD10 (KO), and assessed at 14-16 weeks of age, displayed increased fed glycemia (WT, 8.1±0.1 mmol/L vs KO, 9.7±0.3, p< 0.001), intraperitoneal glucose (1 g/kg; AUC: WT, 1169±34 WT vs KO, 1274±30 mmol/l*min., p=0.034) and insulin (0.75 U/kg; AUC: WT, 875±62 vs KO, 670±34 pM*min., p<0.01) tolerance. Glucose- (3 g/kg) stimulated insulin secretion was also sharply reduced in vivo in knockout mice (AUC: WT, 50.37±22.53 vs KO, 40.57±6.65 pM*min., p=0.026). Correspondingly, glucose- (17 vs 3 mM) induced insulin secretion (WT, 0.86±0.05%; KO, 0.21±0.03% p<0.001) and cytoplasmic Ca2+ increases, assessed using the entrappable fluorescent probe fluo-2, and confocal imaging of whole islets (fold-change: WT, 23.5%±1.7 fold change vs KO, 15.4%±1.9, p<0.001) were reduced in islets from KO mice versus those from WT littermates. Transgenic StarD10 male mice displayed a tendency towards reduced glucose tolerance (AUC: WT, 1251±36; Tg: 1397±69 mmol/l*min., p=0.076) at 16 weeks, but were otherwise normoglycemic. Correspondingly, adenovirus-mediated overexpression of StarD10 in mouse islets exerted no effect on insulin secretion stimulated by glucose (17 vs 3 mmol/L) or KCl (30 mM/L). However, overexpression of StarD10 potentiated apoptosis induced by staurosporine (1 μM) in mouse islets, as assessed by Terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) staining (control: 1.85±0.23%; StarD10: 4.60±0.62%; p<0.0001).

Conclusion: These data indicate that lowered expression of StarD10 gene in risk allele carriers may contribute to exaggerated disease risk, and may act both via changes in insulin secretion and action.

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A novel gene, R3h domain containing-like, might play a role in muscle regeneration

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Background and aims: Skeletal muscles are fundamentally important not only for physiological activities but also for maintaining normal glucose homeostasis. Sarcopenia, a loss of muscle mass that is often seen in the elderly patients with type 2 diabetes, might be caused by the disability of muscles to regenerate, thus inducing frailty and insulin resistance. Although the number of geriatric patients with type 2 diabetes has increased worldwide, the mechanisms underlying sarcopenia and muscle regeneration have not yet been understood. We previously reported that the R3h domain containing-like (R3hdml) gene, a podocyte-specific transcript, inhibited TGF-β-induced p38 MAPK signaling. Furthermore, it has been reported that TGF-β-induced p38 MAPK signaling is important for muscle regeneration. In addition, a meta-analysis of genome-wide association studies has identified a gene locus including the R3hdml gene as a risk allele for type 2 diabetes in East Asians. Therefore, we have begun investigating the function of R3hdml in skeletal muscle, particularly focusing on muscle regeneration.

Materials and methods: R3hdml expressions in skeletal muscle regeneration model mice [10 mM cardiotoxin (CTX) was injected in the tibialis anterior muscle or quadriceps] were evaluated by real-time polymerase chain reaction (qPCR) and western blotting (WB). C2C12 cells, a mouse myoblast cell line, were differentiated into myotubules in the presence of 2% horse serum for 5 days; thereafter, the expressions of R3hdml as well as transcription factors that related to muscle differentiation were examined by qPCR and WB. R3hdml was then silenced with small interfering RNA (siRNA) and the effects of R3hdml on the formation of myotubules were examined. The expressions of R3hdml in skeletal muscle were also studied in wild-type control mice, db/db mice fed or not fed a high-fat diet for 7 weeks.

Results: R3hdml was not detected in skeletal muscle under physiological conditions. However the expressions of R3hdml were increased upon injury of skeletal muscle with CTX indicating that R3hdml expressions were induced by skeletal muscle regeneration. Neither R3hdml mRNA nor protein were detected in undifferentiated C2C12 cells, however their expressions increased along with differentiation of C2C12 cell. The expression of Pax 7, a marker for early muscle differentiation, was decreased and myotubule formation was attenuated by 26.1% when the R3hdml gene was silenced by siRNA in C2C12 cell. Finally, the expressions of R3hdml were also increased in skeletal muscle under diabetic conditions where chronic inflammation and muscle regeneration took place to a lesser extent compared to CTX model.

Conclusion: Since the disability of muscles to regenerate were often observed in geriatric patients with type 2 diabetes, R3hdml, a novel skeletal muscle expressed gene, may play a role not only in glucose metabolism but also in sarcopenia development through the modulation of skeletal muscle regeneration.

PS 012 Prevalence of type 2 diabetes and related factors around the world

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Evaluation of clinical risk factors for diabetes screening in a large cohort of subjects in a primary care setting

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Background and aims: Timely diagnosis of diabetes remains a global challenge as many subjects with diabetes are unaware of their condition. A simple diagnostic strategy which could help decrease the rate of undiagnosed diabetes is needed. The aim of this study was to evaluate the performance of classical clinical risk factors in screening for diabetes in a large group of individuals in a primary care settings.

Materials and methods: In 2014 we conducted a primary care physician-based nationwide screening programme aiming at identifying individuals with undiagnosed diabetes or prediabetes. A total of 561 primary care physicians (PCPs) took part in the programme, and 21 726 subjects were enrolled. The inclusion criteria were following: no earlier diagnosis of diabetes or prediabetes (i.e. IFG and/or IGT) and the presence of at least one classical risk factor: age>45 yrs, family history of diabetes, sedentary lifestyle, smoking, presence of fatty liver disease, hypertension, hyperlipidemia, coronary artery disease, peripheral artery disease, obstructive sleep apnoea syndrome, polycystic ovary syndrome, history of stroke, gestational diabetes or having a child with birth weight> 4 kg as well as BMI>25 kg/m2 or waist circumference>80 cm in women or 94 cm in men. All subjects underwent fasting plasma glucose (FPG) measurement, and those individuals in whom its value exceeded 125 mg/dl were referred to have second FPG measured at least one week later, while those in whom the initial FPG measurement was between 100 and 125 mg/dl had an oral 75 g glucose tolerance test (OGTT) performed.

Results: The prevalence of all risk factors assessed in the examined cohort is presented in Table 1. In the whole group diabetes was diagnosed in 4221 (19.4%), and prediabetes in 5829 (26.8%) of the subjects, thus 10 050 (46.3%) of the individuals in the study were found to have any degree of glucose metabolism abnormalities. The majority of diabetes cases (2825/4221, i.e. 66.9% of the diagnosed group) were diagnosed based on two FPG measurements >125 mg/dl. In 6265 (28.8%) of the subjects the concomitant presence of three main unmodifiable risk factors, which are also simply identified in PCP setting, i.e.: age>45 years, family history of diabetes and BMI>25 kg/m2 were found. In this subgroup 1989 (31.7%) individuals were found to have diabetes and 2101 (33.5%) to have prediabetes, thus, the rate of glucose metabolism abnormalities reached 65.2% in these subjects. The odds ratio for diabetes in this subgroup was 2.757 (95% CI 2.57-2.957), and for diabetes or prediabetes it was 2.998 (2.818-3.189), as compared to those with only one or two coexisting features.

Conclusion: Our data support the use of classical clinical risk factors in screening for diabetes in a primary care setting. Particular effort should be put into testing individuals older than 45 years who have a family history of diabetes and are overweight.

	N	*%*
Age> 45 yrs	17 740	81.7
Sedentary lifestyle	13 814	63.6
Hypertension	13 427	61.8
BMI>25 kg/m2	13 379	61.6
Hyperlipidemia	12 842	59.1
Waist circumference >80 (F) or >94 cm (M)	12 728	58.6
Family history of diabetes	10 890	50.1
Smoking	8 635	39.7
Alcohol abuse	3 122	14.4
Coronary artery disease	4 803	22.1
Fatty liver disease	4 345	20.0
Peripheral artery disease	2 794	12.9
Obstructive sleep apnoea	2 041	9.4
History of stroke	1 344	6.2
Giving birth to child >4 kg	2 118	19.6 (of women)
History of gestational diabetes	1 820	16.8 (of women)
Polycystic ovary syndrome	1 267	11.6 (of women)

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One in four subjects above the age of 20 years could have diabetes in Bangalore

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Background and aims: The number of people with diabetes in India is increasing due to population growth and aging, urbanization, increasing prevalence of obesity and physical inactivity. Despite this, there are no good studies on the prevalence of diabetes and risk factors associated with it. This cross-sectional population survey was undertaken to determine the prevalence of diabetes and impaired glucose in an urban population of Bangalore, India and its association with dietary habits.

Materials and methods: Subjects were invited to attend screening for blood glucose, blood pressure, dietary history and anthropometric measurement at various venues across the city of Bangalore. Local publicity in and around the venue were done few days prior to the event. It was a free event and people could come anytime without appointment.

Results: 3882 subjects were screened over a period of 15 months at 32 venues. Out of this 29 subjects below the age of 20 years and 133 subjects without blood glucose data were excluded from analysis. Diabetes (DM) was defined as history of self-reported condition or random blood glucose of 11.1 mmol/L (200 mg%) or more. Impaired glucose (IG) was defined as those subjects with diabetes or random blood glucose of 7.8 mmol/L (140 mg%) or more. Out of 3720 patients the prevalence of DM was 24.5% (n=913) and that of IG was 30.5% (n=1134). Out of these 846 (21.8%) subjects were already known to have diabetes. Of the remaining 2874 people, 67 (2.3%) had possible diabetes and 288 (10.0%) had IG, which were discovered on the day. Prevalence of both diagnosed and undiagnosed diabetes was high in older subjects (Table 1). There were no relation (p>0.05) in blood pressure (BP), weight and Body Mass Index (BMI) in this population between subjects with or without diabetes. Regarding dietary habvit, there was no difference in BP, weight, BMI and blood glucose between vegetarian and non-vegetarian subjects. Interestingly there were significantly more males in non-vegetarian group (53.1% vs 60.4; p=0.01).

Conclusion: Our data suggest that DM & IG is present in about a quarter of the urban Indian population. 10% of population above the age of 20 years have glucose abnormalities of which they are not aware. Therefore regular screening from the general population in urban area is necessary to avert or delay the progression to DM and reduce diabetes-related morbidity and mortality.

Age Group (Years)	20 - 29	30 - 39	40 - 49	50 - 59	60 - 69	> 70
Total number	730	872	766	694	500	158
Diagnosed Diabetes	19 (2.6%)	83 (9.5%)	185 (24.2%)	262 (37.8%)	219 (43.8%)	78 (49.4%)
Not known to have Diabetes	711	789	581	432	281	80
Undiagnosed Impaired Glucose	20 (2.8%)	59 (7.5%)	77 (13.3%)	76 (17.6%)	43 (15.3%)	13 (16.3%)
Undiagnosed Diabetes	2 (0.3%)	9 (1.1%)	22 (3.8%)	18 (4.2%)	11 (3.9%)	5 (6.3%)

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The prevalence of hypothyroidism is gender-neutrally increased by autoimmune diabetes but unaffected by type 2 diabetes: the population-based HUNT study in Norway

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Background and aims: Associations between autoimmune diabetes and hypothyroidism are known, but insufficiently characterized. Some previous studies suggest that type 2 diabetes is associated with hypothyroidism. The aims were to analyse the impact of autoimmune diabetes on the prevalence of hypothyroidism specifically with regard to gender, and to test for associations between type 2 diabetes and hypothyroidism.
Materials and methods: Cross-sectional population-based studies of adults in two surveys of the HUNT Study. Data were available for 34,287 participants of HUNT2 (1995-97) and 48,598 participants of HUNT3 (2006-08). Prevalence of hypothyroidism was assessed by self-report, TSH measurements and, for HUNT3, linkage with the Norwegian Prescription Database.
Results: In HUNT2, the prevalence of hypothyroidism was in nondiabetics 2.7 fold higher among women (8.3%) than in men (3.1%). Autoimmune diabetes increased the prevalence in women to 15.8% and in men to 10.9%. Thus, compared with having no diabetes, autoimmune diabetes was associated with a 64% higher age-adjusted prevalence of hypothyroidism among women (prevalence ratio [PR] 1.64, 95% confidence interval [CI] 1.15-2.34) but an almost three times higher age-adjusted prevalence of hypothyroidism among men (PR 2.93, 95% CI 1.90-4.52). Type 2 diabetes was not associated with the prevalence of hypothyroidism. In HUNT3 the associations were broadly similar to those in HUNT2.
Conclusion: 1) The enhancing effect of autoimmune diabetes on the prevalence of hypothyroidism was gender neutral, thus decreasing the difference in prevalence between women and men, 2) type 2 diabetes did not incur increased risk of hypothyroidism, thus contradicting the notion that increased surveillance for hypothyroidism is necessary in patients with type 2 diabetes.
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Prevalence of diabetes mellitus and its associated risk indicators in a peripheral region of Bangladeshi population

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Background and aims: A large racial heterogeneity in diabetes suggests that there is a need of conducting epidemiological studies in different communities. There is scarcity of such studies in Bangladesh, particularly in remote rural areas. The objectives of this study were to estimate the prevalence of diabetes and to identify its associated risk indicators in a rural population of Bangladesh.
Materials and methods: In 2008, a large cohort was initiated that followed up in 2011 where we screened a proportion (subset of 63708 participants, aged 31-74) of this cohort for cardiovascular disease related risk factors using WHO CVD Risk Management Package 2002' under a cross-sectional design. The study participants (n=1732, male=834 and female=898) were randomly selected from the subset and screened in a camp setting. Diabetes was diagnosed based on WHO study group Criteria after a 2-hr OGTT, body mass index (BMI), waist-hip ratio (WHR), blood pressure and lipid profile were also estimated.
Results: The prevalence of diabetes was found to be 14.2% (95%CI 12.6-15.9) and that of impaired glucose tolerance (IGT) and impaired fasting glucose (IFG) was 7.9 (95%CI 6.7-9.3) and 4.3 (95%CI 3.4-5.3) respectively. The prevalence of diabetes and impaired glucose regulation differed between males and females ($p<0.005$), but, both increased with age in males as well as in females. After adjusting for potential confounders WHR, hypertension, smoking, triglyceride and cholesterol were found as significant independent risk indicators for the occurrence of diabetes in this population.
Conclusion: Fairly a high prevalence of diabetes was observed in this remote rural Bangladeshi population. Preventive programs, particularly targeted to WHR management, lipid profile control through lifestyle modification should be strengthened in rural areas.Most of the existing research conducted in Bangladeshi rural settings has been in rural areas adjacent to urban spaces, whereas a remote rural area is one geographically and behaviorally distinct from urban influences.

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Nation-wide estimated prevalence of diabetes in Greece: identification of patients with prescribed pharmacological treatment among 94.5% of the total Greek population

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Background and aims: The prevalence of diabetes in Greece is virtually unknown, despite several regional, small-scale epidemiological studies. Since Greece is among the first countries that developed a nationwide electronic drug prescription system, we aimed to identify all patients with prescribed pharmacological treatment for diabetes.
Materials and methods: The Business Intelligence database system of the Greek National Organization for Healthcare Services Provision (EOPYY), which includes big data from e-prescriptions and handwritten prescriptions, was used to provide analytics on these patients (date of birth and gender, based on the unique citizens' social security numbers and the relevant ICD-10 codes). Permission for use of anonymized data was obtained by the administration of EOPYY together with the positive recommendation of the General Secretariat for Public Health of the Greek Ministry of Health, in accordance to the national legislation on Personal Data Protection.
We defined people with diabetes as those who received at least two prescriptions with an ICD-10 code relevant to diabetes (E10, E11 or O24), dispensed by Greek pharmacies between January 1st 2014 and December 31st 2014.
Results: This analysis included 10,222,778 individuals (5,012,226 (49.03%) males and 5,210,552 (50.97%) females, representing 94.5% of the total Greek population, according to the 2011 national census)

and revealed that the prevalence of drug-prescribed diabetes is 7.21% (736,900 individuals). There is no difference between males and females (360,335 vs. 376,565; 7.19% vs. 7.23%, respectively). There is a strong positive association with age, increasing from 0.12% at the age group of 5-14 years, to 3.45% at the age group of 45-54 years and up to 32.11% in those older than 75 years (p for trend<0.001).
Conclusion: In this first nation-wide analysis of data for the prevalence of drug-prescribed diabetes in Greece, it was found that its prevalence is 7.21%, one of the highest in Europe. It is presumed that these data provide reliable estimates of descriptive epidemiology of diabetes, since the analysis of such big databases largely overrides incorrect diagnosis-associated limitations that an electronic prescription system may have.

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Trends in diabetes mellitus in Greece from 2003 to 2014 with a future projection. The SALAMINA study

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Background and aims: Diabetes mellitus (DM) is becoming a worldwide epidemic. The International Diabetes Federation (IDF) recently readjusted its estimations on DM, predicting a higher prevalence, and, according to is data, the prevalence in Greece should be 10.2% for 2013. The aim of the present study is to examine DM prevalence in the adult population of Salamina island, where relevant studies have been carried out in 2003 and 2006 with the same methodology, as well as to estimate DM prevelance in Greece for 2032 according to most recent data.
Materials and methods: Data was derived from three consecutive population-based surveys conducted in Salamina, during three election days (13 October 2002, 15 October 2006 and 25 May 2014). In all three surveys the same selection procedure was used and data was collected using the same questionnaire, completed by almost the same team of interviewers. There were 2805 randomely selected adults (> or =20 years) in 2002, 3478 in 2006 and 2527 in 2014 of similar age and sex distribution to the target population. Demographic and anthropometric data relevant to DM (duration, medication, family history) as well as presence of cardiovascular risk factors or other comorbidities were recorded. Statistical analysis was carried out with SPPS v21.0 (Chicago, Ilinois, USA).
Results: There was no stastistically signifficant difference in age in all three surveys (p=0.251).The overall crude prevalence of diabetes increased significantly, from 245 (8.7%) in 2002 to 358 (10.3%) in 2006 and to 318 (12.6%) in 2014 (p=0.028). The rates per age group for the 2002, 2006 and 2014 surveys were: 0.4%, 0.6% and 0.3% (p=0.128) in individuals aged 20-29, 3.6%, 3.7% and1.9% (p=0.048) in individuals aged 30-39%, 7.3%, 7.2% and 9.4% (p=0.033) in individuals aged 40-49, 21.6%, 21.2% and 26.4% (p=0.026) in individuals aged 50-59, 31.8%, 31.2% and 30.2% (p=0.214), in individuals aged 60-69, and 35%, 36% and 31.8% (p=0.030) in individuals aged over 70. The mean age for diabetes diagnosis was 57.6±11.9 while the mean diabetes duration was 12.3 years for 2014 comparable with the other two surveys (p=0.086). Only 3.2% of participants reported they were under dietary treatment in 2014 (p=0.016), while more diabetes patients were under insulin treatment (26.4%) compared to those in 2002 and 2006 (p=0.012). 79.7% of the individuals who answered the questionnaire had had laboratory tests for glucose levels and lipids during the last 12 months in 2014 which is a signifficantly higher rate compared to higher the rest of the surveys (p=0.022). Using a Markov model to estimate the future diabetes prevalence, a dramatic increase in diabetes prevalence was predicted for 2032 (19.8%)
Conclusion: DM prevalence is higher than the one predicted according to older studies, basically confirming IDF's predictions. An increase of prevalence is observed in younger ages and, according to the prediction for 2032, approximately one out of five people in Greece will have diabetes which indicates a dramatic increase in diabetes prevalence among Greek adults.

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Screening for prediabetes in adults aged 45-55 years in primary care setting should be universal rather than risk-factors based

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Background and aims: Current guidelines recommend yearly screening towards glucose metabolism disturbances in individuals aged >45 years, in whom risk factors for diabetes development are present. However, as prediabetes is a clinically silent condition, its early diagnosis remains a challenge. Middle aged professionally active adults are particularly non-compliant with screening procedures. We conducted a study aiming at assessing the use of risk factors-based screening for impaired fasting glucose (IFG) in primary care setting.
Materials and methods: 5276 diabetes-free individuals (2963 women; 56% women) aged 45-55 years, who had at least one risk factor for diabetes development took part in a nationwide diabetes screening programme conducted at primary care level.The participants underwent full medical examination and had their fasting plasma glucose (FPG) assessed during a visit at their primary care physician office.
Results: Normal FPG (>100 mg/dl) was found in 1421 (27%), while IFG (100-125 mg/dl) in 1860 (35%) persons. FPG>125 mg/dl was found in 1995 (38%) individuals, and they were excluded from further anaysis. The prevalence of well established risk factors for glucose metabolism disturbances in IFG and NFG individuals is presented in the Table. Sedentary lifestyle, family history of diabetes and newly diagnosed hypertension were similarly prevalent in both studied groups, however even when differences in prevalence of other risk factors reached statistical significance, the actual difference was relatively small, with the exception of the history of IFG as it was found almost twice as often in persons with IFG as compared to those with normal fasting plasma glucose.
Conclusion: In conclusion, the prevalence of IFG in adults aged 44-55 years with risk factors is high. Prediabetes screening programmes conducted in high risk population in this age group should be universal rather than risk factors based. In particular, they must not be based on family history of diabetes or sedentary lifestyle as these factors are equally often present in persons with IFG as well as normal fasting glucose.

	IFG (n=1860)		NFG (n=1421)		Absolute prevalence difference (APD)	Relative prevalence difference (APD/IFG in %)	p
	n	%	n	%	%	%	
Waist circumference ≥80 cm (women) or ≥94 cm (men)	1666	89.6	1133	79.7	9.9	11.0	<0.001
BMI ≥25 kg/m²	1653	88.9	1167	82.2	6.7	7.5	<0.001
Sedentary lifestyle	1473	79.2	1109	78.1	1.1	-	NS
Family history of diabetes	1188	63.9	920	64.7	0.8	-	NS
Lipid disorders	1185	63.7	827	58.2	5.5	8.6	<0.01
Hypertension – treated	810	43.5	503	35.4	8.1	18.6	<0.001
Cardiovascular disease	737	39.6	456	32.1	7.5	18.9	<0.001
Hypertension – newly diagnosed	458	24.6	349	24.6	0	-	NS
History of IFG	443	23.8	175	12.3	11.5	48.3	<0.001

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Racial differences in glycaemic marker levels among individuals without diabetes: the coronary artery risk development in young adults study

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Background and aims: Prior studies have reported that glycemic marker levels vary by race and ethnicity among individuals without diabetes. However, it is unclear whether these racial differences occur among both individuals at high-risk for diabetes (i.e., prediabetes) and those with normal glycemic levels and, if so, whether these differences are clinically meaningful. The aim of this study was to assess glycemic marker levels (i.e., fasting glucose, 2-hour post-challenge glucose from OGTT, HbA1c, glycated albumin, fructosamine, and 1,5-anhydroglucitol) by race among individuals with and without prediabetes in a cohort study of adults in the United States.
Materials and methods: The Coronary Artery Risk Development in Young Adults (CARDIA) Study is a prospective cohort study of 5,115 African American and white adults, age 18-30 years at baseline (1985-86), from four field centers. This analysis included 2,465 participants who attended the year 20 examination (2005-06), had glycemic marker data available, and did not have diabetes (defined as fasting glucose ≥126 mg/dL, 2-hour post-challenge glucose≥200 mg/dL, HbA1c≥6.5%, or use of diabetes medications). Prediabetes was defined as a fasting glucose of 100-125 mg/dL, 2-hour post-challenge glucose of 140-199 mg/dL, or HbA1c of 5.7-6.4%. Multiple regression models were used to assess the association of race with each glycemic marker, stratified by prediabetes status.
Results: The mean age of participants was 45 years. Overall, 42% of participants were African American, 55% were female, and 39% had prediabetes. Among those with prediabetes, African Americans had lower fasting glucose (-1.59 mg/dL), but higher HbA1c (0.26%), glycated albumin (0.82%), and fructosamine (8.96 μmol/L) compared with whites after multivariable adjustment. No racial differences were observed for 2-hour post-challenge glucose or 1,5-anhydroglucitol among those with prediabetes (Table). Similarly, among normoglycemic individuals, African Americans had lower fasting glucose (-2.24 mg/dL) and 1,5-anhydroglucitol (-0.66 μg/mL), but higher HbA1c (0.04%), glycated albumin (0.61%), and fructosamine (5.97 μmol/L) compared with whites. No racial difference was observed for 2-hour post-challenge glucose among normoglycemic individuals (Table).
Conclusion: Racial differences in glycemic marker levels were observed across the glycemic spectrum, among both individuals with prediabetes or normal glycemia. These differences were small and not clinically divergent, although African Americans tended to have slightly more adverse glycemic levels than whites for most glycemic markers. Further investigation of the potential prognostic implications, if any, are warranted.

Table. Glycemic marker levels for African Americans and whites, stratified by prediabetes status in the CARDIA Study

	Prediabetes			Normal Glycemia		
	Least Square Means		β (95% CI)	Least Square Means		β (95% CI)
Glycemic Marker	*African American (N=482)*	*White (N=483)*	*African American versus White*	*African American (N=562)*	*White (N=938)*	*African American versus White*
Fasting glucose (mg/dL)	100.57	102.16	-1.59* (-2.86, -0.32)	89.01	91.25	-2.24* (-2.99, -1.50)
2-hr post-challenge glucose (mg/dL)	116.02	118.87	-2.85 (-7.70, 2.00)	93.57	91.97	1.60 (-0.97, 4.16)
HbA1c (%)	5.76	5.50	0.26* (0.21, 0.31)	5.25	5.22	0.04* (0.00, 0.07)
Glycated albumin (%)	12.96	12.14	0.82* (0.66, 0.99)	12.73	12.12	0.61* (0.50, 0.73)
Fructosamine (μmol/L)	228.29	219.33	8.96* (6.46, 11.46)	228.41	222.44	5.97* (4.13, 7.80)
1,5-anhydroglucitol (μg/mL)	19.91	19.87	0.04 (-0.78, 0.87)	18.80	19.46	-0.66* (-1.30, -0.03)

*p<0.05
Models adjusted for age, sex, education, study field center, smoking, body mass index, systolic blood pressure, use of antihypertensive medications, total cholesterol, HDL cholesterol, use of lipid-lowering medications

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Changes in the use of antidiabetic, antihypertensive, and hypolipidaemic drugs within 30 months after Roux-en-Y gastric bypass surgery

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Background and aims: In the majority of patients undergoing Roux-en Y gastric bypass (RYGB) surgery, obesity-related complications such as diabetes and hypertension may improve or remit, with concomitant reduction or discontinuation of medications. Relapse of these complications may occur in some patients after a few years, but studies with long-term follow-up are sparse. We investigated long-term changes in use of antidiabetic, antihypertensive, and hypolipidemic drugs among patients, who underwent RYGB surgery. Furthermore, we compared changes in use of these drugs among RYGB patients with that in a matched comparison cohort of persons without RYGB.
Materials and methods: The study included all 9,908 individuals undergoing RYGB in Denmark during 2006-2010 and a 10:1 matched general population comparison cohort of 99,080 persons, using age, gender, and RYGB/index date as matching variables. We ascertained complete data on medication use from population-based medical databases. All subjects were followed from 6 months prior to the RYGB/index date until 30 months following this date. We computed the proportion of subjects in each cohort with current drug use and prevalence ratios (PRs) comparing use 6 months before and 30 months after the RYGB/index date, using Poisson regression analysis.
Results: The mean age in both cohorts was 40.6 years; 21.7% were male. Figure 1 shows changes in drug use prior to and following the RYGB/index date. Among the RYGB patients 16.5% used antidiabetic drugs 6 months before surgery, while 4.7% were on antidiabetic drugs 30 months after surgery [PR=0.27 (95% confidence interval (CI); 0.27, 0.27)]. In the comparison cohort 2.0% used antidiabetic drugs 6 months before the index date and 2.4% used them 30 months after the index date [PR=1.25 (95% CI; 1.24, 1.25)]. Like antidiabetic drugs, use of hypolipidemic drugs decreased in the RYGB cohort: 14.4% used them 6 months prior to surgery vs. 7.3% 30 months after surgery [PR=0.49 (95% CI; 0.48, 0.49)]. In the comparison cohort 3.8% used hypolipidemic drugs 6 months before the index date, while 5.4% used them 30 months later [PR=1.41 (95% CI; 1.41, 1.42)]. For antihypertensives, 43.2% of RYGB patients were users prior to their surgery compared with 26.1% 30 months after surgery [PR=0.67 (95% CI; 0.67-0.67)]. In the comparison cohort 10.1% used antihypertensives 6 months before and 11.9% 30 months after the index date [PR=1.22 (95% CI; 1.22 -1.22)].
Conclusion: In this nationwide population-based study, we observed large reductions in use of antidiabetic, hypolipidemic, and antihypertensive drugs occurring rapidly after RYGB surgery. This reduction was maintained over 30 months of follow up, with little indication of relapse as indicated by drug use. Nonetheless, use of these drugs remained higher among RYGB patients than in the matched comparison cohort.

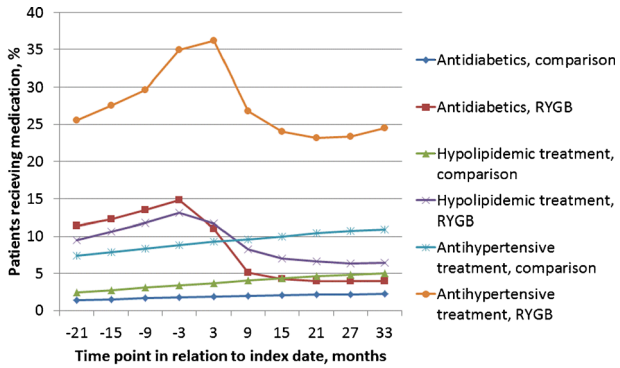

Figure 1. Use of antidiabetic, antihypertensive, and hypolipidemic drugs among 9,908 patients who underwent Roux-en Y Gastric Bypass surgery and 99,080 persons in a matched comparison cohort, from 6 months before and until 30 months after the surgery/index date.

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Impact of pre-existing cardiovascular disease on treatment initiation with SU in Denmark: a registry based population study

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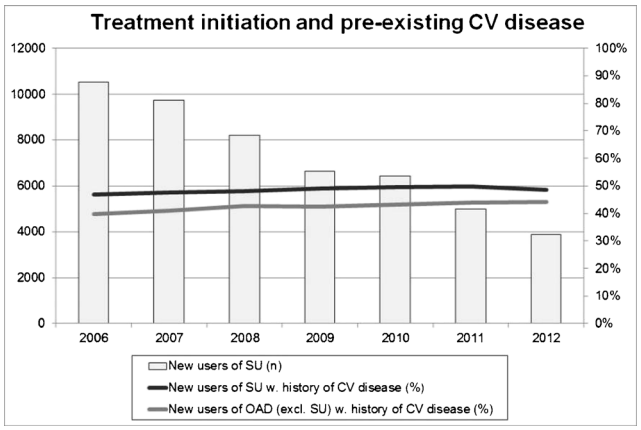
Background and aims: Uncertainties about the cardiovascular (CV) safety of sulphonylureas (SU) are reflected in package labels and treatment guidelines. Utilizing Danish national registries, we examined whether this has affected the prescription patterns for SU in Denmark.
Materials and methods: We analysed the development of new SU users (initiation of SU monotherapy or SU supplementation to any other anti-diabetic drugs) from 2006 to 2012. History of CV disease (ICD-10: I00-I99) 0-13 years prior to initiating SU treatment was assessed in this population and among new users of any other oral anti-diabetic drug (OAD). Since there has been speculation that gliclazide might be associated with a more benign CV profile than other SU´s an additional analysis was conducted where gliclazide was excluded from the SU group. Furthermore the analysis was done separately for two sub-groups of CV disease, namely of coronary heart disease (ICD-10: I20-I25, I46) and myocardial infarct (ICD-10: I21-I22).
Results: We observed a reduction in the number of new SU users during the period from 10.530 to 3.876, representing a decrease of 63.2% (figure 1). The relative proportion of new SU users with pre-existing CV disease increased numerically from 2006 to 2012 (46.9% vs. 48.7%; p=0.06). During the entire period the proportion of new SU users with pre-existing CV disease remained higher than the corresponding proportion for other OAD´s (figure 1, P<0.001 all years). It did not alter the result to exclude the new users of gliclazide (8-12% of the new users of SU). The proportion of new SU users with pre-existing coronary heart disease was also found to be higher than the corresponding proportion for other OAD´s (P <0.05 all years). For patients with pre-existing myocardial infarction the proportion was higher all years although not significant in years 2009 and 2012.
Conclusion: In Denmark the initiation of SU treatment declined from 2006 to 2012. The proportion of new SU users with pre-existing CV disease remained high, approaching 50% over the entire period and was larger than the corresponding proportion for new users of other OADs. Irrespective of definitions of SU group and type of CV disease, we found that initiation of SU therapy was more common among patients with CV disease compared to patients without history of CV disease. This indicates that pre-existing CV disease did not negatively impact clinicians' usage of SU.



Supported by: MSD Denmark

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Medication persistence and adherence in type 2 diabetes patients using fixed- versus loose-dose combination products

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Background and aims: To compare medication persistence and adherence between type 2 diabetes mellitus (T2DM) patients using fixed-dose combination (FDC) and loose-dose combination (LDC) products.
Materials and methods: A retrospective cohort study design using United States' administrative claims data from Jan 1, 2009 through Dec 31, 2013 was employed. Patients with a prescription for at least 1 FDC or 2 LDC medications filled (on the same date) between Jan 1, 2010 and Dec 31, 2012 were identified as the target population, with the index date defined as the fill date. The identified population was restricted to patients with a T2DM diagnosis (non-diagnostic medical claim with ICD-9 CM codes of 250.x0, 250.x2) and at least 1 additional oral anti-diabetic prescription (of the same regimen [FDC/LDC] as the index prescription) within 12 months following the index date. A 12-month pre-index period was used to obtain baseline characteristics. Persistence (no ≥30 day gap) and adherence (medication possession ratio [MPR]) were assessed while the patients were on the index regimen up to a maximum of 1-year (follow-up period) starting on (and including) the index date. Patients on triple therapy over follow-up were excluded. The odds of persistence and adherence [MPR ≥0.8] to therapy were assessed using logistic regression after adjusting for baseline covariates and the hazard of non-persistence was similarly assessed using a Cox proportional hazards model.
Results: Of the 23,361 patients identified, 12,590 (53.9%) were on FDC therapy and 10,771 (46.1%) were on LDC therapy. The majority of patients were male (62.4%) with a mean (SD) age of 54.9 (11.2) years. The mean (SD) number of unique prescription classes was 2.6 (2.9). A statistically significantly greater proportion of FDC patients were persistent (32.1% vs 26.6%, p<0.0001) and adherent to therapy (57.0% vs 50.7%, p<0.0001) when compared to LDC patients. Average duration of persistence was significantly longer among the FDC patients when compared to the LDC patients (207.1 [±136.3] vs 186.3 [±130.0] days, p<0.0001). After adjusting for baseline characteristics, the odds of non-persistence in therapy were 21% lower among T2DM patients on FDC therapy when compared to those on LDC therapy (OR=0.79, 95% CI

[0.74, 0.85], p<0.0001). The odds of being adherent to therapy (MPR/DTMPR ≥0.8) were 28% higher among T2DM patients on FDC therapy when compared to those on LDC therapy (OR=1.28, 95% CI [1.20, 1.36], p<0.0001). Furthermore, T2DM patients on FDC therapy had a 12% lower risk of being non-persistent at any point in time compared to those on LDC therapy (HR=0.88, 95% CI [0.84, 0.91], p<0.0001).
Conclusion: Management of T2DM using FDC therapies provides a compliance benefit to patients when compared to LDC therapies, most likely due to reduced medication burden. This compliance benefit may translate to better glycemic control and possible reduction in healthcare utilization and costs due to improved disease management.

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Use of sodium-glucose co-transporter 2 inhibitors among US patients with type 2 diabetes

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Background and aims: Sodium-glucose co-transporter 2 inhibitors (SGLT-2i) are a new class of oral antihyperglycemic agents (AHA) used to manage hyperglycemia in patients with type 2 diabetes mellitus (T2DM). A retrospective study was conducted using MarketScan database to assess treatment patterns and characteristics of patients using SGLT-2i compared to other oral AHAs.
Materials and methods: Patients initiating oral AHA between 3/29/2013 and 6/30/2013 (index period) qualified for the study. The index date was set as the first oral AHA prescribed in the index period. Included were patients ≥18 years who had continuous enrollment of ≥1 year before and ≥90 days after the index date. Those with T1DM, gestational or secondary diabetes, pregnancy, or metformin use for polycystic ovarian syndrome were excluded. Patients were assigned to SGLT-2i and non-SGLT-2i cohorts based on their index drug. Between-group differences were assessed using Wilcoxon rank-sum tests for continuous variables and $\chi 2$ tests for categorical variables.
Results: Of the 69,511 patients in the study, 2.8% (n=1,917) initiated a SGLT-2i (canagliflozin was the only available SGLT-2i during the study period), and the rest were initiated on other oral AHAs. 30.2% patients were initiated on a 300 mg dose of canagliflozin and of the 68.4% who initiated 100 mg dose, 9.5% escalated to higher dose within 90 days. Compared to patients on other oral AHAs, canagliflozin patients were younger (55.1 vs. 56.1 years, p<0.01) and had a higher proportion of males (56.1% vs. 51.0%, p<0.01). In addition, canagliflozin patients used more concomitant medications during baseline (anti-hypertensive: 77.7% vs. 64.1%; lipid-lowering: 72.8% vs. 49.4%; NSAID: 26.8% vs. 22.8%; beta-blockers: 27.2% vs. 25.0%, all p<0.01) and already had more advanced antihyperglycemic treatment regimens compared to those on other oral AHAs. Furthermore, canagliflozin patients were also more likely to have micro-vascular complications (23.6% vs. 12.7%, p<0.01), including nephropathy (4.4% vs. 2.5%, p<0.01), as well as hypertension (62.0% vs. 56.5%, p<0.01) and hyperlipidemia (62.9% vs. 50.9%, p<0.01).
Conclusion: US patients initiating SGLT-2i were already on multiple AHAs and higher prevalence of comorbidities at baseline compared to patients initiating other AHAs.

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Patient characteristics, treatment and prevalence/incidence of the type 2 diabetes population in Sweden: a nationwide study from 2006 to 2013

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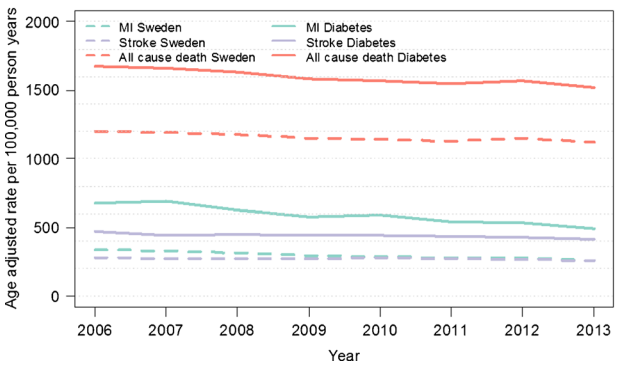
Background and aims: In a modern nation-wide setting characterise patterns of glucose lowering treatment and prevalence and incidence of type 2 diabetes (T2DM) in Sweden.
Materials and methods: Patients who dispensed any glucose-lowering drug (GLD) between 2006 and 2013 were identified in the Swedish Prescribed Drug Register and linked with the Swedish National Patient Register and the Cause of Death Register. Patients with type 1 diabetes and gestational diabetes were excluded. Information on comorbidities and medical events were collected from main and secondary diagnoses at discharge.
Results: In 2013 the prevalence of GLD- treated T2DM was 4.4% (n= 355 114) and incidence 368 per 100 000 patients (28 023). Prevalence of T2DM patients with GLD treatment increased during 2006-2013 by 51% while the incidence remained stable. The mean age was 67 years and 56% were male. Previous cardiovascular disease was present in 34%, micro-vascular disease in 26%, cancer in 19% and 4% had chronic kidney disease. Treatment with statin, antihypertensives and low dose aspirin was present in 56%, 70% and 35% respectively. The most frequently used GLDs were metformin (69%;), insulin (28%) and SU (17%). Incretin based GLDs were used in low proportions (DPP4; 6% and GLP-1; 3%). During 2006-2013 the use of SU decreased by 55% while insulin use increased by 29%. After direct age adjustment, the standardized risk ratio for myocardial infarction, stroke and all-cause mortality was 1.9, 1.6 and 1.4-fold respectively in T2DM patients (Figure).
Conclusion: The number of T2DM patients on glucose lowering drugs in Sweden has increased by 51% the last seven years despite stable T2DM incidence. The incidence of drug treated T2DM was more than twice as high as previously reported which illustrates the general problem to report on true incidence of T2DM accurately with high risk for underestimation. Despite improved medical care the last decade individuals with T2DM still have increased risk of cardiovascular disease and death compared to the general population. The use of SU is declining and seems to be replaced by insulin and less by incretin based glucose lowering drugs.

Figure 2: Age-adjusted rates of myocardial infarction (MI), stroke and all-cause mortality in patients with T2DM and the general Swedish population 2006-2013.



Supported by: AstraZeneca

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First-line and intensified treatment with glucose lowering drugs in the Swedish type 2 diabetes population

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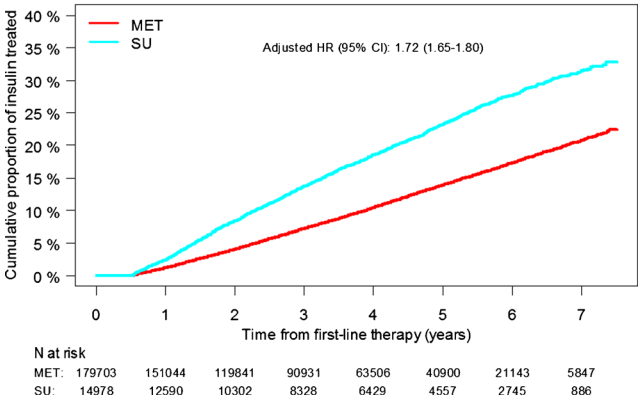
Background and aims: To investigate initiation of first-line glucose lowering drug (GLD) treatment, and subsequent GLD treatment, and prior cardiovascular disease (CVD) for all type 2 diabetes (T2DM) patients in Sweden.
Materials and methods: T2DM patients who were GLD naïve and initiated on their first-line GLD treatment during 2006-2013 were identified in the Swedish Prescribed Drug Register. Patients with type 1 diabetes or gestational diabetes were excluded. Patients were linked with the Swedish National Patient- and Cause of Death registry. Index date was defined as date of patients first GLD dispense. Definition of a non-insulin antidiabetic drug (NIAD) start was any dispense recorded whereas insulin start was defined when treatment duration exceeded 6 months. From 1987 to index date, baseline information on CVDs (coronary heart disease, heart failure, stroke and peripheral artery disease) was collected from the Swedish National Patient Register. Cox survival models adjusted for age, gender, first-line GLD treatment time and any history of CVDs were used to estimate likelihood of early treatment intensification during up to 7 years of follow-up.
Results: In 232,749 T2DM patients, first-line GLD treatment were initiated with either mono NIAD, insulin (alone or in combination with NIAD), or dual NIAD (86%, 13% and 2% respectively). Insulin treated patients, in comparison to mono- and dual NIAD groups were slightly older (64, 63 and 59 years) and had more CVD (36%, 29% and 21%). Despite higher CVD burden, insulin treated patients had significantly less CVD preventive treatment with statin, antihypertensive and low dose aspirin, compared to NIAD groups. Among 199,562 patients initiated on mono NIAD, metformin was most common (90%) followed by sulfonylurea (SU 8%), and other NIADs (2%). Patients initiated with SU were older (72 vs. 62 years), had more CVDs (43% vs. 27%) and were more often treated with low dose aspirin and less with statins, compared to the metformin group. Initiation with SU was associated with earlier intensification to a second and a third-line NIAD, or insulin, compared with metformin; hazard ratio (95% CI): 1.74 (1.69-1.79), 1.76 (1.65-1.88) and 1.72 (1.65-1.80).
Conclusion: In Sweden newly diagnosed T2DM patients are treated much in concordance to diabetes guidelines, beginning with metformin. Noteworthy, patients initiated with SU were associated with earlier insulin treatment intensification. One third of GLD naïve patients had a history of CVD, clearly demonstrating the burden in T2DM patients.

Figure: Likelihood of early insulin start in patients who were either initiated on first-line treatment with sulfonylurea (SU) or metformin (MET).



Supported by: AstraZeneca

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Patient-related predictors for attaining early glycaemic control in type 2 diabetes: a population-based study

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Background and aims: Early glycaemic control in type 2 diabetes (T2DM) may reduce the risk of microvascular and possibly macrovascular complications. However, data on patient-related predictors for achieving HbA1c targets is sparse. We examined predictors of early glycaemic control with particular emphasis on the effects of age and comorbidity.
Materials and methods: We included all individuals registered with a first-time glucose-lowering drug prescription in Northern Denmark between 2000 and 2012. We obtained data on glycated hemoglobin (HbA1c) levels at baseline and within 3 to 6 months after treatment start, comorbidities, and medications from population-based medical databases. Success in reaching HbA1c target (less than 7%) one year after treatment start was examined by Poisson regression analysis, comparing T2DM patients who were frail (i.e., over 65 years with comorbidities) with healthy T2DM patients (less than or equal to 65 years and no comorbidity). Individual patient-related predictors included age, gender, marital status, and presence of non-psychiatric comorbidities (Charlson Comorbidity Index (CCI) level). We adjusted all analyses for age, gender, pre-treatment HbA1c, diabetes duration, and micro-and macrovascular complications at baseline. In a sub-sample, we had information on BMI. We repeated all analyses in this sample.
Results: We identified 38,418 eligible T2DM patients (median age 63 years). Of these, 27,545 (72%) achieved HbA1c target of less than 7% at three to six months after drug initiation. After adjustment for potential confounders mentioned above, there was no difference in achievement of an early glycaemic target of 70 years vs. 60-70 years) or marital status (adjusted RR=0.98 [95% CI 0.96-1.00] comparing widowers with married individuals). Female gender (adjusted RR=0.97 [95% CI 0.96-0.98] for females vs. males) and presence of comorbidity at baseline (adjusted RR=0.94 [95% CI: 0.90-0.97] for patients with CCI≥3 compared to CCI=0) were weakly associated with non-achievement of HbA1c target. The strongest predictor of early glycemic control was the pre-treatment HbA1c level (adjusted RR=0.63 [95% CI: 0.61-0.64] for baseline HbA1c of 7.5-8.9%, and 0.58 [95% CI: 0.57-0.59] for baseline HbA1c of over or equal to 9%, respectively, compared with HbA1c less

than 7.5%). In a sub-cohort with available BMI measurements (n=4,166), results were consistent after additional adjustment for BMI, and BMI per se was not associated with the chance of successful early glycaemic control.

Conclusion: In this large population-based study of incident treated T2DM patients in Denmark, older frail patients had a similar chance of achieving early glycaemic control as 65 years and younger patients without comorbidity. The strongest predictor of reaching HbA1c target less than 7% was the pre-treatment HbA1c level.

Supported by: research grant from Novo Nordisk A/S to Aarhus University

PS 014 Risk marker of type 2 diabetes

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Using diabetes risk scores to select high-risk individuals for diabetes prevention in the United States: a cost-effectiveness analysis

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Background and aims: Several scoring systems have been developed for predicting future risk of type 2 diabetes within the past years. While the performance of these risk scores has been evaluated in detail, few studies have evaluated the application of those scoring systems to identify the targeting population for diabetes prevention. This study aimed to apply the framework of cost-effectiveness analysis to determine the optimal threshold of two previously developed risk scoring algorithms, the German Diabetes Risk Score (GDRS) and the Atherosclerosis Risk in Communities (ARIC) 2009 score while using them for identifying high risk individuals with which to intervene.

Materials and methods: To determine the performance of the two risk scores in a US population, receiver operating characteristic (ROC) analysis was performed combining data from the Atherosclerosis Risk in Communities (ARIC) study and the Cardiovascular Health Study (CHS)(N combined=17,291; aged 44 to 91 years). Survival analysis was applied to determine diabetes incidence rates according to a broad range of risk thresholds (5% to 20% by 1% increments; 25%, 30% and 35%) for the two risk scores. The threshold-specific incidences were incorporated into a Markov-based simulation model. Two scenarios were simulated wherein the 1-stage scenario included stratification by the risk score only and the 2-stage scenario additionally included a glucose test (threshold 100 mg/dl) in the identified high-risk group. Identified high-risk individuals were assumed to receive a low-cost community-based intervention with a risk reduction of 12.5% per year. Simulation samples were obtained from the 2001-2004 National Health and Nutrition Examination Survey (NHANES). Incremental cost per quality adjusted life year gained was calculated by progressively increasing the score threshold.

Results: The area under the ROC curve (ROC-AUC) for the GDRS and the ARIC 2009 score were 0.691 (0.677-0.704) and 0.720 (0.707-0.732), respectively. The incremental cost-effectiveness ratios (ICER) in a 1-stage scenario ranged from $36,447 (GDRS) and $33,404 (ARIC 2009 score) for 35% risk to $51,318 (GDRS) and $60,170 (ARIC 2009 score) for the lowest risk cut-off of 5% risk. The selected risk cut-off for an ICER <$50,000 would be 7% for the GDRS and 9% for the ARIC 2009 score. In the 2-stage scenario with an additional glucose test in the selected high-risk group, ICERS for all cut-offs were below $50,000.

Conclusion: The results of this study suggest that a non-invasive diabetes risk score can be a valuable tool to identify high-risk individuals for a lifestyle intervention. The optimal thresholds of the GDRS and the ARIC 2009 score were 7% and 9%, respectively when using a 1-stage screening with the risk scores only and 5% for both scores when using an additional glucose test with 100 mg/dl threshold in the identified high-risk group in a 2-stage screening.

Supported by: DZD

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A comparison of different glucometabolic markers for prediction of future type 2 diabetes in young and middle-aged subjects: a 20-year follow-up study

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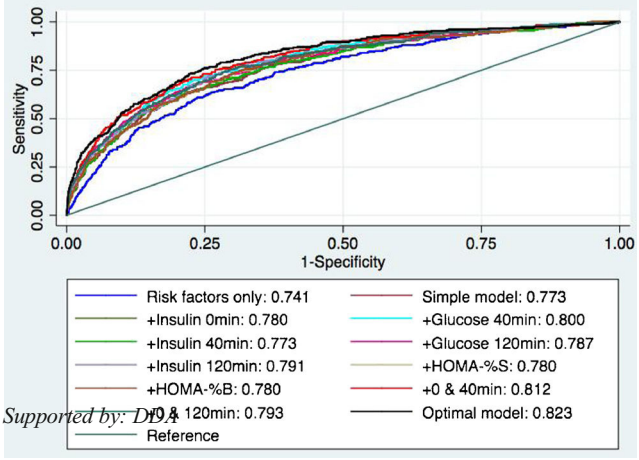
Background and aims: Risk prediction models for type 2 diabetes mellitus (T2DM) often incorporate a few common risk factors (RF) including fasting blood glucose (FBG). Further addition of other glucometabolic risk markers (GRM) incl. multiple measurements during OGTT may improve prediction, but superiority of one over other at long-term follow-up remains unproven. The purpose of this study was to assess the added predictive value of various GRM (fasting plasma insulin, homeostatic model assessment derived insulin sensitivity (HOMA-%S) and beta-cell function (HOMA-%B), and 40 and 120 min BG and plasma insulin during OGTT) to a model with RF (age, BMI, systolic blood pressure, total cholesterol, triglycerides, family history) and FBG.
Materials and methods: We assessed improvement in the prediction of incident T2DM (through re-examination and registries) during 20-year follow-up in a random population sample of 3576 men free from DM and cardiovascular disease at baseline, using the above-mentioned GRM. Their additive value to a model with RF and FBG was evaluated using Cox proportional hazards regression, receiver-operating characteristic (ROC) analysis, and integrated discrimination improvement (IDI).
Results: Median age at inclusion was 48 (IQR 47-48) years. During follow-up (median 20 (IQR 20-20) years), 300 new cases of DM developed. The ROC-curves are depicted in figure 1. Addition of FBG to a model with RF alone resulted in significant improvement (likelihood-ratio X^2=106.62 (p<0.0001); AUC 0.773 vs. 0.741, X^2=10.26 (p=0.001); IDI 0.04 (p<0.0001)). All models with an additional GRM performed significantly better than the model with RF+FBG (AUC 0.780-0.800 vs. 0.773, p<0.05 for all comparisons). The largest improvement was seen for 40 min BG (likelihood-ratio X^2=72.47 (p<0.0001); AUC 0.800, X^2=11.84 (p<0.0001); IDI 0.03 (p<0.0001)), which performed non-significantly better than 120 min BG (p=0.2) and 120 min insulin (p=0.3) and significantly (p<0.05) better than all other GRM. The optimal prediction model (AUC 0.823) included RF, FBG, fasting insulin, BG and insulin at both 40 and 120 min and performed significantly better than the models with RF+FBG+40 min BG and insulin (AUC 0.812, X^2=4.77 (p=0.03); likelihood-ratio X^2=48.62 (p<0.0001)) and RF+FBG+120 min BG and insulin (AUC 0.793, X^2=16.14 (p=0.0001); likelihood-ratio X^2=81.04 (p<0.0001)), respectively. The model with 40 min BG and insulin further performed significantly better than the model with 120 min measurements only (AUC X^2=4.14, (p=0.04)).
Conclusion: GRM provided a significantly better prediction of future DM risk during long-term follow-up than knowledge of RF and FBG alone. Although multiple measurements during OGTT possessed incremental predictive value, the addition of 120 BG and insulin was associated with limited benefit beyond measurements at 0 and 40 min only.



Supported by: DDA

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The association between GAD65 antibody levels and incident diabetes in a population-based cohort: the Hoorn study

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Background and aims: GAD antibodies are autoantibodies against the enzyme glutamic acid decarboxylase (GAD). Up until now it is not clear whether GAD antibody positivity is predictive for the development of diabetes. Prospective studies on whether the presence of GAD antibodies predicts diabetes are rare and provide contradicting results. Therefore, the aim of this study was to assess the association between levels of GAD65 antibodies and incident diabetes after 6.5 years in the general Dutch population.
Materials and methods: We assessed the association in 1302 men and women of the Hoorn study, a population-based cohort. Participants with prevalent diabetes at baseline were excluded. Diabetes status at baseline and follow-up was defined according to WHO criteria 2011, including fasting plasma glucose levels, 2 h post load plasma glucose and HbA1c values. GAD65 antibody levels were measured in serum by means of a standardized radioligand assay and subsequently were expressed as indexes. GAD65 antibody positivity was defined as indexes equal or above the 99th percentile point. Logistic regression analysis was performed to compare GAD antibody positive participants to GAD antibody negative participants.
Results: Of the 1302 participants (age 60.3±7y, 45.8% male), 11.1% had developed diabetes after 6.5 years. For GAD65 antibody positive participants the Odds Ratio (OR) for developing diabetes was 7.09 (95% confidence interval (CI): 2.35-21.40), compared to GAD65 antibody negative participants. After adjustment for age, sex, follow-up duration and glucose metabolism status at baseline, the OR for developing diabetes was 4.86 (95% CI: 1.45-16.29). In sensitivity analyses, the adjusted standardized regression coefficients for HbA1c levels, fasting glucose levels and 2 h post load plasma glucose levels were 0.141 (p<0.001), 0.149 (p<0.001) and 0.058 (p=0.015) in GAD65 antibody positive participants, compared to GAD65 antibody negative participants.
Conclusion: Within our population-based cohort, GAD65 antibody positivity was strongly and independently of glucose metabolism status at baseline associated with an increased risk of developing diabetes during 6.5-year follow-up.

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Continuous glucose monitoring at the early phases of type 2 diabetes

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Background and aims: Little is known about glucose variability in the early phases of type 2 diabetes (T2D). In an effort to improve the risk prediction of T2D, we studied whether a particular pattern of glucose excursion in subjects with impaired glucose tolerance (IGT) would be associated with a decrease in insulin secretion, increase in glucose excursions and/or diabetes after 1.5 (or 5) years follow-up. We also compared a single capillary glucose measurement at 2 hours (2 h) after a standardized test-meal eaten at home with a 2 h oral glucose tolerance test (OGTT) in diagnosis of T2D.
Materials and methods: 36 subjects with IGT and 25 with T2D from the Botnia Study participated in a 2 h OGTT and a one-week continuous glucose monitoring (CGM), during which they ate two test meals at home after 10-12 hours fasting and measured capillary glucose at 0, 60 and 120 minutes after the meal. They filled in a questionnaire and kept a food and exercise diary during the CGM. A follow-up visit will be completed 1-1.5 years after the first visit in March-June 2015, with a similar study protocol except for one of the test meals eaten at the study center with blood sampling. Subjects with known diabetic complications, HbA1c >8% (63.9 mmol/mol), fasting plasma glucose (PG) >10 mmol/l, or treated with insulin, GLP1-analogues, or sulphonylureas were excluded. Metformin was stopped two days before the CGM. None of the participants was on glitazone, gliptine or acarbose medication.
Results: At the first visit, the IGT and T2D groups had similar age, BMI, waist and hip circumference and blood pressure, but fasting PG, 2 h glucose at OGTT and B-HbA1c were significantly higher in those with T2D (6.86 mmol/l and 5.84 mmol/l; 11.44 mmol/l 9.29 mmol/l; 37.7 mmol/mol and 44.7 mmol/mol, respectively) (table). The glucose excursions were significantly larger in the T2D subjects compared with IGT subjects estimated as area under curve above 6 mmol/l (52.58 vs. 84.35, p=0.0041), or as mean or largest amplitude of excursion (p=0.047) (table). As the 2 h glucose was significantly lower after test meal than after OGTT, both insulin and incretin responses will be analyzed during the follow-up visit. Longitudinal changes in glucose excursions, insulin secretion and glucose tolerance will be analyzed in August 2015.
Conclusion: Glucose variability during the CGM was significantly higher in subjects with T2D compared to those with IGT. It will be interesting to see whether a subgroup of IGT subjects with a higher risk for deterioration of glucose tolerance can be identified based on glucose excursions.

Covariate	IGT [*n* = 36]	T2D [*n* = 25]	P-value
Gender (M/F)	21 / 15	10 / 15	
Age (years)	61.99 ± 7.75	63.32 ± 7.06	NS
BMI (kg/m²)	29.25 ± 4.6	28.96 ± 3.14	NS
Waist (cm)	101.86 ± 11.81	100.2 ± 8.91	NS
Systolic BP (mmHg)	143.19 ± 17.16	143.82 ± 17.59	NS
Diastolic BP (mmHg)	80.1 ± 7.74	80.46 ± 8.62	NS
HbA1c (mmol/mol)	37.72 ± 6.66	44.76 ± 4.82	1.69×10^{-5}
Fasting glucose (mmol/l)	5.84 ± 0.62	6.86 ± 0.69	5.05×10^{-5}
2-hours glucose (mmol/l)	9.29 ± 1.8	11.44 ± 3.23	0.034
Glucose variability (CGM)	**IGT**	**T2D**	
Mean glucose (mmol/l)	5.96 ± 0.54	6.68 ± 0.85	0.00049
SD (mmol/l)	1.04 ± 0.26	1.28 ± 0.32	NS
Coefficient of variation (%)	17.5 ± 4.02	19.08 ± 3.52	NS
SD between daily means (mmol/l)	0.42 ± 0.17	0.53 ± 0.19	NS
Range (mmol/l)	6.65 ± 1.47	7.84 ± 1.95	NS
Values in target ≠ (%)	97.62 ± 2.38	95.43 ± 6.92	NS
Values above target (10 mmol/l, %)	1.66 ± 2.16	0.87 ± 2.33	NS
Values below target (3.9 mmol/l, %)	0.72 ± 1.34	3.7 ± 6.65	0.0099
AUC above 6 mmol/l (%)	52.58 ± 27.5	84.35 ± 4.27	0.0041
MAGE (mmol/l)	2.39 ± 0.64	3.02 ± 0.82	0.047
LAGE (mmol/l)	5.44 ± 1.40	6.80 ± 1.55	0.047
MODD (mmol/l)	1.17 ± 0.73	2.15 ± 2.97	NS

First and second column: mean ± SD of covariates and glucose variability indices among IGT and T2D subjects, respectively. Third column: p-value of a Wilcoxon test for differences between IGT and T2D subjects, adjusted for multiple tests with the Bonferroni-Holm method. IGT=impaired glucose tolerance; T2D=type 2 diabetes; NS=statistically non-significant; CGM=continuous glucose monitoring; MAGE=mean amplitude of glycaemic excursions; LAGE= largest amplitude of glycemic excursions; MODD=means of absolute daily differences. ≠ 3.9 mmol/l < glucose < 10 mmol/l.

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Incremental value of a past fasting glucose for the prediction of type 2 diabetes mellitus. The Whitehall II study

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Background and aims: Lifestyle interventions can reduce the risk of type 2 diabetes by more than 50% in high risk populations. In order to effectively identify such high-risk individuals, current diabetes prediction models require improvement. Accordingly, we examined the impact of adding a previous fasting glucose measurement to the Framingham Offspring Diabetes Risk Score (FOS-DRS).
Materials and methods: In an cohort of British government workers taking part in 5-yearly clinical examinations, 338 incident cases of type 2 diabetes were observed (75 g OGTTs or self-report of diabetes or use of antidiabetic medication) in two 5-year baseline-follow-up data cycles (n=3550 and n=4140). FOS-DRS was re-estimated with Weibull regression using baseline data on age, sex, parental history of diabetes, body mass index, systolic blood pressure, hypertensive medication, HDL-cholesterol, triglyceride, and fasting glucose. Then, we added fasting glucose values measured 5 years earlier to the existing model. The value of adding past fasting glucose was examined by C-statistics, net reclassification improvement (NRI), and integrated discrimination improvement (IDI); calibration by the Hosmer-Lemeshow test.
Results: As anticipated, previous fasting glucose concentration was an independent predictor of type 2 diabetes (OR 1.98, 95% CI: 1.56-2.52). Its addition to the FOS-DRS significantly improved discrimination (FOS-DRS vs. additional glucose, C-statistic: 0.774 vs. 0.783, p=0.031; 3-category NRI: 4.7%, 95%CI 0.6-8.8%, IDI: 0.007, P<0.0001); however, calibration was imperfect for both models (both p<0.05). Most of the improvement of NRI was related to an increase in the predicted risk of incident diabetes cases.

Conclusion: In the present study, the addition of a routinely available past clinical measure (fasting glucose) to an optimized clinical diabetes risk score improved its prediction.
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Predicting risk of progression to diabetes from prediabetes defined by HbA_{1c} or fasting plasma glucose in Koreans

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Background and aims: Although both HbA1c and fasting plasma glucose (FPG) criteria have been recommended as screening tests for prediabetes, the performance of these tests in predicting future risk of diabetes remains to be established. We assessed 5-year progression rate to diabetes in individuals identified as having prediabetes by HbA1c and FPG criteria in a large cohort of Koreans.
Materials and methods: We analyzed data of 17,971 Korean adults (age 20-79 years, 39% women) who underwent routine medical check-ups in 2007-2008 (baseline) and followed up in 2012-13 with a median 5.1-year (range 3.1-6.7 years) interval. Patients who had diabetes at baseline were excluded. Prediabetes at baseline was defined as FPG 5.6-6.9 mmol/l or HbA1c 39-46 mmol/mol [5.7-6.4%]. Diagnosis of diabetes at follow-up was based on FPG ≥7.0 mmol/l, HbA1c ≥48 mmol/mol [6.5%], or use of antihyperglycemic medications. Odds ratios (ORs) for developing diabetes were estimated using multiple logistic regression analysis.
Results: Among the 17,971 participants who did not have diabetes at baseline, the prevalence of prediabetes was 30.6% (n=5495) by FPG and 20.4% (n=3664) by HbA1c criteria. A total of 723 subjects (4.0%) developed diabetes during the 5-year follow-up. Only 0.4% of subjects who had normoglycemia (FPG <5.6 mmol/l and HbA1c <39 mmol/mol) at baseline developed diabetes. Individuals with prediabetes identified by HbA1c test had a higher 5-year rate of progression to diabetes compared to those by FPG test (14.7% vs. 10.4%, P <0.001). This trend was consistent regardless of age, sex, BMI, and family history of diabetes. Among individuals diagnosed as prediabetes discordantly by the two tests, subjects diagnosed by HbA1c only had a higher risk of progression to diabetes compared to those by FPG alone (6.0% vs. 3.9%, P <0.001) (Table). Individuals diagnosed as prediabetes by both criteria had much higher rate of progression to diabetes (22.3%). Receiver operating characteristic curve analysis showed that area under the curve was greater for HbA1c (0.855, 95% CI 0.840-0.870) compared to that for FPG (0.830, 0.813-846) (P=0.006). The OR of developing diabetes adjusted for conventional risk factors was also higher for prediabetes by HbA1c (9.91, 95% CI 8.24-11.9) compared with that by FPG (7.29, 5.97-8.89) (P=0.026). When we subdivided the prediabetes group, individuals with higher FPG (6.1-6.9 mmol/l) and HbA1c (42-46 mmol/mol) had substantially higher risk of progression to diabetes compared to those with FPG 5.6-6.0 mmol/l and HbA1c 39-41 mmol/mol, respectively (Table).
Conclusion: Although the HbA1c test identified fewer individuals with prediabetes than did FPG test, the predictive power for progression to diabetes assessed by HbA1c was stronger than that assessed by FPG. However, these two tests could independently and additively predict risk of progression to diabetes, and combined use of both tests could more efficiently identify individuals at risk for diabetes.

Table. Incidence and OR of diabetes after 5 years according to baseline diagnosis of prediabetes

	n	Incident diabetes (%)	Age- and sex-adjusted OR (95% CI)	Multivariate adjusted OR (95% CI)
Prediabetes by				
None (normal)	10761	48 (0.4%)	1 (reference)	1 (reference)
FPG only	3544	137 (3.9%)	8.52 (6.10-11.9)	7.66 (5.39-10.9)
HbA1c only	1713	130 (6.0%)	14.4 (10.1-20.4)	11.9 (8.24-17.3)
Both (FPG & HbA1c)	1951	435 (22.3%)	61.3 (45.0-83.4)	46.7(33.6-64.9)
Fasting plasma glucose				
<5.6 mmol/l	12474	151 (1.3%)	1 (reference)	1 (reference)
5.6-6.0 mmol/l	4062	215 (5.3%)	4.27 (3.45-5.29)	3.66 (2.91-4.60)
6.1-6.9 mmol/l	1433	357 (24.9%)	24.9 (20.2-30.5)	21.1 (16.8-26.3)
HbA1c				
<39 mmol/mol	14305	185 (1.3%)	1 (reference)	1 (reference)
39-41 mmol/mol	2561	216 (8.4%)	6.85 (5.59-8.40)	5.54 (4.47-6.88)
42-46 mmol/mol	1103	322 (29.2%)	30.7 (25.2-37.5)	23.2 (18.7-28.7)

Prediabetes by FPG: FPG 5.6-6.9 mmol/l
Prediabetes by HbA1c: HbA1c 39-46 mmol/mol (5.7-6.4%)
Multivariate adjusted OR: adjusted for age, sex, BMI, smoking, alcohol intake, physical activity, family history of diabetes, hypertension, serum triglycerides and HDL-cholesterol levels.

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FINDRISC score and glycaemic measures, better than HbA_{1c}, as predictor and monitor of diabetes incidence within 5 years

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Background and aims: Diabetes is a rising epidemic, with increasing social and economic impact. PREVADIAB, the first Portuguese nationwide study on the prevalence of diabetes, showed that 11.7% of the general population has diabetes; while nearly 25% already showed prediabetes (IFG, IGT, or both). Five years after, we aimed to evaluate the role of diabetes incidence predictors in a non-diabetic cohort of the initial study.
Materials and methods: From the 5167 individuals recorded on the database of the first PREVADIAB, people evaluated as non-diabetics (either designated with "normal" or "prediabetes" based on both fasting glycemia and/or 2 h post-load challenge) were called for reassessment. Thus, people aged between 23 and 83 years, taken from 73 of the original 122 locations, were recruited 5 years after the initial call, being representative of the non-diabetic distribution observed on the first study. An OGTT was again performed and HbA1c quantified to evaluate glycemic control. WHO criteria were used for the diagnosis of diabetes. For diabetes risk assessment, we applied the Finnish Diabetes Risk Score (FINDRISC) to information from the original study,specific to this subgroup.
Results: The present cohort consisted of 1024 participants. In relation to the deterioration of glycemic control with time, 3.8% of individuals were diagnosed with diabetes in the intervening 5 years, while an additional 5.0% were shown here to have undiagnosed diabetes. In terms of matching the initial diabetes risk score categories to the incidence of diabetes, while no individual initially characterized with the lower FINDRISC score (below 7) progressed to diabetes, 25.9% of individuals initially characterized with the higher risk score (above 20) developed a diagnosis of diabetes within these 5 years. Initial HbA1c was also shown to be related to diabetes incidence, but with a similar sensitivity only at ≥6.3%. In terms of diabetes detection, applying HbA1c diagnostic criteria

(≥6.5%) did not add significantly to criteria based on OGTT measures, while on the other hand merely 25.5% of participants found with undiagnosed diabetes were detected by the HbA1c criteria cut-off.

Conclusion: Primary prevention strategies targeted to high risk populations are usually more cost-effective than the ones targeted to general populations. Initial HbA1c showed to be strongly related to 5 years diabetes incidence only already at a high range, while it was shown to perform poorly in detecting undiagnosed diabetes. Thus, this 5-year prospective analysis argues in favor of the FINDRISC, a non-invasive inexpensive tool, as adequate to evaluate risk of diabetes development in the general population. More, it also argues for the necessity of glycemic measures, and not solely HbA1c, to monitor diabetes incidence in high-risk individuals.

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PS 015 DPP4-inhibitors: pharmacoepidemiology

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Risk of hospitalisation for heart failure between dipeptidyl peptidase 4 inhibitors vs sulfonylureas and saxagliptin vs sitagliptin in a U.S. claims database

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Background and aims: In the SAVOR trial, the risk of hospitalization for heart failure (hHF), a component of the secondary endpoint, was increased with saxagliptin compared to placebo. This observational cohort study used a large U.S. insurance claims database to compare the risk of hHF between patients with type 2 diabetes mellitus (T2DM) treated with dipeptidyl peptidase-4 inhibitors (DPP-4is) vs. sulfonylureas (SUs) and between those treated with saxagliptin vs. sitagliptin.

Materials and methods: Methods followed the U.S. Food and Drug Administration's Mini-Sentinel protocol for active surveillance of antidiabetic agents. Patients initiated treatment between Aug-1-2010 and Aug-30-2013 and had no use of the comparator treatments in the 12 months before treatment initiation (baseline). Each comparison consisted of patients matched 1:1 on a propensity score (nearest neighbor; caliper=0.01) based on demographics, general clinical characteristics, and hHF risk factors measured at baseline. The primary study outcome was time to hHF, defined as the number of days from treatment initiation until hHF or censoring at cessation of the initiated treatment, use of the alternative comparator treatment, loss to follow-up, or 8/30/2013, whichever was earlier. Time to hHF was compared between the matched groups using Cox proportional hazards models. Analyses were stratified by presence of baseline cardiovascular disease (CVD).

Results: The table shows the main study results. The propensity score matches achieved balance on all patient characteristics included within the propensity score models; across the 4 models, the standardized differences for matched covariates ranged from 0%-2.3%; values <10% are considered to be indicative of adequate balance. Within the comparison of DPP-4i vs. SU among patients with no baseline CVD, those who were treated with a DPP-4i had significantly (P=0.013) lower hazards of hHF compared to those who were treated with an SU. In all other comparisons there were no statistically significant differences in the risk of hHF between DPP-4i and SU or between saxagliptin and sitagliptin. Sensitivity analyses on subgroups (patients with no baseline use of loop diuretics, patients with no baseline or follow-up use of thiazolidinediones, and patients with characteristics similar to those included in the SAVOR trial) and using alternative assumptions of time at risk produced consistent results (not shown).

Conclusion: In this analysis of U.S. patients with T2DM in a real-world setting, there was no evidence of increased risk of hHF for DPP-4is relative to SUs or for saxagliptin relative to sitagliptin.

Table. Main Study Results for Propensity Score Matched Cohorts

	DPP-4i vs. SU				Saxa vs. Sita			
	No Baseline CVD		Baseline CVD		No Baseline CVD		Baseline CVD	
	DPP-4i	SU*	DPP-4i	SU*	Saxa	Sita*	Saxa	Sita*
N of patients	82,019	82,019	27,259	27,259	43,402	43,402	13,042	13,042
Mean follow-up (days)	171	164	187	177	181	187	206	206
N of hHF events	35	58	200	202	23	24	82	87
IR per 100 PY	0.091	0.157	1.434	1.527	0.107	0.108	1.116	1.160
HR (95% CI), *P*	0.585 (0.384-0.892), P=0.013		0.946 (0.778-1.151), P=0.580		0.990 (0.560-1.749), P=0.972		0.945 (0.699-1.278), P=0.712	

*Reference category for HR; HR<1 indicates lower risk for DPP-4i or saxagliptin.
CI=confidence interval; CVD=cardiovascular disease; DPP-4i=dipeptidyl peptidase 4 Inhibitors HR=hazard ratio; IR=incidence rate; PY=person-years of follow-up; Saxa=saxagliptin; Sita=sitagliptin; SU=sulfonyiurea

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Pharmacological treatment changes in 4.143 patients with dysregulated type 2 diabetes referred to a tertiary diabetes center

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Background and aims: Changes to the pharmacological treatment are common when addressing dysregulated type 2 diabetes (DT2D). We have previously described improvements in such patients referred from primary care. Our aim here was to describe the most common pharmacological treatment changes implemented in these subjects.

Materials and methods: Pharmacological treatment data before and after a six to nine month treatment program for all patients referred to a tertiary diabetes center between Jan 1st 2001 and Jan 1st 2013 were extracted from the electronic medical records (n=4.143). Patient population and metabolic control data have been described previously; *e.g.* proportion with HbA1c <53 mmol/mol improved from 25% to 47%; blood pressure <140/<85 mmHg from 41% to 52%, and LDL cholesterol <2.6 mmol/l from 56% to 74%. Only between treatment class comparisons were performed, and non-parametric analysis was used throughout.

Results: 61% of patients were on metformin (Met) on referral: 18% prescribed Met alone, 28% a combination of Met and sulphonyl urea (SU), and 14% of Met and insulin (Ins). Any other Met combination, including triple therapy, was virtually non-existent (about 1%). SU alone was seen in 10% of the patients, and 7% of patients were on Ins monotherapy. The combination of SU and Ins was less common at 2%. Total daily insulin dose at referral was 55±47 IE/day (n=924). At discharge the dose in all subjects on insulin was 54±44 IE/day (n=1.462), was increased to 61±50 IE/day in those already on Ins at baseline (n=914; p<0.0001), and 43±31 IE/day (n=548) in those put on Ins. Only 1% was on GLP1-analogue alone and 0.2% on DPP4-i alone at baseline. 9 out of 10 patients who were on Met at referral remained so, and in total 67% were on Met at discharge (Figure 1): Met alone 19%; Met/SU 20%; Met/Ins 18%; and 11% on other combinations: Met/GLP-1 4.2%; Met/GLP-1/Ins 2.1%; Met/DPP4-i 1.3%; Met/SU/Ins 1.1%; Met/SU/GLP-1 1.0%; Met/SU/DPP4-i 0.9%; Met/DPP4-i/Ins 0.4%. SUs were withdrawn more commonly than Met (39%), whereas Ins was rarely discontinued (6%).The HbA1c lowering effect differed significantly between patients receiving different treatment alternatives and was particularly beneficial when including a GLP-1 analogue in triple therapy (-18 *vs.* -12 mmol/mol, p=0.0001). In addition to medication controlling glycaemia we observed increases in the use of antihypertensive drugs (from 63% to 75%), and lipid-lowering drugs (from 55% to 76%), and antithrombotic therapy (from 53% to 75%; p<0.001 for all changes).

Conclusion: When patients are referred from primary care to a tertiary center for DT2D, Ins and Met are the most likely drugs to be continued or newly prescribed, whereas SUs are more likely to be discontinued. The role for newer agents and triple combinations merit further investigation. Even if there are many different drugs available for glycemic control this remains a major task in comparison to the management of lipids and blood pressure where goal attainment is achieved in greater proportions of patients, even with DT2D.

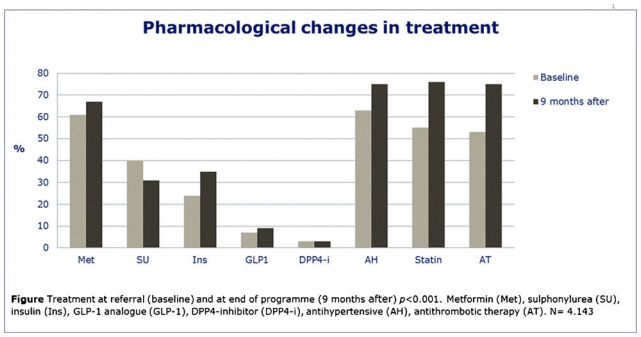

Figure Treatment at referral (baseline) and at end of programme (9 months after) p<0.001. Metformin (Met), sulphonylurea (SU), insulin (Ins), GLP-1 analogue (GLP-1), DPP4-inhibitor (DPP4-i), antihypertensive (AH), antithrombotic therapy (AT). N= 4.143

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Longitudinal changes in type 2 diabetic patient medication treatment patterns from 2006 to 2012

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Background and aims: It is important to understand changes in diabetes treatment patterns over time in order to optimize treatment paradigms. This was a longitudinal study of real-world claims data from a single cohort (N=35017) of patients newly diagnosed in 2006 with Type 2 Diabetes (T2D) which followed changes in their treatment patterns and costs from 2006 to 2012.

Materials and methods: The Truven Health MarketScan® Database was used, from which claims data for only US-based newly diagnosed T2D patients were examined. Inclusion criteria were: at least 2 diagnoses according to ICD-9 codes for T2D, or 1 T2D diagnosis and at least 1 OAD claim, and allowing 1 diagnosis for T1D, ≥18 years of age, continuous enrolment starting from 2006 (index year) to 2012 in a plan with prescription benefits, and with at least 1 anti-diabetic drug prescription during the study. Data were analyzed for drug treatment patterns, and for costs of inpatient and outpatient services, emergency department use, and medications and supplies.

Results: Approximately 28% of newly diagnosed patients had no prescriptions for anti-diabetic drugs in 2007, and this decreased to 22% in 2012. The percentage of patients who received only OADs, but no injectables, during the enrollment period remained between 65% and 69%. Among these patients, the percent receiving 1 OAD fell from approximately 70% to 60%, those receiving 2 OADs rose from 25% to 31%, and patients prescribed 3 OADs increased from 4.5% to 8%. Less than 1% received 4 or more OADs. Monotherapy with Metformin (Met) progressively fell from 35% to 31% of patients. Monotherapy with SUs fell from 7% to 6%, and for TZDs fell from 5% to 1.8% of patients. Dual OAD combinations were used by 17.8% of patients in 2007, progressively rising to 20% in 2012. Insulin monotherapy was initiated by only 0.2% of patients in 2007, rising to 1.7% in 2012. Patients using combined insulin and OAD therapy grew from <1% in 2007 to 6.5% in 2012. Combined GLP-1 agonist/OAD therapy grew from 2.3% to 3.3% of patients in 2012. From 2007 to 2012, total Met use (single and multiple therapy) grew from 56% to 62% of patients. Similarly, total SU use grew from 20% to 27%, TZD use fell from 18% to 10%, and DPP-4 inhibitor use grew from 2% to 15% of patients in the cohort. The total percent of patients using insulin or GLP-1, with and without OADs, rose to 7% and 4% of patients, respectively, in 2012. Total annual costs/T2D patient rose from $9817 to $12551 (based on 2012 levels), with the largest contribution coming from outpatient costs ($4140 to $5485), followed by costs for all drugs ($2897 to $3275), for inpatient services ($1421 to $1695) and emergency department use ($960 to $2096).

Conclusion: From 2006 to 2012, OAD monotherapy in this cohort of newly diagnosed patients declined and dual OAD therapy rose. Costs for all forms of resource utilization increased, resulting in net overall cost increases of $2734 per T2D patient/year.

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DISCOVERing treatment of type 2 diabetes in real world settings using electronic medical record databases

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Background and aims: Despite evidence of improved long term complications with risk factor control in patients (pts) with type 2 diabetes mellitus (T2DM), there is a lack of comparative global data on T2DM treatments (tx) and control from different regions. The availability of existing electronic medical records (EMR) in some countries offers the opportunity to gain insights into the disease, pt characteristics and management patterns, as well as clinical evolution. This study aims to provide descriptive EMR data from the real-world clinical setting on second and further line anti-diabetic tx use among T2DM patients in Canada (CAN), France (FR), Germany (DE), specifically those treated by general practitioners (DE GPs), and the United Kingdom (UK). The main outcomes of interest were changes in T2DM tx, glycaemic control in response to second-line tx and proportion of pts reaching A1c<7% during the follow-up period.
Materials and methods: Retrospective multi-country non-interventional, cohort study utilising longitudinal EMR data from pre-existing databases. Pts with T2DM aged ≥18 years, initiating a second line anti-diabetic tx at baseline, and with a minimum follow-up of 6 months, were included.
Results: A total of 22,272 T2DM pts initiating second-line tx were identified from May 2011 to April 2014. Of these pts, 805 were from CAN, 432 from FR, 8,140 from DE and 12,895 from the UK. Mean A1c at initiation of second-line tx was >7.5% in all countries (CAN: 8.8%; FR: 7.83%; DE GPs: 8.01%; UK: 9.0%). The most common first-line monotherapies were metformin (CAN: 81.7%; FR: 91.7%; DE GPs: 79.40%; UK: 88.6%), and sulphonylureas (SU) (CAN: 13.4%; FR: 4.9%; DE GPs: 12.14%; UK: 10.7%). The most frequently initiated second-line therapies were combination tx: metformin and DPP4 combination (CAN: 38.6%; FR: 38.4%; DE GPs: 45.2%; UK: 21.4%) and metformin and SU combination (CAN: 25.7%; FR: 15.7%; DE GPs: 21.28%; UK: 53.2%). Reductions in A1c were observed from baseline throughout the follow up period in all countries (mean A1c at 6 months in CAN: 7.4%; FR: 6.94%; DE GPs: 7.03%; UK: 7.6%).
Conclusion: Despite availability of guidelines, all countries studied had high A1c at baseline with many pts failing to achieve A1c <7% with second-line therapy. There are large intercountry variations in glycaemic control and second-line tx initiated. After failure of metformin monotherapy, the most common drugs added by physicians were DPP4 inhibitors and SU. Although reductions in A1c were achieved, mean values remained mostly above the recommended target of 7%. Data derived from real-world analyses such as this, where EMR sources are exploited to fill in gaps in evidence, may be useful to inform on the clinical utility of different tx applied in routine practice. The long term glycaemic control in the real world on micro and macrovascular outcomes is being investigated in the DISCOVER program.
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Characteristics and prescription behaviour of insulin glargine users among patients with type 2 diabetes mellitus

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Background and aims: Type 2 Diabetes Mellitus (T2DM) is a progressive disease and treatment intensification is often required. When oral antidiabetic drugs (OAD) fail to maintain glycemic control, initiating insulin therapy is recommended by the ADA and EASD. Published analyses of randomized controlled trials suggest advantages of initiating insulin therapy with long-acting basal insulin (e.g. insulin glargine or insulin determir) compared to conventional intermediate-acting human basal insulin (e.g. NPH). However, target treatment populations and real-world patterns of insulin glargine use have not been well documented. This study examined the characteristics and prescription behavior of patients (pts) initiating insulin glargine in Germany.
Materials and methods: A retrospective cohort study utilized a sample from the Germany IMS Disease Analyzer data. Included were pts with T2DM who initiated any basal insulin (i.e. intermediate-acting or long-acting insulin) in 2009-2013, ≥18 years (yrs), with continuous medical records. Eligible pts were classified into glargine or non-glargine cohort, depending on their index insulin. Between-cohort differences in 1-yr baseline patient characteristics, 1-yr post-index cumulative insulin dose and 1-yr post-index concomitant OAD use were assessed using Wilcoxon rank-sum tests for continuous and $\chi 2$ tests for categorical variables. Multivariable linear regression was used to compare the cumulative insulin dose in the glargine vs. non-glargine cohort, adjusting for baseline HbA1c and other characteristics.
Results: 14,886 basal insulin initiators were included, 47.0% and 53.0% were in the glargine cohort and non-glargine cohort, respectively. Among the glargine cohort, 85.3% initiated glargine only and 14.7% initiated glargine with short-acting insulin. Among the non-glargine cohort, 31.2% initiated NPH only, 16.3% initiated insulin detemir only, and 52.5% initiated either NPH or insulin detemir with short-acting insulin. Compared to the non-glargine cohort, the glargine cohort had higher HbA1C (8.7% vs. 8.6%), and a greater proportion had diabetes-related macro-vascular complications (17.0% vs. 7.7%), hypertension (32.6% vs.15.1%) and hyperlipidemia (15.4% vs. 7.6%) at baseline. Post insulin initiation, compared to the non-glargine cohort, a higher proportion of the glargine cohort used concomitant OADs (DPP-4: 21.3% vs. 11.7%), but they had a lower cumulative insulin dose (9,753 vs. 10,288 IU) (all $p<0.01$). Regression analysis demonstrated that among pts with baseline HbA1c<8% and between 8-9%, the glargine cohort used 124 IU and 963 IU less insulin over 1 year compared to the non-glargine cohort, respectively.
Conclusion: Almost 1/2 of basal insulin initiators initiated insulin glargine. Pts initiating insulin glargine had more comorbidities and T2DM related macro-vascular complications at baseline. In the 1-yr post-initiation, the insulin glargine cohort had higher use of concomitant OAD, but a lower cumulative insulin dose.
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Second-line treatment with sulfonylurea compared to DPP4 inhibitors demonstrated associations with earlier treatment intensification with insulin

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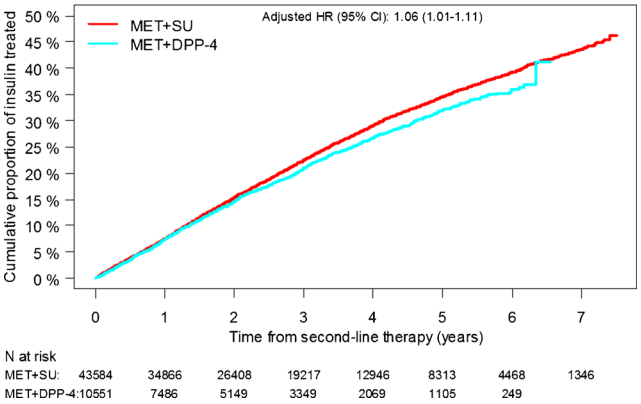
Background and aims: To investigate the initiation of second-line blood glucose lowering drug (GLD) treatment, patient characteristics and subsequent drug intensification for type 2 diabetes (T2DM) patients.
Materials and methods: T2DM patients who were on monotherapy with non-insulin antidiabetic drug (NIAD) and initiated on their second-line GLD treatment during 2006-2013 were identified in the Swedish Prescribed Drug Register and linked with the Swedish National Patient and Cause of Death registries. Index date was defined as the date of patients second GLD dispense after monotherapy on NIAD. Definition of a non-insulin antidiabetic drug (NIAD) start was any dispense recorded whereas insulin start was defined when treatment duration exceeded 6 months. From 1987 to index date, baseline information on comorbidity was collected from the Swedish National Patient Register. Cox survival models adjusted for age, gender, first line duration and prior cardiovascular disease were used to estimate risk of early treatment intensification during up to 7 years of follow-up.
Results: Of the 102,085 T2DM patients initiated on second-line treatment, 73% were on metformin, 22% on SU, 3% on metiglinid and 2% on other treatments in first line. Second-line was initiated by add-on of either one or more NIAD (67%) or switch to insulin (33%). Insulin patients, compared to the NIAD combinations, were older (70 vs. 64), had more history of cardiovascular- (50% vs. 31%) and microvascular disease (29% vs. 17%). In the 68,351 patients (on NIAD only); the most frequent dual-combination was metformin+sulfonylurea (SU) (64%) followed by metformin+DPP4i (15%). Metformin+SU vs metformin+DPP4 were older (65 vs 61 years), less frequently men (59 vs 62%), whereas the history of cardiovascular-, (32 vs 28%) and microvascular disease (18 vs 15%) were similar. Blood pressure-, lipid lowering and low dose aspirin treatment were also similar in the two groups. Compared with metformin+DPP4, metformin+SU was associated with earlier treatment with insulin, hazard ratio (95% CI): 1.06 (1.01-1.11).
Conclusion: In type 2 diabetes patients in Sweden, approximately 70% of second-line treatment was initiated with dual NIADs and more than 30% with insulin. Metformin+SU was the most frequent second-line combination, and demonstrated associations with earlier treatment with insulin compared with the metformin+DPP4 combination.

Figure: Likelihood of early insulin start in patients who were either initiated on second-line treatment with metformin (MET)+sulfonylurea (SU) or MET+DPP4i.



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The beta cell and insulin resistance in polycystic ovary syndrome: new insights into the origins and prevention of type 2 diabetes

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Background and aims: Polycystic Ovary Syndrome is a risk factor for type 2 diabetes (T2D) and cardiovascular disease (CVD) but the mechanisms are poorly understood. The aim of this study was to investigate how the interplay of insulin secretion and action results in glucose dysregulation in PCOS.
Materials and methods: 48 non-diabetic lean and obese women with PCOS (Rotterdam criteria) and 53 BMI-matched controls were studied. Insulin secretion was calculated by a model of insulin secretion and beta cell function from glucose and C-peptide levels, and Insulin Sensitivity Index (ISI) by the method of Matsuda, from a 6-point, 2 hour, 75 g oral glucose challenge. Adiposity was measured by bioimpedance and anthropometry.
Results: 1) Women with PCOS and controls were well matched for BMI (mean [SD]28.83 [6.98] vs 29.42 [7.17] kg/m2; p=0.68) and there was no difference in Beta Cell Glucose Sensitivity (BCGS), which is the mean slope of the dose-response function, representing the static relationship between glucose and insulin secretion (median [IQR] 83.3 [62.2-131.9] vs 102.4 [64.1-145.8] pmol/min/mL/mmol; p=0.57). 2) ISI was strongly related to BMI category (<25, 25-29.9, 30-34.9, >35) (ANOVA; p< 0.001), and waist circumference (WC) and visceral fat (VF) (r=0.50-0.58; p<0.001). Although ISI did not differ in PCOS vs controls, Insulin at 2 hrs was higher in PCOS (371.63 [261.14-578.07] vs 263.91 [150.01-488.93] pmol/l; p=0.03) and strongly associated with VF (r=0.52; p< 0.001) and 2 hr glucose (r=0.66; p<0.001). In both groups, WC and VF correlated strongly with HDL cholesterol (r=-0.42 to -0.64; all p<0.004), systolic blood pressure (r=0.24-0.71; p=0.09-<0.001) and C-reactive protein (r=0.49-0.56; all p<0.002). 3) Women with PCOS and 2 hr glucose >7.5 vs <7.5 mmol/l, had lower BCGS (43.5 [23.6] vs 109.0 [68.5] pmol/min/mL/mmol; p=0.04), higher WC (116.8 (15.8) vs 93.5(15.9) cm; p<0.01) and VF (14.6 (4.2) vs 10.3 (4.6); p=0.05), and despite non-significantly lower ISI (0.42 [0.25-0.96] vs 0.94 [0.53-1.36];p= 0.29) levels of insulin at 2 hrs were much higher (964.0 (579.2-1214.7) vs 328.2 (242.2-475.7) pmol/l; p=0.01). In PCOS, WC was strongly associated with increasing 2 hr glucose, but this was only present in women in the lowest quartile of BCGS (r=0.80; p=0.003).
Conclusion: PCOS predisposes to T2D and CVD as a result of insulin resistance (IR) which is exacerbated by central obesity. Although neither PCOS nor central obesity impair BCGS per se, women who also have poor BCGS possess the critical defect that renders them unable to compensate for increasing IR, and therefore predisposes to T2D. These results show that the pathogenesis of T2D in women with PCOS conforms to the models postulated by DeFronzo and Reaven, in which glucose levels are determiend by an interplay of insulin secretion and resistance, with adiposity being a dominant influence on IR. The findings suggest that waist circumference and 2 hr glucose could be used to stratify risk, and highlight the importance of healthy weight to reduce the risks of T2D and CVD in women with PCOS.
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The shape of plasma insulin and insulin-to-glucose ratio curves during an oral glucose tolerance test and prediction of future risk of type 2 diabetes

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Background and aims: The pattern of glucose curve during oral glucose tolerance test (OGTT) is known to be associated with degree of insulin sensitivity and secretory function as well as with the risk of type 2 diabetes development in adults and adolescents. However little is known about the patterns of insulin or insulin-to-glucose (I/G) ratio curves. In this prospective follow-up study, we aimed to evaluate the risk for future diabetes development according to the shape of plasma insulin and I/G ratio during 75 g OGTT.
Materials and methods: Among subjects enrolled in a community-based cohort named Chungju Metabolic disease Cohort, those who performed OGTT during 2007-2010 and repeated the test during 2011-2014 were included. Subjects with diabetes at baseline were excluded. Blood samples were obtained at 0-, 30-, and 120-min to measure glucose and insulin levels and I/G ratio was calculated for each time points. Compared to the insulin or I/G ratio value at 30-min, those showing lower value at 120-min was classified as having downward-slope, while those with higher value was classified as having upward-slope of insulin or I/G ratio curve, respsectively.
Results: Among 1126 subjects, 117 (10.39%) developed type 2 diabetes after 4 years. Compared to the baseline value, the AUC(0-30 min) of insulin and I/G ratio curves, insulinogenic index and Stumvoll index increased in diabetes-nonconverters after follow-up, which was not shown in diabetes-converters. HOMA-beta and Matsuda index did not show significant change, while BMI and HOMA-IR increased in both groups. Among subjects with downward (n=482) and upward (n=644) slope of insulin curve, 3.9% and 15.2% developed diabetes, respectively. . Among subjects with downward (n=328) and upward (n=798) slope of I/G ratio curve, 3.0% and 13.4% developed diabetes, respectively. Those showing upward slope of insulin curve demonstrated crude odds ratio (OR) of 4.924 (95% CI, 2.541-9.542) and adjusted OR of 3.804 (2.276-6.538) for development of diabetes. Similarly, subjects showing upward slope of I/G ratio curve demonstrated crude OR of 4.924 (2.541-9.542) and adjusted OR of 3.804 (2.276-6.358) for future diabetes.
Conclusion: The shape of insulin or I/G ratio curve during OGTT may help to identify subjects who are at risk of diabetes development. Further studies in different ethnicities are required to confirm out study results.

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Non-alcoholic fatty liver disease predicts type 2 diabetes mellitus, but not pre-diabetes, in Xi'an, China: a 5-year cohort study

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Background and aims: Emerging studies have focused the association between non-alcoholic fatty liver disease (NAFLD) and the risk of type 2 diabetes mellitus (T2DM) but the results were inconsistent. In addition, few studies have put focus on the association between NAFLD and the

risk of pre-diabetes. We aimed to investigate whether NAFLD diagnosed by ultrasonography could predict the risk of T2DM and pre-diabetes in Chinese population.

Materials and methods: The population-base cohort study, which was held in Xi'an, Northwestern China, was based on China National Diabetes and Metabolic Disorders Survey (2007 - 2008). During a follow-up of 5 years, 508 healthy subjects were included as study sample. NAFLD was determined by abdominal ultrasonography. T2DM and pre-diabetes were diagnosed based on oral glucose tolerance test. Cox proportional hazard regression was utilized to examine the association between NAFLD and the development of diabetes and pre-diabetes.

Results: Of 508 subjects, 97 (19.1%) were diagnosed as NAFLD and 411 (80.9%) were as non-NAFLD; 20 (3.9%) developed diabetes and 85 (16.7%) developed pre-diabetes during follow-up. The incidence of diabetes and pre-diabetes in the NAFLD group was 20.6 and 51.6 per 1000 person-years, respectively, whereas that in non-NAFLD group was 4.9 and 29.2 per 1000 person-years, respectively. Cox proportional hazard regression showed that the multivariable-adjusted relative risk (RR) of T2DM and pre-diabetes in the NAFLD group was 4.462 [95% confidence interval (CI): 1.855-10.734, P<0.001] and 1.642 (95% CI: 0.965-2.793, P=0.067), respectively, compared with non-NAFLD group.

Conclusion: NAFLD was a significant predictor for T2DM, but not for pre-diabetes, in Xi'an, China. More future cohort studies are needed to confirm the present findings.

Table Multivariate analysis of non-alcoholic fatty liver disease in predicting type 2 diabetes mellitus and pre-diabetes

Variable	Crude		Adjusted*	
	RR (95% CI)	*P* value	RR (95% CI)	*P* value
Diabetes				
Non-NAFLD	1.000		1.000	
NAFLD	4.247 (1.768-10.204)	<0.001	4.462 (1.855-10.734)	<0.001
Pre-diabetes				
Non-NAFLD	1.000		1.000	
NAFLD	1.788 (1.110-2.881)	0.017	1.642 (0.965-2.793)	0.067

NAFLD: non-alcoholic fatty liver disease; RR: relative risk; CI: confidence interval.

*: Relative risks and 95% CI were calculated using a backward stepwise method. The covariables were age, gender, educational level, cigarette smoking, alcohol drinking, physical activities, family history of diabetes, BMI, blood pressure, fasting glucose level, 2-hour glucose level, serum triglyceride, HDL cholesterol level.

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Serum uric acid predicts incident type 2 diabetes mellitus only in women after adjusting body compositions

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Background and aims: Serum uric acid (SUA) level has been reported to be associated with incident type 2 diabetes mellitus (T2DM) and body composition. However, several previous studies reported combined results for men and women after adjusting for sex and there were no studies that body compositions were taken into account. Thus, we designed this longitudinal sex-specific follow-up study to elucidate the association of SUA levels with incident T2DM including body compositions as an adjusting factor with large scale.

Materials and methods: A retrospective longitudinal study was performed to evaluate the association between SUA levels and the development of T2DM. A total of 21,714 participants (12,307 men and 9,407 women) taking a medical health check-up program without diagnosed T2DM at baseline were enrolled. Separate analyses were performed for men and women including body compositions as a confounding factor. Cox proportional hazards models were used to quantify the independent association between SUA levels and incident T2DM.

Results: During 109,438 person-years of follow-up, there were 1,768 (1,269 in men, 499 in women, respectively) incident cases of T2DM between 2006 and 2012. After full adjustment for multiple associated confounders including body compositions, SUA levels were significantly associated with incident T2DM in women (per 1 mg/dl, hazard ratio (HR)=1.125, 95% confidence interval (CI): 1.005-1.260, p=0.041). In men, however, this association lost statistical significance after adjusting for multiple associated confounders (per 1 mg/dl, HR=1.009, 95% CI: 0.952-1.069, p=0.762).

Conclusion: This study demonstrated that SUA levels are associated with incident T2DM only in women and do not predict incident T2DM in men after adjusting body compositions. Abbreviations: T2DM, type 2 diabetes mellitus; SUA, serum uric acid; BMI, body mass index; BP, blood pressure; LDL, low density lipoprotein; HDL, high density lipoprotein; FFM, fat-free mass; FM, fat mass.

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High triglycerides, low HDL cholesterol and a low LDL cholesterol per apolipoprotein B ratio predict incident diabetes in patients with established CAD

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Background and aims: Patients with type 2 diabetes mellitus (T2DM) exhibit a typical pattern of dyslipidemia with low HDL cholesterol, high triglycerides and a low LDL cholesterol per apolipoprotein B (LDL-C/apoB) ratio reflecting small LDL particles. We hypothesized that high triglycerides, low HDL cholesterol and a low LDL-C/apoB ratio predict incident T2DM among non-diabetic patients with established coronary artery disease (CAD).

Materials and methods: We enrolled 655 non-diabetic patients with angiographically proven stable CAD. Prospectively, the incidence of T2DM was recorded over a mean follow-up period of 6.1±3.7 years. Diabetes was diagnosed according to ADA criteria.

Results: From our non-diabetic coronary patients, 358 (54.7%) at baseline had normal fasting glucose (NFG) <100 mg/dl, and 297 (45.3%) had impaired fasting glucose (IFG) ≥100 mg/dl. During follow-up, T2DM was newly diagnosed in 17.4% of our patients. Baseline IFG compared to NFG was associated with a strongly increased risk of T2DM (26.6% vs. 9.8%; adjusted OR 3.34 [2.17-5.16]; p<0.001). Low HDL cholesterol, high triglycerides, and a low LDL-C/apoB ratio after multivariate adjustment including fasting glucose significantly predicted incident diabetes in the total study cohort (OR 0.65 [0.49-0.86]; p=0.003, 1.40 [1.13-1.74]; p=0.002 and 0.54 [0.41-0.71]; p<0.001, respectively) and also when we separately analyzed patients with IFG (OR 0.67 [0.46-0.97]; p=0.032, 1.42 [1.03-1.96]; p=0.032 and 0.56 [0.39-0.79]; p=0.001, respectively) and NFG (OR 0.62 [0.40-0.96]; p=0.034, 1.38 [1.03-1.86]; p=0.033 and 0.49 [0.32-0.76]; p=0.001, respectively).

Conclusion: We conclude that among patients with angiographically proven stable CAD the incidence of diabetes is high, particularly among those with IFG. Importantly, high triglycerides, low HDL cholesterol and a low LDL-C/apoB ratio significantly predict incident diabetes independently from baseline glycemia.

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A mathematical disease progression model for the effect of diet and exercise in subjects with impaired glucose tolerance in the Finnish Diabetes Prevention Study (FDPS)

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Background and aims: Mathematical modelling is frequently used to characterize and quantify underlying mechanisms of complicated physiological responses. For this purpose, Silber et al developed an integrated glucose-insulin (IGI) model describing dynamic glucose and insulin after glucose provocations in healthy and type 2 diabetic subjects. Although being able to describe the two populations, the progression from healthy to diabetic is missing in the IGI model. The aim of this project was to develop the IGI model to include disease progression for a population with impaired glucose tolerance (IGT), quantifying the effect of diet and exercise intervention.
Materials and methods: Data derived from the randomized Finnish Diabetes Prevention Study (FDPS) and consisted of yearly OGTTs (from baseline, year 0 to the fourth year), one IVGTT at the start of the study (baseline) and one IVGTT at the end of the study (fourth year). One hundred and one out of 552 subjects with impaired glucose tolerance who were randomly assigned to a lifestyle intervention group (diet and exercise) or a control group were studied. Subjects who developed diabetes were excluded from the study at the time of diagnosis. All longitudinal measurements of glucose and insulin after IVGTT and OGTT were used to fit the IGI model with mixed-effect methodology (NONMEM version 7.3). Disease progression was investigated on the pathophysiologically most reasonable parameters of the model. The effect of diet and exercise was investigated on the parameters identified to be involved in disease progression.
Results: Disease progression was found to affect the first phase insulin secretion, the maximal effect of incretin on insulin release and the insulin sensitivity, represented as insulin-dependent glucose elimination (p value <0.001); only the maximal effect of incretin on insulin release was unaffected by changes in lifestyle. For the control group, decreases of 7.5%/year and 4.7%/year were observed on first phase insulin secretion and insulin-dependent glucose elimination. With diet and exercise intervention, the decrease in the first phase insulin secretion was only 1.3%/year and the insulin-dependent glucose elimination increased by 3.1%/year. These values represent patients in the pre-diabetic state as occurrence of diabetes was an exclusion criterion for the study.
Conclusion: The disease progression was successfully included in the IGI model to describe changes in glucose provocation data from subjects with IGT, independent of lifestyle intervention program.
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Circulating concentrations of endothelin-1 predict impaired glucose tolerance and type 2 diabetes in women but not in men: a prospective study in the Vara-Skövde cohort

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Background and aims: Endothelial dysfunction is mechanistically coupled to insulin resistance and is characterised by an imbalance between nitric oxide and endothelin-1 (ET-1). Although ET-1 expression is increased in insulin resistance and type 2 diabetes (T2D) it is unclear whether ET-1 may be a forerunner of these conditions. Our current aim was to investigate whether circulating ET-1 levels may predict the incidence of impaired glucose tolerance (IGT) and T2D in a population based prospective study.
Materials and methods: During 2002-2005, 2816 adult participants (age 30-74 years) were randomly selected from two municipalities in south-western Sweden. At baseline circulating ET-1 levels and metabolic risk factors were measured while glucose metabolism was assesssed by performing an oral glucose tolerance test (OGTT, 75 g). Moreover, blood sampling, including OGTT, being performed in 1338 participants at baseline was repeated at the follow-up between 2012-2014. After excluding participants with IGT, T2D or missing information on ET-1 at baseline, 1152 participants were left for further analysis. General linear models (GLM) were employed to estimate differences in ET-1 levels in participants with or without impaired glucose metabolism. In addition, logistic regressions were used to estimate the odds ratio (OR) for incident IGT or T2D by 1 pg/ml difference in ET-1 level at baseline. All analyses were performed using SPSS 21.
Results: Age- and municipality-adjusted GLM showed that circulating baseline ET-1 levels were significantly higher in women who developed incident IGT and T2D compared with women with normal glucose metabolism at the follow-up (2.8±1.2, n=67 vs 2.4±1.3 pg/ml, n=518, p=0.013). In contrast, we observed no difference in ET-1 levels in men comparing participants with or without IGT/T2D at the follow-up (2.2±1.3, n=83 vs 2.3±1.2 pg/ml, n=483 p=0.589). There was a significant age-independent interaction term between sex and ET-1 (p=0.027). Further, sex-stratified logistic regression was significant in women (OR: 1.3, 95% CI: 1.1-1.6, p=0.013), but not in men (OR: 0.9, 95% CI: 0.8-1.4, p=0.513). Finally, the predictive value of ET-1 for IGT/T2D at the follow-up in women remained significant after further adjustment for age, municipality, waist-hip ratio, CRP, smoking, leisure time physical activity, and systolic blood pressure (OR: 1.3, 95% CI: 1.1-1.7, p=0.010).
Conclusion: This longitudinal population-based study shows that higher circulating ET-1 levels predict development of incident IGT and T2D in women but not in men. ET-1 might be an early marker for increased T2D risk in women, and, thus, a target for future treatment and prevention strategies.
Supported by: ALF-agreement, Swedish Research Council

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Increment of serum bilirubin affects incident type 2 diabetes

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Background and aims: Bilirubin is not only the byproduct of heme catabolism but also an important antioxidant in vivo. Serum bilirubin level has been reported to be associated with incident type 2 diabetes mellitus (T2DM). However, there have been no longitudinal studies to determine the association between changes of serum bilirubin levels and incident T2DM. The aim of current study was to investigate the relationship between baseline serum bilirubin (BB) levels and percentage changes in serum bilirubin (PCB) levels and incident T2DM in a large-scale longitudinal study.
Materials and methods: A total of 24185 participants taking annual medical health check-up program between 2006 and 2012 were enrolled. Multivariable-adjusted Cox regression models were fitted to assess the relative risk of incident T2DM by baseline and percentage change during follow-up in bilirubin levels. PCB levels were defined as follows; changes in serum bilirubin levels were calculated from baseline to the end of follow-up or until a year before the last date of diabetes diagnosis, divided by BB, and multiplied by 100.

Results: After exclusion of subjects with T2DM at the baseline and at the first visit of follow-up, liver disease, and missing data, 20233 participants were investigated and median follow-up duration was 60.23 months. 1275 new cases of T2DM occurred during follow-up. There was no significant difference in BB levels between the group with incident T2DM and the group without incident T2DM ($P>0.05$), while PCB levels of those groups were significantly different ($P<0.001$). When dividing BB and PCB levels into quartiles, the proportions of participants observed to develop T2DM were not different among BB quartiles ($P>0.05$), however, the proportions of incident T2DM significantly increased across quartiles of PCB (from the lowest to the highest quartile, 3.9% in the first, 4.2% in the second, 7.0% in the third, and 9.7% in the fourth; $P<0.001$). After adjusting multiple potential confounders including fasting blood glucose level and HbA_{1c}, the HR of incident T2DM in the highest quartile of PCB was more than two times that of the lowest quartile group (HR 2.038 [95% CI 1.722 - 2.412]; P for trend<0.001). This association was still significant when PCB was analyzed as a continuous variable, per 1% increase, the HR of incident T2DM was 1.006 (95% CI 1.005 - 1.007; $P<0.001$). In subgroup analysis ($n=13394$) with fasting insulin levels, the result remained significant (HR 1.006 [95% CI 1.005-1.008]; $P<0.001$) after additional adjustment of fasting insulin level and homeostasis model assessment of insulin resistance (HOMA-IR).
Conclusion: This study demonstrated that an increase in serum bilirubin level, rather than baseline serum bilirubin level itself, can be associated with incident T2DM.

PS 017 Monogenic diabetes

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A novel insulin mutation in autosomal dominant diabetes

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Background and aims: Diabetes caused by insulin (*INS)* gene mutations is a rare form of neonatal diabetes, prevalence is estimated at 1 of 200.000 live births in Europe. We report a German female patient with a novel form of permanent neonatal diabetes mellitus.
Materials and methods: The second child of non-consanguineous German parents was born at 40 weeks of gestation; BW 3260 g. At the age of 8 days the girl presented with polyuria, sweating and frequent vomiting. Blood glucose showed significant hyperglycaemia >200 mg/dl without ketosis. Insulin (6.9 mU/l) and C-peptide (1.8 µg/l) were detectable and there were no clinical or laboratory signs of exocrine pancreatic insufficiency. Transabdominal ultrasound showed a normal pancreatic structure. No other symptoms or dysmorphic features were evident. Her father had been treated for diabetes mellitus since the age of 3 months, so far classified as type 1 diabetes, while there was no other case of DM in the family history. Genetic testing identified a novel heterozygous *INS* gene mutation (c.128G>A) in both father and daughter. The missense mutation causes an amino acid exchange of the highly conserved position 43 cystein (p.Cys43Tyr), resulting in the loss of one disulfide bond. Bioinformatic tools predict an impairment in protein function (MutationTaster, PolyPhen2, SIFT, AlignGVGD).
Results: We considered that the novel mutant insulin might cause ß-cell toxicity through endoplasmatic reticulum (ER) stress as described for other *INS* mutations. Low dose insulin therapy was started using continuous subcutaneous insulin infusion (CSII) with insulin U10 dilution at the age of 4 weeks in combination with continuous subcutaneous glucose monitoring (CGMS). Significant hypoglycaemia did not occur, blood glucose could be kept in the range between 100 and 170 mg/dl within the first year of life. Bolus insulin was not introduced until the age of 18 months. At the current age of 24 months, the child is thriving well with HbA1c stable between 60-65 mmol/mol under low dose CSII with 0.3 IU/kg/d without hypoglycaemia. The father shows poor glucose control (HbA1c 111 mmol/mol) due to incompliance, and low but significant C-peptide release even after three decades of diabetes.
Conclusion: This novel *INS* mutation leads to a loss of one disulfide bond. Its phenotype has not yet been described. Low dose CSII was used without overt hypoglycaemia aiming to decrease endogenous insulin production and therefore reduce ER stress to minimize progressive loss of beta cell mass. Follow up will show whether low dose insulin infusion will help to maintain ß-cell function due to reduction of ER stress.

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miRNA 224 is readily detectable in urine of individuals with diabetes mellitus and a potential indicator of beta cell demise

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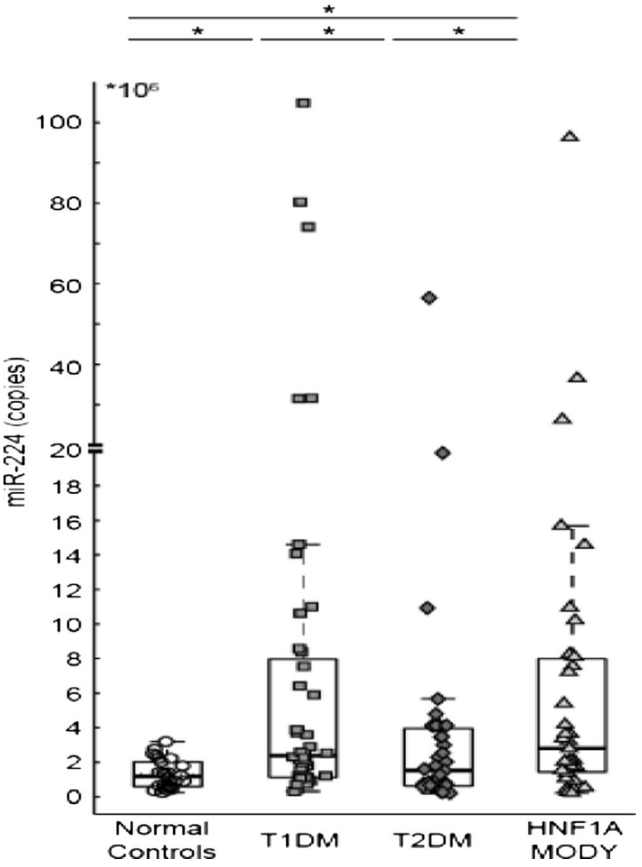
Background and aims: MicroRNA (miRNA) are a class of non-coding, 19-25 nucleotide RNA critical for network-level regulation of gene expression. HNF1A-maturity-onset diabetes of the young (HNF1A-

MODY) is a monogenic form of diabetes resulting from mutations in the gene encoding the pancreatic transcription factor hepatocyte nuclear factor 1α (HNF1A). We recently demonstrated that induced suppression of endogenous HNF1A function in INS-1 cells, a cellular model of HNF1A-MODY increased the levels of two miR-224 and miR-103. Using absolute quantitative PCR analysis, we demonstrated that miR-224 and miR-103 levels were significantly elevated in the serum of HNF1A-MODY carriers when compared to controls. miR-224 is a novel miRNA in the field of diabetes. The aim of this current study was to obtain proof-of-concept that miR-224 and miR-103 are also detectable in the urine of HNF1A-MODY carriers, and to determine whether these diabetes-associated miRNA are also elevated in the urine of patients with type 1 and type 2 diabetes mellitus.

Materials and methods: Absolute levels of miR-224 and miR-103 were determined in the urine of n=144 individuals including HNF1A-MODY carriers, participants with type 1, type 2 diabetes mellitus and normal controls. We also correlated urinary levels of miRNA with serum levels and with clinically relevant indices of renal disease.

Results: miR-224 was significantly elevated in the urine of HNF1A-MODY carriers and participants with type 1 diabetes mellitus (see figure 1). miR-103 was highly expressed in urine across all diabetes cohorts when compared to controls. For both miRNA-224 and-103 we found a significant correlation between serum and urine levels ($p<0.01$). miR-224 and miR-103 levels in HNF1A-MODY, type 1 or type 2 diabetes mellitus cohorts did not correlate with the renal indices; albumin creatinine ratio (ACR) or estimated Glomerular Filtration Rate (eGFR).

Conclusion: We here provide the first proof-of-concept study that miRNA can be readily detected in the urine of participants with diabetes. The utilization of urine as a biofluid has multiple advantages in the clinical setting. Urine sampling is non-invasive and not subject to haemolysis. This is important, as blood contamination and haemolysis are the most common artefacts encountered in miRNA detection in fluids. miR-224 is a novel diabetes-associated miRNA which is highly expressed in the urine of HNF1A-MODY carriers and a type 1 diabetes mellitus cohort. We surmise that the differential expression levels of miR-224 in both insulin deficient states may be an attempt to compensate for beta- cell demise. A further novel finding of the current study is the detection of the well-established diabetes-associated miRNA; miR-103 in urine.



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Permanent neonatal diabetes: new hopes for a dreadful disease

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Background and aims: Of the many possible mutations in the KCNJ11 gene, the Q52R is responsible for a severe form of permanent neonatal diabetes (PND) associated with developmental delay and epilepsy (DEND syndrome). None of the previously 3 cases detected worldwide was successfully switched to sulphonylurea (SU). One of them had only a few months survival and the long-term insulin treatment in the other two patients was associated with poor disease control and persistence of developmental deficits.

Materials and methods: A one year and three months old boy was admitted on 28th July 2014 in our emergency university hospital, for a scheduled attempt on switching from insulin to SU for his PND. He was diagnosed with PND at age 2 months, testing negative for major islet autoantibodies. As switching to SU resulted in ketoacidosis, he has since remained on insulin, currently needing 6 U/day. Recent genetic testing performed in Exeter laboratories (Welcome Trust fund), UK revealed an acquired, rare, but severe point mutation (Q52R) in KCNJ11 gene.

Results: On admission, he had moderate-severe developmental deficit, with moderate hypotonia, but no other significant symptoms. He was just staying horizontally in his bed, without the possibility of rolling over by himself. His weight was 13 Kg, for a 75 cm length, showing no clinical

significant biochemical results except for HbA1c=9.5% (80 mmol/mol), LDH=352 U/L (Normal range: 125-220 U/L), and C-peptide=0.02 ng/ml (intermediate DEND syndrome). He was successfully switched on glibenclamide alone, given every 3-6 h, with a rapid escalation of doses due to lack of initial response. He was discharged from the hospital on 6th August 2014, with a total SU dose around 0.8 mg/Kgc/day. A significant improvement in both metabolic control and developmental deficits was registered after one month. The patient was able to walk and HbA1c was 6.5% at 6 months of SU treatment. Insulin secretion appears to be strongly nutrient dependent, with glucose values dropping after food intake and rising with food deprivation.

Conclusion: This is the first worldwide report of a successful transition from insulin to SU treatment in a patient suffering from PND associated with intermediate DEND syndrome due to Q52R mutation in the KCNJ11 gene. The dramatic improvement in neurologic deficits gives new hopes for what was once considered a dreadful disease.

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The clinical management of hyperglycaemia in pregnancy complicated by maturity onset diabetes of the young

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Background and aims: Women with Maturity Onset Diabetes of the Young (MODY) are often first identified and diagnosed with diabetes during pregnancy. Genetics and hyperglycemia play an important role in determining fetal size in MODY pregnancies .Observational data on glycemic control and fetal outcomes in pregnancies complicated by glucokinase (GCK) and (hepatocyte nuclear factor-1 alpha) HNF-1α MODY mutations are lacking. The aim of the current study was to determine the outcomes and clinical management of hyperglycemia using home blood glucose monitoring (HBGM) in MODY pregnancies.

Materials and methods: A retrospective chart review of 37 women with a GCK or a HNF-1α mutation was conducted. There were 132 pregnancies in these women. Data on variables such as birth weight, mode of delivery and the treatment of hyperglycaemia was recorded. Complications which included congenital anomaly, birth injury, admission to neonatal intensive care unit (NICU) and prolonged neonatal hypoglycemia were recorded. Genotyping of 36 offspring was known. The HBGM recordings of a subset of these women, who were all managed according to our current guidelines for diabetes in pregnancy, with a GCK or HNF-1α mutation referred to one of the three tertiary centres were analysed. The median of the highest and lowest blood glucose value for each day, at each time point, was averaged over a seven day period. This was performed for each trimester.

Results: The birth weight in non-effected GCK offspring was significantly higher than in the effected GCK offspring (4.8 kg (4.1-5.2) vs. 3.2 kg (3.1-3.7), p=0.01). Seven point home blood glucose monitoring (HBGM) over a seven day period in each trimester demonstrated higher fasting and post prandial glycemic excursions in the 1st trimester of GCK pregnancies when compared to HNF-1α pregnancies (Fasting; 5.8 mmol/l (5-6.4) vs 4.7 mmol/l (4.3-4.9), p=0.01 and Post-prandial: 8.6 mmol/l (7.5-10.9) vs 6.2 mmol/l (5.6-7.3), p=0.04) despite insulin treatment. There was a higher percentage of miscarriages in the GCK group when compared to the HNF1A-MODY group (33.3% vs 14%, p=0.07) which was similar to the background population. Insulin initiated at an early gestation appeared to lower the incidence of macrosomia in GCK non-effected offspring.

Conclusion: To date, this is the largest study performed specifically looking at glycemic excursions in pregnancies complicated by GCK and HNF-1α MODY. This study demonstrates that the current management of HNF-1α pregnancies appears to be appropriate. In GCK pregnancies, however, the glycaemic variability that we have clinically noted warrants attention. There was an increased percentage of both macrosomia and miscarriages in GCK pregnancies highlighting the importance of a diagnosis of GCK-MODY in women prior to conception and the necessity for pre-conception care.

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Identification and clinical characteristics of a family with the ARG103PRO mutation in the NEUROD1 gene detected by new generation sequencing

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Background and aims: Until now there have been only a few families with early onset autosomal diabetes due to mutations in the NEUROD1 gene identified. Moreover, only two of them seemed to meet strict MODY criteria. The introduction of next-generation sequencing (NGS) provides a new opportunity to detect more pathogenic mutations in this gene. The aim of this study was to evaluate a segregation of the Arg103Pro mutation in the NEUROD1 gene detected by NGS in the proband from family and to describe the clinical characteristics of mutation carriers.

Materials and methods: We selected 96 diabetic probands of MODY families, among which 42 patients were earlier tested GCK-MODY and HNF1A-MODY by Sanger sequencing with negative results. Genetic testing was performed by the targeted NGS sequencing using a panel of 29 genes in which mutations have been reported to cause monogenic diabetes. This panel included the NEUROD1 gene.

Results: Among genetic variants detected by NGS, one patient had a missense mutation in the gene NEUROD1. It was the Arg103Pro (CGC / CCC) missense mutation. This mutation affected the DNA binding domain of the NEUROD1 transcription factor. We confirmed this sequence difference by Sanger sequencing. The family with the mutation was included into our MODY registry in 1999 and had previously been tested with negative results for mutations in GCK and HNF1A. 17 family members were invited for further genetic testing. In 11 subjects we confirmed the presence of the mutation. Seven adult mutation carriers (all but one) from three generations have been already diagnosed with diabetes and were treated with different therapeutic models. There were 3 individuals with the Arg103Pro mutation diagnosed before the age of 30 years in the family. The range of age of unaffected mutation carriers was 4-48 years. Interestingly, there was a history of neonatal severe hypoglycemia in a pediatric patient who is also a carrier of the mutation. The clinical course reminded episodes typical for HNF4A-MODY.

Conclusion: The use of the NGS method will likely allow for more frequent identification of rarer forms of MODY. The identified MODY family seems to be just the third NEUROD1 family meeting the criteria of MODY.

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Reuptake of secreted carboxyl-ester lipase (CEL) variants, a role in diabetes and exocrine pancreatic disease?

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Background and aims: CEL is a digestive enzyme involved in hydrolysis and absorption of cholesterol and lipid-soluble vitamin esters. We have previously described a syndrome of diabetes and exocrine pancreatic dysfunction caused by mutations in the CEL gene. The mutant protein (CEL-MUT) has a higher tendency to aggregate and undergo endocytosis as compared to the wild-type CEL protein (CEL-WT). Here, we compare the cellular reuptake of CEL-MUT and CEL-WT in HEK293 cells and pancreatic cell lines. We also investigated the intracellular fate of the two protein variants, their effect on viability, as well as their impact on insulin secretion from endocrine cells.
Materials and methods: HEK293 cells stably expressing CEL protein variants were grown for 24 h toward confluence. Conditioned media were added to untransfected cell lines (HEK293, embryonic kidney; INS-1E, insulinoma; 266-6, acinar; PANC-1, ductal) for various durations. Immunostaining and confocal microscopy were performed to investigate endocytosis and degradation of CEL. Cell viability was monitored by quantitation of ATP generated by metabolically active cells and insulin secretion was determined by ELISA.
Results: In HEK293, acinar and INS-1E cells we observed increased uptake of the CEL-MUT protein variant as compared to CEL-WT. After endocytosis the mutant protein was present as intracellular puncta co-localizing with the lysosomal marker LAMP1. The same tendency was noted for CEL-MUT in PANC-1 cells. Exposure to the CEL-MUT protein negatively affected the viability of the investigated cell lines. Preliminary results indicated that long-time exposure of INS-1E cells to the mutated protein leads to a reduced glucose-stimulated insulin secretion compared to when these cells are grown in the presence of the wild-type protein.
Conclusion: Our data indicate that mutated CEL negatively affects insulin secretion. We suggest that extracellular CEL-MUT aggregates are toxic and removed by cell-mediated uptake and degraded by the cells. Taken together, these findings could be relevant for how CEL-MUT cause a disease affecting both the exocrine and endocrine pancreas.
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Serum metabolome changes in healthy subjects with different genotypes of NOS1AP in the Chinese population

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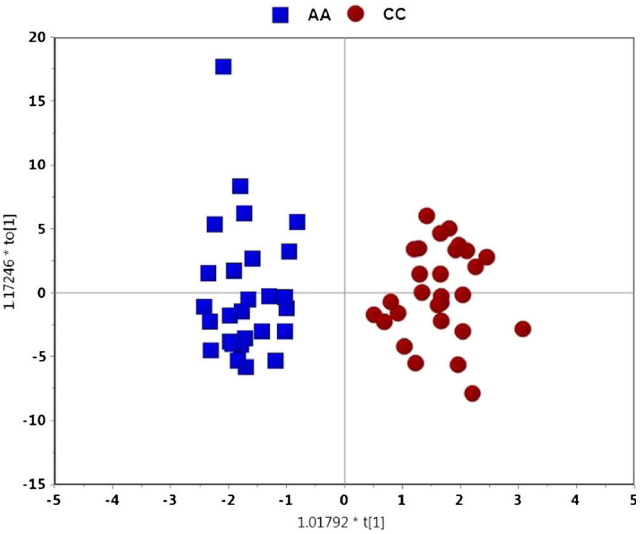
Background and aims: Nitric oxide synthase 1 adaptor protein (NOS1AP), regulates the neuronal nitric oxide synthase (nNOS) activity and has an effect on nitric oxide (NO) release by binding N-methyl-d-aspartate receptors. One functional study showed that rs12742393 could affect NOS1AP gene expression through influencing transcription factor binding. Our previous study showed evidence that rs12742393 in NOS1AP was involved in type 2 diabetes susceptibility in the Chinese population, with C allele as the risk allele (OR 1.17, 95% CI 1.07-1.26; P =0.0005). In this study, we report a comprehensive metabonomic study of different genotypes (AA and CC) of rs12742393 using two complementary analytical platforms, gas chromatography time-of-flight mass spectrometry (GC-TOFMS) and ultra-performance liquid chromatography quadrupole time-of-flight mass spectrometry (UPLC-QTOFMS).
Materials and methods: Fifty-five healthy participants with normal glucose regulation were selected for metabolomic investigation, including twenty-seven CC homozygote and twenty-eight AA homozygote individuals. All the individuals for the metabolomic analysis were matched strictly with age, sex, BMI, glucose, and lipid related parameters. For all the subjects, venous blood samples were obtained after overnight fasting for at least 10 h and subjected to GC-TOFMS and UPLC-QTOFMS. The GC-TOFMS data was analyzed by ChromaTOF software, the UPLC-QTOF-MS ES+ and ES- raw data were analyzed by the MarkerLynx Applications Manager version 4.1 (Waters, Manchester, U.K.) and then metabolites were identified by comparing the accurate mass, mass fragments, characteristic ions with the available reference standards and published reports, in addition to the web-based resources such as the Human Metabolome Database. The data sets were then analyzed and validated by uni- and multi-variate statistical methods, separately. Principle component analysis (PCA) and orthogonal partial least squares-discriminant analysis (OPLS-DA) were carried out (SIMCA-P 12.0, Umetrics, Umeå, Sweden) and Wilcoxon-Mann-Whitney test was selected to measure the significance of each metabolite.
Results: A total 258 metabolites were identified and seven significantly altered serum metabolites identified in AA carriers relative to CC carriers were selected according to the VIP threshold (VIP>1) in OPLS-DA model coupled with the Mann-Whitney U test ($p<0.05$). These including Malic acid, Inosine, maltose, adonitol, ribitol, serine and glycine.
Conclusion: We detected seven metabolites showing significant differences between CC and AA carriers of rs12742393 in NOS1AP. These metabolites might associate with the development of type 2 diabetes in subgroup of patients through the crosstalk with NOS1AP protein, which might provide us a new perspective to the mechanism of type 2 diabetes.



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Integrated analysis of oxidative phosphorylation changes and characteristic metabolic profiles on diabetes with the mitochondrial DNA 3243 (A>G) mutation

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Background and aims: An A-to-G transition at position 3243 of mitochondrial DNA (A3243G) can result in maternally inherited diabetes and deafness (mitochondrial diabetes). The relationship among mutation heteroplasmy, mitochondrial oxidative phosphorylation(OXPHOS) and metabolic profiles are largely unknown, making great challenges for disease prevention and control.
Materials and methods: Five mitochondrial DNA 3243 (A>G) mutation patients, three of them belong to one family linage, were included in this project. The participants' body mass index scores, blood pressures, blood glucose and insulin levels at 0, 30, 60, 120 and 180 min were measured in response to a 75 g oral glucose tolerance test. Also they were undergone pure tone test, brain MRI, visual evoked potential, electrocardiogram. A quantitative method based on pyrosequencing was used to analyze the heteroplasmy of these patient. Quantitative reverse transcription-PCR analysis was used to analysis the expression of all the 13 OXPHOS genes. The serum of five mitochondrial DNA 3243 (A>G) mutation patients, five type 2 diabetes patients, five latent autoimmune diabetes in adult and five normal controls were analyzed using gas chromatography time-of-flight mass spectrometry platform.
Results: We found a variety of clinical conditions in association with mitochondrial DNA 3243 A>G mutation, even in asymptomatic subjects. Levels of the mutation heteroplasmy varied among the different sampled blood, buccal cells and urinary sediment of the mutation carriers. In addition, defects in subunits of the OXPHOS complexes and respiratory chain assembly were observed in the mutation carriers. Using GC-MS profiling methods, we found significant changed metabolites that were associated with mitchodrial DNA 3243A>G mutation.
Conclusion: The metabolic profile of mitochondrial DNA 3243 (A>G) mutation was quiet unique. There are some significant changed metabolites were associated with neurotransmitter and energy transfer. The metabolic profile together with the oxidative phosphorylation changes can partly explain the clinical symptoms of mitochondrial DNA 3243 (A>G) mutation.

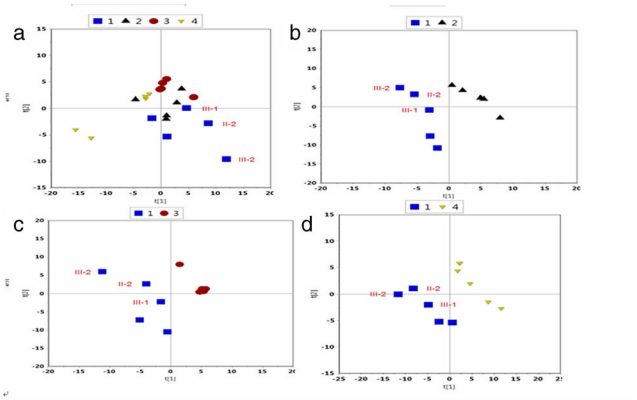

Figure: PLS-DA score plots of ■:mt.3243A>G mutation patients, ▲:Type 2 diabetes patients, ●: LADA patients, ▼: Normal controls. II-2, III-1 and III-2 belong to one family linage: II-2 is the mother of proband III-2, III-1 is the sister of proband III-2.

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Prevalence of retinopathy in adult patients with GCK-MODY and HNF1A-MODY

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Background and aims: Maturity onset diabetes of the young (MODY) is a heterogeneous group of monogenic disorders that result in hyperglycemia of various degree and pattern. It constitutes up to 1 million of patients in Europe. Thus, it is very important to understand the consequences of MODY diagnosis, including the risk of vascular complications. Diabetic retinopathy (DR) is a complication considered to be particularly closely related to the duration and degree of hyperglycemia. We aimed to assess the prevalence of DR in adult patients with GCK-MODY and HNF1A-MODY in Poland and to identify biochemical and clinical risk factors associated with its occurrence.
Materials and methods: We examined 74 *GCK* mutation carriers, 51 with diabetes and 23 with prediabetes, respectively, and 63 patients with HNF1A-MODY. Retinal photographs, twelve for each patient, were done by a fundus camera. Signs of DR were graded according to the DR disease severity scale. Statistical tests were performed to assess differences between the groups and logistic regression was done for the association with DR.
Results: The mean age at examination was 34.5±14.8 and 39.9±15.2 in the GCK-MODY and HNF1A-MODY groups, respectively. Mild nonproliferative DR (NPDR) was found in one patient with the GCK mutation and likely concomitant type 1 diabetes, whereas DR was diagnosed in 15 HNF1A-MODY patients: 9 with proliferative, 3 with moderate NPDR and 2 with mild NPDR. The strongest independent predictors of DR in HNF1A-MODY were markers of glucose control: HbA1c (OR: 2.05, CL%95: 1.2-3.83, p=0.01) and glucose (p=0.006, OR: 1.40, CL%95: 1.12-1.83) analyzed in two separated models. Additionally, arterial hypertension independently predicted DR (OR: 9.06, CL%95: 1.19-98.99, p=0.04) in the model with HbA1c as glycaemic control marker.
Conclusion: In conclusion, DR of any degree was not present in our GCK-MODY group, while in spite of young age almost every fourth subject with HNF1A-MODY showed signs of this complication.
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Variants of the SLC30A8 gene associated with anti-ZNT8 antibody and predisposition to type 1 diabetes

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Background and aims: The SLC30A8 gene encodes the zinc transporter protein (ZnT8), present in the membrane of insulin granules and expressed mainly in pancreatic beta cells. The ZnT8 protein was identified as an autoantigen in T1D and the rs2466293 and rs16889462 variants were associated with susceptibility to type 1 diabetes (T1D) in Caucasians, but there are few data in Latin-America and in the multi-ethinic Brazilian population. The objective was to evaluate the influence of the variants rs2466293 and rs16889462 in the predisposition to T1D and in the frequency of anti-ZnT8 autoantibody (Ab) which was previously found to be 48.3% in 479 T1D patients living in Sao Paulo city.
Materials and methods: We evaluated 623 patients T1D (age: 25± 12.7 years; 367 F / 257 M, 80% white) and 650 controls (age: 29± 11.2 years; 243 F / 407 m, 63.4% white). The variants rs2466293 and rs16889462 were genotyped by the Vera Code Golden Gate (Illumina)

methodology. Autoantibodies against zinc transporter (anti-ZnT8) were determined by ELISA (Kronus, USA CV of <7%) and against glutamic acid decarboxylase (anti-GAD65) and tyrosine phosphatase (anti-IA2) by radioimmunoassay (RSR Limited, UK, CV <7%). The genotypic associations were analyzed using the chi-square test or Fisher exact test. P <0.05 was considered significant.

Results: The frequencies of genotypes of rs2466293 and rs16889462 variants were in Hardy-Weinberg Equilibrium , were similar in patients and controls and were independent of gender. However, in non-whites, the rs2466293 AA genotype conferred protection to T1D, prevailing in controls (57.1%) compared to T1D patients (43.7%); p=0.0233; OR= 0.5821; CI=.3716 to .9117. The rs1688946 AG genotype , although rare, was associated with higher frequency of anti-ZNT8 Ab (75%) when compared to GG (48%); p=0.0415; OR=3.245; CI=1.023 to 10.288. None of SLC30A8 variants influenced on age at diagnosis of T1D or on anti-GAD65 and anti-IA2 Abs frequency .

Conclusion: The rs2466293 and the rs16889462 variants in SLC308A gene were related to T1D predisposition and to anti-ZnT8 Ab frequency respectively in our population.

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PS 018 Genetic diversity in type 1 and type 2 diabetes

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GWAS-implicated in type 1 and type 2 diabetes loci: insight into their relative roles in the pathogenesis of Latent Autoimmune Diabetes in Adults (LADA)

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Background and aims: The genetic etiology of adult-onset autoimmune diabetes, including 'latent autoimmune diabetes in adults' (LADA), remains unresolved. LADA exhibits clinical features of both type 1 (T1D) and type 2 (T2D) diabetes. LADA cases present initially as adult-onset diabetes, but not requiring insulin, yet with circulating T1D-associated islet autoantibodies and comprise 5-10% of all adult-onset diabetes in Europe and China.

Materials and methods: Leveraging existing genome-wide SNP genotyping data generated on the Illumina Infinium II OMNI Express platform, we assessed the association of all GWAS-implicated T1D and T2D loci reported to date in 965 LADA subjects and 1134 healthy control subjects recruited from a European multicenter study. Through strict phenotyping criteria, diagnosis was defined by subjects aged 30-70 years, positive for glutamic acid decarboxylase autoantibodies without initial insulin therapy.

Results: The MHC region was strongly associated with LADA (sentinel SNP rs1063355; odds ratio (OR)=1.58; P=3.24×10-10). Strong evidence of association was also observed for other T1D-associated loci, including SH2B3 (rs3184504 and rs1265564, OR=1.45; P=1.18×10-6), IL7R (rs6897932, OR=1.31; P=4.34×10-5 & rs1445898; OR= 1.23; P=2.94×10-4), PTPN22 (rs2476601 and rs6679677, OR=0.71; P=2.62×10-4) and INS (rs689, OR=1.45; P=3.46×10-4). Despite these observations, it was interesting to note that there was no association with the strong T1D-associated locus, CLEC16A or, contrary to previous reports, the key T2D TCF7L2 locus. However, when we constrained the dataset on positivity for the IA2 antibody, the TCF7L2 locus did yield nominal evidence of a paucity of the T2D-associated rs7903146 T allele (n=367 cases; OR=0.78; P=0.014). Conversely, also in the IA2 constraint setting, the HNF1A locus yielded significant (including survival for multiple correction) evidence of an excess of the T2D-associated rs7957197 T allele (OR=1.55; P=1.04×10-4). These novel observations require follow-up studies to elucidate the mechanism of action in this setting.

Conclusion: In conclusion, LADA, the major form of adult-onset autoimmune diabetes as defined here, reveals a genetic etiology very similar to childhood-onset T1D, with the striking exception of CLEC16A. Absence of the genetic locus CLEC16A could account for a later age at presentation of T1D, notably LADA.

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Genes previously associated with Polycystic Ovary Syndrome (PCOS) in the Asian population and variability in PCOS population from central Europe

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Background and aims: Recent investigations by genome-wide association approach in an Asian population identified many genes associated with polycystic ovary syndrome (PCOS), as LHCGR (luteinizing hormone/choriogonadotropin receptor), DENND1A (DENN/MADD domain containing 1A), YAP1 (Yes-associated protein 1), TOX3 (TOX high mobility group box family member 3) and insulin receptor (INSR) genes. In this context we explored variation of these genes and that of KLF14 (Kruppel-like factor 14) gene in PCOS in a population of 401 cases and 143 controls from Central Europe well characterized at the endocrine and metabolic levels, aiming to test gene association to PCOS in a population of Caucasian origin.
Materials and methods: Genetic markers used in the study were the SNPs rs2479106 A/G, rs1894116 A/G, rs4784165 T/G, rs2059807 (A/G) and rs1562398 for DENND1A, YAP1, TOX3 and INSR, respectively. Genotyping of SNPs was carried-out by KASPar technology while genetic association and genotype-phenotype correlations were calculated by logistic regression and ANOVA, respectively using StatView and GoldenHelix programs, respectively.
Results: We observed gene-dose effect of LHCGR (rs1340572) with diastolic blood pressure (P=0.014; GG (n =1) 100 mmHg, AG: (n=33) 73,8±3.2 mmHg, AA: (n=224) 69.7±0.8 mmHg) while DENND1 (rs2479106) displayed a trend of gene-dose effect with plasma insulin at 120 minutes during the OGTT test (P=0.053; AA (n=84): 73.8± 7.2 mU/L, AG (n=101): 102.0±10.9 mU/L, GG (n=22) 128.3± 39.2 mU/L). However none of genes were associated with PCOS phenotype in our investigation.
Conclusion: This study allowed observing the association of genes with metabolic components of PCOS with some variability from data previously reported in the Asian population, which highlights one more time the necessity of considering differences between populations when studying the genetic susceptibility to complex disorders.
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Haplotype mapping of GCKR gene indicates the association with PCOS and reveals the role of BCAA metabolism

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Background and aims: Polycystic ovary syndrome (PCOS) is one of the most common endocrine disorders characterized by reproductive and metabolic disorders including insulin resistance (IR) and metabolic syndrome (MetS). It has been shown that dysregulated branched-chain amino acids (BCAA) metabolism may be associated with IR. High plasma level of BCAA was also noted in women with PCOS. At the genetic level, some genes were correlated to BCAA plasma variation, type 2 diabetes (T2D), IR and obesity, among which the GCKR (glucokinase regulatory protein) is one of the candidates. The aim of this work was to investigate the potential association of GCKR with PCOS and its components.
Materials and methods: We studied a population from Central Europe (401 cases and 143 controls) with PCOS diagnosed according to Rotterdam criteria, using the leader SNP (Single nucleotide polymorphism) rs1260326 (C/T) as well as 200 SNPs at this locus. The leader SNP was genotyped in all population by KASPAR technology. Haplotype mapping was performed in a nested study to determine the Linkage Disequilibrium (LD) pattern in population. The locus of GCKR gene was screened with 200 SNPs in 55 cases and 48 controls by Affymetrix technology. LD pattern and haplotypes reconstruction were carried-out by HAPLOVIEW 4.2 and PHASE 2.1, respectively while gene association and genotype-phenotype correlations were assessed by logistic regression and ANOVA using StatView and GoldenHelix.
Results: We found an association of the leader SNP rs1260326 TT genotype with protective effect against PCOS (P<0.03, OR 0,49 95%CI [0.25 - 0.96]). This result was specifically observed in lean PCOS women (P=0.036) and PCOS without metabolic complications or MetS (P= 0.045). The nested study showed a block of LD including SNPs rs2293572, rs2293571, rs1260326 and rs3817588 while reconstruction of haplotypes in population revealed 7 haplotypes, among which 5 were more frequent: H3 (CGTT) (49%), H5 (GACT) (27.7%), H2 (CGCC) (15.0%), H1 (CGCT=ancestral haplotype) (4.9%) and H6 (GACC) (2.4%). The T variant of rs1260326 was found in the most frequent haplotype.
Conclusion: Our finding indicates that GCKR is a good candidate for PCOS and is in accordance with some previous studies albeit with some variations and reinforces the interest in BCAA and our approach in studying SNPs variation in ethnic groups in Europe.

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Impact of known type 2 diabetes associated variants on circulating levels of branched-chain amino acids during an oral glucose tolerance test

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Background and aims: A detailed understanding of the pathophysiology of type 2 diabetes (T2D) and identification of early metabolic alterations is essential for identifying individuals at high risk of this disease. Our aim is to investigate the branched-chain amino acids (BCAAs; that is leucine, isoleucine, valine) level in various stages of pre-diabetes and their association with known T2D risk variants.
Materials and methods: ADDITION-PRO is a population based longitudinal cohort study of individuals at low to high risk for diabetes, nested in the ADDITION-Denmark study. A stratified sub-sample of individuals were invited to a follow-up health examination (2009-2011) and 2,082 participants attended. A total of 1,490 participants without known diabetes were given a standard 75 g oral glucose tolerance test (OGTT) after an overnight fast of ≥8 hours. The blood samples were drawn at 0, 30 and 120 min for assessment of serum insulin, plasma glucose, and plasma BCAAs concentrations. Plasma BCAA levels were measured by NMR spectroscopy. Previously reported 68 T2D risk SNPs were genotyped using the Illumina Infinium HumanCoreExome Bead chip or imputed with high quality into a 1000 genomes phase 3 panel. Genotype-phenotype correlations were studied using the linear mixed model test implemented in the EPACTS software package.
Results: Circulating BCAA levels decreased during an OGTT (30, 120 minutes) compared to fasting (0 minutes) levels. Additionally, fasting

and stimulated BCAA levels were higher in men compared to women ($P < 0.001$), higher in obese compared to lean subjects ($P < 0.001$), and higher in individuals with impaired fasting glucose (IFG) ($P < 0.04$) and impaired glucose tolerance (IGT) ($P < 0.001$) compared to normal glucose tolerance. BCAA levels were positively correlated with sex, BMI, insulin levels, HOMA-IR and negatively correlated with Matsuda indices. Genetic association with fasting and/or stimulated BCAA levels (after adjusting for age, sex and BMI) suggested that the known T2D risk variants in *JAZF1* (rs864745, effect size -2.7%), *DUSP9* (rs5945326, effect size +2.6%), *IGF2BP2* (rs1470579, effect size +2.4%) and *GCKR* (rs780094, effect size -2.4%) were associated significantly ($P < 0.05$) with Isoleucine levels. *PPARG* (rs1801282, effect size +3.0%), *JAZF1* (rs864745, effect size +1.9%), *DUSP9* (rs5945326, effect size +2.2%), *PAM* (rs35658696, effect size +3.5%) variants were significantly ($P < 0.05$) associated with leucine levels. And the variants in *ADAMTS9* (rs4607103, effect size +7.6%), *DUSP9* (rs5945326, effect size +5.9%), *IGF2BP2* (rs1470579, effect size +2.8%), and *GRB14* (rs3923113, effect size -2.2%) were significantly ($P < 0.05$) associated with valine levels.

Conclusion: Our large population-based study shows that BCAA levels were significantly higher in individuals with impaired glucose regulation. Several T2D risk SNPs were significantly associated with BCAA levels; however, biological functions of these associated variants are largely unknown. Further investigations are needed to find the casual relation of the T2D risk variants with BCAA levels.

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Genome-wide association studies of diabetic kidney disease in patients with type 2 diabetes

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Background and aims: Diabetes mellitus is associated with a range of chronic complications including diabetic kidney disease (DKD), a leading cause of end-stage renal disease (ESRD). To date few robustly associated genetic factors for these traits have been identified. As part of the SUMMIT consortium we performed a meta-analysis of genome-wide association studies (GWAS) in patients with type 2 diabetes (T2D) from European backgrounds to identify genetic determinants of DKD.

Materials and methods: GWAS were performed on 7 case control phenotypes based on the severity of diabetic nephropathy (DN). DN was characterised by micro-albuminuria, macro-albuminuria, end stage renal disease (ESRD), or chronic kidney disease (CKD; eGFR <60 ml/min) in 4 European studies with type 2 diabetes comprising up to 3,345 cases and 2,372 controls. We analysed 9,213,894 single nucleotide polymorphisms, imputed based on the 1000 Genomes (March 2012) reference panel, using EMMAX, adjusting for sex, age at onset, duration of diabetes and kinship matrix. Meta-analyses were performed using a fixed effects model and SNPs with $p < 5 \times 10^{-6}$, from any of the binary phenotypes, were selected for *in silico* replication in 2 European cohorts comprising 3,247 cases and 906 controls.

Results: From the meta-analyses we identified 139 SNPs for *in silico* replication. After joint analysis of the discovery and replication cohorts 9 SNPs showed nominal replication ($p < 0.05$ with same direction of effect), however no loci reached genome wide significance ($p < 5 \times 10^{-8}$). The strongest joint signals were rs4977388 (ESRD vs all other phenotypes, $p = 2.25 \times 10^{-7}$, near *MLLT3*), rs7916840 (DN, $p = 6.2 \times 10^{-7}$ intronic to *GP158*), rs9942471 (DN, $p = 9.8 \times 10^{-7}$, near *GABRR1*), rs6865390 (Macro-albuminuria + ESRD, $p = 2.05 \times 10^{-7}$, near *RA114*) and rs2206136, (CKD, $p = 5.42 \times 10^{-7}$, intronic to *PLCB4*).

Conclusion: To follow up on these findings we are now performing *de novo* genotyping in 3,867 additional individuals with T2D. In addition we are also integrating these data with T1D data from SUMMIT and the JDRF DNCRI, T2D data from FIND and other sources, as well as exome sequence data. By combining and comparing these different analyses we hope to gain a broader picture of the biology of DKD.

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The protective effect of cathepsin H on beta cell apoptosis is mediated through the Jak-STAT and NFkappaB signalling pathways

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Background and aims: In type 1 diabetes (T1D) the pancreatic β-cells are specifically destroyed by the immune system. Genome-wide association studies have identified >50 loci that are associated with the risk of T1D, however, only little is known about how these variants modify disease risk. We recently showed that T1D-associated single nucleotide polymorphisms (SNPs) in *CTSH* (lysosomal protease cathepsin H) affect the expression level of CTSH. By combining *in vitro* and *in vivo* experiments with clinical studies we demonstrated that CTSH regulates β-cell function and disease progression in children with newly-diagnosed T1D. Interestingly, overexpression of CTSH protected insulin-secreting INS-1 cells against cytokine-induced apoptosis, which was associated with decreased activation of phospho-JNK and phospho-p38, reduced expression of the pro-apoptotic factors *Bcl2l11* (Bim), *Myc* and *Hrk* (DP5), and increased insulin transcription. The aim of this study was to identify the mechanisms through which CTSH mediates its protective effect.

Materials and methods: INS-1 cells ($n = 3$) stably transfected with a plasmid encoding CTSH (pCTSH) or an empty control vector (pcDNA) were left untreated or stimulated with 150 pg/ml recombinant mouse IL-1β and 5 ng/ml recombinant rat IFN-γ for 6 h or 16 h. RNA was extracted and microarray analysis performed using the Affymetrix Rat Genome 230 array. Data was analyzed in R using the Bioconductor affy package and normalized using the RMA method. Comparisons between pCTSH and pcDNA cells were done using moderated t-statistic and p-values were adjusted to control the false discovery rate using the Benjamini-Hochberg procedure. Genes were considered differentially expressed when the adjusted p-value was below 0.05 and the fold change was below -1.2 or above 1.2.

Results: 39 genes were differentially expressed between the pCTSH and the pcDNA cells in the untreated condition, whereas 46 and 38 genes were differentially expressed between the pCTSH and the pcDNA cells after cytokine treatment for 6 h and 16 h, respectively. The top candidate genes are involved in the KEGG pathways: apoptosis, Jak-STAT signaling, NFκB signaling, insulin signaling, and chemokine signaling. Among the most interesting candidates is *Trib3* which previously has been implicated in endoplasmic reticulum stress-induced β-cell apoptosis. The expression of *Trib3* was decreased by 1.48 fold in pCTSH cells compared to pcDNA cells after 6 h of cytokine treatment ($p = 1.68 \times 10\text{-}4$).

Conclusion: The current data support our previous findings that CTSH protects against cytokine-induced apoptosis by regulating apoptotic signaling pathways in the β-cell, such as the Jak-STAT and NFκB signaling pathways. Our data indicate that higher CTSH levels in β-cells may protect against immune-mediated damage and preserve β-cell function, thereby representing a possible target for β-cell therapy in T1D.

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Generation of epigenetic and transcriptome maps for insulin-producing cells in pancreas, gallbladder and the brain

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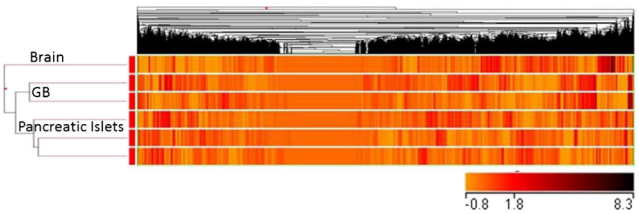
Background and aims: Islet transplantation is currently the only established cell-based therapy for treatment of diabetes. However due to the limited availability of pancreas and low yield of islets isolated - it is important to find alternate sources of potential insulin-producing cells. We're the first to identify insulin-producing cells in adult human gallbladder epithelium. Although, the levels of pro-insulin gene transcripts in gallbladder are thousand-fold less than in pancreatic islets, it is still significantly higher than the reported levels in differentiated stem cells. Insulin-producing cells have been also identified in brains of fruit-flies, mice and a clinical case of insulinoma from a patient with congenital brain malformation. The aim of this research is to generate epigenetic and transcriptome maps of the insulin-producing cells in pancreas, gallbladder and the brain. This will help understand the lineage commitment and function of insulin in different tissues.
Materials and methods: We implemented a range of molecular techniques including Next Generation Sequencing (NGS), Ultra high throughput TaqMan-based real-time mRNA and miR PCR, immunocytochemistry, single cell PCR, Chromatin IP (ChIP) and DNA methylation to map the transcriptome and epigenome expression of mouse and human islets, gallbladder and brain cells.
Results: Our analyses using NGS indicate similarities between the transcriptome in the brain, gallbladder and pancreatic islets (Figure 1). Insulin transcript is detected in the human gallbladders, but at several million-fold lower than in islets. Similar levels were found for glucagon in the gall bladder and brain in comparison to the higher levels in islets. Somatostatin is lower by an order of magnitude in the brain while expression of Pdx1 in the gallbladder is at ~50% of the abundance seen in human islets. Expression of the glucose transporter Glut1 is significantly higher in human islets than Glut2- at comparable levels in human gallbladder. Urocortin3, a recently described candidate associated with maturation of insulin -producing cells was seen at ~8-fold lower in gallbladder than human islets and around 20-fold lower in human brain. miRNAs important in islet beta-cell function were found in the human gallbladder epithelium (correlation, R2=0.716). ChIP data indicates progenitors of gallbladder epithelial cells continue to retain open chromatin conformation at the insulin promoter region, with H3K4 trimethylation- significantly higher compared to the insulin promoter conformation in the negative control (cord-blood cells).
Conclusion: Similarities and differences in transcriptome and epigenome maps of human gallbladder and pancreas samples are identified through NGS, real-time PCR technologies and DNA methylation epigenetics.



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PS 019 Multiple organs influence complication risk

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Risk factors of new-onset diabetes after liver transplantation and whether the interleukin-2 receptor antagonists can reduce its incidence

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Background and aims: To investigate risk factors for new-onset diabetes after transplantation (NODAT) in patients undergoing a liver transplantation and whether interleukin-2 receptor antagonists (IL-2Ra) can reduce the incidence of NODAT.
Materials and methods: We retrospectively analyzed the preoperative and postoperative clinical data of patients aged >18 years who underwent a liver transplantation in our hospital between April 2001 and December 2013. Patients with diabetes mellitus or taking glucocorticoids before transplantation, with incomplete data, or undergoing the second transplantation or being died within 3 months after transplantation were excluded. Finally, 705 non-diabetic patients who underwent a liver transplantation were included for analysis. The incidence of NODAT was analyzed according to the fasting plasma glucose (FPG) after live transplantation. All the patients were divided into 2 groups (NODAT and N-NODAT groups)and the possible risk factors of NODAT were analyzed with One-Way ANOVA and also with logistic regression analysis, including age, gender, hepatitis virus infection, living donor, preoperative FPG level, BMI, preoperative liver function, the severity of liver cirrhosis, acute rejection (AR), postoperative immunosuppressant regimen, metabolic factors, especially the effect of IL-2Ra on NODAT. Statistical analysis was done by SPSS 19.0.
Results: Of the 705 eligible study patients, 224 patients suffered from NODAT while 481 patients did not. The incidence of NODAT was 31.77%. One hundred and four patients (46.4%) in the NODAT group received IL-2Ra, while 276 patients(57.4%) in the N-NODAT group received IL-2Ra. Single factor analysis indicated that age, preoperative FPG were positive related to NODAT ($P < 0.05$), while living donor, IL-2Ra were negative related to NODAT ($P < 0.05$). Logistic regression analysis indicated that age (OR=1.025, $P = 0.006$), preoperative FPG (OR=1.305, $P = 0.012$) can increase the risk of NODAT, while IL-2Ra (OR=0.555, P=0.001) and living donor (OR=0.121, P=0.041) can reduced the risk of NODAT.
Conclusion: Age and preoperative FPG level was independent risk factors for NODAT, while the IL-2Ra and living donor can reduce the risk of NODAT after liver transplantation.

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Low cumulative incidence of end-stage renal disease in young patients with type 1 diabetes in Sweden: a population based study

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Background and aims: A previous study from our group showed a low cumulative incidence of end-stage renal disease (ESRD) in a Swedish

cohort of type 1 diabetes (T1D) patients with median duration of 20 years. We speculated that a good diabetes health care system might have postponed the peak incidence of ESRD and that young age at onset of T1D can postpone the development of diabetic nephropathy (DN) and ESRD. Moreover, diabetes onset during puberty may promote the development of diabetic complications. Our previous study also indicated differences by sex in ESRD development and a possible interaction with age at onset. Female patients who developed T1D after puberty had similar risk of ESRD as those with onset before 10 years of age. Male patients had the same high risk with onset during puberty and after puberty, those with onset before 10 years had the lowest risk. The aims of the present study are to assess the cumulative incidence of ESRD due to DN in a large prospective population-based cohort of T1D patients at maximum 36 years of diabetes duration and to study the effects of sex and age at onset of T1D.

Materials and methods: Since 1977 all incident cases of T1D in the ages 0-14 years are recorded in the Swedish Childhood Diabetes Register (SCDR). The Swedish Renal Registry (SRR) started in 1991 and collects data on all patients with active uraemia treatment, ESRD. We decided to include patients with diabetes duration ≥14 years. In total 9381 patients from the SCDR were included. We have recently received permission to include data from the Swedish National Diabetes Register, a national quality register, and are awaiting data to include patients with age at onset 15-34 years.

Results: For the childhood onset cases the median diabetes duration was 23.8 years, maximum 36.7 years, and 154 patients had developed ESRD due to diabetes. The cumulative incidence was 4.5%. There was no statistical difference between male and female patients with age at diabetes onset before 15 years of age, males 5.0%, females 3.8%. We confirm that onset of diabetes before 10 years of age postpones the development of ESRD when compared to onset during 10-14 years, HR 2.3 (95% CI= 1.7-3.3). Further analyses will be available for presentation in September.

Conclusion: The cumulative risk of ESRD due to diabetic nephropathy in Swedish T1D patients at maximum 36 years of diabetes duration is still exceptionally low. There is no difference in the development of ESRD between male and female patients with onset of diabetes before 15 years of age.

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Sarcopenia is associated with micro- and macro-albuminuria independently of hypertension and diabetes: nationwide surveys (KNHANES 2008-2011)

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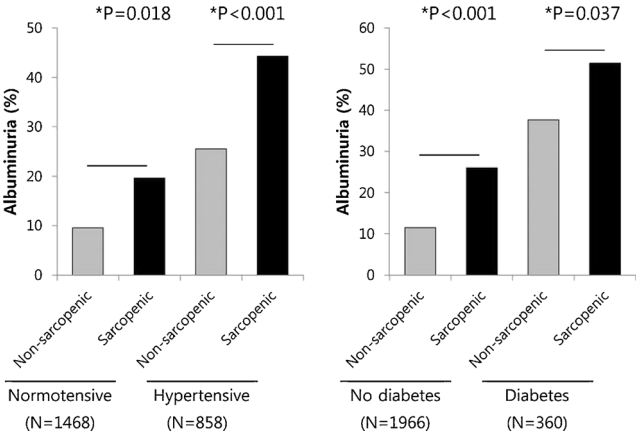
Background and aims: Sarcopenia is known to be associated with obesity-related comorbidities such as hypertension and diabetes. However, its influence on albuminuria or nephropathy has not been determined. Therefore, the aim of this study was to identify the relationship between sarcopenia and albuminuria or nephropathy in the general population.

Materials and methods: This was a population-based, cross-sectional study using nationally representative samples of 2,326 subjects aged≥ 20 years from the Korea National Health and Nutrition Examination Surveys (KNHANES) 2008-2011. The nephropathy was defined more than 30 μg/mg of a urinary albuminuria-to-creatinine ratio (UACR) in spot urine or more than one positive for proteinuria in urine dip stick tests. The skeletal muscle index (SMI) [SMI(%)=total appendicular skeletal muscle mass (kg) / weight (kg) x 100] measured by dual-energy X-ray absorptiometry was used to diagnose sarcopenia with cut points of 32.2% for men and 25.5% for women according to the previous study (J Clin Endocrinol Metab. 2014;99:3879-3888).

Results: A total of 385 (16.5%) subjects were classified as having a micro- or macro-albuminuria. Individuals with sarcopenia showed a higher proportion of albuminuria compared to subjects with non-sarcopenia (22% vs. 8%, odds ratio [OR]=3.26, 95% confidential intervals [CI]=2.32-4.58, P<0.001). Sarcopenic subjects had a higher risk of albuminuria, after stratification with hypertension (all ORs=2.32, all Ps< 0.05) or diabetes (ORs=1.76 to 2.71, all Ps<0.05), which are known as established risk factors for albuminuria (Figure 1). Multiple logistic regression analysis also demonstrated that sarcopenia was independently associated with micro- or macro-albuminuria after adjusting for confounding factors related to albuminuria and renal function (OR=1.70, 95% CI=1.11-2.59, P=0.014).

Conclusion: Sarcopenia is associated with increased risks of micro- and macro-albuminuria independently of hypertension or diabetes. This suggests that sarcopenia may be considered as a novel risk factor for albuminuria.



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The mortality risk in post transplantation diabetes mellitus is better predicted by glucose than HbA_{1c} based diagnostic criteria

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Background and aims: Current diagnostic criteria for posttransplantation diabetes mellitus (PTDM) are either a fasting plasma glucose (fPG)≥ 7.0 mmol/L (≥126 mg/dL) or a 2-hour post-challenge plasma glucose≥ 11.1 mmol/L (≥200 mg/dL) during an oral glucose tolerance test (OGTT). Recently the diagnostic criteria for type 2 diabetes of glycosylated haemoglobin (HbA1c)≥6.5% (≥48 mmol/mol) was proposed also for PTDM. We examined HbA1c based criteria compared with conventional glucose criteria in the early phase after transplantation as a predictor of mortality.

Materials and methods: In this retrospective cohort study of 1996 renal transplant recipients, transplanted between 1999 and 2011, we estimated mortality hazard ratios for patients diagnosed with PTDM. The study participants underwent weekly fPG measurements during the first 10 weeks after transplantation, followed by an OGTT and HbA1c measurements at 10 weeks post-transplant.

Results: During a median follow-up of 5.4 years, 314 patients died. Both PTDM detected by an OGTT (OGTT criteria) and persistent hyperglycaemia throughout the first 2 months post-transplant (manifest PTDM) were associated with mortality (OGTT criteria: adjusted hazard ratio [HR] 1.64, 95% confidence interval [CI] 1.02 - 2.63, p=0.04.

Manifest PTDM: adjusted HR 2.21, 95% CI 1.40 - 3.49, p=0.001). PTDM detected by HbA1c≥6.5% (≥48 mmol/mol) was not associated with mortality (adjusted HR 0.96, 95% CI 0.61 - 1.51, p=0.86).

Conclusion: Our findings confirmed that, in the early phase after renal transplantation, PTDM diagnosed by conventional glucose criteria, as opposed to the HbA1c criterion, predicted mortality.

Mortality risk according to diabetes category

		Crude			*Multivariate*		
Diagnostic category,							
Model 1	**n**	**HR**	**95% CI**	**p**	**HR**	**95% CI**	**p**
PTDM by OGTT criteria	91	1.84	(1.17-2.89)	0.01	1.64	(1.02-2.63)	0.04
Manifest PTDM	73	2.44	(1.58-3.77)	<0.001	2.32	(1.49-3.67)	<0.001
Pre-transplant diabetes	365	1.63	(1.26-2.11)	<0.001	1.75	(1.31-2.34)	<0.001
Model 2							
PTDM by HbA1c criterion	88	1.54	(1.01-2.36)	0.05	0.96	(0.61-1.51)	0.86
Manifest PTDM	73	2.44	(1.58-3.77)	<0.001	2.21	(1.40-3.49)	0.001
Pre-transplant diabetes	365	1.63	(1.26-2.11)	<0.001	1.66	(1.24-2.22)	0.001

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Impact of renal transplantation on glucose tolerance in Japanese recipients with impaired glucose tolerance

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Background and aims: New-onset diabetes after transplantation (NODAT) is a serious and frequent complication related with renal transplantation. Insulin resistance and impaired insulin secretion could contribute to the pathogenesis of glucose intolerance after renal transplantation, but it has been controversial which component of them is more relevant. In the current study, we investigated the natural history of glucose tolerance, insulin secretion and insulin sensitivity by analyzing the results of oral glucose tolerance test (OGTT) in Japanese recipients before and one year after renal transplantation.

Materials and methods: We performed a retrospective study of Japanese recipients without diabetes who received renal transplantation at our hospital. A 75-g OGTT was performed before and one year after renal transplantation. NODAT was defined according to the OGTT result (2-h glucose≥200 mg/dL; fasting glucose≥126 mg/dL). Normal glucose tolerance (NGT), impaired fasting glucose (IFG) and impaired glucose tolerance (IGT) were defined according to the World Health Organization Expert Committee. Insulin sensitivity was estimated by the use of the Matsuda index and homeostasis model assessment of insulin resistance (HOMA-IR). Insulin secretion was evaluated based on the insulinogenic index and oral disposition index.

Results: Of the 62 recipients, 31 were diagnosed as NGT, while the remaining 31 were diagnosed as IGT before renal transplantation. In the NGT group, 9.7% of the recipients developed NODAT, 32.3% developed IFG and/or IGT, and 58.1% remained NGT one year after renal transplantation. In the IGT group, after one year, 12.9% developed NODAT and 29.0% remained IGT. Unexpectedly, 58.1% of the IGT group turned to NGT. When the patients in the IGT group were classified into an amelioration group (the changes in area under the curve of the glucose excursion during the OGTT (ΔAUCglu)<0) and a non-amelioration group (ΔAUCglu>0), there were no differences in parameters reflecting insulin secretion and insulin sensitivity between the two groups before transplantation. After one year of renal transplantation, Matsuda index was significantly increased, with no significant changes in insulinogenic index or oral disposition index in the amelioration group. By contrast, insulinogenic index and oral disposition index were significantly reduced, with no significant changes in atsuda index or HOMA-IR in the non-amelioration group. To reveal the factors influencing glucose tolerance before and after renal transplantation in the IGT group, we examined the association between ΔAUCglu and the changes in parameters reflecting insulin secretion and insulin sensitivity. The changes in Matsuda index (r=-0.5580, p=0.0014), insulinogenic index (r=-0.4674, p=0.0092) and oral disposition index (r=-0.7788, p<0.0001) had a significant negative correlation with ΔAUCglu.

Conclusion: More than half of Japanese recipients with IGT turned to NGT after one year follow-up of their renal transplantation. Our results suggested that the increase of insulin sensitivity without decreased insulin secretion is associated with the improvement of glucose tolerance in these recipients. Further studies are needed to elucidate the mechanism of the increase of insulin sensitivity and maintaining of insulin secretion after renal transplantation.

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Coronary artery disease as a risk for developing type 2 diabetes mellitus

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Background and aims: Diabetes mellitus is a major risk factor for coronary artery disease (CAD); whether conversely CAD confers an increased risk for diabetes is unclear.

Materials and methods: We prospectively recorded incident diabetes over 6.1±3.7 years in 829 consecutive non-diabetic Caucasian patients undergoing coronary angiography for the evaluation of stable CAD, covering 5057 patient years.

Results: During follow-up, diabetes was newly diagnosed in 133 patients, i.e. in 16% of the study population or in 2.6% per year. Patients with significant CAD (n=444) when compared to subjects who did not have significant CAD at the baseline angiography were at a strongly increased diabetes risk (20.3 vs. 11.2%; p<0.001). The relationship between CAD and incident diabetes was confirmed after multivariate adjustment including metabolic syndrome status (OR 1.85 [1.23-2.79], p=0.003).

Conclusion: We conclude that the presence of CAD indicates a strongly increased risk for incident diabetes. Repeated diabetes screening of coronary patients and targeted programs to prevent diabetes in these high-risk patients are warranted.

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Fatal complications and causes of death in patients with type 2 diabetes mellitus comparing with general population by results of autopsies

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Background and aims: To assess the frequency of fatal complications and the risks of death from different causes in patients with type 2 diabetes mellitus (DM) comparing with general population.

Materials and methods: On the basis of autopsy of 439 patients with type 2 diabetes mellitus had died in the Clinical Hospital №50 of Moscow during 2006-2009 we studied the main causes of their death and fatal complications. We compared our results with similar data from the Moscow City Statistics for the general population: autopsies of all dead

patients in hospitals of Moscow during 2007-2009. Chi-square test was used and relative risk (RR) with 95% confidence interval [95% CI] was calculated. Differences were considered statistically significant at p <0.05.

Results: Patients with type 2 DM significantly were more likely to die from diseases of circulatory system compared to general population: 338 subjects (77,0%) vs.45586 subjects (57,7%), p<0,001, RR 1,33 [1,26-1, 40]. Diabetics more often died from ischemic heart disease - 211 subjects (48.1%) vs.20007 subjects (25.3%), p <0,001 RR 1,89 [1,71-2,09], from myocardial infarction - 83 subjects (18.9%) vs.10739 subjects (13.6%), p=0,001 RR 1,39 [1,13-1,69], from cerebrovascular disease - 154 subjects (35.1%) vs.20084 subjects (25.4%), p <0,001 RR 1,38 [1,20-1,56] and from ischemic stroke - 89 subjects (20.3%) vs.11725 subjects (14.8%), p=0,001 RR 1,36 [1,12-1,65]. Patients with type 2 DM had a greater risk (p 0,001) of death also from ischemic bowel disease 19 subjects (4,3%) vs.1287 subjects (1,6%), RR 2,65 [1,65-4,19], cholelithiasis - 12 subjects (2.7%) vs.469 subjects (0,59%), RR 4,60 [2,48-8,25], chronic pancreatitis - 5 subjects (1.14%) vs.104 subjects (0,13%), RR 8,65 [3,12-21,78], and pyelonephritis - 18 subjects (4.1%) vs.1034 subjects (1,3%), RR 4.1 [1,92-5,00]. The groups (diabetics and general population) did not differ (p 0,05; RR≈1) in frequency of such causes of death as hemorrhagic stroke (26 subjects (5.9%) vs.4971 subjects (6.3%)), cancer (67 subjects (15, 3%) vs.10240 subjects (13.0%)), chronic obstructive pulmonary disease (8 subjects (1.8%) vs.1264 subjects (1.6%)), gastric or duodenal ulcer (3 subjects (0.6%) vs.938 subjects (1.2%)) and non-alcoholic cirrhosis (9 subjects (2.0%) vs.1565 subjects (2.0%)). Patients with type 2 DM were more likely to develop fatal complications: pneumonia - 138 subjects (31,4%) vs.4023 subjects (7,37%), p 0,001 RR 4,26 [3,67-4,9]; pulmonary embolism - 100 subjects (22,8%) vs.3481 subjects (6,38%), p=0,000 RR 3,53 [2,93-4,21]; gastrointestinal bleeding - 36 subjects (8, 2%) vs.2801 subjects (5,14%), p=0,005 RR 1,59 [1,14-2,19]; peritonitis - 28 subjects (6,37%) vs.1462 subjects (2,68%), p 0,001 RR 2,38 [1,62-3, 44].

Conclusion: In comparison with general population the patients with type 2 DM more often died not only from diseases of circulatory system, but also from a number of diseases of gastrointestinal tract and pyelonephritis. Diabetics had a greater risk of severe complications such as pneumonia, pulmonary embolism, gastrointestinal bleeding and peritonitis. Along with the appropriate management of DM and the correction of cardiovascular risk factors, the timely diagnostics and treatment of non-cardiovascular comorbidities are necessary. An autopsy is an objective method of obtaining reliable information on the structure of mortality.

PS 020 Progenitor cells and pancreas differentiation

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Use of epigenetic inhibitors to modulate endocrine differentiation

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Background and aims: In the developing pancreas, the endocrine differentiation program is initiated in multipotent pancreatic progenitors by expression of the basic helix-loop-helix (bHLH) transcription factor Neurogenin3 (Neurog3). Posttranscriptional modifications of histones constitute an epigenetic mechanism that is closely linked to both gene silencing and activation events. A key mark in differentiation processes is the trimethylation of Histone3 at lysine 27 (H3K27me3), which is associated with transcriptional repression. Previously, we showed that ectopic Neurog3 promoted the removal of H3K27me3 marks at promoter regions of some of its target genes, suggesting a functional interaction between Neurog3 and regulators of this chromatin mark. In the present study, we aimed to specifically evaluate the contribution of these epigenetic regulators to the proendocrine activity of Neurog3. To this objective, we used chemical inhibitors for the H3K27 methyltransferase EZH2 and the H3K27 demethylases Jmjd3/UTX and studied their effects in the activation of the pancreatic endocrine differentiation program in several in vitro models.

Materials and methods: We used the mouse pancreatic duct cell line mPAC as a Neurog3-dependent endocrine differentiation model. Mouse embryonic fibroblasts (MEFs) were obtained from E12.5-E14.5 mouse embryos. To activate endocrine differentiation, we used a recombinant adenovirus expressing Neurog3 in mPAC cells and a recombinant adenovirus expressing Pdx1+MafA+Neurog3 in MEFs. Chemical inhibitors used were: EI-1/GSK-126 (EZH2) and GSK-J4 (UTX/JMJD3). Total H3K27me3 mark levels were analyzed by Western blotting, gene expression was quantitated by Real Time PCR and chromatin marks were assessed by chromatin immunoprecipitation assays (ChIP).

Results: In mPAC cells, GSK-J4 treatment led to marginal increases in total levels and enrichment of H3K27me3 marks at key endocrine gene promoters. By contrast, EI-1 treatment significantly reduced total levels and enrichment of H3K27me3 at promoter regions. Remarkably, this decrease was accompanied by enrichment in acetylated H3K27 (H3K27ac) but not by transcriptional activation of these genes. Upon ectopic expression of Neurog3, GSK-J4 dramatically blocked whilst EI-1 improved transcriptional activation of the endocrine program. However, pre-treatment with EI-1 (before Neurog3 expression) was not sufficient to impact Neurog3 function, suggesting functional interaction between EZH2 and Neurog3. Lastly, in MEFs, chemical inhibition of EZH2 also resulted in a higher level of activation of selected endocrine genes (NeuroD1, Nkx2-2, Pax6, Insulin) in response to combined ectopic expression of Pdx1, Neurog3 and MafA.

Conclusion: These results demonstrate the participation of epigenetic regulators of the H3K27me3 mark in the activation of the endocrine transcriptional cascade promoted by Neurog3. Inhibition of EZH2 activity may be a useful strategy to improve the efficiency of endocrine differentiation and beta cell transdifferentiation protocols in vitro.

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Examination of sorted ALDHhi and ALDHhi/CD133+ cells in the developing human pancreas

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Background and aims: The enzyme aldehyde dehydrogenase (ALDH) has been previously determined to regulate endocrine differentiation via retinoic acid signaling in the developing human pancreas. CD133, a surface membrane protein associated with the ductal cells in the fetal and adult human pancreas, has also been used in prior studies to identify and isolate stem cells. However, the separation of cells with high ALDH activity (ALDHhi) and the analysis of putative stem cell markers associated with ALDH expression has not yet been reported. This study aims to characterize transcription factors and stem cell surface markers in sorted ALDHhi cells in the human fetal pancreas, and examines the differentiation potential of ALDHhi or ALDHhi/CD133+ cells in vitro.
Materials and methods: Human fetal pancreata (18 to 22 weeks of fetal age), composed mainly of undifferentiated epithelial cells, were dissociated for fluorescence-activated cell sorting (FACS). Cells sorted based on ALDH activity alone (ALDHhi and ALDHlo populations) or ALDH colocalization with CD133 (ALDHhi/CD133+ and ALDHlo/CD133- populations) were examined for transcription factors (TFs), hormones, and pancreatic cell lineage markers immediately after cell sort, after in vitro expansion culture, or following differentiation of cluster formation using immunohistological and qRT-PCR approaches.
Results: FACS demonstrated that CD133 was highly expressed in ALDHhi cell populations, while CD34 was expressed mainly in ALDHlo cells ($p<0.001$). The sorted ALDHhi population cells contained higher levels of islet-associated TFs (SOX9, PDX-1, and NKX6.1, $p<0.01$) compared to ALDHlo sorted cells, as well as hormones insulin and somatostatin ($p<0.05$), but not glucagon. It was also noted that vimentin + and CD31+ cells were predominantly observed in ALDHlo population immediately after cell sorting. Expanded culture of sorted ALDHhi or ALDHhi/CD133+ cells resulted in loss of nuclear endocrine TFs, except SOX9, and displayed mesenchymal-like structure. The loss of ductal cell phenotype in ALDHhi cells and increased vimentin+ staining in both expanded cell cultures may indicate that sorted cells enter epithelial-to-mesenchymal (EMT) transition. A similar result was observed in sorted ALDHlo or ALDHlo/CD133- cells during the expanding culture. Differentiation of expanded ALDHhi or ALDHhi/CD133+ cells led to formed islet-like clusters, restored CK19 ductal cell marker, and increased endocrine TFs gene expression; however, no endocrine hormones were detected in the clusters.
Conclusion: This study demonstrated that ALDHhi cells contain a CD133 enriched endocrine progenitor cell pool in the developing human pancreas, indicating that sorted ALDHhi/CD133+ cells may be fated towards islet cell commitment. However, future experiments require optimal culture environments for inducing progenitors to differentiate into insulin-producing islet cells.
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MicroRNA expression dynamics during human induced pluripotent stem cells differentiation into insulin-producing cells

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Background and aims: In a previous study we demonstrated that human induced pluripotent stem cells (HiPSCs) are able to differentiate into insulin-producing cells following the stages of pancreatic organogenesis. Moreover, mice transplanted with differentiated HiPSCs secreted human C-peptide in response to glucose, suggesting that these differentiated cells can engraft and secrete insulin in-vivo. MicroRNAs (miRNAs) are a class of small non-coding RNA molecules, which control gene expression by inhibition of messenger RNA translation. MiRNAs are involved in the control of several biological processes such as cell differentiation or insulin secretion.
Here, we aim at characterizing miRNA expression profiles during differentiation of HiPSC into insulin-producing cells
Materials and methods: HiPSC were derived from fibroblast preparations from adult healthy donors and from human fetal fibroblast cell line IMR90, using a retroviral cocktail of reprogramming factors. HiPSC differentiation into insulin-producing cells lasted 18 days and was performed following Viacyte protocol with slight modifications. Total RNA was extracted using miRvana Kit at different stages during differentiation (day 0-native HiPSCs, day 4-definitive endoderm, day 10-posterior foregut, day 18-endocrine pancreas). Gene expression was analyzed by RT Real Time PCR using Taqman assays. The expression of 768 miRNAs was analyzed using Taqman human microRNA array cards PanelA+B and normalized using small RNAs RNU48 and RNU6. Differentially expressed miRNAs were further analyzed by RT-stem loop Real Time PCR. MiRNA predicted target genes were identified using Targetscan 6.2 and Gene Ontology classification was performed using online software DAVID 6.7
Results: A progressive upregulation of islet hormonal genes (insulin, glucagon, somatostatin) and of endocrine/beta-cell specific transcription factors NGN3, MAFA, MAFB, NeuroD1 and ISL-1 was detected at day-4, -10 and -18 of differentiation vs native HiPSC, paralleled by increased expression of glucose sensing apparatus genes GCK and GLUT2. MiRNA expression profiles analysis of native HiPSC during differentiation into insulin-producing cells revealed that 347/768 miRNAs were expressed at least at one time point of all samples; in addition, 19 miRNAs resulted as differentially expressed during differentiation: 12 upregulated (miR-9, miR-9#, miR-10a, miR-99a#, miR-124a, miR-135a, miR-138, miR-149, miR-211, miR-224, miR-342-3p and miR-375) and 7 downregulated (miR-31, miR-127, miR-143, miR-302c#, miR-373, miR-518b and miR-520c-3p). Following Targetscan 6.2 analysis of predicted target genes of differentially expressed miRNAs, Gene Ontology classification revealed that such target genes belong to categories of major significance in pancreas organogenesis such as cell morphogenesis at day 4, organ/tissue development at day 10 or pancreas development and exocytosis at day 18
Conclusion: We detected a specific miRNA signature in HiPSC during their differentiation into insulin producing cells and demonstrated that differentially expressed miRNAs putatively control the expression of genes involved in pancreas organogenesis

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The role of miRNAs in adult cells reprogramming along the endocrine pancreatic lineage

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Background and aims: Cell replacement therapy may offer a cure for diabetes as long as new sources of insulin producing cells (IPCs) are found. Transdifferentiation of liver cells to IPCs may offer a new therapeutic source for diabetic patients. We have reported that ectopic expression of pancreatic transcription factors (pTFs) in the liver induces insulin production, both *in vivo* and *in vitro*. The key transcription factor is Pancreatic-duodenal-homeobox-1 (PDX1) which plays a dual and central role in regulating pancreas organogenesis in embryo and β-cell function

in adults. Regardless to the use of several pTFs combined with specific growth and soluble factors, transdifferentiation efficiency could be further improved. MicroRNAs (miRNAs) are short non-coding RNAs (20-25 nucleotides) that regulate gene expression. miRNAs have been shown to participate in almost every cellular process investigated so far. Several specific miRNAs involved in β-cells' development and function and also with the upkeep of insulin transcription have been identified. It was shown that miRNAs are indeed involved in the transdifferentiation process between several tissues. The present study is designed to analyze the role of miRNAs in the transdifferentiation process of extra-pancreatic tissues along the pancreatic ß-cell lineage.

Materials and methods: Primary human liver and fibroblast cultures are generated from>10 separate donors. Transdifferentiation induction is performed by ectopic expression of pTFs sub-cloned in adenoviral vectors. Soluble factors are used to increase transdifferentiation efficiency. Transdifferentiation is evaluated at the molecular, cellular and functional levels.

Results: Using global analyses in miRNA arrays (QuantiMir™, NanoString) our data suggest that pTFs induced transdifferentiation is associated with alterations in the expression profile of miRNAs in both human fibroblasts and liver cells. IPCs originated from fibroblasts exhibited numerous changes in the miRNA profile, substantial activation of 9 miRNAs and the suppression of more than 100 miRNAs. Many of the β-cell specific miRNAs including miR-375 expression was not increased, possibly suggesting that not all the transcriptional repressors have been removed from the IPC cells. Ectopic complementation of miR-375, the major miRNA in the pancreas, indeed increased insulin gene expression.A potential role of miRNA in the transdifferentiation process is further exemplified by increases in Dicer expression; Pdx-1 but none of the other pancreatic pTFs used, specifically activated Dicer expression. The increased activation of Dicer expression during transdifferentiation may suggest a crucial role for miRNA biogenesis during reprogramming and the alteration in cells' fate.

Conclusion: Our data suggest that pTFs induced reprogramming alters the profile of the miRNA expression in part by increasing Dicer expression. Although it is well known that Dicer is ubiquitously expressed among many cell types, its activation during transdifferentiation potentially suggest an important role of controlling miRNA biogenesis during the reprogramming process. MiRNAs may lead profound insights into the underlying molecular and cellular pathways which regulate cell-fate transitions, to be used in regenerative medicine approaches.

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Epithelial to mesenchymal transition process is a frequent event in vitro in all pancreatic endocrine cell types

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Background and aims: The concept of epithelial to mesenchymal transition (EMT) of cultured pancreatic beta cells has been carefully demonstrated by lineage tracing. In vitro, adult human beta cells transition into a highly proliferative mesenchymal cell population. The presence of EMT in other pancreatic endocrine cell populations is unknown. The aim of this study was to determine the EMT process in endocrine pancreatic cells.

Materials and methods: Human islets were isolated from pancreas of 6 multiorgan donors (age 47±7 y.o; BMI 27±2; islet purity 67±6%). Islets were dissociated into single cells by enzymatic and mechanical disruption. Single endocrine cells (34±7% endocrine cell purity) were purified by magnetic cell sorting using PSA-NCAM antibody. The resulting cell fraction (74±6% endocrine cell purity) was seeded for monolayer culture and split 1:2 weekly. Expression of the mesenchymal marker vimentin (determined by immunofluorescence) in human endocrine cells was used to evaluate EMT from day 0 to passage 4 (P4). Gene expression was determined by quantitative real time PCR. Beta cell apoptosis was quantified by double-staining using TUNEL assay and insulin antibody.

Results: A progressive reduction of cells expressing endocrine markers (insulin, glucagon, somatostatin and pancreatic polypeptide (PP)) was found along the four passages of culture. PP positive cells were identified up to P2 (0.3±0.2%), and the presence of insulin (0.6±0.2%), glucagon (0.2±0.1%), and somatostatin (0.2±0.1%) positive cells was residual at P4. The percentage of apoptotic beta cells was low (day 0: 0.56±0.26%; day 4: 0.04±0.01%; day 8: 0.03±0.02%; day 12: 0.05±0.04%; P2: 0.06±0.06%) suggesting that the reduction in insulin expressing cells was not due to increased beta cell death. Co-expression of endocrine and mesenchymal markers was detected after 4 days in culture and increased subsequently (insulin+vim+ 6±4% at P1; 58±4% at P4). Other endocrine populations were also found to co-express vimentin (glucagon+vim+ 62±4% and 93±4%, somatostatin+vim+ 18±6% and 95±5%, at P1 and P4 respectively; PP+vim+ 81±9% at P1 and 92±8% at P2). Changes in gene expression were also suggestive of EMT process with a significant increased expression of mesenchymal markers (cdh2, snai2, vim, nt5e and acta2) and reduction of cdh1.

Conclusion: All four types of adult human pancreatic endocrine cells underwent EMT in vitro. Lineage tracing will be used to quantify the contribution of the different endocrine cell populations to the proliferative mesenchymal pool.

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Haematopoietic CD34+ stem-cell differentiation to insulin-producing cells: possible role of betatrophin

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Background and aims: Though human pancreatic islet transplantation has raised great expectation, the shortage of pancreas donors remains a main limitation. Stem cell therapy is, therefore, being a matter of continuous investigation. We have investigated the potential of betatrophin to improve generation of insulin-producing cells (IPCs) from circulating hematopoietic CD34+ stem-cell of normal subjects as well as type 1 (T1DM) and type 2 diabetic (T2DM) patients.

Materials and methods: Hematopoietic CD34+ stem cells were isolated by immunomagnetic technique from the blood of 15 non-diabetic (ND: 46±4 yrs; 5 M/10 F; BMI 24.7±1.5 Kg/m2), 10 T1DM (45±5 yrs; 7 M/3 F; 23.7±1.6 Kg/m2) and 15 T2DM (62±4 yrs; 8 M/7 F; 26.5±1.4 Kg/m2) subjects. Differentiation into insulin-producing cells was obtained by a protocol, modified in our laboratory, in presence or absence of betatrophin (10 μmol/l). Insulin-producing cells were identified by colorimetric staining and immune-fluorescence technique. Real-Time R-PCR was used for assessment of insulin gene expression and telomere length (TL) as a marker of cell senescence. Insulin was measured in culture media by IRMA assay.

Results: The number (ND: 2.71E+4±5.19E+3; T1DM: 3.26E+4±1.43E+4; T2DM 4.21E+4±1.16E+4 cell/ml) and viability (ND 42.38±6.68%; T1DM 43.29±6.18%; T2DM 44.55±7.09%) of CD34+ stem cells was similar in the 3 groups and was not affected by age. After 10-day culture in vitro expansion (ND 1.12E+5±2.03E+4, T1DM 3.08E+5±9.22E+4, and T2DM 1.31E+5±1.92E+4 cell/ml; all $p<0.05$ vs. day 1) and viability remained comparable among the 3 groups. TL was lower in T2DM (4.62E+1±3.44 bp) as compared to ND (2.78E+7±1.15E+7 bp) and T1DM (9.58E+7±6.18E+7 bp; both $p<0.05$) at baseline and it did not change after 10 day cell replication. Insulin gene expression in CD34+ stem cells increased in all groups being higher in T2DM (15.91±4.44 2^-ΔCt) than in ND (2.30±1.25 2^-ΔCt) and T1DM (1.84±0.77

2^-ΔCt; both p<0.05). The increase in insulin mRNA expression was associated with a mild increase in insulin concentration in the medium. Addition of betatrophin to the culture medium further increased insulin mRNA expression in all groups (ND 13.9±2.93 2^-ΔCt, T1DM 9.12± 3.41 2^-ΔCt, and T2DM 50.63±2.17 2^-ΔCt; all p<0.05 vs. no betatrophin) with no effect on insulin release in the medium.
Conclusion: Although circulating stem cells from normal and diabetic subjects can be manipulated to increase insulin mRNA expression, insulin release remains insufficient. Betatrophin enhances mRNA expression without affecting insulin release. Although our results support a hypothetical generation of insulin producing cells from circulating stem cells, the relevance of this approach remains questionable.

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Elucidating the role of Pancreatic Stellate Cells (PaSCs) during islet cell development in the human foetal pancreas
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Background and aims: Pancreatic stellate cells (PaSCs) are non-endocrine, mesenchymal-like cells that reside within the peri-pancreatic tissue of the rodent and human pancreas. PaSCs regulate extracellular matrix (ECM) turnover in maintaining the integrity of pancreatic tissues architecture. Although there is evidence indicating that PaSCs are involved in islet cell survival and function, its role in islet cell differentiation during pancreatic development remains unclear. In the present study, the expression pattern and functional role of PaSCs was examined in the early to mid-gestation human fetal endocrine pancreas.
Materials and methods: The presence of PaSCs in the human fetal (8-22 weeks fetal age) pancreas was characterized by quantitative RT-PCR, western blotting, and ultrastructural and immunohistological approaches. Isolated human fetal islet-epithelial cell clusters (17-21 weeks fetal age) were directly and indirectly co-cultured with active or inhibited PaSCs to examine the developmental effects of PaSC on islet cell differentiation in vitro.
Results: Using microarray, qRT-PCR, and western blot approaches, we found PaSCs to be highly proliferative, with significantly higher gene and protein levels for PaSC markers during the 1st trimester of pregnancy compared to the 2nd. Ultrastructural and immunofluorescence analysis of PaSCs demonstrated a population of PaSCs near ducts and newly formed islets. A subset of PaSCs co-localized with transcription factors (PDX-1, SOX9, and NKX6.1) that are critical for maintenance of the pancreatic progenitor pool and endocrine differentiation. Human fetal PaSCs were isolated from pancreata collected during the 2nd trimester of pregnancy and identified by expression of stellate cell markers and associated products. Inhibition of PaSC activation using ATRA resulted in reduced PaSC proliferation and production. Co-culture of human fetal islet-epithelial cell clusters directly on PaSCs or indirectly using Millicell® Inserts showed a significant reduction in NGN3, NKX6.1, and insulin expression, while PDX-1 and SOX9 expression remained unchanged. Interestingly, high levels of Notch signalling, significantly increased PTF1A mRNA and the number of amylase+ cells were observed during the co-culture.
Conclusion: We have investigated the expression and potential function of PaSCs in the early to mid-gestation human fetal pancreas, and have determined that PaSCs are abundant during early stages of pancreatic development around ductal and islet structures. Direct and indirect co-culture of islet-epithelial cell clusters with PaSCs suggests that PaSCs are required for maintaining pancreatic progenitor pool. However, over-activation of PaSCs plays a negative role with respect to endocrine cell differentiation in the developing human fetal pancreas.
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The core trithorax protein Wdr5 is essential for specification of pancreas progenitors into endocrine and exocrine cell lineages
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Background and aims: All cells of the pancreas arise from Pdx1+Sox9+ pancreas progenitors that begin differentiation into endocrine, exocrine and ductal cells starting at around embryonic day 12.5 (E12.5). Various transcription factors are critical for this process; for example, Ngn3 is essential for specification of endocrine cells, which will mature into the islet cell types, including insulin-producing beta-cells. However, exactly how these transcription factors are appropriately regulated during pancreas development is still largely unknown. We have previously compared the chromatin state of mouse pancreatic islets and ES cells using chromatin immunoprecipitation sequencing data and found that cis-regulatory regions associated with pancreas critical transcription factors need to gain activating marks to become expressed; however, when and how this occurs is still not clear. Trithorax group (TrxG) complexes promote gene activation by catalyzing histone methylation via Mll/Set1 methyltransferases and we hypothesized that these complexes are necessary for induction of factors critical to progenitor cell specification during pancreas development.
Materials and methods: To test this hypothesis, we used lentiviral short hairpin RNA-mediated knockdown of the core TrxG protein Wdr5 (shWdr5) in an in vitro model of mouse pancreas development where E13.5 Pdx1+Sox9+Ngn3- progenitor cells are expanded into spheroids and then differentiated for seven days.
Results: We first examined the expression of select TrxG complex proteins, Wdr5, Dpy30, Mll1 and Mll3, at E12.5 and E14.5 of mouse development by immunohistochemistry. These proteins were widely expressed in the mouse embryonic pancreas and were co-expressed with both Pdx1+ and Ngn3+ cells. Suppressing Wdr5 using our in vitro pancreas progenitor sphere differentiation model, we found that shWdr5 treatment significantly reduced the number of spheres generated per pancreas (n=4, p<0.001) as well as the diameter of the spheres produced (n=4, p<0.01). Interestingly, shWdr5 also almost completely prevented expression of markers of endocrine cell lineages, including Ngn3, Ins1, Ins2 and Gcg (n>4, p<0.01). This effect was confirmed by experiments in mouse insulin promoter-GFP spheres, which had significantly reduced production of GFP+ cells in shWdr5-treated spheres (n=4, p<0.01). To determine the effect of shWdr5 on the other pancreas lineages, we performed RNA-seq on pancreas spheres treated with shScramble and shWdr5. Consistent with our gene expression data, the RNA-seq results confirmed that knockdown of Wdr5 impairs endocrine cell specification. To our surprise, exocrine cell specification was also impaired, but development of ductal cells was not.
Conclusion: These results suggest that the core TrxG complex protein Wdr5 is essential for pancreas progenitors to be specified into endocrine and exocrine, but not duct, cell lineages. This implies that in pancreas progenitors, factors necessary for duct cell specification and differentiation have already attained histone methylation at their cis-regulatory regions, while factors necessary for endocrine and/or exocrine specification, such as Ngn3, have not.
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PS 021 Intracellular signalling in beta cells

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Induction of selective unresponsiveness to glucose but not nutrient secretagogues in general by depolarisation in the absence of nutrients

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Background and aims: The mechanisms underlying the metabolic signals which amplify glucose-induced insulin secretion while circumventing plasma membrane depolarization are still poorly understood in spite of considerable research efforts. Here, an experimental protocol is presented which may prove useful to identify the relevant metabolites.
Materials and methods: All parameters were measured in freshly isolated mouse islets. Insulin secretion was measured by ELISA of the fractionated perifusion efflux, the oxygen consumption rate (OCR) was measured by a fluorescence quench technique, and the ATP/ADP ratio by the luciferase technique.
Results: A 60 min perifusion of isolated islets with 500 μM tolbutamide in the absence of glucose abolished the insulinotropic effect of 30 mM glucose but not of 10 mM alpha-ketoisocaproic acid (KIC). The same dissociation was seen when 15 mM K+ was used instead of 500 μM tolbutamide. When the perifusion period prior to the glucose stimulation was 40 min instead of 60 min, the loss of the insulinotropic efficacy of 30 mM glucose was incomplete. Similarly, when the KATP channel activity was only partially inhibited by 50 μM tolbutamide, a gradual increase of secretion resulted in response to 30 mM glucose. This pattern resembled the effect of glucose after 60 min perifusion in the absence of glucose (control). In SUR1 KO islets the moderate insulinotropic efficacy of glucose and KIC remained unaffected by 60 min perifusion with 500 μM tolbutamide in the absence of glucose. The loss of the insulinotropic efficacy of glucose was paralleled by the inability of glucose to raise the OCR. Under the same condition KIC increased OCR in parallel with secretion. Under control condition glucose increased the OCR, even faster than it increased secretion. The discrepancy between the preserved insulinotropic efficacy of KIC and the loss of efficacy of glucose was not due to different ATP/ADP ratios.
Conclusion: These observations suggest that the combination of moderate depolarization, either by KATP channel closure or 15 mM K+, and the absence of basal nutrient supply decreases the concentration of one or several metabolites critical for glucose-induced secretion, but leaves the exocytotic machinery of the beta cell unimpaired. This situation should facilitate the identification of the relevant amplifying metabolites.
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Beta cell action potentials and Ca^{2+} currents during K^+ depolarisation

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Background and aims: The concept of the amplifying pathway of insulin secretion is mainly based on experimental observations where the nutrient-induced depolarization via K_{ATP} channel closure is replaced by K^+ depolarization. Additionally, diazoxide is used to clamp the K^+ conductance at a constant level, thus precluding insulinotropic effects via changes in the activity of voltage-dependent ion channels. Earlier, diazoxide was described to exert direct effects on the mitochondrial membrane potential; recently, discrepancies were described that exist between depolarization via K_{ATP} channel closure and K^+ depolarization.
Materials and methods: Using mouse islets insulin secretion was measured by batch perifusion and ELISA and the free cytosolic Ca^{2+} concentration ($[Ca^{2+}]_i$) by the Fura technique. Primary mouse beta cells were used for electrophysiological experiments. The membrane potential was measured by utilizing the perforated patch mode. Simultaneous measurements of $[Ca^{2+}]_i$ were performed by loading with Fluo 4. To specifically investigate currents through voltage-dependent Ca^{2+} channels in voltage-clamp experiments using the conventional whole cell configuration K^+ channel blockers were added to the bath solution and intracellular KCl was replaced by CsCl in the pipette solution.
Results: Raising the extracellular K^+ concentration of perifused islets to 15 and 40 mM led to a strong further increase of the insulin secretion even if the beta cells were already depolarized by K_{ATP} channel closure. This effect of high K^+ was accompanied by an increase of $[Ca^{2+}]_i$, which could be shown by simultaneous measurements of the plasma membrane potential. It could completely be antagonized by application of L-type Ca^{2+} channel blockers. In voltage-clamp experiments the Ca^{2+} influx appeared as nifedipine-inhibitable inward action currents when outward K^+ currents were blocked. Under these conditions 15 mM K^+ induced prolonged action currents and 40 mM K^+ transformed the action current pattern into a continuous inward current. Under current-clamp conditions the increase of the K^+ concentration to 15 mM in the presence of 500 μM tolbutamide led to a broadening of the action potentials. This was due to the fusion of several action potentials, separated by prolonged phases of electrical silence. A similar pattern of fused action potentials was caused by 1 μM BayK8644, an opener of L-type Ca^{2+} channels. In contrast to BayK8644 high extracellular K^+ did not affect the current-voltage relation when conditions were used to selectively measure currents through voltage-dependent Ca^{2+} channels.
Conclusion: High extracellular K^+ concentrations do not simply activate Ca^{2+} influx via voltage-dependent Ca^{2+} channels, they also affect the pattern of Ca^{2+} influx as becomes visible in the presence of K^+ channel blockers. In contrast to the action of Ca^{2+} channel openers this effect does not result from a direct interference with channel function.
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Pancreatic beta cell dedifferentiation involves the voltage-gated calcium channel subunit gamma4

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Background and aims: Voltage-gated Ca2+ channels (CaV) are fundamental for insulin secretion. They are heteromeric complexes comprised of the pore-forming alpha1 subunits, and the accessory modulatory subunits beta, alpha2delta and gamma. The impact of gamma subunits in the pancreatic beta-cell is unexplored, especially the gamma4 (CaVγ4) isoform, which has been widely detected in many tissues. Importantly, CaVγ4 exhibits differential down-regulation in human diabetic islets and was therefore investigated in detail. The aim of this study is to investigate the physiological mechanism through which CaVγ4 influences beta-cell function.
Materials and methods: Roles of CaVγ4 in INS-1 cells, rat islets, human islets and beta-cell specific MafA knockout mice (MafAΔβcell) islets were identified by systems biology approaches in combination with physiological validation experiments: microarray, siRNA silencing, real-time quantitative PCR, western blot, patch clamp, immunostaining and radioimmunoassay.
Results: In human diabetic islets, CaVγ4 exhibits differential 15% down-regulation (by microarray collected from 113 donors, $P<0.05$). The gene and protein expression of CaVγ4 were also reduced by gluco-/lipotoxic

conditions (35% and 34v respectively, n=3). CaVγ4 exerted surprisingly strong effects on beta-cell Ca2+ signaling, by an action specific to L-type CaV channels. Silencing of CaVγ4 reduced expression of L-type Cav1.2 and Cav1.3 (28% and 32% respectively, n=3), and also suppressed voltage-gated Ca2+ entry (888.5±49.8A.U.C. vs. 1084.5± 48A.U.C.(negative control); n=3) and insulin exocytosis (389±35fF n=14 vs. 997±206fF(negative control); n=15). This is reminiscent of a dedifferentiated diabetic beta-cell phenotype. Interestingly, microarray analysis of co-regulated genes identified the transcription factor MafA that is responsible for the final steps of beta-cell differentiation, as a potential regulator of CaVγ4 (r=0.649, P<0.001, from 128 donors). This was verified by MafA silencing and in MafAΔβcell (55% reduction of CaVγ4 protein levels, n=3).

Conclusion: In conclusion, gamma4 affects beta-cell function stronger than expected. CaVγ4 is important for maintaining a normal differentiated beta-cell phenotype, and is down-regulated by glucose, palmitate and in human diabetes. Treatment aiming at restoring CaVγ4 may contribute to correcting the dedifferentiated beta-cell phenotype in diabetes.

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The type 2 diabetes associated K^+ channel TALK-1 modulates pancreatic beta cell electrical excitability and 2nd-phase insulin secretion

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Background and aims: Glucose-stimulated insulin secretion (GSIS) requires Ca^{2+} influx through voltage-dependent Ca^{2+} channels (VDCCs). VDCC activity is modulated by the plasma membrane potential (V_m), which is tuned by K^+ channels. The two-pore domain K^+ (K2P) channel TALK-1 is linked to type-2 diabetes risk through a non-synonymous polymorphism (TALK-1 A277E), however, its physiological function has remained elusive. The aim of this study was to assess the role of β-cell TALK-1 channels on electrical excitability, Ca^{2+} influx, GSIS, and glucose homeostasis.

Materials and methods: Whole-cell or single channel electrophysiological recordings were used to assess TALK-1 currents and electrical excitability of mouse and human β-cells. TALK-1 currents were inhibited by expression of a dominant negative (DN) TALK-1 channel variant or eliminated via a knockout mouse model (TALK-1 KO). Islet Ca^{2+} levels were determined with the Ca^{2+} dye Fura-2. Insulin secretion was measured from pancreatic islets and serum. Blood glucose was monitored for changes in glucose tolerance.

Results: Genetic ablation of TALK-1 reduces mouse β-cell K2P currents (11.7±1.0 pA/pF, n=25) compared to controls (18.3±1.2, n=23). Human β-cells also express functional TALK-1 channels (36.7±4.5 pA/pF, n=10) and their K2P currents are significantly attenuated following expression of a TALK-1 DN (22.1±2.3 pA/pF, n=11; P=0.008). Interestingly, we find that TALK-1 A277E channels show increased open probability (P_O; 0.15±0.01, n=6) compared to TALK-1 A277 (0.09±0.01, n=5; P<0.05), suggesting that TALK-1 A277E may hyperpolarize the β-cell V_m and limit GSIS. This is supported by our finding that TALK-1 KO β-cells are depolarized by 6.2±1.4 mV in low glucose (2 mmol/l) and 5.2±1.1 mV in high glucose (14 mmol/l) compared to controls (n=7/genotype; P<0.03). The plateau fraction (the fraction of time spent in the electrically active phase) is also increased in TALK-1 KO β-cells by 0.13±0.02 compared to controls (P=0.03; n=7/genotype). Furthermore, we observe a 0.28±0.06 peak/minute increase in the frequency of Ca^{2+} oscillations in TALK-1-deficient islets relative to controls (n>125 islets/genotype; P<0.005). Consistent with these findings, we observe augmented 2nd-phase insulin secretion from TALK-1 KO islets, which secrete more insulin (4.02±1.07 ng/100 islet equivalents) than control islets stimulated with 11 mmol/l glucose (P<0.05; n=4 islet preparations/genotype). Ablation of TALK-1 channels modestly increases serum insulin levels in chow-fed mice but does not alter glucose tolerance. However, after three weeks on a high-fat diet, fasting blood glucose is significantly reduced in TALK-1 KO mice (169.5±5.6 mg/dl) relative to control mice (221.3±8.4 mg/dl; n=10 mice/genotype; P<0.0005), revealing that beta-cell TALK-1 plays a critical role in adapting to metabolic stress.

Conclusion: TALK-1 channels hyperpolarize the β-cell V_m, modulating 2nd-phase GSIS by limiting the plateau fraction and reducing the frequency of Ca^{2+} oscillations. Furthermore, TALK-1 channels impact glycemia under environmental conditions that promote diabetes. As we find that TALK-1 A277E channels have increased activity, this suggests that TALK-1 A277E may exacerbate hyperglycemia and accelerate diabetes pathogenesis under metabolically stressful states.

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Ca^{2+}-dependent expansion of cortical ER in insulin-secreting beta cells

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Background and aims: The endoplasmic reticulum (ER) is responsible for protein and lipid synthesis and Ca^{2+} homeostasis. Dysfunctional ER is associated with β-cell failure and death in diabetes. The ER morphology is complex with numerous domains making contacts with other membranous organelles, including the plasma membrane (PM). It is becoming increasingly clear that such membrane contact sites are important for the normal function of the ER. The ER-PM contacts are generated by the ER-localized protein family Extended-Synaptotagmins (E-Syts), which contains multiple C2-domains with Ca^{2+} and lipid-binding properties. The aim of this study was to investigate the distribution and dynamics of E-Syt-generated ER-PM contacts in β-cells.

Materials and methods: TIRF and confocal microscopy was used to study subcellular distribution kinetics of fluorescently tagged E-Syt1, E-Syt2, E-Syt1 mutants and the cytosolic fragment of Synaptotagmin-1 (Syt-1) together with the cytosolic Ca^{2+} concentration in intact or permeabilized MIN6 cells. An optogenetic approach was used to rapidly deplete the PM of the lipid $PI(4,5)P_2$ by light-induced PM recruitment of a 5'-phosphatase.

Results: Immunoblotting showed high expression of both E-Syt1 and E-Syt2 in MIN6 β cells and mouse islets. Fluorescence-tagged E-Syt1 localized to the reticular ER and was rapidly recruited ($t_{1/2}$ 21±2 s) to the PM upon depolarization-induced Ca^{2+} influx. When overexpressed alone, E-Syt2 was constitutively bound to the PM but showed depolarization-induced PM binding after co-expression with E-Syt1, demonstrating E-Syt1/2 heterodimer formation. Elevation of the glucose concentration from 3 to 20 mM resulted in oscillatory binding of E-Syt1/2 heterodimers to the PM and expansion of cortical ER. Both responses were completely inhibited by the omission of extracellular Ca^{2+} and by blocking L-type Ca^{2+} channels with 100 μM verapamil. Experiments in permeabilized cells revealed half-maximal binding of the E-Syt1/2 heterodimer to $PI(4,5)P_2$ in the PM at 1.3±0.2 μM Ca^{2+}. Deletion studies showed that the central C2C domain and the C-terminal C2E domain of E-Syt1 function as an intramolecular coincidence detector. Both domains were thus required for efficient PM binding within the physiological Ca^{2+} range (1-10 μM). E-Syt1 and the cytosolic fragments of Syt1, which is the Ca^{2+}-sensor for insulin granule exocytosis, showed nearly identical Ca^{2+}-induced PM-binding with half-maximal effects at 7.6±0.9 μM and 10.2±0.7 Ca^{2+}, respectively, in permeabilized MIN6 β cells. The PM binding of the proteins was reduced by 95±4% (E-Syt1) and 79±9% (Syt1) after light-induced $PI(4,5)P_2$ depletion, demonstrating a role of this lipid for PM-binding of both ER and insulin granules.

Conclusion: The ER undergoes expansions in the cortical regions during glucose-stimulation of insulin secreting cells. The expansion is driven by

Ca^{2+}-induced interactions between the ER-localized proteins E-Syt1/2 and $PI(4,5)P_2$ in the PM. The lipid- and Ca^{2+}-binding properties of E-Syt1/2 strongly resembles those of granule-localized Syt1, indicating ER expansion at sites for insulin granule exocytosis.

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Activation-dependent translocation of Epac2 to granule docking sites at the beta cell plasma membrane

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Background and aims: Epac2 is an important mediator of cAMP-dependent processes in pancreatic β-cells. Activation of Epac2 results in its translocation from the cytoplasm to the β-cell plasma membrane, but it is not known how the membrane interaction is mediated. Epac2 consists of multiple domains organized in a regulatory and a catalytic region. The regulatory region contains a low- and a high- affinity cAMP-binding domain (CNB1 and CNB2) and a DEP (disheveled, Egl-10 and pleckstrin) domain. The catalytic region contains, apart from the catalytic domain, a Ras association domain that previously has been found to interact with the plasma membrane. The aim of the present study was to elucidate how different domains in the regulatory region contribute to membrane localization of Epac2 in β-cells and to investigate how localization of Epac2 is related to insulin secretory granule docking and exocytosis.
Materials and methods: Epac2 binding to the plasma membrane was monitored with total internal reflection fluorescence (TIRF) microscopy of intact or α-toxin-permeabilized MIN6 β-cells co-expressing wildtype and mutant versions of Epac2 tagged with the fluorescent proteins GFP and mCherry, respectively. Secretory granules and exocytosis were visualized by TIRF imaging of cells expressing fluorescent protein-tagged neuropeptide Y (NPY) as granule marker along with Epac2.
Results: Addition of cAMP to permeabilized β-cells evoked concentration-dependent translocation of Epac2 to the plasma membrane with half-maximal and maximal translocation at ~30 μM and >100 μM, respectively. Removal of the CNB1 domain reduced the maximal translocation amplitude to 45% of the wildtype control, but markedly increased the cAMP sensitivity (half-maximal translocation at 5.6 μM). After removal of the DEP domain, a protein module often involved in membrane targeting, the cAMP-induced translocation was reduced by more than 90%. Epac2 translocation was also investigated in intact cells during stimulation with the cAMP-elevating hormone glucagon-like peptide-1 (GLP-1). At 1 nM, GLP-1 triggered pronounced translocation of wildtype Epac2 to the plasma membrane. The translocation response was significantly increased by CNB1 deletion (274% of wildtype), or a point mutation preventing cAMP-binding to the CNB1 domain (133%). In contrast, translocation was decreased by a point mutation preventing cAMP-binding to the CNB2 domain (28%), as well as by DEP deletion (46%). The cAMP-increasing agent forskolin induced clustering of Epac2 at sites of docked insulin secretory vesicles in 40% of the β-cells and this clustering required an intact CNB1 domain. When cells were depolarized with 75 mM K^+, exocytosis events were seen as disappearance of granular NPY-mCherry fluorescence immediately after onset of the stimulation. The Epac2 signal was significantly elevated at granules undergoing exocytosis, but indistinguishable from background at granules that failed to release.
Conclusion: cAMP-induced translocation of Epac2 to the plasma membrane requires nucleotide binding to the high-affinity CNB2 domain and presence of an intact DEP-domain. The low-affinity cAMP-binding CNB1 domain is not required for membrane targeting, but modulates the cAMP-sensitivity of the membrane interaction and contributes to the specific association of Epac2 with granule docking sites. The data indicate that Epac2 translocation to the plasma membrane promotes secretory granule priming and exocytosis.

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Eriodictyol stimulates insulin secretion through cAMP/protein kinase a signalling pathway in mice pancreatic islets

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Background and aims: Very recently our group has undertaken a long-term program to identify new insulin secretagogue(s) using in-house compounds available at a 'Compound Bank'. Among 179 tested compounds, eriodictyol (ED), a flavanoid, isolated from Lyonia ovalifolia was found as a potent insulin secretagogue in in-vitro assay. In the present study, we explored possible mechanism(s) of insulin secretory activity of ED in isolated mice islets and MIN6 cells.
Materials and methods: Islets were isolated from BALB/c mice pancreas by collagenase digestion followed by hand picking. Batches of three size matched islets or MIN6 cells (passages 23 - 26) were incubated in KRB buffer containing 3 or 16.7 mM glucose in the presence or absence of test substance(s). Secreted insulin from islets and MIN6 cells were measured by mouse insulin ELISA kit.
Results: ED stimulated insulin secretion from mice islets and MIN6 cells only at stimulatory glucose concentrations distinctly different from sulfonylurea. At 16.7 mM glucose, ED significantly induced insulin secretion from isolated islets at 50-200 μM concentrations, maximum effect at 200 μM. At 3 mM glucose when islets were incubated with 200 μM ED and 50 μM diazoxide to keep K-ATP channels open, ED-induced insulin secretion was not affected; however, at 16.7 mM glucose ED-induced insulin secretion was inhibited significantly by diazoxide (ng/islet/h; M±SEM; 1.75±0.32 vs. 39.76±2.71, $P<0.001$). When islets were depolarized by 25 mM KCl in the presence of diazoxide, insulin secretory ability of ED was enhanced at both 3 mM (ng/islet/h; M±SEM; 21.5±1.42 vs 7.37±1.6, $P<0.001$) and 16.7 mM (ng/islet/h; M±SEM; 70.51±1.83 vs. 24±1.64, $P<0.001$) glucose. In the presence of 200 μM verapamil, an L-type Ca2+ channels blocker, ED-induced insulin secretion was not affected at 3 mM but inhibited at 16.7 mM (ng/islet/h; M±SEM; 5.2±0.54 vs. 50.55±3.61, $P<0.001$) glucose. In the Ca2+ free media, ED-induced insulin secretion was significantly inhibited at 16.7 mM (ng/islet/h; M±SEM; 10.13±0.88 vs 50.62±3.61, $P<0.001$) glucose. IBMX, a phosphodiesterase inhibitor, at 100 μM significantly ($P<0.001$) enhanced ED-induced insulin secretion (70.22±2.91 ng/islet/h) at 16.7 mM glucose in comparison to ED (41.61±2.06 ng/islet/h) and IBMX (50.22±2.04 ng/islet/h) alone. H-89, a protein kinase A (PKA) inhibitor, at 30 μM almost completely inhibited ED-induced insulin secretion at 16.7 mM glucose (ng/islet/h; M±SEM; 13.81±0.61 vs. 41.13±1.96, $P<0.001$). Calphostin-C, a protein kinase C (PKC) inhibitor, at 2.5 μM partially inhibited ED-induced insulin secretion at 16.7 mM glucose (ng/islet/h; M±SEM; 29.74±1.37 vs. 41.13±1.97). When islets were pretreated with pertussis toxin (100 ng/ml), ED-induced insulin secretion was not affected at both 3 and 16.7 mM glucose.
Conclusion: ED, a novel insulin secretagogue, exerts an exclusive glucose-dependent insulinotropic effect in isolated mice islets and MIN6 cells. ED-induced insulin secretion seems to be mediated through cAMP/PKA signaling pathway, distal to the K-ATP channels and L-type Ca2+ channels, coupled with stimulatory glucose.
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Chronic exposure to fructose exaggerates glucose-stimulated insulin secretion in INS-1E beta cells and human islets

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Background and aims: Fructose massively appeared as an additive in the Western diet in the late 70's, preceding high incidences of obesity and type 2 diabetes and raising questions regarding putative mechanistic links. Upon fructose administration, energy sensing mediated by AMPK activation is impaired in the hypothalamus, increasing feeding behaviour in rodents. In pancreatic beta-cells, AMPK is activated in conditions of nutrient deprivation and renders the beta-cell more sensitive to glucose. Although fructose does not acutely stimulate insulin secretion, we decided to investigate the effects of chronic exposure to fructose on beta-cell function.
Materials and methods: INS-1E cells and freshly isolated human islets from different donors (n=10, each donor provided written informed consent) were exposed for 4 days to 5.5 mM fructose. Culture at standard 11.1 mM (INS-1E) and 5.6 mM glucose (human islets) served as control culture condition. At the end of the 4-day pre-treatment period, we performed immunodetections of Kir6.2 and P2Y1, measured AMPK phosphorylation, ATP levels, and insulin secretion.
Results: Chronic exposure of INS-1E cells and human islets for 4 days to fructose potentiated insulin secretion in response to intermediate 8.3 mM glucose (1.6-fold, $p<0.05$). Paradoxically, fructose pre-treatment reduced intracellular ATP levels by 20% and induced AMPK phosphorylation (1.3-fold, $p<0.05$). The latter effect was mimicked by the stable cell permeant ADP analogue 2-methyl-S-ADP, although not additive to fructose. Correlating with intracellular energy deprivation and AMPK activation, fructose caused translocation of K+-ATP Kir6.2 channels to the cell membrane, exhibiting starving-like phenotype. Upon glucose stimulation, ATP and ADP are co-secreted with insulin and may activate purinergic receptors on the plasma membrane. Western blot and immunofluorescence analyses showed the presence of the calcium-mobilizer P2Y1 receptor in INS-1E cells. Accordingly, in the fructose-treated cells, higher intracellular calcium oscillations were observed. Addition of P2Y1 agonists potentiated insulin secretion stimulated by 8.3 mM glucose on control INS-1E cells and human islets, mimicking the effects of fructose pre-treatment. Conversely, the P2Y1 antagonist MRS2179 reversed the potentiated secretory response induced by fructose exposure. Moreover, clearance of extracellular ATP and ADP by apyrase also inhibited fructose-potentiating effects.
Conclusion: Fructose treatment induced starving-like phenotype in INS-1E cells and human islets, resulting in the potentiation of glucose-stimulated insulin secretion. This effect was mediated by activation of purinergic P2Y1 receptor through increased release of cellular ATP/ADP.
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Of mice and men: a comparative analysis of G-protein coupled receptor expression in mouse and human islets of Langerhans

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Background and aims: G-protein coupled receptors (GPCRs) have proven to be very successful drug targets and are responsible for the actions of approximately 30% of all prescribed therapeutics. The attractiveness of GPCRs as drug targets can be attributed to their involvement in numerous biological processes including the regulation of islet function. However, despite the large pool of potential GPCR targets only a small proportion of these are the targets of current type 2 diabetes clinical projects (GLP1R, GPR119, FFAR1). This can partly be attributed to the lack of knowledge of GPCR expression homology in translation models required to assess the suitability of such potential targets. The aim of this study was to compare the expression of GPCR mRNAs in mouse and human islets to evaluate to what degree studies on mouse islet GPCR function are translatable to human islet physiology.
Materials and methods: GPCR mRNA expression in isolated mouse and human islets was quantified relative to GAPDH using quantitative SYBR green based real-time PCR (qPCR). Human islets were isolated from heart beating donors at the islet isolation centre at King's College London. Mouse islets were isolated from 8 week old male ICR mice using collagenase digestion and handpicked. mRNA was extracted from islets using the TRIzol method and reverse transcribed into cDNA for qPCR screening.
Results: 327 functional GPCRs present in both human and mouse genomes were included in the study. GPR56 was the most abundant GPCR mRNA in human islets (15.6±1.6% of GAPDH, n=3), while the most highly expressed GPCR in mouse islets was Cckar (78.4±12.4% of Gapdh, n=4). 99 GPCRs (30.3%) were absent or expressed at trace levels in both mouse and human islets. 114 GPCR mRNAs (34.9% of GPCRs studied) were expressed above trace levels in both mouse and human islets. 62 of these (54.4%) showed >200% higher mRNA expression relative to Gapdh in mouse islets compared to their human counterparts, including 9 GPCRs (S1PR3, GALR1, OXTR, VIPR2, TACR3, CCKAR, GABBR2, TAAR1, PTGER3) whose mRNA expression was greater than 5000% more abundant in mouse islets than in human islets. On the other hand, mRNAs encoding 18 GPCRs were at least twice as abundant in human islets compared to mouse islets, including 3 GPCRs (SCTR, GPR135 and C5AR1) which were over 500% more abundant in human islets compared to mouse islets. The remaining 34 GPCRs were expressed at similar levels in both human and mouse islets. Of particular interest, 80 GPCRs (25.2%) were expressed only in human islets (e.g. HTR1F, GPR63) and 34 GPCRs (10.3%) were present only in mouse islets (e.g. GALR3, LPAR6). Additionally, 4 GPCRs (GPR148, OXER1, CCRL1, P2RY8) which are not present in the mouse genome, were expressed above trace levels in human islets.
Conclusion: This study found that approximately one third of GPCR mRNAs are expressed at similar levels in mouse and human islets, while the rest show significant differences in expression between mouse and man. These clear species differences in GPCR expression are relevant to appropriate interpretation of functional studies using mouse islets, as they indicate whether results obtained using mouse islets are relevant to human physiology.
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Insulin secretion from human and rodent islets is modulated by adrenergic alpha2A receptor-selective antagonists

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Background and aims: Insulin secretion from the pancreatic islet is regulated by a plethora of neurotransmitters and hormones including catecholamines such as adrenaline. Recently, the adrenergic alpha 2A receptor (AdrA2A) has been associated to type 2 diabetes risk with a phenotype of reduced insulin secretion and receptor overexpression at cellular levels in human islets. In this study we therefore evaluated to which extent an AdrA2A-selective antagonist modulates insulin secretion from human and rodent islets in the absence or presence of catecholamines.
Materials and methods: The in vitro pharmacology of a novel carboxamide (Cpd250) was first evaluated in functional and binding assays that assess its AdrA2 receptor family subtype specificity. After confirming that Cpd250 was a >50× selective AdrA2A antagonist compared to AdrA2B and AdrA2C, we evaluated its effects on insulin secretion in human and rat islets in the presence of catecholamines as well as its pharmacokinetic properties.
Results: Cpd250 reversed receptor activation by 300 nM noradrenaline in a cell line overexpressing human AdrA2A with a relative IC_{50} of 57 nM. In binding assays using membranes from cells overexpressing human AdrA2A, AdrA2B or AdrA2C, Cpd250 bound to AdrA2A with 64× higher affinity than the closest family member. Binding K_i:s were 23 nM, 9220 nM and 1480 nM for AdrA2A, AdrA2B and AdrA2C receptors, respectively. Binding K_i:s for human AdrA1A or AdrA1B were both >25000 nM. Cpd250 had a slight affinity for human 5HT2B (K_i=3390 nM) but the K_i:s for all other 5HT receptors tested were >5000 nM. In rat islets 300 nM noradrenaline inhibits insulin secretion elicited by 11.2 mM glucose by 50-70%, an inhibition that is fully reversed by Cpd250 at doses of 3000 nM or higher. Similar observations were made in human islets; inclusion of 1000 nM noradrenaline suppressed insulin secretion induced by 11.2 mM glucose by 60%, an effect which was fully reversed by Cpd250 with an approximate EC_{50} of 1000 nM Cpd250. We next evaluated the pharmacokinetics of Cpd250 in rats; however, we failed to observe any measurable plasma exposure. Cpd250 had very poor stability in rodent plasma, suggesting that Cpd250 is a useful in vitro tool but cannot be used to evaluate AdrA2A receptor function in vivo.
Conclusion: Here we show that adrenergic inhibition in rodent and human islets can be reversed by an AdrA2A specific antagonist which further validates previously published data on the importance of the AdrA2A receptor in the regulation of insulin secretion. We have also shown that it is possible to identify compounds that show selectivity for AdrA2A. However, Cpd250 will require further optimization to be a useful tool for in vivo studies.

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G-protein coupled receptor dynamics in insulin secreting cells

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Background and aims: The alpha 2A adrenergic receptor (ADRA2A) is a G-protein coupled receptor (GPCR) whose activation has multiple effects on signalling processes inside the pancreatic β cell, ultimately leading to reduced insulin exocytosis. Polymorphisms in ADRA2A are also associated with reduced insulin secretion and the development of type II diabetes. We aimed to identify proteins that interact with ADRA2A and to characterise the movement of ADRA2A single molecules in the membrane of insulin secreting cells.
Materials and methods: Insulin secreting INS-1 cells were co-transfected with ADRA2A-GFP and potential interacting proteins tagged with mRFP or mCherry and imaged using TIRF microscopy. For single particle tracking, INS-1 cells were transfected with ADRA2A-PAtagRFP and imaged using PALM microscopy.
Results: ADRA2A-GFP was observed as bright spots in the INS-1 cell membrane which did not associate with docked secretory granules expressing NPY-mCherry. However, membrane associated ADRA2A-GFP did colocalise with caveolin-1-mRFP, a marker of caveolae. ADRA2A-GFP colocalisation with caveolin-1-mRFP was reduced following stimulation of INS-1 cells with the ADRA2A agonist norepinephrine (10 μM). ADRA2A-PAtagRFP was observed both as single molecules and forming larger brighter clusters in the INS-1 cell membrane. The fluorescence intensity distribution of ADRA2A-PAtagRFP molecules changed following stimulation with 10 μM norepinephrine, with two peaks observed for stimulated cells compared to a single peak for unstimulated control cells. Mean squared displacement analysis of ADRA2A-PAtagRFP tracks revealed that fractions of ADRA2A-PAtagRFP molecules exhibited normal, anomalous or corralled diffusion. This indicates that while some ADRA2A-PAtagRFP molecules move freely in the membrane, others are impeded in their range of motion. Overall ADRA2A-PAtagRFP single molecules moved rapidly, with a median speed of 0.075 μm2/s, however the peak speed was 0.025 μm2/s, indicating the presence of a slower moving receptor population. Following stimulation with 10 μM norepinephrine, ADRA2A-PAtagRFP speed was slightly increased to a median value of 0.083 μm2/s.
Conclusion: ADRA2A exhibits a range of dynamics in the membrane of insulin secreting cells, which we predict are under the control of agonist stimulation and/or interaction with other membrane proteins, such as caveolin-1. ADRA2A association with caveolae may stabilise the receptor and influence its ligand stimulated interactions with G-proteins and downstream effectors. Norepinephrine stimulates ADRA2A movement away from caveolae, most likely into the cell via endocytosis, which would control the amount of membrane bound receptor available for further stimulation.
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GPR75 stimulation to insulin secretion: identification of a signalling pathway in beta cells

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Background and aims: We have previously identified that the atypical chemokine G-protein coupled receptor GPR75 is the most abundantly expressed chemokine receptor in mouse and human islets, is localized to beta cells, and is activated by the pro-inflammatory chemokine ligand 5 (CCL5). Furthermore, we have also found that the conventional CCL5 receptors, CCR1, CCR3 and CCR5, are expressed at very low levels in islets. We have previously demonstrated that CCL5-induced elevations in intracellular calcium ([Ca2+]i) and insulin secretion are significantly diminished upon GPR75 down-regulation. However, identification of further downstream GPR75 signalling pathways in rodent beta cells remains unknown. Therefore, this study aimed to identify components of this pathway in MIN6 beta cells.
Materials and methods: Changes in [Ca2+]i were measured by single cell microfluorimetry of Fura-2 (5 μM) loaded MIN6 beta cells (50,000 cells per coverslip). Insulin secretion from MIN6 beta cells (30,000 cells per well) was determined in static incubation experiments and quantified by radioimmunoassay.
Results: Phospholipase C (PLC) inhibition in MIN6 beta cells diminished CCL5-induced elevations in [Ca2+]i (0.25 nM CCL5: 149±33% of tolbutamide response; +10 μM U73122: 65±16%, n=29, P<0.05) and

insulin secretion (20 mM glucose: 6.75±0.58 ng/30,000cells/h; +10 nM CCL5: 8.88±0.57; +10 nM CCL5+10 μM U73122: 6.90±0.11, P>0.2 vs 20 mM glucose). Blockade of L-type Ca2+ channels with nifedipine abolished CCL5-induced elevations in [Ca2+]i in MIN6 beta cells (P>0.2 vs basal), and inhibited CCL5-induced insulin release (20 mM glucose: 3.57±0.25 ng/30,000cells/hr; +10 nM CCL5: 6.35±0.37, P<0.001; + 10 nM CCL5+10 μM nifedipine: 4.93±0.43). Depletion of DAG-sensitive PKC isoforms by 24 hour exposure to 200 nM 4-beta PMA inhibited the stimulation of insulin secretion by CCL5 (Control: 20 mM glucose: 7.54±0.41 ng/30,000cells/hr; +25 nM CCL5: 9.12±0.50, P< 0.05: PKC-depleted: 20 mM glucose: 3.13±0.11; +25 nM CCL5: 3.42 ±0.12, P>0.2 n=6-8). Furthermore, CCL5-induced insulin secretion was also inhibited upon CAMK II inhibition (20 mM glucose: 6.75±0.58 ng/ 30,000cells/hr; +10 nM CCL5: 8.88±0.57; +10 nM CCL5+10 μM KN62: 5.07±0.43, P<0.001 vs 10 nM CCL5).

Conclusion: These data indicate that activation of the GPR75 signalling pathway by CCL5 in MIN6 beta cells permits Ca2+ influx via L-type voltage operated Ca2+ channels by activating PLC and subsequent activation of DAG-sensitive PKC isoforms. Furthermore, the identification of a calcium influx component upon GPR75 activation likely promotes the exocytosis of insulin secretory granules by activating CAMK II. This novel role for CCL5 in improving beta cell function via the GPR75 signalling pathway provides further insight into its therapeutic potential for improving insulin secretion in type 2 diabetes.

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The adhesion receptors GPR56 and GPR126 and their ligands are expressed by islets and regulate beta cell function

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Background and aims: GPR56 and GPR126 are members of the adhesion class of G-protein coupled receptors that play key roles in organ development. Collagen III is the agonist of GPR56 and we have shown that GPR56 is one of the most abundant GPCRs in human islets. Collagen IV increases cAMP in GPR126 transfected HEK293 cells and stimulates an increase in [Ca2+]i in tumour cells. The roles of GPR56 and GPR126 in islets is not known, so the aim of this study was to investigate their expression and functions in islets.

Materials and methods: Expression of GPR56, GPR126, collagens III and IV was determined by RT-PCR, Western blotting, and immunohistochemistry of mouse and human pancreases. The effects of 100 nM collagens on insulin gene expression and β-cell adhesion were determined by qPCR and adhesion assay respectively. Insulin secretion was quantified by radioimmunoassay after 3 days exposure of mouse islets to collagens III and IV. Changes in [Ca2+]i of Fura-2 loaded MIN6 β-cells on exposure to 100 nM collagens III and IV were measured by single cell microfluorimetry.

Results: GPR56, GPR126, collagens III and IV were detected in mouse and human islets. GPR56 was abundantly expressed by β-cells but was absent from glucagon-secreting α-cells of mouse islets. Unlike collagen IV, collagen III was not expressed by β-cells, but was confined to islet capillaries. Collagens III and IV significantly increased insulin gene expression (by 178.7±5.9% and 211.5±5.3% respectively, p<0.001) and enhanced β-cell adhesion (control: 0.08±0.01 absorbance units; +Col III: 0.13±0.02; +Col IV: 0.23±0.03, p<0.01, n=5). Pre-exposure of isolated mouse islets to 100 nM collagens III and IV for 3 days led to significantly enhanced glucose-induced insulin secretion (20 mM glucose: 5.91± 1.22 ng/islet/h; +Col III: 10.30±0.94; +Col IV: 13.26±0.99, p<0.01, n=7-8). Chronic exposure to collagen III did not increase basal insulin release (2 mM glucose: 0.24±0.02 ng/islet/h; +Col III: 0.20±0.01, p=0.1, n=7), but islets exposed to collagen IV for 3 days did show significantly increased basal insulin release (2 mM glucose: 0.24±0.02 ng/islet/h; +Col IV: 0.38±0.05, p<0.05, n=7). Collagen IV induced elevations in β-cell [Ca2+]i at 2 mM glucose (100 nM Col IV: 60±4% tolbutamide response, n=24) and it also had a small stimulatory effect at 20 mM glucose (100 nM Col IV: 133±10% 20 mM glucose response). Collagen III was without effect on [Ca2+]i in Fura 2-loaded β-cells at 2 mM glucose (n= 32).

Conclusion: Mouse and human islets express the adhesion receptors GPR56 and GPR126 and their ligands, collagen III and IV, suggesting that these receptors may be activated in a paracrine/autocrine manner. Our data further suggest that these receptors may be the mechanism through which extracellular matrix collagens regulate β-cell adhesion, insulin synthesis and insulin secretion.

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Characterisation of the sweet taste receptor electrophysiology in pancreatic beta cells

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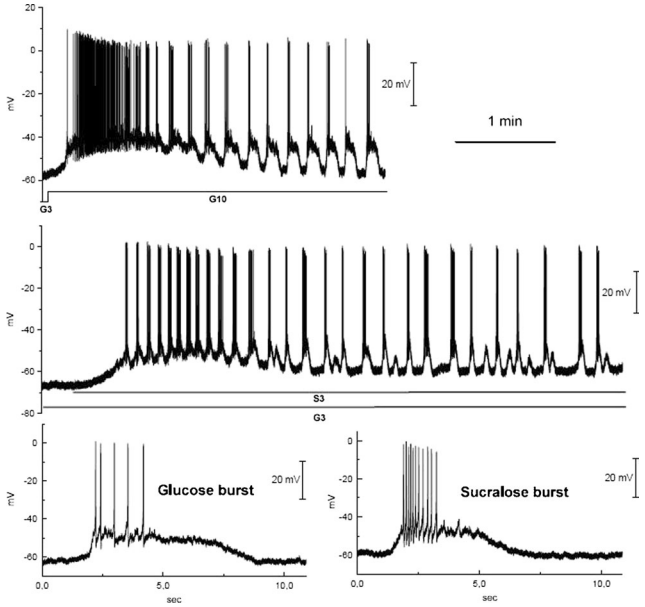
Background and aims: According to the so-called "fuel hypothesis" the stimulation of insulin release by glucose and other nutrient secretagogues may be attributed to their catabolism resulting inter alia in an increased generation of ATP. However, recent work based among others on the effects in beta cells of non-metabolizable agents, such as sucralose, has revived interest on a "receptor hypothesis", apparently involving the activation of the sweet taste receptor T1R3. The present study aimed at characterizing the electrophysiological effects of sucralose in mouse insulin-producing cells.

Materials and methods: The beta cell membrane potential was recorded in isolated mouse pancreatic islets fixed with micropins to the bottom of a perifusion chamber. The superfusion medium contained, as required, glucose and/or sucralose.

Results: When tested in the absence of glucose, sucralose (5 to 10 mM) did not affect membrane potential. At a substimulatory glucose concentration (3 to 5 mM), sucralose evoked a biphasic electrophysiological response. The first phase lasted for a few minutes and consisted of a depolarizing wave with overimposed bursting activity. No obvious difference was found between bursting induced by either glucose or sucralose. Incidentally, the response to sucralose displayed some heterogeneity. Thus, 15% of the cells were unresponsive. Moreover, In the 85% responsive cells, a second phase of electrical activity was only apparent in 11 out of 17 cells. Interestingly, when the sucralose concentration was increased during the second phase initially evoked by a lower concentration of the artificial sweetener, the cells displayed an unresponsive behavior, suggesting an apparent desensitization. At a suprastimulatory glucose concentration (5 to 10 mM), sucralose also excited the cells in a dose-dependent manner. Lower sucralose concentrations increased bursting duration at the expense of hyperpolarized periods. Higher glucose concentrations are able to drive the cell into continuous activity. The effect reverted immediately after sucralose withdrawal even for high sucralose concentrations (30 mM).

Conclusion: The present findings reveal that sucralose exerts a stimulatory effect on beta cell electrical activity. This effect is closely related to the glucose concentration. Thus, sucralose is unefficient in the absence of glucose, while potentiating the electrical response recorded in the presence of the latter hexose. These data support the view that the activation of a sweet taste receptor may stimulate and modulate glucose responses. The similarities between glucose and sucralose actions may suggest a distal convergence in their mode of action. The data presented constitutes a strong evidence of the receptor hypothesis as far as sucralose, an agonist of the homodimeric T1R3 taste receptor in the pancreatic beta cell, is able to induce an excitatory effect of the pancreatic beta cell electric activity.



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Palmitate stimulates insulin secretion by enhancing mitochondrial respiration via intracellular metabolism and FFAR1 signalling

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Background and aims: Free fatty acids (FFAs) play an important role in the regulation of insulin secretion. At high glucose, FFAs enter glycerolipid/free fatty acid cycle and generate insulinotropic metabolic coupling factors such as long-chain acyl CoAs, phosphatic acids, diacylglycerol, etc. FFAs enhance insulin secretion also by interacting with G-protein coupled receptors (GPCRs) on the plasma membrane. One of the GPCRs highly expressed in beta cells is the free fatty acid receptor 1 (FFAR1 or GPR40). We have reported that palmitate potentiates insulin secretion via a synergistic action of intracellular metabolism and FFAR1 signalling. Given the importance of mitochondria in insulin secretion, we investigated the effects of fatty acid metabolism and FFAR1 signaling on mitochondrial function.

Materials and methods: Study was performed on mouse insulinoma MIN6 cells. Cells were treated for 1 hour with XF assay media containing 25 mM glucose and 0.5 mM palmitate in the absence or presence of FFAR1 antagonist, ANT203. Cells were also exposed to FFAR1 agonist TAK875. During treatment, insulin secretion and mitochondrial oxygen consumption rate (OCR) were measured. Insulin was measured by ELISA whereas OCR was measured by Extracellular Flux Analyzer XF96e. Palmitate oxidation and glucose utilization were determined by including during culture [3H]palmitate and d-[5-3H]glucose, respectively.

Results: MIN6 cells cultured in the presence of palmitate secreted 50% more insulin than in the absence of the fatty acid. This was accompanied by a 35% rise in OCR. Inhibition of the FFAR1 pathway with ANT203 prevented the palmitate-induced rise in insulin secretion. The effect was associated with a significant reduction in OCR to levels close to those observed in control cells. Stimulation of another Gαq-coupled receptor, M3 muscarinic acetylcholine receptor, with agonist carbachol in the presence of ANT203 reverted OCR back to levels observed in the presence of palmitate alone. Stimulation of FFAR1 with TAK875 in the absence of extracellular palmitate did not enhance OCR. By inhibiting fatty acid or glucose oxidation we showed that the increase in OCR in palmitate-treated cells was largely due to increased glucose oxidation. In line with this, glucose utilization in the presence of palmitate was increased compared to control cells. However, FFAR1 antagonist did not reduce palmitate-induced glucose utilization. Also, FFAR1 agonist TAK875 in the absence of extracellular palmitate did not elevate glucose utilization.

Conclusion: During short-term exposure palmitate stimulates insulin secretion by enhancing mitochondrial respiration via complementary action of intracellular metabolism and Gαq-dependent signaling of FFAR1

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Kisspeptin and the adaptation of islets to pregnancy: a role for islet serotonin

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Background and aims: During pregnancy, the pancreatic islets of Langerhans undergo adaptive changes in order to compensate for the normal progression of insulin resistance that occurs during gestation. These adaptive changes include both increased glucose-stimulated insulin release and increased beta-cell mass. The signals underlying these changes are not fully understood, however it is established that prolactin and placental lactogen play an important role. Recently beta-cell serotonin (5-HT) has been found to mediate the effects of lactogenic hormones on beta-cell proliferation and glucose-stimulated insulin release via autocrine or paracrine effects at the Htr2b receptor. We have previously shown that placental kisspeptin (KP), and its G-protein coupled receptor, GPR-54, may also play a key contributory role in mediating the adaptation of rodent islets to pregnancy. However, the downstream mechanism by which KP causes these compensatory changes is not understood. We therefore investigated a possible role of the islet serotonin system, as well as the involvement of key pro-proliferative genes, in mediating the effects of KP.

Materials and methods: Islets were isolated from female ICR mice and cultured in either 2 mmol/L or 20 mmol/l glucose RPMI, with or without KP-10 (1 μmol/l) for 48 hrs. Islet samples were then taken and mRNA expression of key genes investigated using quantitative RT-PCR analysis.

Results: KP treatment dramatically increased the mRNA expression of the 5-HT synthesis enzyme *Tph-1* within islets cultured in stimulatory glucose concentrations (20 mmol/l glucose: $0.4\pm0.06\times10^{-3}$, 20 mmol/l glucose + 1 μmol/l KP-10: $1.5\pm0.2\times10^{-3}$; relative to *Gapdh*; n=4; p=0.063), but no change was observed for *Tph-2* expression (20 mmol/l glucose: 0.032±0.004, 20 mmol/l glucose+1 μmol/l KP-10: 0.053±0.006; relative to *Gapdh*; n=4). Additionally no change in expression of the 5-HT receptor *Htr2b* was observed in islets treated with KP-10 at either glucose concentration (20 mmol/l glucose: $0.2\pm0.01\times10^{-3}$, 20 mmol/l glucose+1 μmol/l KP-10: $0.2\pm0.009\times10^{-3}$; relative to *Gapdh*; n=4). Exposure of isolated mouse islets to KP-10 had no effect on mRNA expression levels for the proliferative gene markers *Cyclin E1*, *Cyclin D1*, and *Ki67* (20 mmol/l glucose: 3.27±0.74, 69.1±4.35, $1.3\pm0.2\times10^{-3}$; 20 mmol/l glucose+1 μmol/l KP-10: 5.21±0.97, 94.0±1.48, $1.4\pm0.2\times10^{-3}$; expression levels of *Cyclin E1*, *Cyclin D1* and *Ki67* relative to *Gapdh* respectively; n=3) or the pro-survival gene marker, *Birc5* (20 mmol/l glucose: $0.8\pm0.3\times10^{-3}$, 20 mmol/l glucose+1 μmol/l KP-10: $0.8\pm0.2\times10^{-3}$; relative to *Gapdh*; n=3).

Conclusion: These results suggest that in addition to an established role mediating the effects of the lactogenic hormones, KP signalling may also be mediated through beta-cell 5-HT. Specifically KP increases the expression of the 5-HT synthesis enzyme Tph-1, but not Tph-2, suggesting an increase in 5-HT production. However, contrary to previous studies, there was no change in expression levels of the 5-HT receptor Htr2b. Furthermore, at present these data do not show which proliferative or pro-survival genes may be involved in mediating effects of KP on beta-cell mass, further measurements are required to determine the downstream signalling pathways involved in these effects.

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Foetal endocannabinoids orchestrate the organisation of pancreatic islet microarchitecture

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Background and aims: 2-Arachydonoylglycerol (2-AG) and anandamide, major endocannabinoids are implicated in the control of glucose utilization and energy homeostasis by orchestrating pancreatic hormone release. Moreover, in many cell niches, endocannabinoids regulate cell proliferation, fate determination and migration. Nevertheless, endocannabinoid contributions to the development of the endocrine pancreas and, consequently, to long-lasting changes in hormone release postnatally remain unknown.
Materials and methods: To dissect the role of endocannabinoid signaling during endocrine pancreas development we combined the genetic ablation of main components of endocannaboinoid signaling, (e.g., CB_1 cannabinoid receptor (CB_1R), monoacylglycerol lipase (MAGL) or transient receptor potential vanilloid 1 channels (TRPV1) knock-out models), modifications to the maternal diet during pregnancy (enrichment in ω-3 polyunsaturated fatty acids) and pharmacological probing of endocannabinoid signaling in 'pseudoislets', α/β cell aggregates that model cell sorting in pancreatic islets *in vitro*.
Results: Our results show that α cells produce 2-AG in mouse fetuses and human pancreatic islets, which primes the recruitment of β cells by CB_1R engagement. By using subtractive pharmacology we extend these findings since exogenous anandamide, a promiscuous endocannabinoid/endovanilloid ligand, impacts both the determination of islet size by cell proliferation and α/β cell sorting by differential activation of transient receptor potential TRPV1 channels and CB_1Rs. Accordingly, genetic disruption of TRPV1 channels increases islet size, while CB_1R knockout augments cellular heterogeneity by inducing α/β cell 'mixing' and favors insulin over glucagon release. Dietary enrichment in ω-3 fatty acids during pregnancy and lactation in mice, which permanently reduces endocannabinoid levels in the offspring, phenocopies $CB_1R^{-/-}$ islet microstructure and improves coordinated hormone secretion. This is significant since both $CB_1R^{-/-}$ and ω-3 fatty acid-exposed offspring are lean.
Conclusion: Our study identifies fundamental roles for endocannabinoids acting at CB_1R and TRPV1s in determining cellular diversity, structural complexity and life-long plasticity of the endocrine pancreas. Furthermore, it highlights that maternal dietary choices during pregnancy can program fetal pancreas development by altering endocannabinoid bioavailability which prospectively determines the offspring's sensitivity to metabolic stressors.
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PS 023 Mechanisms of insulin secretion

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The PIN protein participates in and modulates exocytosis of insulin secretory granules in the INS-1 cell line

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Background and aims: PIN (Protein Inhibitor of Neuronal NO synthase) or LC8 (Light Chain 8) is a hub protein able to interact with multiple partners including neuronal NO synthase (nNOS) and cytoskeletal proteins like myosin V and dynein. We have previously shown that PIN is a positive modulator of glucose-induced insulin secretion. In order to better understand the role of PIN, we developed an inhibitory peptide, able to block interactions between PIN and its proteic partners and rendered cell-permeant by coupling with the Tat-peptide.
Materials and methods: Glucose-induced insulin secretion was measured in INS-1 β-cells in the presence of the Tat-peptide alone (GRKKRRQRRR), - the inhibitory peptide (GRKKRRQRRRGGIDVGIQVDWD) or - an irrelevant one (GRKKRRQRRRGGKAVDLSHQPS). For Western blotting, monoclonal anti-α-tubulin (Sigma Aldrich) or anti-PIN (BD Biosciences) antibodies were used. Immunoprecipitation was performed using a polyclonal anti-PIN (Santa Cruz) or the monoclonal anti-α-tubulin antibody. GST-pull down was performed using recombinant GST-PIN as bait on INS-1 extracts. Potential partners of PIN were identified by Orbitrap XL mass spectrometry thanks to the Functional proteomics platform of Montpellier. For TIRF microscopy experiments, cells were transfected with NPY-EGFP reporter and continuous perifused at 32°C with KRB buffer containing 75 mM KCl to stimulate insulin secretion. Cells were imaged using a custom-built lens-type TIRF microscope based on an Axiovert 135 microscope with a 100×/1.45 objective (Carl Zeiss).
Results: In the INS-1 cell line, the inhibitory peptide decreased insulin secretion induced by 2.8, 5.6 and 8.3 mM glucose, by 33% at 20 μM, and by 58% at 40 μM ($p<0,001$). Such an inhibitory effect has also been observed using a PIN siRNA, which decreased PIN expression and reduced 5.6 and 8.3 mM glucose-insulin secretion by respectively 16 and 20% in response to ($p<0,001$). Surprisingly, in the presence of the inhibitory peptide, we observed, by Western blotting, a decreased level of soluble α-tubulin (-22% at 20 μM and -95% at 40 μM), suggesting an increased polymerization of tubulin. Interestingly, using GST-pull down with PIN as bait, revealed an interaction of PIN with α1C-tubulin and β5-tubulin. Direct interaction of PIN and α-tubulin was confirmed by immunoprecipitation experimentations. Finally, in TIRF microscopy experiments, we found the average release time of insulin granules, during single exocytosis event, increased by 3.5 fold in the presence of the inhibitory peptide.
Conclusion: Interaction of PIN with cytoskeletal proteins appears to be involved in the control of insulin granules exocytosis, possibly via modulation of α-tubulin polymerization.

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LKB1 is required for normal glucose-signalling, but not insulin secretion, in pancreatic beta cells

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Background and aims: Liver kinase B1 (LKB1/STK11), a tumour suppressor inactivated in Peutz-Jeghers syndrome, exerts its biological

effects largely by the direct phosphorylation of AMP-activated protein kinase (AMPK) and AMPK-related kinases. Deletion of LKB1 from pancreatic beta cells using a variety of Cre deleter strains has previously been shown to improve insulin content and secretion in mice. Here, we explore the impact of LKB1 deletion in beta cells on glucose-induced changes in mitochondrial metabolism and Ca^{2+} signalling using islet imaging approaches.

Materials and methods: Mice deleted selectively in the beta cell for LKB1 were generated by breeding animals bearing flox'd alleles at exons 3-6 with the novel and highly beta cell-selective Ins1Cre deleter line. Total and released insulin was measured by radioimmunoassay. Changes in intracellular ATP/ADP ratio ($[ATP/ADP]_{cyt}$) and Ca^{2+} ($[Ca^{2+}]_{cyt}$) were measured by confocal microscopy of whole islets expressing the recombinant GFP-based probe Perceval, or loaded with the fluorescent Ca^{2+} dye, fluo-2, respectively. An LKB1-encoding adenovirus (Vector Biolab), titred using qPCR to assess LKB1 overexpression, was used to rescue the effects of LKB1 deletion. Metabolite profiling was performed on isolated islets after extraction in 16.7 mM glucose on an Agilent 6890 N gas chromatograph coupled with a Leco Pegasus III TOFMS electron impact time of-flight mass spectrometer.

Results: Whereas insulin secretion in response to elevated glucose (11 vs 3 mM) was largely unaffected (4 mice/ genotype, 6 size matched islets/ well, $p>0.05$), glucose-stimulated increases in $[ATP/ADP]_{cyt}$ (12 islets/ genotype, $p<0.05$) and $[Ca^{2+}]_{cyt}$ (12 islets/4 mice/genotype, $p<0.01$) were severely impaired in islets from LKB1 KO mice, as was the percentage of islets responsive to glucose-stimulation ($n=12$ islets, $p<0.05$, $p<0.01$, respectively). $[Ca^{2+}]_{cyt}$ signalling was largely rescued after LKB1 over-expression in Ins1Cre::$LKB1^{flox/flox}$ islets (12 islets, 3 mice/genotype, $p>0.05$), excluding developmental effects of LKB1 deletion as responsible for the observed signalling abnormalities. Metabolomic profiling showed that fatty acids (C12, C16, C18), glycerol and glycerol-3-phosphate were lowered in LKB1 null islets, as were lactate and fumerate ($n=3$ mice/genotype, $P<0.05$).

Conclusion: Despite the improvement of glucose tolerance previously observed in this model, and preserved insulin secretion, deletion of LKB1 significantly impairs normal glucose-induced $[ATP/ADP]_{cyt}$ and $[Ca^{2+}]_{cyt}$ signalling in beta cells. Our findings suggest that LKB1 deficiency stimulates metabolic changes in beta cells which activate a robust amplifying pathway. This appears to override defects in the classical triggering mechanisms of insulin secretion that rely on oxidative metabolism of glucose towards ATP.

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S561f cdkal1 variant, identified by whole exome sequencing of congenital hyperinsulinism patients, affects insulin content and release in ins1-e cells

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Background and aims: Congenital hyperinsulinism (CHI) is a rare disorder (MIM#256450), characterised by inappropriate insulin secretion that is responsible of hypoglycaemia, which can lead to neurological damage. In a previous work conducted in our laboratories 17 patients of Congenital Hyperinsulinism were recruited for a Whole Exome Sequencing (WES) study. We selected all the genes presenting rare or novel potentially damaging single nucleotide variants to perform candidate gene prioritization analysis. We found that CDK5 Regulatory Subunit Associated Protein 1-Like 1 (CDKAL1) was one of the most significantly enriched genes by the bioinformatics analysis. CDKAL1 is a methylthiotransferase anchored to the endoplasmic reticulum membrane, that modifies the tRNALys (UUU) ensuring the correct translation of the codons AAA, AAG into Lysine residue. A Lysine residue is crucial for the correct cleavage of pro-insulin and the folding of mature insulin protein. Different studies have previously associated CDKAL1 to type 2 diabetes development risk and to impaired glucose homeostasis phenotype in knockout (cdkal1 -/-) mice. The S561F-CDKAL1 variant identified in our study results in the substitution of a residue of serine by a phenylalanine, more hydrophobic residue at the level of the transmembrane domain. The aim of this work is to understand the consequences of the CDKAL1 variant S561F on the protein itself and on the insulin content and release of pancreatic beta cells.

Materials and methods: Clonal INS1-E cells expressing Wild Type (WT) or S561F CDKAL1 were generated and used as model to characterise S561F-CDKAL1 impact on beta cell function. The localisation of the mutated protein was monitored by immunofluorescence and insulin content and release was measured with ELISA assays.

Results: Wild type CDKAL1 overexpressed in INS1-E cells localized as expected in the reticular compartment, diffused in the cell cytoplasm. The genetic variant S561F was similarly confined in the reticular compartment, although its localization was enriched in vesicular-like structures distributed in the perinuclear region. Insulin content was increased by overexpression of WT CDKAL (2 fold over INS1E, $p<0.05$) while it was decreased by S561F-CDKAL1 variant, thus suggesting a different insulin processing in the mutant CDKAL1. Insulin release measured in overnight culture medium in basal conditions showed that S561F-CDKAL1 overexpression increased the constitutive insulin release (2 to 4 fold over INS1E in clones 3 and 7, respectively; $p<0,05$, $p<0,005$ vs WT).

Conclusion: The S561F-CDKAL1 variant leads to an abnormal localisation of the protein. An increase of insulin release was observed as consequence of the overexpression of the mutated CDKAL1 protein. Our findings support the importance of CDKAL1 in insulin release and propose novel mechanisms leading to beta cells dysfunction that can participate to the development of congenital hyperinsulinism.

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Protein disulfide isomerase ameliorates beta cell function in pancreatic islets overexpressing human islet amyloid polypeptide

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Background and aims: Human islet amyloid polypeptide (hIAPP) is the major component of amyloid deposits in pancreatic islets of patients with type 2 diabetes. The process of hIAPP misfolding and aggregation is one of the factors that may lead to beta-cell dysfunction and death. Endogenous chaperones have been described to be important for the folding and proper function of proteins. Here, we examine the effect of the endoplasmic reticulum chaperone protein disulfide isomerase (PDI) on insulin and IAPP secretion in hIAPP Tg mouse islets.

Materials and methods: Human islet lysates were immunoprecipitated for hIAPP and interaction with major chaperones was analyzed. hIAPP Tg islets were transduced with an adenovirus encoding for the chaperone PDI and cultured with 16 mM glucose and 400 μM palmitate (HG+PA) for 24 hours. Islet function was determined by glucose-stimulated insulin and IAPP secretion and gene expression and protein levels of PDI and apoptosis marker caspase 3 were determined by real-time RT-PCR and immunohistochemistry, respectively.

Results: Immunoprecipitation of human islet lysates indicated that, among the most important chaperones of the ER (BiP, CCT4, TCP1a and PDI), only PDI was interacting with hIAPP. Islet transduction with an adenoviral vector encoding for chaperone PDI demonstrated that PDI could be overexpressed in mouse pancreatic islets. PDI overexpression at a dose of 20 MOI had no effect in glucose-stimulated insulin secretion in untreated wild-type islets. When PDI was overexpressed in hIAPP Tg islets, PDI was able to restore beta cell dysfunction associated with hIAPP overexpression by increasing insulin secretion levels. Furthermore, treatment of islets with HG+PA for 24 hours decreased glucose stimulated insulin and hIAPP secretion in hIAPP Tg mice. Nevertheless, PDI overexpression was able to counteract the decrease in insulin and hIAPP secretion in hIAPP Tg islets.
Conclusion: Overexpression of chaperone PDI was able to rescue HG+PA-induced beta cell dysfunction. This approach could reveal a new therapeutic target and aid in the development and evaluation of strategies to decrease beta cell dysfunction in type 2 diabetic patients.
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Generation of the transgenic INS-SNAP (SOFIA) pig for the high-throughput high-content study of insulin granule biogenesis and turnover in isolated islets

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Background and aims: Age-distinct pools of insulin secretory granules (SGs) display different propensity for exocytosis, with newly synthesized SGs being preferentially secreted. Recently we exploited an insulin-SNAP chimera (Ins-SNAP) as a reporter for conditional fluorescent labelling and imaging of SGs generated at different times in insulinoma cells and *mINS2-SNAP* knocked-in SOFIA (Study OF Iinsulin Aging) mice. With this approach we provided formal evidence for the preferential exocytosis of young SGs and their progressive loss of mobility with ageing. These findings are relevant for the understanding of type 2 diabetes, in which reduction of the 1st phase of insulin secretion and increased proinsulin release point to deficits in SG biogenesis and turnover. Here we report the generation of an *INS-SNAP* transgenic pig (SOFIA pig) as a large animal model for studying in greater detail the biogenesis and turnover of insulin SGs through the performance of high-throughput high-content (HTHC) screenings of compounds affecting these processes.
Materials and methods: Pools of stably *INS-SNAP* transfected porcine kidney cells were used for somatic cell nuclear transfer (SCNT), and reconstructed embryos were transferred to recipient gilts. The resulting progeny was examined for transgenic status by PCR and southern blotting and then for INS-SNAP protein expression in β-cells by immunocytochemistry on pancreatic tissue sections. Correlative light-electron microscopy (CLEM) was performed on pancreatic tissue sections of a mature founder pig. Pseudoislets obtained from *hINS-SNAP* insulinoma INS-1 cells were used to optimize a semi-automated HTHC pipeline in a 384-well format. Neonatal islet-like cell clusters (NICC) isolated from newborn SOFIA pigs were used for a pilot HTHC screening for compounds affecting SG biogenesis and turnover.
Results: A 2-year-old SOFIA founder pig was sacrificed to verify the expression of INS-SNAP protein in situ and the β-cell ultrastructure. Electron microscopy did not reveal morphological alterations of the ER, SGs or other β-cell compartments. The restricted expression of INS-SNAP protein in β-cells and its correct targeting to SGs were confirmed by CLEM. Optimization of the HTHC platform allowed for the homogenous dispensing of ~50 *hINS-SNAP* INS-1 pseudoislets or NICC/well in 384-well plates and their retention throughout the SNAP labelling and immunolabelling procedures. Parameters for automated confocal imaging, recognition and analysis of fluorescently labelled INS-SNAP^{+} SGs were validated, thus enabling a first pilot screen for compounds affecting the turnover of age-defined SGs.
Conclusion: The successful generation of a transgenic SOFIA pig shall enable for the first time the performance of HTHC studies on the biogenesis and age-dependent turnover of insulin SGs within islets, hence providing novel insight into the biology of β-cells and treatment opportunities for type 2 diabetes.

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ERK7: a novel regulator of insulin secretion and lipid metabolism in Drosophila

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Background and aims: Mitogen Activated Protein Kinases (MAPKs) are Ser/Thr kinases which regulate a wide variety of cellular processes. Although this family is very well studied, some of the members like ERK7 (ERK8/MAPK15) are poorly characterized. ERK7 is an atypical MAP kinase which was shown to be activated by amino acid and serum starvation in *Drosophila* S2 cells. We have developed *in vivo Drosophila* models to further characterize its physiological functions.
Materials and methods: dILP2-Gal4; UAS-GFP driver line was used for Insulin Producing Cells (IPC) specific genetic manipulations. CG-Gal4 was used for fat body specific experiments. RNAi lines and p53 overexpression lines were obtained from VDRC, Austria and Bloomington Stock Center, USA. qRT-PCR assays were used to measure mRNA levels of insulin like peptides and lipogenic genes. Immunostainings using anti-dILP2 antibody were used to assess secretion of insulin like peptides. Gas chromatography was used for quantitative lipidomics.
Results: We hereby show that ERK7 is an important regulator of growth and lipid metabolism in *Drosophila*. We observed that upon starvation, ERK7 is expressed in median neurosecretory cells (IPCs) which regulate larval growth by secreting insulin-like peptides (dILPs) in diet-dependent manner. We demonstrate that overexpression of ERK7 in IPCs strongly inhibits dILP secretion which consequently leads to reduced body size and a delay in larval development. We also identify p53 as an upstream activator of ERK7 in IPCs. Further, we establish that ERK7 function in IPCs is necessary for efficient starvation response. Finally, we also show that ERK7 has important metabolic functions outside IPCs. We show that ERK7 function in lipogenic tissues of *Drosophila* is important for normal lipid homeostasis. Overexpression of ERK7 in these tissues caused qualitative and quantitative changes in lipid levels. We are currently generating ERK7 mutants to try and decipher the pathways it regulates by using RNA-seq analysis.
Conclusion: ERK7 regulates starvation response by inhibiting insulin secretion from insulin producing cells. It also has a role outside IPCs in regulation of lipid homeostasis.
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Reciprocal changes in glucose tolerance after pancreatic beta cell selective deletion or over-expression of Slc30a8/ZnT8 in mice

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Background and aims: The incorporation of two zinc (Zn^{2+}) ions into the insulin hexamer is fundamental for insulin maturation. Zinc Transporter 8 (ZnT8, encoded by *SLC30A8*) is highly, and almost exclusively, expressed within pancreatic beta and alpha cells where it facilitates the importation of cytosolic zinc into secretory granules. Co-secretion of Zn^{2+} from secretory granules is an important paracrine regulator of islet hormone secretion and has also been shown to regulate insulin bioavailability. Moreover, a common polymorphism (R325W) within *SLC30A8*, which lowers transporter activity, is associated with higher risk of developing type 2 diabetes (T2D). Recently, however, rare inactivating mutations in *SLC30A8* have been described which appear to protect against T2D development. Here, we explore the effects of either ZnT8 deletion or over-expression specifically in the beta cell in mice.
Materials and methods: Beta cell specific deletion of ZnT8 was achieved by crossing mice harbouring LoxP sites either side of exon 1 of *Slc30a8* with an Ins1Cre deleter strain. Transgenic mice were generated by pronuclear injection of DNA encoding c-myc tagged ZnT8 downstream of a tetracycline regulated promoter. Beta cell overexpression of ZnT8 was achieved by crossing transgenic mice with mice containing the tetracycline transactivator gene under the control of the insulin promoter. Glucose tolerance was assessed using glucose tolerance tests and insulin granule structure by electron microscopy.
Results: Beta cell selective deletion: Male $Ins1Cre^{+/-}::ZnT8^{fl/fl}$ mice displayed significant impairments in glucose tolerance at 10 weeks of age *vs* $Ins1Cre^{-/-}::ZnT8^{fl/fl}$ littermate controls (13.6±0.74 mmol/L *vs* 11.5±0.59 mmol/L at 30 min. respectively; P<0.05; n=8/11). Granule morphology was dramatically altered following ZnT8 deletion, with an increased percentage of granules from $Ins1Cre^{+/-}::ZnT8^{fl/fl}$ mice lacking dense cores (36.3±2.48 *vs* 85.6±1.37, $Ins1Cre^{+/-}::ZnT8^{fl/fl}$ *vs* $Ins1Cre^{-/-}::ZnT8^{fl/fl}$, P<0.001, n=8 beta-cells/genotype) or having 'rod-like' structures (31.9±3.42 *vs* 0.6±0.28, $Ins1Cre^{+/-}::ZnT8^{fl/fl}$ *vs* $Ins1Cre^{-/-}::ZnT8^{fl/fl}$, P<0.001, n=8 beta-cells/genotype), insulin granule diameter was also increased in ZnT8 null mice (377.467 ± 5.12 nm *vs* 328.09 ± 5.46 nm, *Ins1Cre+/-::ZnT8 fl/fl vs Ins1Cre-/-::ZnT8 fl/fl*, P<0.001, n=204/226 granules). Beta cell selective overexpression: Female mice overexpressing ZnT8 ($Rip7rTTa^{+/-}::ZnT8Tg^{+/-}$; doxycycline treated from 5 weeks) showed significant improvements in glucose tolerance compared to $Rip7rTTA^{+/-}::ZnT8Tg^{-/-}$ littermate controls at both 10 (12.2 ±0.51 mmol/L *vs* 13.9±1.1 mmol/L; respectively; P<0.05; n=6) and 14 weeks of age (8.84±0.59 mmol/L *vs* 11.7±1.01 mmol/L; respectively; P<0.01; n=6). Overexpression of ZnT8 also caused a significant reduction in glucose-stimulated insulin secretion in isolated islets (0.40±0.05 ng/mL *vs* 0.94±0.21 ng/mL, $Rip7rTTa^{+/-}::ZnT8Tg^{+/-}$ *vs* $Rip7rTTa^{+/-}::ZnT8^{-/-}$ respectively; p<0.05; n=10-13).
Conclusion: Using in vivo models to manipulate gene expression we show that down- or up-regulation of ZnT8 in the beta cell respectively worsens and improves glucose tolerance in the mouse. These studies support the view that ZnT8 activation may be therapeutically useful in T2D treatment.
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A pre-obese state accelerates beta cell dysfunction in patients with type 2 diabetes: a glucagon stimulation test-based cross-sectional study

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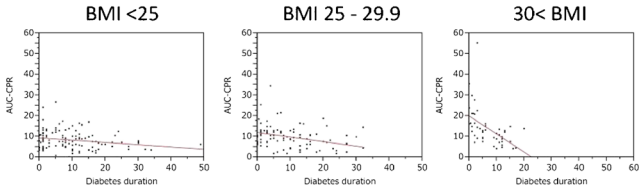
Background and aims: Type 2 diabetes (T2D) is a progressive disease in which β-cell function declines. The loss of β-cell function results in poor glucose control in T2D patients, and it accelerates the progression of diabetic complications. Some preclinical studies have shown links between obesity and β-cell dysfunction. Therefore, we aimed to investigate the relationship between obesity and the progress of β-cell dysfunction in T2D patients.
Materials and methods: We conducted a cross-sectional study on T2D patients whose β-cell function were assessed using 1-mg intravenous glucagon stimulation test (GST) from June 2010 through March 2015. Their BMI and known diabetes duration were also collected. After reviewing their medical records, the onset of diabetes was primarily defined as the time of a new T2D diagnosis after a 75-g oral glucose tolerance test (75 g-OGTT) was performed. In cases with missing 75 g-OGTT data, we defined the onset as the first finding of an HbA1c level ≥48 mmol/mol. The β-cell function was assessed using the area under the curve for serum C-peptide immunoreactivity (AUC-CPR) during the GST. Patients were stratified into the following groups: non-obese (BMI, <25 kg/m^2), pre-obese (BMI, 25-29.9 kg/m^2), and obese (BMI, ≥30 kg/m^2). Univariate regression analysis was performed to determine the relationship between the AUC-CPR and diabetes duration for each group. The primary endpoint was to assess the effect of obesity on the annual declining rate of the AUC-CPR.
Results: After screening, 256 consecutive T2D patients were included in our analysis. Of those, 120 patients (47%) were non-obese, 88 (34%) were pre-obese, and 48 (19%) were obese. The obese group showed the highest AUC-CPR levels (non-obese: 8.25±4.48 ng/mL•min, pre-obese: 9.81±5.48 ng/mL•min, obese: 13.83±9.11 ng/mL•min; p <0.001). Each group had a negative regression between the AUC-CPR and diabetes duration (non-obese: β=-0.11, 95% confidence interval [CI]: -0.20 to -0.02, p=0.02; pre-obese: β=-0.22, 95% CI: -0.36 to -0.02, p=0.002; obese: β=-0.87, 95% CI: -1.30 to -0.44, p <0.001). However, as shown in figure 1, the annual declining rate of the AUC-CPR was significantly accelerated in the pre-obese group (-1.84% per year, p=0.02 vs. non-obese group) and the obese group (-4.37% per year, p <0.001 vs. non-obese group) compared to the non-obese group (-1.17% per year).
Conclusion: In this cross-sectional study, negative regression was confirmed between β-cell function and diabetes duration according to the AUC-CPR. The annual declining rate of β-cell function was already accelerated in the pre-obese state prior to reaching the obese state. These findings suggest that antidiabetic therapy aimed at body weight reduction should be chosen from the pre-obese state in order to preserve β-cell function in T2D patients.

Figure 1. Univariate regression analysis between AUC-CPR and diabetes duration



BMI	<25	25-29.9	30<	p value
β	-0.11	-0.22	-0.87	<0.001
R^2	0.04	0.11	0.25	
95% CI	-0.20 to -0.02	-0.36 to -0.08	-1.30 to -0.44	
AUC-CPR % decline / year	1.17	1.84	4.37	
p value	0.02	0.002	<0.001	

P values were determined by ANOVA or ANCOVA.

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Deletion of PKCε in islet-associated macrophages improves beta cell function

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Background and aims: Islet inflammation is an increasingly accepted contributing factor to β-cell dysfunction in the context of Type 2 Diabetes. This is thought to involve the actions of macrophages which are increased in islets of animals fed a high-fat diet (HFD), as well as multiple models of diabetes. Whole body deletion of the serine/threonine kinase PKCε in HFD mice improves β-cell function in vivo. PKCε is important in toll-like receptor signalling, and is involved in macrophage function and activation. We hypothesise that PKCε is an important regulator of macrophage function in islet-associated macrophages and is a target to alleviate macrophage-mediated β-cell dysfunction in HFD mice.
Materials and methods: To study the specific actions of PKCε we developed a floxed PKCε mouse (PKCεf). This was crossed with Pdx-cre or Rip-cre mice for β-cell specific deletion and LysMcre mice for macrophage specific deletion . Control mice were the corresponding cre alone, or PKCεf mice. Bone marrow chimeras were generated by irradiation of congenic donor mice, which then received bone marrow cells from PKCε WT or null mice.
For all models mice were fed normal chow or HFD for varying times and glucose tolerance tests (GTTs) and insulin tolerance tests (ITTs) were performed. Insulin and c-peptide excursions were also monitored during GTTs, using ELISA. Circulating cytokines, islet gene expression, and islet morphology were characterised in LysMcrePKCεf mice. Additionally, primary peritoneal and bone marrow-derived macrophages from PKCε WT and null mice were isolated to characterise the role of PKCε in macrophage function and activation in vitro.
Results: β-cell specific PKCε deletion did not improve glucose tolerance or β-cell function in HFD mice compared to controls. Bone marrow chimera mice reconstituted with PKCε null bone marrow exhibited improved glucose tolerance ($p<0.05$, two-way ANOVA), and insulin and c-peptide excursions ($p<0.05$, two-way ANOVA) during the GTT at 20 weeks of high fat feeding, but not before. At 12 weeks of high fat feeding LysMcrePKCεf mice exhibited significantly improved glucose tolerance ($p<0.05$, two-way ANOVA), an elevated insulin profile ($p<0.05$, two-way ANOVA) and significantly improved c-peptide response ($p<0.05$, t-test) during the GTT. This effect was not seen in chow-fed mice. Islets from HFD fed LysMcrePKCεf mice showed improved glucose-stimulated insulin secretion ex vivo ($p<0.05$, one-way ANOVA). Insulin resistance as measured by an ITT was not altered, and no gross changes in circulating cytokines or inflammatory gene expression were observed. β-cell area was not changed in LysMcrePKCεf mice. In vitro stimulation of primary PKCε null macrophages with common activators (e.g. LPS, IFNγ, palmitate) showed no obvious alteration in macrophage activation as measured by production of nitric oxide and release of IL-1β, IL-1α, TNFα, IL-6, MCP-1, IL-12, IL-23, IL-10 and PGE2.
Conclusion: Deletion of PKCε in the haematopoietic compartment, and more specifically macrophages, improves β-cell function in HFD mice. This improvement occurs after increased macrophage accumulation and does not appear to be due to any gross alterations in canonical activation pathways. These results suggest that a subtle alteration in macrophage biology independently of currently known mediators can preserve β-cell function under a HFD stress.
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TLR4 triggered inflammation involves an interplay between macrophages, alpha and beta cells in human pancreatic islets

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Background and aims: Inflammatory signals are strong mediators of metabolic failure in fat, liver, brain, muscle as well as in pancreatic islets. Toll-like receptor-4 (TLR-4) signaling is one of the major pro-inflammatory pathway; its ligands as well as downstream products, i.e. cytokines and chemokines are increased systemically in patients with T2D as well as in at-risk individuals. TLR4 knockout mice are protected from the metabolic consequences of a high fat diet. In the present study we investigated the mechanism of TLR4 induced inflammation in isolated human islets.
Materials and methods: Isolated human islets from healthy donors were plated onto ECM-coated dishes and exposed to the natural TLR4 ligand Lipopolysaccharide (LPS) for 3 days. Depletion of islet resident macrophages was achieved by treatment with clodronate liposome. Expression and secretion of pro-inflammatory cytokines/chemokines was evaluated by qRT-PCR and ELISA. Glucose-stimulated insulin secretion(GSIS) from islet supernatants and insulin content from human islets were examined by insulin ELISA. Beta cell apoptosis and IL-8 localization were determined by double labeling for the TUNEL assay or by anti-IL-8 and anti-insulin or anti-glucagon antibodies. Human monocytes were enriched from human PBMCs and used for the migration assay through a Boyden chamber.
Results: Treatment of isolated human islets with LPS reduced GSIS by 71.6% and insulin content by 76.6% and increased beta cell apoptosis by 2.5-fold, compared to untreated control. In parallel, mRNA levels of pro-inflammatory cytokines/chemokines, including IL-1alpha, IL-1beta, IL-6, TNFalpha, CCL2 and IL-8, were elevated by LPS. Depletion of resident macrophages in human islets by clodronate showed that LPS-induced IL-1beta and IL-1alpha expression was macrophage dependent, while IL-6, TNFalpha, CCL2 and IL-8 expression remained unchanged, suggesting the endocrine cells themselves were the major source of these cyto-/chemokines. Immunohistochemical staining showed intra-islet production of IL-8 from alpha cells. In contrast, human macrophages differentiated from human primary monocytes produced high levels of IL-8 as well as IL-1beta in response to LPS. Migration of primary human monocytes was induced by supernatants from LPS-treated human islets; this was highly dependent on IL-8, since an IL-8 neutralizing antibody completely blocked the migration.
Conclusion: Our results show that TLR4 activation leads to beta cell dysfunction, beta cell apoptosis and islet inflammation.This depends on the interplay between islet-associated resident macrophages, alpha cells and beta cells, while recruited islet macrophages may exacerbate inflammation and amplify islet damage.
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An emerging role for autophagy in the regulation of beta cell survival by glucagon-like peptide-1

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Background and aims: Loss of pancreatic beta- cell mass is central to the development of Type 2 diabetes. Autophagy is a self-degradative process, important for recycling cell components in response to stress and, under most conditions, is considered a cell survival mechanism. However, deregulation of autophagic flux can lead to cell death. Whether beta-cell autophagy has a protective or detrimental role in the development of T2D is contentious. Glucagon-like peptide-1 (GLP-1) improves glycaemic control through multiple actions including preventing beta cell loss, although the mechanisms are not well understood. The aim of the current study was to determine whether the protective effect of the GLP-1 analog exendin-4 on glucolipotoxic-induced cell death is due to alterations in autophagic flux.
Materials and methods: INS-1E cells were treated with glucolipotoxic stress (25 mM glucose, 0.5 mM palmitate) in the absence or presence of 100 nM exendin-4 and/or 10 μM BAPTA, 10 μM ESI-09 for 6-16 h. Immunohistochemical staining of p62 both in fixed INS-1E and pancreas sections from control and T2DM donors was used to assess autophagy impairment. Autophagic flux was assessed by LC3-I to LC3-II conversion and by using a mCherry-GFP-LC3 construct.
Results: Treatment of INS-1E cells with glucolipotoxic stress increased cell death as evident by increased PI staining (3.7-fold±0.4 n=3, $p<0.001$) and also increased LC3-I to LC3-II conversion (2.5-fold±0.1 n=3, $p<0.01$), a distinct marker of autophagy. Glucolipotoxicity increased p62 puncta accumulation (2.0-fold±0.2 n=6, $p<0.01$), which indicates defective autophagy. In accordance, further studies using mCherry-GFP-LC3 showed that treatment of INS-1E with glucolipotoxic stress leaded to accumulation of autophagosomes and impaired autophagic flux. An increase in p62 puncta accumulation was also found in pancreatic beta cells from Type 2 diabetic donors, indicating that autophagy may also be impaired in human diabetes. Co-treatment with exendin-4 protected INS-1E cells from glucolipotoxic induced cell death (37.3% decrease n=3, $p<0.01$). Exendin-4 further exacerbated LC3-I to LC3-II conversion (4.1-fold±0.2 n=3, $p<0.01$), while reducing p62 puncta accumulation (17.9% decrease n=6, $p<0.05$) suggesting further stimulation and, at least partial, restoration of autophagic flux. The Ca^{2+} chelating agent BAPTA and ESI-09, an Epac inhibitor, suppressed the increase in LC3-I to LC3-II conversion driven by exendin-4 (59.0% decrease n=3, $p<0.05$ and 64.7% decrease n=4, $p<0.05$, respectively), suggesting a role for calcium in the exendin-4 regulation of autophagy.
Conclusion: Glucolipotoxicity causes impairment in autophagic flux in beta cells, resulting in accumulation of autophagosomes inside the cells. Co-treatment with exendin-4 causes partial restoration in autophagosome clearance through an EPAC/calcium-dependent mechanism.
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Modulation of beta cell function by the atrial natriuretic peptide

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Background and aims: Recent studies suggest that the cardiac hormone ANP (atrial natriuretic peptide) is an important regulator of metabolism. It may provide a link between cardiovascular and metabolic dysregulation in obesity and (pre)diabetes. The aim of the present study was to investigate the effect of ANP on beta-cell stimulus-secretion coupling.
Materials and methods: Ion currents and the membrane potential (V_m) of mouse beta-cells were measured with the patch-clamp technique. Mitochondrial membrane potential ($\Delta\Psi$) was determined with Rh-123 fluorescence. Protein expression in pancreatic islet lysates was assessed by Western blotting. For insulin secretion measurements, batches of 5 islets were incubated for 60 minutes at 37°C with the indicated substances. Insulin was determined by radioimmunoassay.
Results: ANP (10 nM) decreased the open probability of the K_{ATP} channel in beta-cells of GC-$A^{flox/flox}$ mice to 29±11% (n=6, $p\leq0.01$). This effect was absent in mice with specific deletion of the GC-A receptor in beta-cells (Rip-$Cre^{+/-}$ GC-$A^{flox/flox}$) (119±15%, n=6, n.s.). A membrane-permeable analog of the second messenger cGMP, 8-Br-cGMP (250 μM), mimicked the effect of ANP on the K_{ATP} channel in beta-cells of GC-$A^{flox/flox}$ mice (reduction to 72±8% open probability vs.

100% control, n=7, p≤0.05), indicating that ANP acts in a cGMP-dependent manner. Determination of specific current density (GC-$A^{flox/flox}$: 21±2 pA/pF, n=33 vs. Rip-$Cre^{+/-}$ GC-$A^{flox/flox}$: 23±1 pA/pF, n=34, n.s.) as well as Western blotting (n=6, n.s.) revealed no significant difference in the number of K_{ATP} channels or protein expression in beta-cells of Rip-$Cre^{+/-}$ GC-$A^{flox/flox}$ mice and their wild type littermates. ANP (10 nM) did not influence mitochondrial membrane potential (15 mM glucose: 1232±35 a.u. vs. +ANP: 1240±32 a.u., n=60, n.s.), indicating that it does not affect ATP production. In accordance with closure of K_{ATP} channels, ANP increased glucose-induced electrical activity measured as fraction of plateau phase (FOPP=percentage of time with spike activity) (ANP: 65±5% vs. glucose 10 mM: 47±5%, n=6, p≤0.05). Insulin secretion was increased by ANP at a subthreshold glucose concentration (6 mM glucose) in islets of GC-$A^{flox/flox}$ mice (ANP: 0.33±0.07 ng insulin/(islet*h) vs. 6 mM glucose: 0.24±0.06 ng insulin/(islet*h), n=4, p≤0.05), whereas the effect was absent in islets of Rip-$Cre^{+/-}$ GC-$A^{flox/flox}$ mice (ANP: 0.23±0.06 ng insulin/(islet*h) vs. 6 mM glucose: 0.24±0.04 ng insulin/(islet*h), n=4, n.s.).

Conclusion: These results provide evidence that ANP exerts cGMP-dependent specific actions in the endocrine pancreas via activation of the GC-A receptor and that the hormone is involved in the regulation of beta-cell function.

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Ghrelin has a direct inhibitory effect on insulin secretion in human islets from both healthy subjects and type 2 diabetic patients

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Background and aims: Ghrelin is an insulinostatic hormone expressed in the gastro-intestinal tract and in islet ghrelin cells. In rodents most of the circulating ghrelin originates from the fundus but in humans a larger portion originates from the islets and intestine, demonstrating the necessity of experimental data from humans in addition to other experimental models. The effect of ghrelin is well documented in several experimental models, as well as *in vivo* in humans. However, the direct effect of ghrelin on human islets is not well known. Although IVGTTs have shown decreased insulin-, as well as C-peptide release after ghrelin infusion it has hitherto not been clarified if this is a primary response. Thus, our aim was to perform *in vitro* secretion assays to study the direct effect of ghrelin on human islet insulin secretion in islets from normoglycemic and type 2 diabetes (T2D) donors. Furthermore it is not known how islet expression of ghrelin and its receptor GHS-R1a are affected by T2D. We aimed to investigate ghrelin and GHS-R1a expression levels to establish the effects of prolonged hyperglycemia on ghrelin signaling. This was to be done using microarrays on islets from healthy subjects and T2D patients.

Materials and methods: Human islets from the Nordic Network for Clinical Islet Transplantation, delivered through the Human Tissue Lab (LUDC), were considered being from normoglycemic (HbA_{1c}<6.0) or T2D (diagnosed or HbA_{1c}>6.0) donors. The islets were incubated in 2.8 or 16.7 mM glucose for 1 hour with or without different concentrations of ghrelin. Insulin secretion was determined by ELISA (Mercodia). Micro-array data was collected from 128 donors using the Affymetrix GeneChip Human Gene 1.0 ST array.

Results: 1 hour incubation of human islets from T2D donors (HbA_{1c}>6.0 with 1, 10 and 100 nM ghrelin at 16.7 mM glucose dose-dependently decreased insulin secretion with a 31.2±15.7% reduction at 10 nM (p=0.042 for n=3). At 2.8 mM glucose ghrelin did not effect insulin secretion. In islets from normoglycemic donors the same pattern was seen with a dose dependent decrease in insulin secretion at 16.7 mM glucose with 31.5±7.27% at 100 nM (p=0.022 for n=6). Thus indicating that the *in vivo* effect of ghrelin on insulin secretion is predominantly due to a direct effect on the beta-cells. The micro-array data from human islets revealed that islet ghrelin expression was negatively correlated with HbA_{1c} (p=0.007 for n=128) and that ghrelin expression was lower in T2D islets (p=0.03). Islet ghrelin also correlated positively with insulin, glucagon and somatostatin expression ($p=1*10^{-10}$, p=0.001 and $p=2*10^{-5}$ respectively for n=128. The ghrelin receptor (GHS-R) expression did not correlate significantly with known diabetes risk-factors such as BMI, insulin secretion index or HbA_{1c}. Nevertheless, GHS-R correlated with PDX-1 expression ($p=3*10^{-13}$ for n=128) and somatostatin ($p=1.6*10^{-16}$ for n=128) indicating a previously unknown connection to both beta-cell transcription and delta-cell function.

Conclusion: We conclude that: 1) ghrelin directly inhibits insulin secretion in islets from both healthy subjects and T2D patients. 2) ghrelin expression levels correlate negatively with both HbA_{1c} and diagnosed T2D and positively with insulin, glucagon and somatostatin. 3) the ghrelin receptor also correlates positively with somatostatin. These findings suggest that inhibition of ghrelin signaling may be a therapeutic avenue for treatment of T2D.

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The role of Epac2 in bile acid-mediated modulation of insulin secretion

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Background and aims: We have shown recently that bile acids enhance glucose-stimulated insulin secretion via activation of the farnesoid X receptor (FXR). FXR activation leads to closure of K_{ATP} channels, however, the underlying mechanism is still unknown. The aim of the present study was to test whether Epac2 (exchange protein directly activated by cAMP 2) plays a role in the bile acid-mediated regulation of glucose homeostasis via FXR.

Materials and methods: Experiments were performed with beta-cells from wild-type (WT) and Epac2-knockout (KO) mice. Ion currents were measured with the patch-clamp technique; the cytosolic Ca^{2+} concentration $[Ca^{2+}]_c$ was determined by fura-2.

Results: Chenodeoxycholate (CDC, 500 nM) and the synthetic FXR agonist GW4064 (500 nM) enhanced $[Ca^{2+}]_c$ in beta-cells of WT mice to 130±12%, n=12, p≤0.05 and 119±5%, n=18, p≤0.01, respectively. Interestingly, both substances did not alter $[Ca^{2+}]_c$ in beta-cells of Epac2-KO mice (CDC: n=41, GW4064: n=22). The Epac-selective cAMP analog Sp8 (1 μM) evoked an elevation of $[Ca^{2+}]_c$ in WT beta-cells (122±4%, n=22, p≤0.001) but not in Epac2-KO beta-cells (n=17) indicating that Epac activation augments $[Ca^{2+}]_c$ in beta-cells. We further tested whether the K_{ATP} channel density is altered in beta-cells of Epac2-KO mice. Whole-cell K_{ATP} current measurements were performed without ATP in the pipette solution to induce maximum K_{ATP} current. The experiments revealed a significant decrease in K_{ATP} channel density in beta-cells lacking Epac2 (WT: 26±2 pA/pF, n=15 vs. Epac2-KO: 18±2 pA/pF, n=22, p≤0.05). To investigate whether the ATP sensitivity of the K_{ATP} channel is modified by FXR activation, we tested different ATP concentrations in the pipette solution in the absence and presence of GW4064. The FXR agonist increased ATP sensitivity of the channels in WT beta-cells by about 40% (14±1 pA/pF n=7 vs. 8±1 pA/pF, n=6, p≤0.01 for 1 mM ATP and 11±1 pA/pF, n=9, vs. 6±1 pA/pF, n=9, p≤0.01 for 2 mM ATP with and without GW4064, respectively). The FXR agonist did not affect ATP sensitivity of K_{ATP} channels in Epac2-KO beta-cells (n=5-8).

Conclusion: The experiments provide evidence for a link between bile acid signaling and the activation of Epac2 which seems to increase K_{ATP} channel sensitivity towards ATP. It is suggested that FXR stimulation activates Epac2 which in turn decreases K_{ATP} channel activity.

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Decorin: A new positive component of skeletal muscle to beta cell communication?

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Background and aims: Inter-organ crosstalk including possible communication between skeletal muscle and beta cells is an important component of normal physiology that may contribute towards the pathophysiology of type 2 diabetes. Indeed, skeletal muscle cells secrete different sets of soluble proteins (myokines) which could act positively or negatively (in health and disease respectively) on the function of distant tissues including pancreatic islets. We have observed that conditioned medium from skeletal muscle of healthy individuals has positive effects on beta cell function. Decorin, a chemokine that has been shown to be a component of the extracellular matrix of a variety of connective tissues, was identified as a myokine regulated by muscle fiber type. The major aim of this study was to explore the impact of decorin on primary sorted beta cells under control conditions and after exposure to TNF-α or cytomix.
Materials and methods: All studies were performed using sorted rat primary beta cells exposed to decorin for 24 h. Glucose-stimulated insulin secretion (GSIS) was measured by radioimmunoassay after 1 h at 2.8 mmol/l (basal) and 1 h at 16.7 mmol/l (stimulated) glucose. Confocal immunofluorescence microscopy was used to study focal adhesion and actin remodeling (after 15 min glucose stimulation). Cell death and proliferation were measured by TUNEL assay and BrdU incorporation respectively. To study the potential beneficial/protective effects of decorin, beta cells were exposed to decorin for 24 h alone or with addition of cytomix (20 ng/ml each TNFα, IFNγ, IL1-β) or 20 ng/ml TNF-α alone. Data are mean±SE for 5-8 independent experiments.
Results: Decorin treatment of rat primary beta cells for 24 h increased GSIS (control: 12.4±3.2% content/h; 1 μg/ml decorin: 15.08±3.8%*; 10 μg/ml decorin: 14.1±1.8%*; *p<0.05 vs. control) and decreased cell death (TUNEL-positive beta cells control: 0.7±0.2%; 1 μg/ml decorin: 0.38±0.1%*; 10 μg/ml decorin: 0.4±0.1%*; *p<0.05 vs. control). Decorin had no effect on beta cell proliferation. Confocal microscopy showed a modification of glucose-induced formation of focal adhesions containing paxillin and actin after 24 h decorin (1 μg/ml). Decorin (100 μg/ml) induced a slight increase of paxillin phosphorylation and presented a denser actin network in both basal and stimulated conditions depending on the decorin concentration (1 μg/ml-100 μg/ml). Decorin protected against the adverse effects of TNF-α on GSIS (control: 10.20± 0.8% content/h; TNF-α: 8.6±0.8%*; TNF-α + 1 μg/ml decorin: 11.03± 0.9%**; *p<0.05 vs. control; **p<0.05 vs. TNF-α alone) and blocked the effects of cytomix on apoptosis (control: 0.75±0.1% TUNEL-positive beta cells; cytomix: 5.9±0.12%; cytomix + 1 μg/ml decorin: 0.95± 0.35%).
Conclusion: We demonstrate for the first time that decorin has a positive effect on beta cells, increasing glucose-stimulated insulin secretion and decreasing cell death in the basal state. The myokine affects also beta cell focal adhesion and actin remodeling. Moreover, decorin protects beta cells from the adverse effects of TNF-α and cytomix, indicating the potential for a specific impact of decorin on cytokine signaling pathways in beta cells.
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Impact on beta cells of myokines secreted by skeletal muscle of differing insulin sensitivity and fiber type

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Background and aims: Decreased beta-cell functional mass and insulin resistance are hallmarks of type 2 diabetes (T2D). We have studied crosstalk between skeletal muscle of different insulin sensitivity and beta-cells, and suggested that myokines secreted by insulin-resistant skeletal muscle could contribute towards decreased beta-cell function/mass in T2D. In obese and T2D individuals, the distribution of muscle fiber types is shifted toward faster, more glycolytic fibers. We have now explored the impact on beta-cells of secreted products from human myotubes of different fiber type with varying insulin sensitivity.
Materials and methods: Human skeletal muscle biopsies of different fiber type where collected from soleus (S; slow twitch fiber) and triceps (T; fast twitch fiber); cells were expanded in culture and differentiated into myotubes. mRNA expression were measured by unbiased RNA sequencing on biopsies and corresponding cell preparations. Human myotubes where treated for 24 h with or without (control) 20 ng/ml TNF-α to induce insulin resistance. Glucose uptake was measured in response to insulin. Conditioned media (CM) from the fiber types (test: TNF-α-CM-S or T; control: C-CM-S or T) were collected and analyzed to identify their myokine composition by protein arrays. Sorted rat and human primary beta-cells were used to test effects of exposure to CM for 24 h on death (TUNEL), proliferation (BrdU) and glucose-stimulated insulin secretion (GSIS). Data are mean+/-SE with p<0.05 considered significant for all indicated differences.
Results: Biopsies from soleus and triceps revealed a unique gene signature with 2497 genes (S vs.T; p<0.01) differentially expressed in these 2 groups, while primary cells derived from soleus and triceps showed 1682 differentially expressed genes (S vs.T; p<0.01). Moreover, cells isolated from triceps and soleus secreted a different panel of myokines in control condition and after treatment with TNF-α allowing for identification of fiber specific, TNF-α and fiber/TNF-α dependent myokines. Interestingly, TNF-α failed to induce insulin resistance in cells isolated from triceps. CM-S and -T increased whereas TNF-α-CM-S but not -T decreased GSIS. Beta-cells treated with TNF-α-CM-S showed increased death (11+/-2-fold increased TUNEL-positive cells) and decreased proliferation (1.1+/-0.4 vs. 7.3+/-1.7% BrdU positive cells vs. C-CM-S) whereas TNF-α-CM-T treatment had no impact on these parameters vs. C-CM-T treatment. Finally, CM from either fiber type without prior exposure to TNF-α increased beta-cell proliferation (Control: 4.7+/-0.3; CM-S: 7.3+/-0.6*; CM-T: 8.6+/-1.1* % BrdU positive cells; *p<0.05 vs. Control).
Conclusion: Taken together these results show that skeletal muscle cells from biopsies with different fiber composition present unique gene expression and myokine signatures. Moreover the impact on pancreatic beta-cells of human skeletal muscle cells is fiber specific, with both positive and negative effects depending on different insulin sensitivity. The identification of the fiber specific myokines and their molecular targets on beta-cells may lead to new therapeutic strategies for preservation of functional beta-cell mass in T2D.
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PS 025 Regulating islet gene expression

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Identification of novel targets of the miR-212/132 cluster involved in Wnt-signalling and regulation of insulin secretion in the pancreatic beta cell

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Background and aims: We have recently shown the miR-212/132 cluster to be highly overexpressed in diabetic Goto-Kakizaki (GK) rat islets. In the present study we aimed to validate targets of miR-212 and miR-132 and investigate their effects on insulin secretion.
Materials and methods: Conservation of microRNA 3'UTR target predictions was evaluated using TargetScan. Rat miR-212 and miR-132 were overexpressed using mirVana Mimics in INS-1 832/12 clonal beta-cells by transfection and analysed with RT-qPCR and Western Blot.
Results: In silico analysis of 249 predicted miR-212 and miR-132 target genes conserved in human, rat and mouse revealed several genes of interest to the study of type-2 diabetes mellitus (T2DM). Among these were MAPT and two transcription factors (TFs) targets of Wnt-signalling, ISL1 and SOX6. ISL1 binds to the enhancer region of the insulin gene and is a primary target of TCF7L2; SOX6 is known to function in transcriptional regulation through interaction with beta-catenin and is down-regulated in human islets from donors with high HbA1c; and MAPT, which is understood to be hyper-phosphorylated in neurofibrilar tangles in insulin deficient mice, to a large extent by glycogen synthase kinase 3 beta (GSK-3beta), a central kinase that is inactivated by canonical Wnt-signalling. Moreover, clustering of miR-212/miR132 predicted targets shows enrichment within the Wnt-signalling pathway. We have confirmed down-regulation of MAPT (p=0.0118, N=3), ISL1 (p=0.0057, N=3) and SOX6 (p=0.0287, N=3) at the mRNA level in the insulin secreting cell line INS-1 832/13 when miR-212 are overexpressed. Likewise, after overexpression of miR-132 mRNA expression of SOX6 (p=0.0008, N=3), ISL1 (p=0.0009, N=3) and MAPT (p=0.0001, N=3) was reduced. We have also obtained preliminary Western blot data showing down regulation of MAPT at the protein level.
Conclusion: These results give evidence that MAPT, SOX6 and ISL1, all three genes relevant to type-2 diabetes, are likely to be targets of miR-212 and miR-132. In addition, these results indicate that these microRNAs are involved in the translational regulation of genes affected by canonical Wnt-signalling. We are currently aiming to investigate the effect of these targets on insulin secretion and to verify additional targets of miR-212/132 involved or affected by Wnt-signalling by using an Argonaute-2/RIP (Ago2-RIP) approach.
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Circulating levels of microRNAs predict residual beta cell function and glycaemic control the first 12 months after diagnosis in children with type 1 diabetes mellitus

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Background and aims: MicroRNAs have gained intensive research focus in order to explain pathophysiological processes leading to many complex disorders including autoimmune diseases. Type 1 diabetes mellitus is the final result of T-cell driven beta-cell loss leading to lifelong insulin treatment and regular control of metabolic status. In this study it is hypothesized that miRNA expression profile over time predicts residual beta-cell function and metabolic control in newly diagnosed children with type 1 diabetes mellitus.
Materials and methods: Children and adolescents with newly diagnosed type 1 diabetes mellitus from The Danish Remission Cohort (N=129) were intensively followed with blood samples the first 12 months after diagnosis. A subset of 40 children from this cohort were followed up 5 years after diagnosis. In this subgroup RNA was extracted from serum samples obtained at 1, 3, 6, and 12 months. At the same time points meal stimulated C-peptide and HbA1c were measured and IDAA1c calculated. miRNA profiling was performed using a predefined panel of 179 human miRNAs. Normalization is based on the global mean of all expressed miRNAs. Statistical analysis of miRNA prediction on disease progression with stimulated C-peptide, HbA1c and IDAA1c as endpoint was performed by multiple linear regression analysis adjusted for age and sex. Comparisons were further adjusted for multiple testing by the Benjamini-Hochberg approach for the 172 miRNAs at all time points.
Results: miRNA profiling was performed in a total of 182 serum samples. 172 different miRNAs were identified and further analyzed. Stimulated C-peptide at 12 months was predicted by hsa-mir-197-3p at 3 months. A doubling in miRNA level corresponded to a 3.5 fold higher stimulated C-peptide level (p=0.03). In addition a doubling of hsa-mir-24-3p and hsa-mir-146a-5p levels at 3 months corresponded to a 4.2% and 3.5% decrease in IDAA1c 12 months after diagnosis, (p=0.01) (p=0.02) respectively. A doubling in hsa-mir-375 at 3 months corresponded to a 0.75 fold lower stimulated C-peptide level at 6 months (p=0.02). Furthermore a doubling of hsa-mir-301a-3p at 3 months corresponded to a 1.1% decrease in HbA1c at 6 months (p=0.02).
Conclusion: A combination of circulating novel miRNAs expressed in sera from children newly diagnosed with type 1 diabetes mellitus can predict beta-cell function and metabolic control 12 months after disease onset. miRNAs could potentially be used as predictive biomarkers in attempt to individualize the beta-cell regenerative treatment at a crucial timepoint in this group of patients.

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Modulation of long non-coding RNAs in islets of high fat diet induced obesity mice

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Background and aims: The genome transcribes a great number of long non-coding RNAs (lncRNAs). Despite being involved in diverse gene-regulatory mechanisms and their dysregulation being implicated in many diseases, only few lncRNAs have been functionally characterized and little is known about their contribution to type 2 diabetes. Type 2 diabetes is characterised by a combination of beta-cell failure and insulin resistance. The mechanisms leading to beta-cell dysfunction are complex and still largely unknown. The aim of this project is to identify novel lncRNAs, study their deregulation in the islets of mice fed a high fat diet and to investigate their role in the regulation of beta-cell functions.
Materials and methods: Islets were isolated from 13 weeks old C57Bl/6 mice after being fed a standard (SD) or a high fat (HFD) diet for 8 weeks. 100 nucleotide pair end sequencing was performed on cDNA libraries prepared from rRNA-depleted islet RNA using the Illumina platform. Reads were mapped with TopHat software version 2.0.8 to UCSC mm9 mouse genome. *Ab initio* transcript reconstruction was performed using Cufflinks version 2.1.1 and novel lncRNAs transcripts were classified from known lncRNAs and protein-coding mRNAs using Cuffmerge version 2.1.1. Differential lncRNAs of selected novel lncRNAs was confirmed by quantitative real-time PCR.

Results: RNA sequencing yielded around 500.000.000 reads par sample of which on average 75% were mapped to the mouse genome. We identified a total of 14874 protein-coding transcripts of which 1366 were upregulated and 396 downregulated in HFD mice. Functional annotation of the differentially expressed protein-coding genes showed a strong enrichment for endoplasmic reticulum, mitochondrion, protein biosynthesis and transport processes. We also detected 1761 previously annotated lncRNAs, of which 23 were upregulated and 104 downregulated. Finally, *de novo* annotation identified around 1805 novel lncRNAs, 39 of which were upregulated and 107 downregulated. Amongst the 1805 novel lncRNAs, only 237 overlapped with previously published transcripts. In agreement with previous studies, the expression of lncRNAs was lower than that of protein-coding genes, with the majority displaying an expression below an FPKM of 5. Amongst the differentially expressed genes, we confirmed in additional samples the upregulation and downregulation of two selected lncRNAs. XLOC_010971 was 35±21 (p=0.0205) fold higher in HFD versus SD while XLOC_013310 was 50% lower (p=0.0051) in HFD versus SD.
Conclusion: We used high throughput sequencing to study the differences in gene expression in islets of a well characterized model of diet-induced obesity and glucose intolerance. We detected many changes in protein-coding genes and previously annotated lncRNAs. Furthermore, we identified a new set of novel lncRNAs, a group of which displayed changes in expression between islets of lean and diet induced obesity mice, suggesting that lncRNAs could play an important role during the pathogenesis of type 2 diabetes. These data provide the basis for future studies on the contribution of lncRNAs to beta-cell dysfunction during the development of type 2 diabetes.
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Influence of environmental factors on the repression of "disallowed" genes in mouse islets

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Background and aims: The concept of "disallowed" genes - a special group of housekeeping genes that is expressed in all tissues except one - was introduced in the context of low Km hexokinases and monocarboxylate transporters, genes that would interfere with normal glucose-induced insulin release when expressed in the beta cell. Subsequent mRNA analysis using freshly isolated islets and a panel of tissues from the same mice indicated that a small set of "disallowed" genes is part of the phenotype of adult mouse islets as a result of repressive changes of chromatin structure and the finetuning influence of microRNA's. This phenotype is established during the neonatal period when beta cells mature. It is unknown to what extent environmental factors can reverse this epigenetic signature so that "disallowed" genes are induced in beta cells. The aim of the present study was to examine the potential influence of important environmental factors (age, diet, pregnancy) on the expression of the "disallowed" genes in mouse islets.
Materials and methods: For the ageing experiment, we compared mRNA signals in collagenase isolated islets from male C57Bl6/J mice (Janvier) of 1 month, 2 months, 6.5 months, 16.5 months and 26 months of age. We next studied mRNA from islets of male mice fed a high fat diet (45% fat) or standard diet (9% fat) for 16 weeks, starting at 6 weeks of age. Islets from female mice (age 10-12 weeks) were analysed at day 0, 9.5 and 15.5 of pregnancy. Via quantitative RT-PCR (N=4 per tested condition) we quantified islet mRNA expression signals of a signature of 14 genes considered "disallowed" in islets. Chromatin immunoprecipitation was used to test histone-3 epigenetic markers for activation (H3K9ac) and repression (H3K27me3) of gene expression.
Results: Age-dependent repression of the 14 "disallowed" genes follows two patterns with significant correlations between genes within each group (e.g. R2=0.98 between Zyx and Ldha; R2=0.96 between Pdgfra and Itih5). Expression of the largest group (Arghdib, Cat, c-Maf, Ldha, Lmo4, Smad3, Zfp36l1 and Zyx) was independent of age in animals of 1 month or older. In the second group (Itih5, Oat, Pdgfra, Cxcl12, Igfbp4 and Slc16a1) mRNA expression signals decreased significantly in function of age (e.g. 1 vs 6.5 months, Pdgfra: 0.91±0.22 vs 0.09±0.03; p=0.004; Itih5: 1.1±0.34 vs 0.2±0.02, p=0.04). For none of the 14 "disallowed" genes, the repression was weakened when comparing 2 yrs old with 1 month old mice. In line with this result, the epigenetic H3K9ac H3K27me3 marks were well preserved in function of age. High fat diet feeding for 16 weeks led to small alterations of expression of three genes: Itih5 (0 vs 16 weeks HFD; 1.43±0.32 vs 2.1±0.36, p=0.03) and Zyx (0.97±0.11 vs 1.3±0.19, p=0.02) and Ldha (1.01±0.32 vs 0.57±0.1, p=0.04). At day 15.5 of pregnancy we observed between 1.5 and 2.4 times more repression of Cxcl12 (p=0.01), Igfbp4 (p=0.003), Itih5 (p=0.01) and Oat (p=8.10-5); genes associated with the control of proliferation of cells.
Conclusion: With some minor exceptions, the phenotypic islet signature of 14 "disallowed" islet genes appears stable during ageing, after high fat diet and during pregnancy indicating that maintenance of an epigenetic repressive signature is very effective in islets against the influence of environmental factors.
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DBP and Nfil3/E4BP4 play pivotal roles in circadian regulation of beta cell function

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Background and aims: Disturbances in circadian rhythm regulation have been implicated in the development of metabolic disorders, including diabetes mellitus. The most convincing evidence has emerged from the study in mice with tissue-specific ablation of Bmal1, one of the core clock components. However, the detailed links between circadian regulators and downstream targets are poorly understood. Recently, we reported that the expression level of Dbp was decreased and E4bp4 was increased simultaneously in the islets of Wfs1-/- Ay/a mice, a model of diabetes. Dbp and E4bp4 are clock-controlled output genes. DBP activates, whereas E4BP4 reciprocally suppress downstream target genes by competing the same binding site, D-box. Here, we investigated the roles of Dbp and E4bp4 in the circadian regulation of ß-cell function.
Materials and methods: We investigated the circadian expression of Dbp and E4bp4 in pancreatic islets under normal, and restricted feeding (RF). We generated transgenic mice with beta-cell specific E4bp4 overexpression (MIP-E4BP4), and examined islet morphological changes and ß-cell function.
Results: RF caused 6-h phase advances in Bmal1 and Clock expression and a 12-h phase advance in Dbp expression and the bathyphase of E4bp4 expression. The higher sensitivity of the Dbp and E4bp4 to RF compared with Bmal1 and Clock suggests a role of the Dbp transactivity in the adjustment in pancreatic ß-cells to changes in food availability. In MIP-E4BP4 ß-cells, E4BP4 should suppress the expression of targets of the DBP. We generated 2 MIP-E4BP4 lines, TG-C and TG-D. TG-C and TG-D have 10 and 3 transgene copies, respectively. Real-time RT-PCR confirmed 150- or 50- fold up-regulation of E4bp4 in the isolated islets, with increased corresponding nuclear protein levels. Circadian expression of Bmal1 and Clock was not altered. Glucose tolerance test revealed markedly impaired glucose tolerance in both of the TG mice with severely diminished insulin secretion. Glucose excursion in TG-C was higher than

that in TG-D, suggesting gene-dose dependent effect. We then analyzed insulin secretion in perfused pancreas. TG-C had markedly reduced and delayed insulin secretion to 17 mM glucose. MIP-E4BP4 mice were indistinguishable in body weight from control littermates. We confirmed a 50% reduction in insulin content in TG-C. The ratio of beta to alpha cells was significantly decreased and the proportion of islets with alpha-cells in center was obviously increased in TG-C. Electron microscopy revealed the total number of insulin granules to be reduced in TG-C ß-cells. RNA expression of several genes involved in insulin secretion rises around the beginning of the feeding period and sustains during that period. In contrast, circadian RNA expression of these genes was diminished in TG-C islets during the same period. Increase in those RNA transcription might get beta-cells ready to rapidly release insulin preceding feeding time.

Conclusion: Findings from this genetic model support the premise that DBP/E4BP4, downstream of the core clock genes, plays essential role in the regulation of beta-cell function, and therefore occupies a pivotal position in beta-cell failure following circadian misalignment.

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Glucose-dependent expression of Alx3 in alpha cells inhibits glucagon gene expression via interactions with Pax6

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Background and aims: The regulation of glucose homeostasis to maintain normoglycaemia requires the coordinated activity of insulin and its counter regulatory hormone glucagon. Defects in insulin synthesis, secretion and/or function resulting in hyperglycaemia have been at the centre of diabetes research since its discovery. However, the notion that abnormally increased glucagon production and secretion may play an important role in the etiopathogenic mechanisms of this disease has gained recognition in recent years. Although glucagon secretion in response to fluctuations in the concentrations of glucose has been widely studied, relatively less attention has been paid to investigate how changes in glucose levels regulate glucagon gene transcription. The regulation of glucagon gene expression in alpha cells involves the binding of several transcription factors to at least four regulatory elements, named G1-G4, located in the proximal promoter. Among these transcription factors the paired-like homeoprotein Pax6 plays a prominent role as a key factor for alpha cell function. In addition, Alx3, another paired-like homeoprotein known to be important for glucose homeostasis, appears to regulate glucagon gene transcription because glucagon expression is decreased Alx3-deficient mice. In the present study, we aimed to determine the mechanism by which Alx3 regulates glucagon gene expression in alpha cells.

Materials and methods: Isolated mouse islets and alphaTC1 cells were used. Protein-DNA binding was tested by chromatin immunoprecipitation (ChIP) and electrophoretic mobility shift assays (EMSA). Quantitative RT-PCR and siRNA were used for expression studies. Student's t test was used for statistical analysis.

Results: ChIP assays and EMSA demonstrated that Alx3 binds the glucagon promoter at the G3 but not at the G1 element, whereas Pax6 binds both elements as previously reported. Immunoprecipitations from alphaTC1 cells as well as GST-pull down assays showed that Alx3 interacts and forms heterodimers with Pax6 via the homeodomain. Alx3-Pax6 heterodimers bind G3, although Alx3 alone is unable to bind this site. In contrast, Alx3-Pax6 heterodimers cannot bind G1, so that Alx3 inhibits Pax6 binding to this site. Consistent with this, overexpression of Alx3 in transfected alphaTC1 cells resulted in decreased glucagon promoter activity and inhibition of Pax6-dependent transactivation. Expression of Alx3 in mouse islets and alphaTC1 cells increased after raising the concentration of glucose. This was accompanied by decreased binding of Pax6 to G1. In cultured islets from wild type mice, glucagon expression was lower in the presence of 16 mM glucose than in the presence of 2.8 mM glucose. In contrast, changes in glucose concentration had no effect on glucagon expression in islets from Alx3-deficient mice. In alphaTC1 cells in which Alx3 expression was silenced by siRNA, expression of glucagon in the presence of 2.8 mM glucose was lower than in controls, but in the presence of 16 mM glucose the effect was reversed so that glucagon mRNA levels were higher than in controls, reflecting availability of Alx3-free Pax6 for binding to G1.

Conclusion: Our data indicate that glucose stimulates the expression of Alx3 in alpha cells, resulting in glucose-dependent inhibition of glucagon gene expression via Alx3-Pax6 interactions on the G1 element. Thus, Alx3 contributes as a key component to the mechanisms that regulate glucose-dependent glucagon expression in pancreatic islets.

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Regulation of glucose-stimulated TXNIP expression by histone acetyltransferase (HAT) p300 and histone deacetylases (HDACs) in pancreatic islet cell

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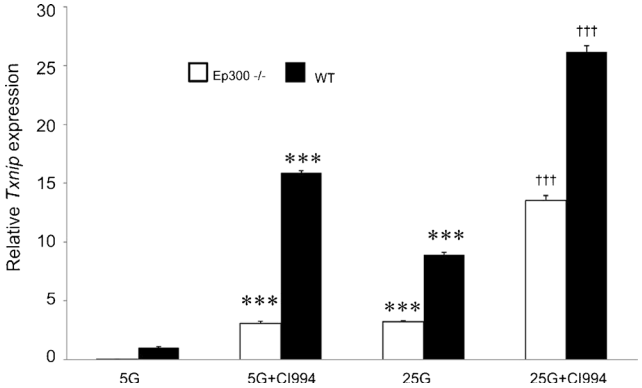
Background and aims: Uncontrolled hyperglycemia has been considered a main reason for beta-cell deterioration over time and referred to as glucotoxicity. It has been suggested that glucose through epigenetic mechanisms, i.e. effect on histone marks, influences gene expression. Here we have used the TXNIP gene as a candidate to study such mechanisms as it is known to be upregulated by glucose in islets where it can induce beta cell apoptosis. We tested whether glucose induced TXNIP expression by stimulating activating histone mark but also if these effects of glucose on TXNIP expression could be prevented by a HAT inhibitor or by mutating the HAT by CRISPR.

Materials and methods: We applied qPCR to determine Txnip mRNA expression and chromatin immunoprecipitation (ChIP) to study acetylation at histone 3 lysine 9 (H3K9ac). We also used CRISPR with sequence-specific gRNAs to mutate HAT p300 in an insulin-producing rat beta cell line INS1 832/13 cells as an attempt to prevent the effect of glucose on histone acetylation.

Results: Treatment of INS1 832/13 cells with high glucose (25 mM) for 24 hours resulted in a 30-fold increase in Txnip mRNA expression, and accordingly a 20% increase of H3K9ac at the 5'-coding region of Txnip. We then tried to prevent the effect of glucose on Txnip expression by using the HAT p300 inhibitor C646 to reduce acetylation. Txnip expression was significantly inhibited by C646 at both 5 mM (by ~40%) and 25 mM glucose (by ~75%). The involvement of HAT p300 in Txnip expression was further confirmed by ChIP-qPCR using primers flanking the 5'-coding region of Txnip and showed a 70% down-regulation of H3K9ac by C646 at 5 mM glucose. However, no reduction in H3K9ac by C646 was detected at 25 mM glucose. This suggests that high glucose may also elevate H3K9ac by inhibiting HDACs. To confirm this hypothesis, we mutated p300 in INS1 832/13 cells by creating an indel deletion at exon1 of the p300 gene (Ep300) using CRISPR/Cas9. Compared to the wild-type INS1 cells, the Ep300 knockout cells showed almost abolished Txnip expression (96% reduction) at 5 mM glucose (see Fig), which correlated with a 70% reduced acetylation at H3K9ac Txnip, indicating direct involvement of p300 in glucose-induced Txnip expression and histone acetylation. Furthermore, in these cells Txnip expression was partially recovered by the HDAC inhibitor CI994 or high glucose with elevated H3K9ac to the same levels as in wildtype cells. This suggests that in the absence of p300, glucose-stimulated H3K9ac and thereby Txnip expression can also be achieved by inhibition of HDACs.

Conclusion: We demonstrate that glucose can stimulate Txnip expression in beta-cells by stimulating an activating histone mark, H3K9ac, involves

activity of both HAT p300 and HDAC. This study thus points at the potential of reversing adverse effects of glucose on gene expression by treatments that can modify these histone effects.



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Beta cell serotonin during pregnancy: Fact or artifact?

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Background and aims: In pregnant mice, placental lactogen induces Tph1, the rate-limiting enzyme of serotonin biosynthesis, in a subpopulation of pancreatic beta cells. This physiological process is recapitulated in non-pregnant mice by the artificial expression of the human growth hormone (hGH) minigene in many commonly used transgenic driver lines for islet and diabetes research. hGH has lactogenic effects in the mouse, by binding to prolactin receptors on beta cells. The physiological process during pregnancy and the common artifact in genetically modified mouse models raises the question what the function is of beta cell serotonin. It was suggested (but not confirmed) that serotonin acts locally on beta cell proliferation and insulin release. In order to find an answer, we examined the phenotype of Tph1-deficient mice.
Materials and methods: Beta cell-specific Tph1 knockout mice (Tph1betaKO) were generated by crossing Tph1fl/fl mice (Dr. Yadav, Cornell University, US) with Rip-creERR26LacZ mice that do not express hGH. Second, we re-analysed the total body Tph1KO mice which have serotonin-negative islets during pregnancy. Quantitative PCR was performed for islet transcripts encoding Ki-67 and for the 12 most induced genes during pregnancy ("islet pregnancy gene signature"). Islet serotonin was measured using HPLC and normalized for islet GABA content. Pancreas sections were used for serotonin immunohistochemistry and quantification of Ki-67/insulin double positive cells as an index for beta cell proliferation.
Results: To our surprise, the Tph1betaKO line maintained high islet [serotonin]/[GABA] ratios during pregnancy (Tph1betaKO versus Cre-control $p=0.74$, Flox-control $p=0.27$), despite an efficient and islet-specific recombination of the gene. The underlying mechanism is that genetic recombination in islets removes exons 2 and 3 of the gene, but leaves a downstream methionine codon in the correct reading frame for initiation of translation. Thus, Tph1betaKO beta cells produce a truncated but catalytically active TPH1. Taking a step back, we therefore used total Tph1KO mice for further studies. mRNA analysis in pregnant mice showed that Tph1 was not induced during pregnancy and there was no compensatory increment in Tph2 in WT mice. All but one of the genes of the islets pregnancy gene signature remained unchanged in islets from pregnant Tph1KO mice, the exception being the gene encoding isovalerate dehydrogenase (Ivd) an enzyme needed for the catabolism of leucine. Importantly, neither Ki-67 mRNA signal, nor the percentage Ki-67 positive beta cells was decreased in pregnant Tph1KO mice.
Conclusion: The Tph1betaKO mouse which has already been used in literature, still has islet serotonin production during pregnancy and therefore cannot be used to investigate a serotonin-deficient islet phenotype during pregnancy. Total body Tph1KO mice with complete disappearance of islet serotonin production during pregnancy were statistically the same as WT mice in terms of islet Ki-67 mRNA signal and number of Ki-67-positive beta cells. Together, our data do not support the idea that islet serotonin stimulates beta cell proliferation during pregnancy.
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PS 026 Beta cell injury: cellular compartments

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Proinflammatory cytokines disrupt islet gap junction channels and calcium homeostasis early in development of diabetes in NOD mice

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Background and aims: Pro-inflammatory cytokines mediate β-cell decline and islet dysfunction in type1 and type2 diabetes. Cytokines disrupt islet Ca2+, and disruption to islet Ca2+,are observed in models of type 1 diabetes. In addition, there is a strong Ca2+ dependence to apoptosis mechanisms. Gap junction channels between islet β-cells regulate Ca2+, and studies in models of type2 diabetes have indicated their disruption during disease progression. The goal of this study was to understand the role of altered Cx36 gap junction coupling in islet dysfunction during the progression of type1 diabetes and how pro-inflammatory cytokines may disrupt coupling.
Materials and methods: Ca2+ signaling, insulin secretion, cell viability and Cx36 gap junction coupling and Cx36 phosphorylation were measured in mouse and human islets treated with a cocktail of pro-inflammatory cytokines (10 ng/mL TNF-α, 5 ng/mL IL-1β,100 ng/mL IFN-γ), or in islets from NOD and NOD-RAG1ko mice at >6 weeks of age. Glucose tolerance tests (GTT) were administered in these mice. Genetic modulators of Cx36 and pharmacological modulators of Cx36, KATP, PKCδ, iNOS and NO donors/scavengers were also applied. Statistical significance was determined using ANOVA with Tukey's post-hoc analysis ($\alpha=0.05$).
Results: Low levels (up to 1/100 dilution) of pro-inflammatory cytokines disrupted Ca2+ signaling in terms of disrupted oscillations and increased basal activity ($p<0.005$). Cx36 gap junction coupling was also reduced under these conditions in mouse and human islets ($p<0.05$). This disruption was dependent on nitric oxide generation, PKCδ activation and phosphorylation of Cx36 (all $p<0.005$). Altered Ca2+ signaling ($p<0.05$) and decreased Cx36 gap junction coupling ($p<0.05$) was similarly observed as early as 6 weeks of age in NOD mice prior to the emergence of diabetes but in the presence of disruptions to glucose tolerance. Gap junction coupling, Ca2+ dynamics and glucose tolerance further declined with increasing age, prior to the onset of diabetes. Increased gap junction coupling in mouse and human islets reduced cytokine-induced disruptions to insulin release and β-cell death ($p<0.05$). Decreased gap junction coupling in mouse islets increased cytokine-induced disruptions to insulin release and β-cell death ($p<0.05$). This increase was reversed through inhibiting depolarization-induced Ca2+ elevations by KATP activation (p&gt0.1).
Conclusion: This study shows that gap junction coupling and Ca2+ regulation is highly-sensitive to disruption by conditions associated with the development of type1 diabetes, and discovers the mechanisms underlying its disruption in mouse and human models. This study also suggests this loss of gap junctions exacerbates cytokine-induced islet dysfunction and β-cell death later in disease progression through Ca2+ dependent mechanisms. As elevated gap junctions protects against this, this may provide a therapeutic target for delaying the onset of diabetes. Defining the mechanisms underlying gap junction disruption, may provide a means to modulate gap junctions for such a therapeutic strategy.
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Overexpression of Fis1 leads dose-dependently to alterations of mitochondrial morphology and impairment of glucose-stimulated insulin secretion in INS1E beta cells

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Background and aims: Mitochondria in living cells exist as a dynamic network that continuously cycle through fusion and fission events. Mitochondrial fission is mainly regulated by three proteins, the fission protein 1 (Fis1), the dynamin-related protein 1 (Drp1) and the mitochondrial fission factor (Mff). Numerous studies indicate that Fis1 is the key regulator for mitochondrial dynamics and, thus, essential to maintain mitochondrial function. Recent data questioned the need of Fis1 for mitochondrial network integrity and eventually proper insulin secretion in pancreatic beta cells. Therefore, the aim of this study was to investigate changes in the mitochondrial network structure and cellular function after overexpression of Fis1 in INS1E cells.
Materials and methods: Overexpression of Fis1 in INS1E cells was achieved by the pLUX lentiviral vector system. Scrambled lentiviral particles were used as control. Gene expression of Fis1 was carried out by quantitative Real-Time PCR and the protein level of Fis1 was determined using western blot and immunofluorescence analyses. The ATP content was measured using the ATPlite assay and glucose-stimulated insulin secretion by ELISA. The mitochondrial membrane potential and mitochondria morphology was analyzed by means of TMRE and MitoTracker Green, respectively.
Results: Lentiviral Fis1 overexpression of resulted in significantly enhanced gene and protein expression of Fis1 compared to control infected cells. We observed an impairment of ATP content and Glucose-stimulated insulin secretion was significantly impaired after enhancement of Fis1 in INS1E cells, whereas the control cells respond to the glucose stimulus. The ATP content was markedly reduced in Fis1 overexpressing cells compared to control infected cells. Likewise, the mitochondrial membrane potential and glucose content was significantly reduced in cells overexpressing Fis1 compared to control infected cells. INS1E cells as well as control infected cells showed an interconnected mitochondrial network with a homogenous structure. In contrast, a 10-fold overexpression of Fis1 resulted in fragmentation of mitochondria. Higher level of overexpression evoked dose-dependently formation of mitochondrial perinuclear clusters. In addition, we observed enhanced loop formation of mitochondria in cells with overexpression of Fis1.
Conclusion: Our results suggest that Fis1 is certainly important for beta cell metabolism and proper glucose-stimulated insulin secretion. Because the enhanced expression of Fis1 evoked a strong change in the mitochondrial morphology of INS1E cells, we propose to further characterize the role of Fis1 in regulation of mitochondrial dynamics and function in pancreatic beta cells in healthy and type 2 diabetic subjects.

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Oxidative stress induced-autophagy might involve in pancreatic beta cells remodelling during neonatal mice through p53/pAMPK/mTOR/ Beclin1 pathway

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Background and aims: Pancreatic beta cells undergo dynamic remodeling during neonatal period to meet the increased physiological demands after weaning, with decreased proliferation, neogenesis and enhanced

apoptosis. Autophagy, a catabolic process of the lysosomal degradation of cellular components, has been found to be crucial in beta cell survival/function and body metabolism. However, whether autophagy involves in neonatal beta cells remodeling is unknown. This study was designed to explore the function and related molecular mechanisms of autophagy in beta cells remodeling during neonatal period.

Materials and methods: Mice islets at 1-, 3-, 8-week after birth were isolated by collagenase. The morphological structure of autophagosome was observed by electron microscope.Double immunofluorescence staining was performed for insulin with LC3. The expression of autophgy-related genes were detected by real-time quantitative PCR.The expression of the p53/pAMPK/mTOR/Beclin1/LC3-II proteins was detected by Western blotting. The expression of malondiadehyde (MDA) and nicotinamide adenine dinucleotide phosphate (NADPH) oxidase subunits p22phox of islet cells were detected by lipid oxidation kit and immunohistochemistry respectively. Hydrogen peroxide (H2O2)- stimulated INS-1 cells were applied as a cell model of oxidative stress on beta cells. INS-1 cells were treated with PFT-α (the inhibitor of p53), the change of protein expression of p53/pAMPK/mTOR/Beclin1/LC3-II was detected by Western blotting.

Results: (1) Compared with 1 w and 8 w, the typical doubled-membrane autophagosome appeared within cytoplasm of islet cells at 3 w, the expression of p22phox , MDA and insulin+/LC3+ cells was significantly increased (P<0.01) (2) The mRNA level of p53 was higher at 3 w than that at 1 w and 8 w (P<0.05), while mTOR was lower than that at 1 w and 8 w (P<0.01). The mRNA expression of the autophagy-related genes dynamically changed. Compared with 1 w and 8 w, the mRNA levels of ULK1, Becn1 and Map1lc3b markedly increased at 3 w (P<0.05). Compared with 1 w, the levels of pik3c3, Atg5, Map1lc3a and Atg16l1 increased at 3 w (P<0.05); the mRNA level of Atg4d was significantly increased at 3 w and 8 w (P<0.05). At 3 w, the mRNA expression of Atg4c was higher than that at 8 w (P<0.05). (3) Western blot showed that compared with 1 w and 8 w,the protein levels of p53, pAMPK, Beclin1 and LC3-II were markedly increased at 3w (P<0.01), while mTOR protein significantly decreased at 3 w (P<0.01). (4) The MDA level of INS-1 cells after H2O2 stimulation was markedly increased in a dose dependent manner (P<0.01), along with the markedly increased expression of LC3-II and p53. With the inhibitor of p53 to block the signaling pathway, the protein expression of p53/pAMPK/Beclin1/ LC3II were significantly decreased, but the expression of mTOR increased in H2O2-stimulated INS-1 cells (P<0.05).

Conclusion: Both autophagy and oxidative stress increase in islet cells at 3-week after birth. Oxidative stress induced-autophagy might involve in pancreatic beta cells remodeling during neonatal mice through p53/pAMPK/mTOR/Beclin1 pathway.

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Evidence of beta cells dedifferentiation in human type 2 diabetes

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Background and aims: Diabetes is associated with a deficit of insulin-producing β cells. Animal studies show that β cells become dedifferentiated in diabetes, reverting to a progenitor-like stage, and partly converting to other endocrine cell types. To determine whether similar processes occur in human type 2 diabetes, we surveyed pancreatic islets from 15 diabetic and 15 non-diabetic organ donors.

Materials and methods: We scored dedifferentiation, by immunohistochemistry, using markers of endocrine lineage (Synaptophysin and Chromogranin A), β cell-specific transcription factors (NKX6.1, FOXO1, MafA), and a newly identified endocrine progenitor cell marker, aldehyde dehydrogenase 1A3 (ALDH1A3).

Results: Dedifferentiated cells, defined as Synaptophysin-positive/hormone-negative cells, accounted for 31.9% of β cells in type 2 diabetics vs. 8.7% in controls (p<0.001). The expression and localization of transcription factors required for maintenance of β cells in rodents (FOXO1, NKX6.1, and MAFA) were altered in 84% of insulin positive cells in diabetics (p<1×10-5) and associated with the expression of the progenitor cell marker ALDH1A3. Interestingly, insulin secretion in response to glucose was inversely correlated with the dedifferentiation score (r=0.55, p<0.05). Moreover, β cell-specific transcription factors were ectopically found in glucagon- and somatostatin-producing cells of diabetic subjects.

Conclusion: The data support the view that pancreatic β cells become dedifferentiated and convert to α- and δ-"like" cells in human type 2 diabetes. We envision dedifferentiation as a mechanism to protect β cells from apoptosis by stealth, preserving them for re-differentiation under more favorable metabolic conditions. The findings should prompt a reassessment of goals in the prevention and treatment of β cell dysfunction.

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Palmitate-induced impairment of glucose-stimulated insulin secretion by pancreatic beta cells is disconnected from concomitant mitochondrial respiratory defects

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Background and aims: Pancreatic beta cell mitochondria couple the oxidative breakdown of glucose to the synthesis of ATP and are essential for glucose-stimulated insulin secretion (GSIS). Indeed, mitochondrial respiratory activity might prove a good indicator of functional beta cell integrity, and insight in islet respiration may thus benefit transplantation protocols. With this study we aimed to clarify the connection between mitochondrial function and GSIS in pancreatic beta cells subjected to glucolipotoxic conditions.

Materials and methods: Islets isolated from C57BL/6 mice and INS-1E insulinoma cells were exposed for 24 or 48 hr to BSA-conjugated palmitate (free concentration 20-40 nM) in the presence of 11 mM glucose. Glucose sensitivity of oxidative phosphorylation was derived from absolute oxygen uptake rates measured in a Seahorse XF24, and insulin was quantified by ELISA. Respiratory activity and insulin secretion were normalised to islet DNA or INS-1E cell number determined from PicoGreen and DAPI fluorescence, respectively. Significance of palmitate effects was evaluated with Student's t-tests.

Results: Islets exposed to palmitate for 48 hr consume oxygen at a rate of 3.9±0.5 pmol oxygen x min-1×ng DNA-1 when incubated at 5.5 mM glucose. This respiratory rate is corrected for non-mitochondrial oxygen uptake and is indistinguishable from that exhibited by control islets exposed to BSA alone. Basal mitochondrial respiration increases to 8.2±1.5 and 6.4±0.8 pmol oxygen × min-1×ng DNA-1 in control and palmitate-exposed islets, respectively, when glucose is raised to 28 mM. Normalised to basal mitochondrial oxygen uptake, palmitate lowers the islets' respiratory response to glucose from 2.5- to 1.7-fold (P<0.05). This respiratory defect is echoed by GSIS impairment: 48-hr palmitate exposure lowers the islet insulin secretory response to glucose from 10.5-fold±1.8 to 3.4-fold±0.6 (P<0.001). GSIS impairment results from an inhibitory palmitate effect (P<0.01) on absolute insulin secretion at 28 mM glucose and a stimulatory effect (P<0.001) on absolute basal insulin release at 5.5 mM glucose. GSIS impairment is also apparent after 24-hr palmitate exposure when the islet insulin secretory response to glucose has been lowered from 6.5-fold±0.5 to 3.5-fold±0.6 (P<0.01). Importantly, after this relatively short exposure, palmitate has not yet significantly affected islet mitochondrial respiration. A disconnect between palmitate-induced

respiratory defects and GSIS impairment is also seen in INS-1E cells. After 24-hr exposure, palmitate lowers the insulin secretory response (secretion at 28 mM glucose normalised to insulin release by nutrient-starved cells) from 4.4-fold±1.1 to 1.2-fold±0.1 ($P<0.05$). This GSIS annulment results from palmitate stimulation of basal insulin release ($P<0.05$) and a small non-significant inhibition ($P=0.45$) of insulin secretion at 28 mM glucose. Concomitantly, palmitate lowers the normalised mitochondrial respiratory response to glucose of nutrient-starved INS-1E cells from 1.7±0.1 to 1.2±0.1 ($P<0.05$). This respiratory defect is exclusively caused by an inhibitory effect ($P<0.01$) of palmitate on absolute respiration at 28 mM glucose.

Conclusion: Palmitate-induced GSIS impairment in pancreatic beta cells is disconnected from mitochondrial respiratory defects.

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Improved human type 2 diabetes beta cell function and survival by autophagy induction

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Background and aims: Cells degrade and recycle their components by autophagic processes. Autophagy alterations have been proposed to also cause beta cell dysfunction thato lead to type 2 diabetes (T2D). We evaluated the effects of autophagy modulation in islets prepared from T2D and non-diabetic (ND) human donors, studied under several different experimental conditions.

Materials and methods: Islets were isolated from 5 T2D (age: 77±7 yrs; gender: 3 M/2 F; BMI: 23.9±3.7 Kg/m2) and 17 non-diabetic (ND; age: 65±21 yrs; gender: 5 M/12 F; BMI: 23.4±3.3 Kg/m2) organ donors. T2D and/or ND islets were then cultured for 1-5 days with 10 ng/ml rapamycin (autophagy inducer), or 5 mM 3-methyl-adenine (3MA) and 1.0 nM concanamycin-A (ConcA) (autophagy blockers), either in the presence or absence of metabolic (0.5 mM palmitate) or chemical (0.1 ug/ml brefeldin A) inducers of endoplasmic reticulum (ER) stress.

Results: Compared to untreated T2D islets, rapamycin exposed (24 h) diabetic islets showed improved insulin secretion (glucose-induced insulin stimulation index from 1.6±0.8 to 2.1±1.1, $p=0.05$), reduced percentage of β cells showing signs of apoptosis (from 6.6±1.7 to 2.0±0.8% by electron microscopy, $p<0.05$) and better preserved insulin granules, mitochondria and ER ultrastructure. This was associated with significant reduction of PERK, CHOP and Bip gene expression (qRT-PCR). As expected, in ND islets palmitate exposure (5 days) induced a 4-5 fold increase of beta cell apoptosis (from 0.4±0.2 to 2.0±0.8%, $p<0.01$); this deleterious action was prevented by rapamycin (0.3±0.3%) and significantly exacerbated by 3-MA (7.0±1.1%) and ConcA (3.1±1.1%). Substantially similar results were observed with brefeldin treatment (24 h). Glucose-stimulated insulin secretion from ND islets was reduced by palmitate (40-50%) and brefeldin (60-70%); rapamycin prevented palmitate cytostatic action, but affected brefeldin effects marginally. Both palmitate and brefeldin induced PERK, CHOP and Bip gene expression, which was partially, but significantly prevented by rapamycin.

Conclusion: This study emphasizes the importance of autophagy in human beta cell function and survival, particularly in situations of ER stress. Modulation of autophagy could be a tool for beta cell protection strategies.

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Mitochondrial dysfunction and diabetes predisposition

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Background and aims: Pancreatic β-cells couple mitochondrial oxidation of glucose to ATP with insulin secretion. The Cohen diabetic sensitive rat (CDs), a genetic model of diet-induced diabetes, develops hyperglycemia when fed a diabetogenic, high sucrose diet (HSD) but maintains normoglycemia on regular-diet (RD). We previously demonstrated in CDs rats reduced glucose stimulated insulin secretion (GSIS) in association with altered mitochondrial morphology. We aim to examine islet-mitochondrial function and biogenesis in CDs and its role in diabetes development in CDs rats.

Materials and methods: Pancreatic islets were isolated from CDs and non-diabetic control rats fed 30 days RD, or a HSD. Activity of islet-mitochondrial respiratory chain enzyme, cytochrome-c oxidase (COX, complex IV), normalized to activity of citrate-synthase (CS) was determined spectrophotometrically. Gene expression was analyzed by qRT-PCR. Protein level was determined by Western-blot analysis. ATP content was measured by the luciferin-luciferase luminescence assay.

Results: Complex IV activity was ~50% reduced ($P<0.01$ vs. control) in islets isolated from normoglycemic-CDs fed RD, maintaining only small residual activity (~15%) in islets of hyperglycemic-CDs rats fed HSD. ATP content was 20% lower ($P<0.01$ vs. control) in islets of normoglycemic-CDs markedly reducing on HSD (60%, $P<0.01$ vs. control). Expression of complex IV subunits; COX2, COX4 and COX5b was decreased ($P<0.01$) in islets of normoglycemic-CDs-rats, decreasing furthermore on the diabetogenic-diet. Gene-expression of complex IV assembly chaperones (COX10, COX11, COX17 and SCO1) was unaltered in islets of normoglycemic-CDs rats. COX17 expression was 50% reduced in islets of hyperglycemic-CDs while the expression of the other the COX assembly factors were unchanged. COX1 protein levels was lower in islets of normaglycemic-CDs decreasing furthermore in islets of hyperglycemic-CDs rats. CDs-islet expression of mitochondrial biogenesis activators (NRF1, NRF2 and TFAM) was decreased; while PGC-1α expression and protein levels as well as islets mtDNA content were unchanged.

Conclusion: We found a tight association between the significant reduction in islets-COX activity and GSIS in normoglycemic-CDs rats. Our data suggest that combination between reduced COX activity and mitochondrial biogenesis sets a congenital low mitochondrial threshold in islets of CDs rats. The low islet-COX threshold probably plays a critical role in the inability of the β-cells to secrete sufficient insulin to maintain the plasma glucose concentrations within the normoglycemic-range when exposed to a diabetogenic diet. The Cohen diabetic rat represents an interesting model to study molecular pathways underlying reduced GSIS and provides a good model to examine novel therapeutic intervention aimed at improving β-cell function.

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Coxsackievirus B5 infection and CVB5 proteases 2A and 3C both increase caspase 3/7 activity independently from apoptosis and reduce insulin granule cargoes in beta cells

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Background and aims: Type 1 Diabetes (T1D) results from the autoimmune destruction of pancreatic beta cells. Major cargoes of the beta cell secretory granules (SGs), namely proInsulin/Insulin, ICA512/IA-2 and ZnT8/SLC308A are dominant autoantigens of T1D, but the reason(s)

for their preferential targeting by autoimmunity is unknown. Human Enteroviruses (HEVs), like Coxsackievirus B (CVB) are among the candidate environmental agents triggering and/or accelerating the development of T1D. However, the relationship between HEV infection and loss of self-tolerance toward SG cargoes is unclear. We previously showed that CVB5 infection of rodent islets and insulinoma cells inhibited the glucose-induced rapid up-regulation of total protein biosynthesis, but not that of SG precursor proteins, including proInsulin, proICA512, proPC1/3, proPC2 and proChromograninA. The corresponding mature SG cargoes and Insulin release were instead dramatically reduced. Thus, we investigated if and how HEV infection affects the expression of SG autoantigens that may favor their targeting by the immune system.
Materials and methods: Mouse insulinoma MIN6 cells were infected with Faulkner (F) or MIN6 cell adapted (MCA) CVB5 serotypes and compared to non-infected cells 96 h post-infection. Mouse pancreatic islets were harvested 72 h post-infection with CVB5 strains. MIN6 cells transfected with CVB5 proteases 2A and 3C were compared to mock transfected cells 96 h post-transfection. Proteasome activities were measured by luminescence assays and protein expression by immunoblotting.
Results: The reduction of mature SG proteins upon CVB5 infection was insensitive to proteasome inhibitors MG-115 and MG-132. However, in CVB5-infected cells the increased Caspase-, Trypsin- and Chymotrypsin-like proteolytic activities associated with the proteasome were insensitive to these inhibitors. CVB5-infection increased also Caspase 3/7 activity, which however was mostly insensitive to Caspase 3/7 inhibitor and not associated with enhanced apoptosis, as assessed by TUNEL and Annexin V staining, while necrosis was increased. Accordingly, cleavage of apoptotic markers Caspase 3/7 and Poly (ADP-ribose) polymerase (PARP) was not detected. Finally, the levels of Major Histocompatibility Complex class I molecules (MHC class I) were unchanged in CVB5 infected insulinoma cells, but increased in CVB5-infected mouse islets. Remarkably, expression of CVB5 proteases 2A and 3C, similarly to CBV5 infection, was sufficient to reduce the levels of mature SG proteins in MIN6 cells. Likewise, in proteases 2A and 3C transfected cells Caspase-, Trypsin- and Chymotrypsin-like activities associated with the proteasome as well as Caspase 3/7 activity were increased, albeit not apoptosis.
Conclusion: Our findings suggest that CVB5 proteases 2A and 3C target SG cargoes to massive degradation in conditions of reduced translation of housekeeping proteins, thus poising infected beta cells for preferential presentation of SG protein-derived peptides on MHC class I.
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PS 027 Beta cell injury: lipotoxicity

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Role of PIAS1 in palmitate mediated beta cell dysfunction
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Background and aims: Elevated circulating free fatty acid levels, in particular palmitate, have been connected with development of type 2 diabetes mellitus (T2DM). The notion is supported by observed beta-cell dysfunction in islets after long-term treatment with palmitate. The protein inhibitor of activated STAT (PIAS) family consists of four members. Initially identified as negative regulators of STAT mediated signaling, they have since been implicated in regulating many transcription factors by blocking DNA binding activity, recruiting transcriptional co-repressors or co-activators and promoting protein sumoylation through SUMO E3 ligase activity. In a recent study PIAS1 suppressed the transcriptional activity of liver X receptor (LXR) in hepatocytes. LXR is involved in integrating lipid metabolism and the potential role of PIAS1 in lipid metabolism made us investigate the role of PIAS1 in islet dysfunction induced by chronically elevated palmitate levels.
Materials and methods: Human islets were cultured in the presence or absence of palmitate for 7 days. After culture, glucose-stimulated insulin secretion was measured by ELISA and levels of various proteins by western blot. PIAS1 interacting proteins were pulled down and identified using LTQ-Orbitrap mass spectrometer.
Results: Glucose-stimulated insulin secretion was decreased by 50% and apoptosis increased by 40% in human islets after long-term palmitate treatment. PIAS1 was present at the protein level in control islets and the level almost doubled after treatment with the fatty acid. PIAS1 interacting proteins included ATP citrate lyase (ACLY) and ATP synthase subunit 5 beta (ATP5B). In islets treated with palmitate the two proteins were significantly reduced. Overexpressing PIAS1 in beta-cells significantly reduced ACLY and ATP5B. Consistent with reduced ACLY levels in islets exposed to palmitate, levels of acetyl-CoA carboxylase (ACC) and fatty acid synthase (FAS) were also reduced in islets treated with the fatty acid.
Conclusion: Long-term palmitate treatment of islets increased levels of PIAS1, which in turn decreased ACLY and ATP5B protein levels implicating decreased lipogenesis as well as ATP synthesis. Elevated PIAS1 levels may thus contribute to altered lipid and energy metabolism reducing GSIS and increasing apoptosis.
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Eukaryotic translation initiation factor 2A (eIF2A) protects pancreatic beta cells from ER stress-induced apoptosis by selective regulation of translational initiation
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Background and aims: Endoplasmic reticulum (ER) stress plays important role in beta cell death in diabetes. ER stress leads to activation of the unfolded protein response (UPR), which inhibits protein translation via phosphorylation of eIF2α, and if unresolved induces apoptosis. eIF2A has been implicated in the translation of specific mRNAs under stress conditions, such as viral infection, when general translation is suppressed. Therefore, our specific hypothesis was that eIF2A plays a key role in regulation of mRNA translation during UPR in beta cells.

Materials and methods: Thapsigargin (Tg, 1 μmol/l), palmitate (PA, 0.5 mmol/l in BSA) were used to induce ER stress in mouse insulinoma cells (MIN6), primary mouse and human islets. Real-time RT-PCR and Western Blot were used to assess gene expression. Spinning disc confocal microscopy and ER tracker Blue-White DPX (Molecular Probes) were used for subcellular localization studies. Plasmid encoding GFP-tagged eIF2A was transfected using electroporation and cells were FACS sorted 72 hr later for the experiments. Apoptosis was assessed by live cell imaging of propidium iodide stained cells. Insulin secretion assay was performed using RIA. To assess protein synthesis cells were treated with 35S Met/Cys DMEM for 30 min, protein samples were collected and separated using SDS-PAGE, and radiation quantified.
Results: We showed that mRNA (3.7±0.5-fold, n=3) and protein (2.3± 0.3-fold, n=6) expression of eIF2A was up regulated in MIN6 beta cells treated with Tg, suggesting an association with the UPR. PA also increased protein expression of eIF2A by 2.1±0.1 fold (n=3). The findings were confirmed using isolated mouse and human islets treated with Tg and PA. We found that eIF2A was predominantly localized in the ER under basal and UPR conditions (67.1±13% n=10). We showed 94.3± 5.1% (n=3) reduction in thapsigargin-induced apoptosis in MIN6 cells overexpressing eIF2A (4-fold). On the protein level reduced death of beta cells was associated with 90.5±6.1% (n=3) decrease in protein expression of UPR pro-apoptotic marker CHOP. However eIF2A overexpression had no effect on induction of transcription of CHOP mRNA during UPR, indicating specific role of eIF2A in the inhibition of stress-induced translation of CHOP mRNA. Tg decreased insulin secretion by 54±11% (n=3) in MIN6 cells, however it was only decreased by 21±17% in cells overexpressing eIF2A. MIN6 cells showed decreased protein synthesis rate by 80.2±10% (n=3) after 2 hours of Tg treatment. However protein synthesis rate was unchanged in cells overexpressing eIF2A, despite normal induction of eIF2α phosphorylation after Tg treatment. Immunoprecipitation of eIF2A showed increase in the interaction of eIF2A with eIF2α during UPR in MIN6 cells with the maximum of 5.9±1.1 fold increase (n=3) after 24 hr of Tg treatment, which might explain uncoupling of translational initiation from eIF2α phosphorylation in cells overexpressing eIF2A.
Conclusion: We conclude that eIF2A improves function of beta cells and prevents UPR-induced apoptosis. We identified a novel protective role for eIF2A in the context of ER-stressed beta cells via the selective inhibition of CHOP mRNA translation and prevention of global translation attenuation. Thus, eIF2A may potentially serve as a new therapeutic target in diabetes.
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Lipotoxic stress induces pancreatic beta cell apoptosis through modulation of BCL-2 proteins by the ubiquitin-proteasome system
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Background and aims: Previous studies have shown induction of endoplasmic reticulum (ER) stress, increased levels of ubiquitinated proteins, and deregulation of the Bcl-2 protein family in the pancreas of type 2 diabetic patients. Here, we evaluated the role of the saturated free fatty acid (FFA) palmitate in modulation of the ubiquitin-proteasome system (UPS) and β-cell apoptosis.
Materials and methods: UPS activity was assessed by immunohistochemistry in lean and obese human and mouse samples. Real time PCR, Western blot, and viability assays were performed to study the molecular mechanism of palmitate-induced β-cell death in human and mouse islets and MIN6 cells. β-cell function and islet apoptosis triggered by diabetogenic conditions were analysed in Bcl-2 transgenic mice (RIP-Bcl-2).
Results: To examine the effect of obesity on the UPS in islets, we stained pancreatic sections of lean and obese [body mass index (BMI)>25 kg/m2] humans with anti-ubiquitin antibody. In obese human samples, there was an increased prevalence of ubiquitinated proteins in pancreatic islets when compared to lean controls. Similar results were obtained in pancreata from mice fed a high fat diet for 24 weeks. To determine whether exposure to increased FFAs had a role in the increased ubiquitin staining observed in obese subjects, human islets were treated with palmitate and levels of ubiquitinated proteins were measured by Western blot analysis. We observed an increase in the levels of ubiquitinated proteins after palmitate treatment in human islets. Similar results were obtained in mouse islets and MIN6 cells (1.5 fold increase in ubiquitination, n=4, $p<0.05$). Moreover, after 24 h treatment with either palmitate or the UPS inhibitor MG132, there was a significant increase in cell death (15% cell death in palmitate-treated cells, 28% cell death in MG132-treated cells, 1-2% cell death in control cells, n=5, $p<0.001$). Palmitate or MG132 treatment induced ER stress in β-cells (ATF4, Chop and Bip activation), resulting in decreased expression of the pro-survival proteins Bcl-2 and Bcl-XL, and upregulation of the pro-death BH3-only protein PUMA. On the other hand, pharmacological activation of the UPS by sulforaphane (SFN) ameliorated ER stress, upregulated anti-apoptotic Bcl-2 proteins and protected β-cells from palmitate-induced cell death (21% cell death in palmitate-treated cells, 7% cell death in palmitate and SFN-treated cells, 3-4% cell death in control cells, n=5, $p<0.05$). Furthermore, transgenic overexpression of Bcl-2 protected islets from apoptosis in vitro (45% cell death in palmitate-treated wild-type islets, 32% cell death in palmitate-treated RIP-Bcl-2 islets, 16-22% cell death in control islets, n=4, $p<0.05$) and improved glucose-induced insulin secretion in vivo.
Conclusion: Our results suggest that the FFA palmitate inhibits the UPS, causing β-cell apoptosis, and that this effect could be reversed by activation of the proteasome. The mechanism of β-cell death involves the deregulation of Bcl-2 proteins via activation of ER stress signaling. In this context, overexpresison of the pro-survival protein Bcl-2 improved β-cell function in a mouse model of obesity. Overall, our data clarify the mechanism by which FFAs induce β-cell death and provide therapeutic targets to improve glucose homeostasis in type 2 diabetes.
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Role of Sirtuin 3 (Sirt3) in the regulation of pancreatic beta cell function
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Background and aims: Pancreatic beta cells play an important role in the pathogenesis of type 2 diabetes (T2D). Beta cells are extremely vulnerable to pathological conditions such as hyperglycemia and hyperlipidemia. Sirt3 is a mitochondrial deacetylase which regulates a series of cell metabolic pathways including glucose and fatty acid oxidation. We aimed to investigate the role of Sirt3 in the regulation of beta cell function.
Materials and methods: Sirt3 mRNA and protein expression levels of MIN6 pancreatic beta cell line and isolated mouse islets were measured after free fatty acid (FFA) treatment. Sirt3 was overexpressed or knocked down in MIN6 cells and primary islets to evaluate its role on beta cell function. Sirt3 knockout (KO) mice and wild type (WT) mice were treated with high fat diet (HFD) or standard diet (STD) for 3 months (n=7 for each STD feed group, n=8 for each HFD feed group). Body weight and blood glucose levels were measured every week. Oral glucose tolerance test

(OGTT) and insulin tolerance test (ITT) were performed after mice were fed with STD or HFD. During OGTT, blood samples were collected for insulin level determination in addition to blood glucose measurements.
Results: Exposure of MIN6 cells and primary islets to FFA decreased Sirt3 expression and impaired glucose stimulated insulin secretion (GSIS). However, overexpression of Sirt3 partially rescued FFA induced impairment on insulin secretion. This protective effect was abolished after intervention with protein kinase A (PKA) specific inhibitor H89. Knock down of Sirt3 in MIN6 cells attenuated glucose stimulated ATP generation (7.00±0.49 vs. 4.71±0.38 pmol/μg protein, $p<0.001$), CREB phosphorylation and PPARγ expression. Besides, the GSIS of MIN6 cells was also decreased after Sirt3 siRNA transfection (1.13±0.32 vs. 0.58± 0.15 ng/μg protein, $p<0.05$). Similar changes were observed in islets isolated from Sirt3 KO mice. However, these impairments in beta cells function were ameliorated after re-expression of Sirt3 in Sirt3 KO islets via Sirt3 adenovirus infection. After 3 months of HFD feeding, glucose tolerance of both Sirt3 KO and WT mice were impaired with a higher magnitude observed in Sirt3 KO mice (glucose AUC: HFD feed WT vs. KO=1102±88.42 vs. 1328.25±52.22 mmol•L^{-1}•min^{-1}, $p<0.05$). In addition, the fasting serum insulin level was decreased in Sirt3 KO mice compared with WT mice after both STD and HFD feeding (STD feed WT vs. KO=0.033±0.012 vs. 0.0042±0.00060 ng/ml, $p<0.05$. HFD feed WT vs. KO=0.042±0.015 vs. 0.0039±0.00023 ng/ml, $p<0.05$). The GSIS of Sirt3 KO mice was also significantly reduced compared with WT mice after both STD and HFD feeding (STD feed WT vs. KO=0.160±0.029 vs. 0.031±0.023 ng/ml, $p<0.01$; HFD feed WT vs. KO=0.240±0.040 vs. 0.130±0.027 ng/ml, $p<0.05$).
Conclusion: Sirt3 is involved in the regulation of insulin secretion in pancreatic beta cells under physiological condition and after HFD. Over-expression of Sirt3 protects pancreatic beta cells from FFA induced dysfunction possibly through a PKA/CREB dependent pathway.

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Downregulation of TxNIP under glucolipotoxic conditions in insulin secreting cells

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Background and aims: Glucose dependent stimulation of TxNIP is speculated to play a role in glucotoxicity, by inhibiting the ROS scavenger thioredoxin, thereby, increasing oxidative stress. Upregulation of TxNIP by glucose occurs via ChREBP dependent transcriptional activation. This activation is negatively regulated by the transcription factor FOXO1. The glucose dependent stimulation of TxNIP is counteracted by fatty acids. We have previously observed that palmitic acid lowers TxNIP mRNA levels both in a FFAR1 dependent and independent manner. Although the FFAR1 dependent pathway does not involve PKCδ or FOXO1, INS-1E cells overexpressing PKCδ contain significantly lower TxNIP mRNA levels than control INS-1E cells. The present study attempts to identify the detailed mechanisms of TxNIP mRNA regulation.
Materials and methods: INS-1E cells were cultured under standard culture conditions (11 mmol/l glucose, 10% FCS) and protein expression was downregulated by siRNA. After 48 h of culture, cells were treated with palmitic acid (60 and 600 μmol/l) or the FFAR1 agonist TUG-469 (3-10 μmol/l). Inhibitors were added 1 h prior to palmitic acid. Changes in expression were analysed by qRT-PCR and western blot.
Results: To examine the role of FOXO1 in TxNIP mRNA regulation, INS-1E cells were incubated with the pan protein kinase B (Akt/PKB) inhibitor Akti-1/2, which triggers dephosphorylation and nuclear accumulation of FOXO1. Akti-1/2 (10 μmol/l) did not lower but rather increased glucose (11 mmol/l) induced TxNIP mRNA levels. In accordance, in PKCδ overexpressing INS-1E cells, which have low TxNIP mRNA levels, phosphorylation of Akt/PKB and nuclear phospho-FOXO1 was significantly higher than in control INS-1E cells. These observations suggest that glucose induced TxNIP expression might be antagonized by phosphorylated but not by dephosphorylated FOXO1. Since palmitic acid transiently reduced Akt/PKB phosphorylation, palmitic acid induced TxNIP downregulation is probably mediated by other pathways. FFAR1 induced enhancement of insulin secretion depends on the stimulation of phospholipase C (PLC) and protein kinase D1 (PKD1) and therefore, we examined whether these enzymes exert effects on TxNIP mRNA levels. While the PLC inhibitor U73122 (1 μmol/l) counteracted FFAR1 dependent reduction of TxNIP mRNA levels, the downregulation of PKD1 by siRNA had no effect. In addition, the inhibition of ERK1/2 by PD98059 (10 μmol/l) did not affect TxNIP mRNA levels. Finally, the stress kinase JNK, which plays a crucial role in palmitic acid induced β-cell death, was inhibited by SP600125 (10 μmol/l). The inhibitor did not counteract the effect of palmitic acid but itself reduced TxNIP mRNA levels.
Conclusion: In insulin secreting cells, TxNIP mRNA levels are rapidly and reversibly regulated by a variety of pathways including Akt/PKB, JNK and PLC.
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Role of very long chain fatty acid elongase 2 (Elovl2) in the regulation of pancreatic beta cell dysfunction induced by gluco-lipotoxicity

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Background and aims: The IMIDIA program (http://www.imidia.org/) aims to identify new targets involved in ß cell dysfunctions induced by obesity. To achieve this goal, RNAseq analyses have been performed on isolated islets of Langerhans from mice under high fat diet and controls. Using bioinformatical analysis, we found that expression of Elovl2 was correlated with glucose intolerance. This enzyme is involved in the production of poly-unsaturated fatty acids, such as decosahexaenoic acid (DHA). Therefore, our project aimed to determine the role of Elovl2 in ß cell dysfunction induced by saturated fatty acids and high glucose concentrations, a phenomenon defined as gluco-lipotoxicity.
Materials and methods: Pancreatic cell lines (INS-1 and MIN6) or islets of Langerhans have been incubated with 0.4 mM of palmitate and 30 mM of glucose. Elovl2 expression has been determined by real-time PCR and Western-Blot. The role of Elovl2 has been determined using a stable cell line over-express it or by adding its product, DHA, on the β cells. Insulin secretion (ELISA test) and apoptosis (detection of caspase 3/7 activity and PARP cleavage by western blot) of pancreatic β cells have been determined. Ceramide production has been quantified through a DAG kinase test and lipidomics.
Results: We found that gluco-lipotoxicity inhibits Elovl2 expression in β cells. Addition of DHA to the incubation medium can partially restore insulin secretion induced by glucose. DHA also inhibits caspases-3/7 activation and PARP cleavage induced by gluco-lipotoxicity. Interestingly, stable Elovl2 over-expressing cells are partially protected from apoptosis induced by glucolipotoxicity. We also found that adding DHA and over-expressing Elovl2 inhibit ceramides production induced by gluco-lipotoxicity.
Conclusion: Our results show that Elovl2, through DHA production, can counteract the deleterious effects induced by gluco-lipotoxicity by blocking ceramide synthesis.
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Enhancer of Zeste Homolog 2 (EZH2) is a molecular switch in HDAC3-mediated glucolipotoxicity in beta cells

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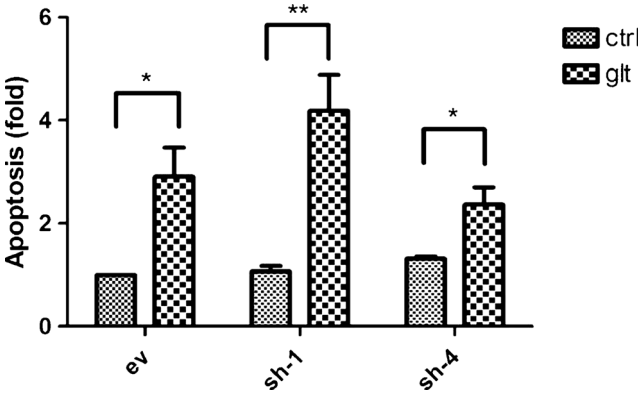
Background and aims: Type 2 diabetes (T2DM) is caused by relative insulin deficiency arising when pancreatic β-cell insulin secretion fails to compensate for insulin resistance. Elevated blood free fatty acids and later glucose are mediators of progressive β-cell failure in T2DM. Posttranslational modifications by acetylation and methylation of the histone backbone in chromatin are important epigenetic modulators of gene transcription. We previously showed that inhibition of histone deacetylase (HDAC) 3 reverts deleterious effects of β-cell glucolipotoxicity (GLT), but the molecular mechanisms are unclear. The aim of this study was to investigate the mechanisms by which β-cell dysfunction and apoptosis by HDAC3-mediated glucolipotoxicity (GLT) can be reverted by HDAC3-inhibition.
Materials and methods: mRNA microarray was performed on samples from the rat β-cell line INS-1E exposed to a diabetic milieu (25 mM glucose and 0.5 mM palmitate) in the presence or absence of the HDAC3-selective inhibitor BRD3308 and validated by RT-qPCR. Microarray data was analyzed by fold-change and the 400 top scoring genes from each set and the intersecting gene sets were analyzed to find enriched transcription factors (TF) by employing the ENCODE ChIP-Seq Significance Tool. EZH2 inactivation was performed by overexpression of four different EZH2 shRNAs in INS-1E cells, confirmed by real-time PCR and Western blotting. Empty vector (EV) served as control (ctrl). EZH2 knock-down cells were exposed to 25 mM glucose and 0.5 mM palmitate, and β-cell apoptosis was analyzed by quantitative in vitro determination of cytoplasmic histone-associated DNA fragments (Roche). Data was analyzed by two-tailed, paired Student's t-test.
Results: The mRNA microarray data revealed 52 genes differentially regulated by nutrient overload (glucolipotoxicity, GLT) with or without specific HDAC3-inhibitor. The microarray was validated by qRT-PCR of 6 genes selected for their differential expression pattern which was confirmed in 5/6 cases. By unbiased bioinformatics analysis of the mRNA microarray data we found that the histone methyltransferase (HMT) and TF Enhancer of zeste homolog 2 (EZH2) is a key transcriptional regulator in HDAC3 inhibitor treated insulin-producing cells exposed to GLT. We achieved ~70% knock down of EZH2 with sh-1 and ~40% knock down with sh-4 and somewhat lower knock-down by sh-2 and -3. Pronounced shRNA mediated knock-down of EZH2 (sh-1) aggravated whereas partial knock-down (sh-4) protected against GLT-induced β-cell apoptosis (p-value of n=4: ** $p<0.01$, * $p<0.05$, Figure 1).
Conclusion: Our results identified EZH2 as a novel molecular switch regulating glucolipotoxicity-induced β-cell dysfunction and apoptosis. The possibility that the bimodal role of EZH2 on β-cell viability is conferred by the dual action of EZH2 as HMT and TF is under investigation.



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PKCdelta is a key regulator of palmitate-induced beta cell death

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Background and aims: High glucose and fatty acid (FA) levels induce beta cell dysfunction and cell death, leading to a condition known as glucolipotoxicity. Both FAs and glucose are metabolized to diacylglycerol which activates novel protein kinase C (PKC), including PKCdelta. Although activated PKCdelta is implicated in cell death, the molecular mechanisms involved in the role of PKCdelta in beta cells are yet to be fully defined. The aim of this study was to elucidate PKCdelta's molecular mechanism during glucolipotoxicity in beta cell death.
Materials and methods: Mouse insulinoma (MIN6) cells were incubated with 25 mmol/l glucose with or without 0.4 μmol/l palmitate for 72 h. Phosphorylation of PKCdelta and protein expression of Pdx1 were analyzed by Western blotting. Translocation of PKCdelta was studied by PKCdelta staining and Western blotting. Cell death was assessed with Annexin V and propidium iodide. PKCdelta knockdown was performed using a lentiviral vector followed by a cell death assay. To study the molecular mechanism in vivo, we created beta cell specific Pkcd−/− mice (bPKCDKO) and treated them with 50 mg/kg streptozotocin (STZ) in the neonatal period (type 2 diabetes model). These mice were fed either a normal diet (ND) or a high fat diet (HFD). Intraperitoneal (i.p.) glucose tolerance tests were performed after a 16-h fast. Alpha/beta cell architecture and mass were assessed by insulin/glucagon staining, and beta cell death was assessed by TUNEL staining.
Results: Palmitate phosphorylated PKCdelta, with no change in whole PKCdelta protein expression in MIN6 cells, suggested PKCdelta activation. Although in the normal state, PKCdelta was localized in the cytoplasm, palmitate induced a nuclear translocation of a full length and a catalytically active fragment of PKCdelta. During palmitate treatment, the number of Annexin V positive cells significantly increased. Furthermore, palmitate phosphorylated Pdx1 at T11, and reduced the expression of Pdx1 protein, implicating PKCdelta induced MIN6 cell death via Pdx1 down regulation. To confirm the involvement of PKCdelta in palmitate induced MIN6 cell death, PKCdelta was knocked down. PKCdelta deficiency prevented the development of palmitate induced Annexin V-positive cells by 28% (p 0.05). bPKCDKO mice treated with STZ and HFD showed improved glucose tolerance compared with mice fed ND, accompanied by improved insulin secretion. Although HFD-fed control mice treated with STZ showed reduced beta cell mass and disturbed alpha/beta cell architecture, PKCdelta deficiency in beta cells ameliorated beta cell mass to about 70% ($p<0.05$) and disturbed alpha/beta cell

architecture. PKCdelta deficiency in beta cells also showed a reduction in beta cell apoptosis by 46% ($p<0.05$).
Conclusion: Under glucolipotoxic conditions, PKCdelta is activated, followed by the downregulation of Pdx1, which results in beta cell death. In vivo, beta cell specific PKCdelta deficiency ameliorates beta cell mass and insulin secretion, as a result of reduced beta cell apoptosis. Our results suggest that palmitate induces beta cell death via a PKCdelta/Pdx1 dependent pathway. It may serve as fundamental knowledge pertaining to novel approaches toward diabetes, by targeting the PKCdelta/Pdx1 pathway.

PS 028 Beta cell injury: apoptosis

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NPY Y1 receptor stimulation protects against cytokine-induced apoptosis in mouse islets

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Background and aims: NPY receptor stimulation has proliferative actions and protective effects against programmed cell death in a number of tissues, but little is known about the protective effects of NPY receptor activation on beta cell survival. We have identified Y1 receptors as the most abundant sub-type in mouse islets. Therefore, we investigated the potential protective role of Y1 receptor activation in mouse islets.
Materials and methods: Isolated mouse islets were treated for 24-48 h with the Y1 receptor agonist $Leu^{31}Pro^{34}$-NPY (1 nmol/l-1 μmol/l) alone or in combination with the Y1 antagonist BVD10 (100 nmol/l-1 μmol/l) or the PLC inhibitor U73122 (10 μmol/l) in the absence or presence of a cytokine cocktail (IL-1β, 50 U/ml; TNF-α, 1000 U/ml, IFN-γ, 1000 U/ml) for the last 24 h of incubation. Apoptosis was measured by caspase glo 3/7 activity and insulin secretion by RIA.
Results: Treatment of mouse islets for 48 hours with $Leu^{31}Pro^{34}$-NPY (1 nmol/l-1 μmol/l) protected against cytokine-induced apoptosis (mixed cytokine response: 100%; 60±2%, 67±5%, 54±2%, 69±7% with 1 nmol/l, 10 nmol/l, 100 nmol/l and 1 μmol/l $Leu^{31}Pro^{34}$-NPY respectively, $p<0.01$- $p<0.001$, $n=8$). Furthermore, pre-treatment with 1 nmol/l $Leu^{31}Pro^{34}$-NPY for 24 h followed by cytokine exposure alone and 1 nmol/l $Leu^{31}Pro^{34}$-NPY treatment in the presence of cytokines for 24 h without pre-treatment significantly protected mouse islets against cytokine-induced apoptosis (mixed cytokine response: 100%; pre-treatment: 61±5%, $p<0.001$; no pre-treatment: 71±5%, $p<0.01$, $n=8$). The selective Y1 receptor antagonist BVD10 did not inhibit islet apoptosis induced by cytokines when administered alone (100 nmol/l BVD10: 98±22%; 1 μmol/l BVD10 139±27% of the mixed cytokine response, $p>0.2$, $n=8$), but reversed the anti-apoptotic effect of $Leu^{31}Pro^{34}$-NPY (1 nmol/l $Leu^{31}Pro^{34}$-NPY + 100 nmol/l BVD10: 78±13%; 10 nmol/l $Leu^{31}Pro^{34}$-NPY + 1 μmol/l BVD10: 109±14% of the mixed cytokine response, $p<0.05$-$p<0.01$, $n=8$), indicating the protective effect is likely via Y1 receptors. Inhibiting PLC activity with U73122 reversed the anti-apoptotic effects of $Leu^{31}Pro^{34}$-NPY (10 μmol/l U73122+1 nmol/l $Leu^{31}Pro^{34}$-NPY: 110±13% of the mixed cytokine response, $p<0.01$, $n=6$), whilst having no protective effect on cytokine-induced apoptosis when administered alone (10 μmol/l: 109±17% of the mixed cytokine response, $p>0.2$, $n=6$), suggesting that the anti-apoptotic properties of Y1 receptor activation may be mediated through a PLC signalling cascade. As expected, acute exposure to $Leu^{31}Pro^{34}$-NPY inhibited glucose-induced insulin secretion when used at 1 μmol/l (2 mmol/l glucose: 0.6±0.05 ng $islet^{-1}$ h^{-1}; 20 mmol/l glucose: 2.5±0.5; 1 μmol/l $Leu^{31}Pro^{34}$-NPY: 0.9±0.3, $p<0.05$, $n=8$), but it had no effect at 1 nmol/l-100 nmol/l (all $p>0.2$, $n=8$). In addition, 48 h exposure to $Leu^{31}Pro^{34}$-NPY (1 nmol/l-100 nmol/l) had no significant effect on glucose stimulated insulin secretion (all $p>0.2$, $n=8$).
Conclusion: These results indicate that NPY Y1 receptor stimulation can protect against cytokine-induced apoptosis in mouse islets at low concentrations without inhibiting glucose-stimulated insulin secretion.
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Hypoxia induces beta cell death by inhibiting the adaptive UPR

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Background and aims: Hypoxia is implicated in the loss of functional beta cell mass in type 2 diabetes and with islet transplantation, although the mechanisms are unknown. The adaptive unfolded protein response (UPR) is required for endoplasmic reticulum (ER) homeostasis and beta cell integrity. Here we investigated the influence of hypoxia on the adaptive UPR and the role it plays in apoptosis.
Materials and methods: Isolated mouse islets and MIN6 cells were exposed to various O2 tensions. Ddit3 (Chop) and Hif1α were inhibited using siRNA. Hspa5 (Bip) was overexpressed using a plasmid vector. JNK was inhibited using SP600125. UPR and hypoxia-response gene expression was assessed in islets from prediabetic and diabetic db/db mice and age-matched lean control mice. mRNA and protein levels were measured by real-time RT-PCR and western blot. Apoptosis was measured by DNA fragmentation ELISA.
Results: Deprivation of O2 (1% vs 20%) for 4-24 h markedly reduced the mRNA and protein levels of adaptive UPR genes, including Hspa5, Hsp90b1 and Fkbp11 as well as the activation of Xbp1 (splicing) and PERK (phosphorylation). Opposing effects were observed in MEF cells suggesting that hypoxia specifically inhibits the adaptive UPR in beta cells. This was accompanied by upregulation of integrated stress response (ISR) genes, including Ddit3, Atf3 and Trb3 along with increased phosphorylated EIF2A. Interestingly, Ddit3 knockdown significantly increased adaptive UPR gene expression in association with partial protection against hypoxia-induced apoptosis ($p<0.05$). Moreover, Hspa5 overexpression alone partially protected against hypoxia-induced apoptosis ($p<0.01$). JNK inhibition, but not Hif1α knockdown, partially prevented the hypoxia-mediated loss of adaptive UPR gene expression and protected against hypoxia-induced apoptosis ($p<0.01$). Finally, mRNA levels of hypoxia-response genes, including Hyou1, Tpi1, Gapdh and Eno1, were markedly upregulated in vivo in the islets of diabetic db/db mice, but not in prediabetic db/db mice, suggesting that islet hypoxia correlates with beta cell failure. Interestingly, the upregulation of hypoxia-response genes further correlate with downregulation of adaptive UPR gene expression in diabetic db/db islets.
Conclusion: Hypoxia inhibits the adaptive UPR in beta cells partially via Ddit3 and JNK activation, but independently of Hif1α. Downregulation of the adaptive UPR contributes to hypoxia-induced beta cell apoptosis and may play a role in the loss of functional beta cell mass in type 2 diabetes.
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The L-type calcium channel beta1 subunit is essential for preventing apoptosis in beta cells

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Background and aims: Voltage-gate calcium channels (Cav) play a pivotal role as triggers of insulin secretion. Genetic variants in genes encoding the pore-forming Cavα1 subunits determine the human beta-cell phenotype and affect the risk of type 2-diabetes. The functions of the auxiliary Cavβ subunits are only patchily explored, but they are proposed to modulate of translocation and stability of the α1 subunits. Supporting this idea, Cavβ3 was previously shown to suppress Ca2+-oscillations in mouse beta-cells. Furthermore, Cavβ2 is subject to palmitoylation, which could serve as a regulatory signal for α1 function. To clarify this we have investigated gene co-expression with Cavβ in human islets and explored the effects of all Cavβ subunits (1-4) in beta-cell function.
Materials and methods: Insulin secretion. Calcium imaging. Immunoblotting. Viability assay.
Results: Microarray analysis of gene expression in human islets revealed that the different Cavβ subunit genes (CACNB1, CACNB2, CACNB3, CACNB4) significantly correlated primarily with genes encoding other Cav subunits, structural proteins, as well as apoptosis-regulating proteins. For example, CACNB1, -2, -4 correlated with EIF3E that was recently demonstrated to control translocation of Cavα1. These results support the idea that Cavβ may interfere with beta-cell function and viability. In addition, CACNB1 and CACNAB2 both exhibited a negative correlation vs. HbA1c of the islet donors. Next, we investigated the effects of silencing the different Cavβ subunits on glucose-stimulated insulin secretion in INS(832-13)cells. Gene silencing (72 h) was efficient as verified on the protein level by immunoblotting and for e.g. Cavβ1 it amounted near >95%. Interestingly, silencing of Cavβ2, Cavβ3 and Cavβ4 failed to affect insulin release under either basal or stimulatory conditions. By contrast, reduced Cavβ1 expression resulted in increased insulin release under both basal and stimulated conditions. Based on these results, we next focused on Cavβ1 and explored depolarization-evoked increases in cytoplasmic Ca2+ concentration ([Ca2+]i) in Cavβ1-silenced cells. Surprisingly, this resulted in a 76% decrease in [Ca2+]I evoked by high K+ (9.8±1.09 vs. 2.3±0.23 fluorescence intensity; $P<0.001$), whilst not affecting basal [Ca2+]I. This result contrasts with the observed increased insulin release. In an attempt to explain this we next investigated cell viability (EthD-1 & Calcein AM) and found that this was severely compromised in Cavβ1-silenced cells (0.46±0.21 vs. 7.8±0.098 percentage cells; $P<0.001$). This was due to increased apoptosis as assessed by immunoblotting of cleaved caspase 3, which increased by 300% in Cavβ1-silenced cells.
Conclusion: Cavβ1 affects Ca2+-homeostasis in the beta-cell, but is essential for beta-cell viability. Silencing of Cavβ1 results in increased apoptosis in a non-Ca2+ dependent fashion.
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A dual approach with Siglec-7: it inhibits macrophage infiltration into human islets and restores beta cell function and survival

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Background and aims: In both, type 1 and type 2 diabetes mellitus cytokine and chemokine production and infiltrating immune cells are hallmarks of islet inflammation. Siglecs (Sialic-acid binding immunoglobulin like lectins) are cell surface receptors expressed on haematopoietic cells, they balance immune responses by immunoreceptor tyrosine-based inhibitory motifs (ITIMs), which recruit tyrosine phosphatases and eventually inhibit activation signals transduced from pattern recognition receptors. Siglec-7 was found to be downregulated in diabetic islets as well as in activated PBMCs. Here, we aimed to investigate whether such changes in Siglec-7 expression directly modulate islet immune cell infiltration, β-cell function and survival.
Materials and methods: To investigate the role of Siglec-7 in β-cell function and survival, over-expression was carried out in isolated human islets with or without diabetic stimuli (glucose/palimitic acid, cytokine mixture IL-1β/IFNg/TNFα), followed by glucose stimulated insulin secretion (GSIS) and TUNEL assay. Cytokine secretion was analyzed by the Meso Scale Discovery® multi array technology. To ascertain its role in immune cells, Siglec-7 expression was analyzed by RT-PCR and FACS in freshly isolated PBMCs from non-diabetic individuals cultured with the same diabetogenic conditions or 100 ng/ml Lipopolysaccharide (LPS) for 12 hours.

Results: Siglec-7 mRNA was markedly down-regulated in islets isolated from patients with T2D and in pancreases from autopsy from patients with T2D. Over-expression of Siglec-7 improved β-cell function and survival in human islets isolated from patients with T2D. The overexpression also prevented glucolipotoxicity- and cytokine-induced β-cell apoptosis. In isolated human islets from patients with T2D, Siglec-7 overexpression caused a reduction in basal IL-1β (40%) and TNFα (50%) expression. In human monocytes, LPS, glucose/palmitic acid, as well as the cytokine mixture IL-1β/IFNg/TNFα induced their activation as seen by the induction of IL-6 mRNA and CD25 expression; a simultaneous decrease was observed in Siglec-7 mRNA levels. Induced migration of enriched monocytes was observed under conditions of elevated glucose/palmitate as well as by islets from patients with T2D. This demonstrates the triggering of inflammation in islets under diabetogenic conditions. Conditioned media from islets overexpressing Siglec-7 majorly inhibited cell migration of immune cells.
Conclusion: Our data suggest that Siglec-7 is regulated in islets and immune cells under diabetogenic conditions and influences immune cell migration, β-cell function and survival.
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Reduced expression of the transcription factor, STAT6, in beta cells may contribute to their loss in human type 1 diabetes
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Background and aims: It is widely accepted that immune cells infiltrate the pancreatic islets in human type 1 diabetes and contribute to beta-cell loss by releasing pro-inflammatory cytokines. In addition, the production and influence of anti-inflammatory cytokines (e.g. IL-4 or IL-13) is correspondingly down-regulated in this disease, which may also contribute to beta cell loss. IL-4 and IL-13 each signal via their cognate cell surface receptor to induce the phosphorylation and activation of the transcription factor, STAT6; which then mediates their effects. In the current study we have evaluated the extent to which STAT6 acts as a regulator of beta-cell viability and have studied whether islet STAT6 expression is altered in human type 1 diabetes.
Materials and methods: An archival collection of formalin fixed paraffin embedded (FFPE) pancreas samples from both healthy controls and individuals with type 1 diabetes was interrogated using STAT6 antisera. Both immunofluorescence (IF) and immunohistochemical (IHC) staining approaches were employed. Cell culture studies were also undertaken to explore STAT6 phosphorylation and beta-cell viability by Western blotting and flow cytometry respectively.
Results: IF staining of FFPE human pancreas tissue revealed a robust expression of STAT6 within the endocrine pancreas in non-diabetic individuals. Importantly, STAT6 was found at high levels in insulin-containing beta-cells but expression was negligible in other islet endocrine cell types. To assess the role of STAT6 in the beta-cell, IL-4 and IL-13 were employed as stimuli in INS-1E cells and isolated human islets. Treatment of these with IL-4 or IL-13 induced a rapid, time-dependent rise in STAT6 phosphorylation at Tyr641, indicating increased STAT6 activation. In accord with this, the anti-inflammatory cytokines potently protected the cells against a range of cytotoxic insults including serum starvation, saturated fatty acids and pro-inflammatory cytokines (10% foetal calf serum: 11.3±0.9%; no serum: 35.5±1.3% cell death; no serum plus 20 ng/ml IL-13: 23.8±0.8%; $p<0.001$). Pharmacological inhibition of Janus kinases (which act immediately upstream of STATs) prevented cytokine-induced STAT6 phosphorylation and reversed the improved cell viability caused by IL-4 or IL-13 (no serum: 30.0±1.3% cell death, IL-13: 16.9±0.8%, IL-13+P6: 30.3±1.3%; $p<0.001$). Finally, we investigated whether the expression of STAT6 is altered within the islets of pancreases of patients with type 1 diabetes compared to controls. Pancreas sections from six healthy and six age- and sex-matched patients with recent-onset type 1 diabetes were immunostained for STAT6 and islet hormones. Images of five islets per section were captured at identical microscope and camera settings. The mean fluorescence intensity of STAT6 expression within each islet was calculated and analysis of these data revealed that total STAT6 levels were significantly decreased in the islets of individuals with recent-onset diabetes (MFI for STAT6; healthy control 14.22±1.23, T1D 2.31±0.64; $p<0.001$).
Conclusion: STAT6 is expressed in human beta-cells and its activation improves the viability of these cells in response to various cytotoxic insults. Expression of STAT6 is reduced in the beta cells of individuals with type 1 diabetes and this may contribute to the enhanced vulnerability of these cells in the face of the pro-inflammatory milieu found in inflamed islets.

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Robust amyloid deposition with M1 macrophage infiltration in the islets in super-elderly Japanese type 2 diabetic subjects
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Background and aims: Dramatic increase in elderly type 2 diabetic patients (T2DM) is a serious problem in modern society. Although pathogenesis of elderly T2DM can be distinct from that in younger ones based on their different energy metabolism, differences in the islet pathology between elderly and less old diabetic patients are yet to be addressed. In this study, we attempted to characterize the islet changes in elderly T2DM patients.
Materials and methods: Pancreata from 13 young non-diabetic (Y-ND) (mean 25.8 y.o.), 22 middle-aged non-diabetic (M-ND) (61.3), 23 middle-aged diabetic (M-DM) (59.4; onset of DM 48.8),30 super-elderly non-diabetic (SE-ND) (89.5), and 10 super-elderly diabetic subjects (SE-DM) (88.5; onset of DM 80.3) were investigated. On the tetra immunostained sections, morphometric analysis was conducted on each endocrine cell as to their volume density, mass and occupancy in the islet. Immunohistochemical expressions of insulin transcriptional factors (TFs) such as PDX1 and MafA or in situ hybridization for (pro)insulin mRNA, and infiltration of macrophages positive for CD68 (M1-M) and CD163 (M2-M) were assessed for insulin transcriptional activity and inflammatory changes, respectively .
Results: Pancreas weight (PW) was comparable among groups except for SE-DM which showed 50% decrease compared to SE-ND. In accord with this result, pancreatic parenchyma of SE-DM displayed severe atrophy. In addition, we found a high frequency of amyloid deposition (amyloid volume density>0.1%) in SE-DM (70%) compared to M-DM (27%) and SE-ND (3%). Compared to M-ND, volume density of β cells (Vβ) was reduced ca 30% in M-DM. In contrast, there was a significant increase in volume density of α cells (Vα in M-DM. Vβ in SE-ND was increased to 130% of M-ND accompanied by an increase in islet volume density (Vi). Vβ in SE-DM was dramatically reduced 35% compared to SE-ND ($p<0.01$). There were no significant changes in either δ or PP cell volume densities among all cohorts. The expressions of PDX1 and MafA were the most prominent in Y-ND and preserved in M-ND, whereas those in M-DM, SE-ND, and SE-DM were all suppressed to similar extents to those in M-ND, together with reduced insulin mRNA expression ($p<0.05$). Both M1-M and M2-M infiltrated into the islets of M-DM and further M1-M infiltrates were conspicuous in SE-DM, while M2-M infiltrates in SE-DM were less marked compared to that of M-DM.
Conclusion: This study disclosed that the islet pathology in SE-DM is distinct from those in M-DM, characterized by pancreas atrophy, amyloid deposition, macrophage infiltrates, and less implication of TFs. Thus, environmental insults may more strongly be implicated in the onset of SE-DM compared to usual M-DM.

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A role for the Nrf2-antioxidant pathway in intrinsic beta cell repair after resolution of hyperglycaemia

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Background and aims: It is well established that sustained hyperglycemia causes beta cell necrosis and apoptosis. However, very little information exists about whether damaged beta cells can undergo intrinsic repair once treatment resolves the hyperglycemic state, and if so, what mechanisms might be involved. To address these issues, we studied euglycemic ZDF rats that become hyperglycemic when fed a high fat diet (HFD). Observations were made during a baseline non-HFD period, during a HFD, and after returning to a non-HFD with particular emphasis on Nrf2, a protein that binds to the promoter elements of genes involved with synthesis of antioxidant proteins.
Materials and methods: 3 groups (n=12 each) of normoglycemic ZDF female rats were fed non-HFD (16.5% fat) for 2 weeks. Thereafter, one group each was fed the HFD (48% fat) for 9, 18, and 28 days, respectively. Animals (n=4) from each of the groups were sacrificed for pancreatectomy at 3 time periods: at baseline; on the 9th, 18th, and 29th day of HFD; and at the end of a 3 week return to non-HFD. Non-fasting blood samples were taken throughout the study for glucose and insulin. Pancreases were used for insulin immunostaining and beta cell mass as well as for immunoflourescent staining for Ki67, apoptosis, PDX-1, superoxide dismutase (SOD-1), catalase (CAT), glutathione peroxidase-1 (GPx-1), hemoxygenase-1 (HO-1) and nuclear factor erythroid-derived 2-related factor (Nrf-2).
Results: 9 days HFD caused hyperglycemia (348 +/- 16 mg/dl) and during the remaining weeks after return to non-HFD, elevated glucose levels returned to baseline levels (147 +/- 10 vs 135 +/- 3 baseline, mg/dl; p=ns). After 18 days HFD and hyperglycemia (513+/- 34), elevated glucose values partially returned to baseline levels (215 +/- 37 vs 144 +/- 6 baseline; p=ns). After 28 days HFD and hyperglycemia (546 +/- 63) and during the remaining weeks after return to non-HFD, elevated glucose levels failed to return to baseline levels 294 +/- 69 vs 133 +/9; p< 0.03). Corresponding insulin levels failed to respond to the higher glucose levels by the end of the experiments: 18 day study=20 +/- 4 vs. 38 +/- 4 microU/ml baseline; 28 day study=14 +/- 2 vs. 26 +/- 4 baseline. Insulin immunostaining revealed beta cell damage after 9, 18, and 28 days of HFD. However, islets were healthy-appearing after a return to non-HFD in the 9 day HFD study. Nonetheless, there was a lesser improvement in beta cell imagery during return to non-HFD after 18 days HFD and very little improvement in beta cell imagery after return to non-HFD after 28 days HFD. Nrf2, HO-1, and SOD-1 were prominent in the nucleus during HFD in the 9 day study compared to pre-HFD images, although less so in the 18 and 28 day experiments. No changes in apoptosis, Ki67, GPx-1, or catalase were observed.
Conclusion: These data demonstrate that HFD in female ZDF rats causes hyperglycemia and associated beta cell damage that is reversible after a return to non-HFD in a time-related fashion wherein longer exposure to HFD is accompanied by lesser recovery of beta cell mass. The immediate appearance of intranuclear Nrf2 and antioxidant proteins that accompany the recovering beta cell morphology after the shortest but not the longest exposure to HFD suggests an early response role of the Nrf2-antioxidant pathway in beta recovery from hyperglycemia-induced damage.
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Protection against inflammatory beta cell damage by lysine deacetylase inhibition and microRNA expression

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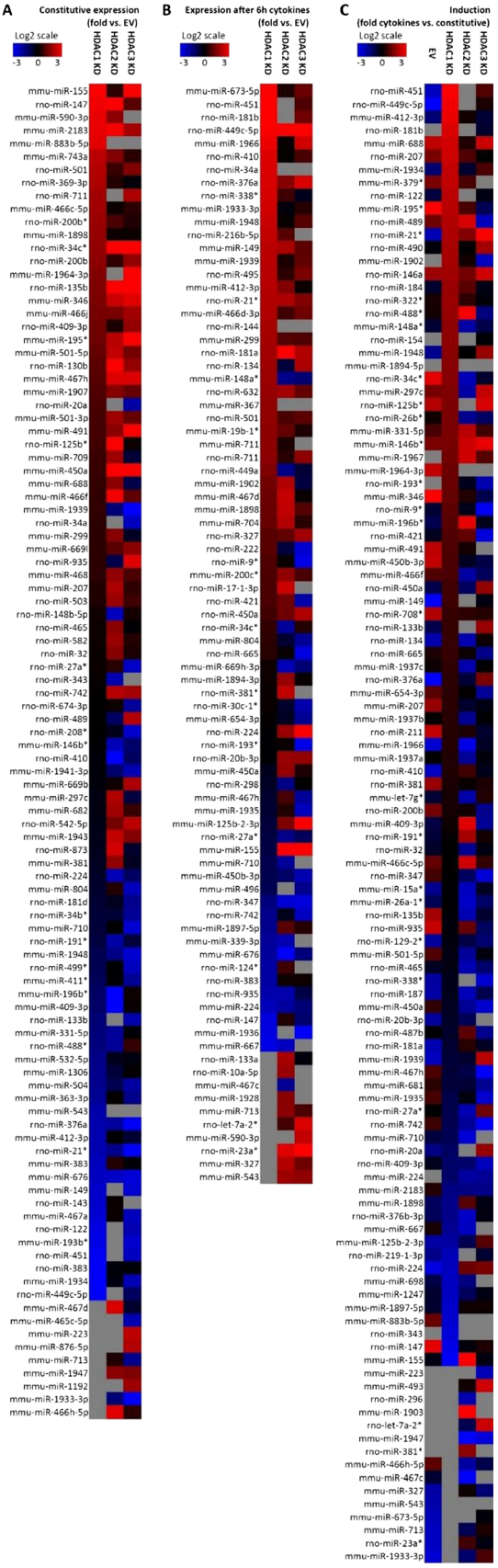
Background and aims: Pro-inflammatory cytokines contribute to pancreatic β-cell apoptosis in type 1 and 2 diabetes mellitus. The detrimental effects resulting from cytokine-induced signaling in the β cell can be reduced by inhibition of class I classical lysine deacetylases (KDACi), especially HDAC1 or HDAC3, and is associated with down-regulation of inflammatory gene expression, only in part through hyperacetylation of NFκB. We therefore hypothesize that HDACi-mediated hyperacetylation of histones and/or other proteins upregulate expression of microRNAs (miR), which repress translation of oxidative stress proteins responsible for β-cell death. The aim of the study is to identify novel and specific therapeutic targets for β-cell protection by mapping the miR profile of β cells rescued from inflammatory assault by inhibition of lysine deacetylation, thereby identifying miR that repress cytokine-induced mitochondrial and oxidative β-cell stress.
Materials and methods: Stable INS-1 knockdown (KD) clones of HDAC1, -2, and -3 (class 1 KDACs) or empty vector were generated by Lentiviral shRNA transduction. The cells were incubated 6 h with or without cytokines (150 pg/mL IL-1β + 5 ng/mL IFNγ), and RT-qPCR-based miR array was performed. Regulation of several miR was verified by TaqMan RT-qPCR, and medium nitrite was determined with Griess' reagent.
Results: Following systematic analysis using NormFinder, miR-103 was chosen for normalization of the qPCR array data. Constitutive expression of 103 miR, and cytokine-induced expression of 84 miR were up- or down-regulated more than 3-fold by KD of HDAC1, -2, and/or -3 (see figure). MiR-146a, -146b, -21 and -34a were chosen for further analysis, and their expression was assessed by RT-qPCR normalized to U6. Cytokine exposure induced a 15-fold expression of miRNA-146a, which was reduced by 65% in HDAC1- but not in HDAC2- or HDAC3-deficient INS-1 cells ($p<0.05$, n=5). Expression of miRNA-146b, miR-21 and miR-34a were unaffected by HDAC1-3 deficiency, while cytokine-induced NO production was reduced by 27% and 44% in the HDAC1 (n=8) and HDAC2 (n=5) but not HDAC3 deficient clones respectively. The qPCR array analysis revealed several additional novel miR potentially important for understanding the beneficial effects of KDAC inhibition, which are currently being functionally validated.
Conclusion: The interesting association between HDAC1 KD-mediated protection against cytokine-induced INS-1 cell destruction, and the inhibitory effect of HDAC1 knockdown on the β-cell pro-apoptotic miR-146a and other miR under investigation, warrants further investigations aiming to show a causal role of miR in HDAC inhibitor-mediated protection of pancreatic β cells. The perspective of this study is to develop novel anti-diabetic drugs targeting HDAC1 and/or associated miR.



Supported by: Danish Research Agency Grant #1331-00349B and the Novo Nordisk Foundation

PS 029 Beta cell mass imaging and regulation

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Graft revascularisation is essential for non-invasive monitoring of transplanted islets with radiolabeled exendin

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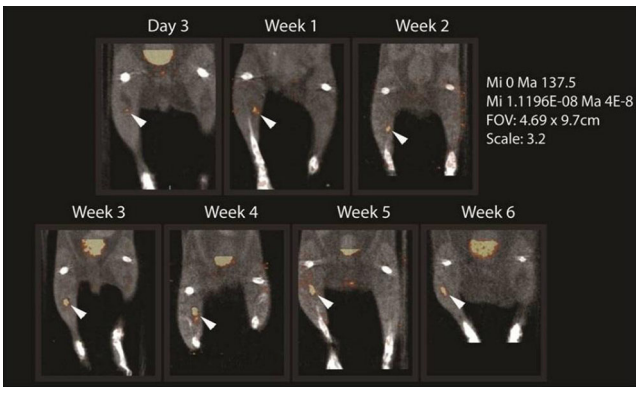
Background and aims: Pancreatic islet transplantation provides insulin independence for type 1 diabetes patients. However, the clinical outcome is still poor due to decline of islets survival in response to inflammatory and non-inflammatory processes. Clearly, development of non-invasive imaging tools visualizing islet transplants would improve the therapy by predicting islets survival. ^{111}In-exendin-3 is a promising tracer to non invasively visualize β-cells by SPECT (Single Photon Emission Computed Tomography). However, after isolation, islet vasculature is disrupted and therefore, delivery of the radiotracer to islet grafts early after transplantation may depend on their revascularization. Here we investigated the role of islet revascularization dynamics in promoting exendin-3 delivery to islet grafts and to image them after transplantation by SPECT.

Materials and methods: Syngeneic transplantation was performed in calf muscle of C3H mice with 800 islets. Mice were injected intravenously with 15 MBq of ^{111}In-exendin and scanned 1 h post-injection. SPECT was acquired at 3 days, 1, 2, 3 or 4, 5, 6 weeks. Exendin uptake was determined by SPECT quantitative analysis and immunostaining was performed against insulin and VEGFR-2 to confirm presence of β-cells and to quantify intra-islet vascular density, respectively. Data were expressed as means ± SEM.

Results: After 3 days, 0.013±0.003 kBq of the tracer accumulated in the transplants, which significantly increased up to 0.06±0.005 kBq at 4 weeks ($p<0.001$) and remained similar up to 6 weeks after transplantation (0.07±0.003 and 0.07±0.002 kBq, for week 5 and 6, respectively). First signs of revascularization coincided with low exendin-3 uptake by 3-day old grafts and increase in islets vascular density between 3 days and 3 weeks ($p<0.001$) correlated with the uptake of exendin-3 by the islets (Pearson Correlation coefficient $r=0.85$)

Conclusion: Islet revascularization is a key physiological process when monitoring islet transplants after in-vivo targeting of the β-cells with radiotracers. Stable and reproducible uptake of exendin was achieved starting 4 weeks post-transplantation, indicating that longitudinal assessment of islet survival by SPECT can reproducibly be performed after this time point.



Supported by: The People Programme (Marie Curie Action)

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Re-routing of insulin signalling in response to knockdown of PI3K-C2alpha promotes pancreatic beta cell proliferation

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Background and aims: Signaling via insulin receptor B-type (IR-B) and PI3K-C2α is required for glucose-stimulated insulin secretion and keeps the beta cell in a glucose-sensitive, differentiated state. We have recently observed that knock-down of PI3K-C2α in pancreatic beta cells, in contrast to most other cells, leads to increased proliferation and survival. The aim of this study was to evaluate the mechanism underlying the increased beta cell proliferation and survival after knockdown of PI3K-C2α.
Materials and methods: MIN6 cells, primary mouse and human beta cells were treated with control siRNA or siRNA against PI3K-C2α. Cell proliferation was measured by incorporation of labeled DNA precursors into nuclei. Protein expression and phosphorylation were evaluated by Western blot. Apoptosis rate was determined by triple staining with Hoechst 33342, propidium iodide and AlexaFluor488-annexinV. IR-B/Shc interaction was evaluated by FRET analysis and co-immunoprecipitation.
Results: Knockdown of PI3K-C2α led to increased cell proliferation of MIN6 cells (1.48±0.11fold), mouse (1.88±0.24fold) and human (1.92±0.21fold) primary beta cells. Cells treated with PI3K-C2α siRNA were more resistant to apoptotic stimuli (48.4±4.9% less apoptotic MIN6 cells when treated with 20 μM H_2O_2 and 32±2.9% less apoptotic MIN6 cells when treated with 6 μM staurosporine). The positive effect of PI3K-C2α knockdown on cell proliferation was insulin-dependent and was abolished both by IR-B-blocking antibodies ($p \leq 0.01$) and IR-B-blocking aptamers ($p \leq 0.01$). Knockdown of PI3K-C2α led to increased IR-B/Shc interaction (1.8±0.3fold). Knockdown of PI3K-C2α led to decreased signaling via the IR-B/PKBα-pathway resulting in a less glucose-sensitive/differentiated phenotype and to increased signaling via the IR-B/Shc/ERK-pathway resulting in enhanced proliferation.
Conclusion: Insulin resistance in the signaling cascade via IR-B/PI3K-C2α leads to partial loss of the differentiated, glucose-responsive state of the beta cell but at the same time promotes beta cell proliferation via a different signaling cascade involving Shc/ERK. PI3K-C2α represents a key regulatory element allowing the beta cell to switch between IR-B signaling leading to differentiation respective proliferation in response to insulin.
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Beta cell amount, function and turnover in non-diabetic obese vs lean subjects

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Background and aims: To better understand the relative roles of beta cell amount, function and turnover in the maintainance of glucose homeostasis in non-diabetic (ND) obese subjects (OB), we compared several beta cell characteristics in 13 OB (age 66±13 yrs; 4 M/9 F; BMI: 32.0±3.3 kg/m2) and 14 lean [LEAN; 64±15 yrs; 4 M/10 F; 22.5±1.8 kg/m2 ($p<0.01$ vs OB)] ND organ donors.
Materials and methods: Immunofluorescence staining was performed with pancreatic sections; glucose stimulated insulin secretion was assessed from isolated islets by the batch incubation method; laser capture microdissection (LCM) was used to acquire beta cell enriched samples from frozen specimens.
Results: Pancreatic insulin (INS) and glucagon (GLUC) areas were 0.71±0.34% and 0.27±0.25% in OB, and 0.85±0.50% and 0.22±0.15% in LEAN, with the respective INS/GLUC values of 3.0±1.1 and 4.1±1.3 ($p=0.06$). Cells positive for both INS and GLUC were 10.5% in OB and 7.0% in LEAN islets. Beta cell clusters (<4 cells) per mm2 were 7.8±4.9 in OB and 7.6±7.0 in LEAN (>90% in acinar tissue, the remaining in/close to ducts); similar proportions of OB and LEAN clusters contained only INS+ (around 60%) or GLUC+ (around 35%) cells; costained INS+ and GLUC+ cells were seen in 7.6 and 3.9% of clusters in OB and LEAN, respectively. TUNEL or Ki67 positive beta cells were similarly rare in the two groups. Glucose stimulated (16.7 mM glucose) INS release (μU/islet/min) was 0.093±0.048 from OB and 0.058±0.031 from LEAN (both $p<0.01$ vs basal) islets; INS stimulation index was higher with OB islets (3.4±1.2 vs 2.3±1.1, $p=0.03$), with a similar effect also seen with glibenclamide (3.4±1.1 vs 2.5±1.2) but not with arginine (2.4±0.9 vs 2.2±1.0). LCM was applied to islets and acinar or duct clusters. RIN values were >6 for islet beta cells and >4 for cluster beta cells, with respective concentrations of >4 ng/μl and approximately 100 pg/μl.
Conclusion: Beta cells in ND OB show greater insulin secretion to glucose and glibenclamide, but not arginine; the morphometric fetures of OB vs LEAN beta cells requires further studies; LCM use could allow detailed molecular investigation of OB beta cell properties.
Supported by: JDRF and IMIDIA

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In vitro imaging of GLP-1R as an approach to non-invasive in vivo pancreatic islet imaging

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Background and aims: Recently, exendin-4 derivatives targeting glucagon like peptide -1 receptor (GLP-1R) are emerging as promising candidates for the imaging of pancreatic β cell mass. Our project aims at developing and evaluating 1) multimodality imaging probes with exendin as the targeting ligand; 2) multivalent probes with 1 to 4 exendin to increase the avidity of the probe to receptor; and 3) inclusion of short and long PEG linkers to reduce the kidney uptake and enhance circulation half-lives for in vivo studies. In our studies, a defined cyclopeptidic scaffold was selected as delivery vehicle for these probes. All probes were labeled with Cy5 for fluorescence imaging.
Materials and methods: Dose response assays were carried out to determine the binding affinity (IC50) of exendin derivatives. Then, competition binding assays were designed to study the specificity to the GLP-1R. Finally, cell imaging experiments at 37°C and 4°C were conducted to visualize internalization of the probes. Assays were performed in CHL/hGLP-1R cells that ectopically express hGLP-1R and compared to the GLP-1R negative native CHL cell line.
Results: Dose response assays: All compounds showed affinities in the nanomolar range to CHL/hGLP-1R cells. The cyclopeptide carrying 2 exendins and short PEG linker showed the lowest IC50 values (1.0±0.1 nM) in CHL/hGLP-1R cells (see table below) No binding was observed in GLP-1R negative CHL cell line or other human control cell lines (HeLa, Panc-1). Competition binding assays: At high concentrations of unlabeled exendin, tagged exendin derivatives were unable to displace the untagged ligand. Cell imaging experiments: At 37°C Internalization of the probes into the cytoplasm was observed. However, at 4°C was mostly confined to the cell membrane indicating an active transport of the probes.
Conclusion: GLP-1R targeting cyclopeptide carrying multiple exendin copies show high binding affinities to the GLP-1R similar to exendin-4 (39aa). The competition assays and fluorescence imaging confirm the specificity of the probes to the receptor for receptor-mediated internalization

Exendin-4 derivatives with Cy5	IC_{50} (nM)
Exendin	2.6 ± 0.2
Cyclopeptide + 1 Exendin short PEG	7.1 ± 0.5
Cyclopeptide + 1 Exendin long PEG	2.9 ± 1.5
Cyclopeptide + 2 Exendins short PEG	1.0 ± 0.1
Cyclopeptide + 2 Exendins long PEG	2.3 ± 0.6
Cyclopeptide + 4 Exendins short PEG	2.8 ± 1.7
Cyclopeptide + 4 Exendins long PEG	3.0 ± 1.4

Supported by: SNSF, JDRF, BETAIMAGE, IMIDIA, BETATRAIN

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Cathelicidin antimicrobial peptide: a novel regulator of islet function and beta cell regenerative capacity

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Background and aims: Cathelicidin antimicrobial peptide (CAMP) is a naturally-occurring secreted peptide primarily expressed in immune cells and in epithelia that are in contact with the environment. CAMP has numerous pleiotropic roles in immunomodulation, wound healing and cell growth. We have previously demonstrated that Camp expression is up-regulated in the gut when type 1 diabetes (T1D)-prone BBdp rats are protected from diabetes development. Unexpectedly, we identified CAMP expression in the pancreatic β-cell, a previously unreported finding. Thus, we sought to characterize the novel roles of CAMP in the pancreatic islet and investigate its potential as a novel therapeutic for the treatment of diabetes.
Materials and methods: Immunohistochemistry was used to detect CAMP expression in the pancreatic islet. Changes in Camp gene were analysed by qRT-PCR in diabetes-prone BBdp and control BBc rats. Dispersed islets were loaded with an intracellular calcium indicator, FURA-2 AM, to assess calcium mobilization following treatment with the CAMP peptide (LL-37). Glucose-stimulated insulin secretion and glucagon secretion were evaluated under high or low glucose conditions, respectively, in the presence or absence of the CAMP peptide. Finally, a cohort of BBdp rats was treated daily for one week with either the CAMP peptide or saline control and duct-associated insulin+ cells were quantified to assess β-cell regenerative processes.
Results: CAMP expression colocalizes with insulin+ but not glucagon+ cells, indicative of the novel expression of CAMP in the β-cell but not the α-cell. Camp gene expression was significantly down-regulated in the islets of young diabetes-prone BBdp rats compared with control BBc rats prior to the onset of insulitis, suggesting early involvement in T1D pathogenesis. CAMP treatment of dispersed islets from both BBc and BBdp rats resulted in a significant increase in intracellular calcium levels. This effect was both delayed and blunted in the absence of extracellular calcium, indicating that CAMP promotes the mobilization of both extracellular and intracellular calcium stores. Consistent with the critical role of calcium mobilization in islet hormone secretion, CAMP treatment similarly promoted both insulin and glucagon secretion from islets isolated from BBc and BBdp rats. Daily treatment with the CAMP peptide for 7 days resulted in enhanced β-cell neogenesis. In addition, we identified changes in the gut microbiota in BBdp rats compared with BBc rats. In vivo CAMP treatment alleviated this gut microbiota dysbiosis and resulted in a shift in abundance in specific bacterial populations towards control BBc levels.
Conclusion: Our data indicate for the first time that CAMP is expressed in the islet β-cell and demonstrate a novel role for CAMP in pancreatic islet function and β-cell regenerative capacity. Importantly, by stimulating both insulin and glucagon secretion, CAMP may be promoting islet paracrine signalling thereby enhancing overall islet function and glucoregulation. Furthermore, changes in expression in diabetes-prone BBdp rats indicate that CAMP could play an important role in T1D pathogenesis. Taken together, these findings strongly suggest that CAMP could be used as a novel therapeutic target for diabetes.
Supported by: CIHR

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GCN2, a type 2 diabetes mellitus susceptibility gene, is associated with the regulation of pancreatic beta cell mass

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Background and aims: Single-nucleotide polymorphism (SNP) analysis of Japanese diabetes patients has revealed a significant correlation between a general control nonderepressible 2 (*GCN2*) SNP and type 2 diabetes mellitus (T2DM). GCN2 is a molecule activated by amino acid deficiency. Amino acid deprivation leads to accumulation of uncharged transfer RNAs (tRNAs), which induce the activation of GCN2. A comparison of GCN2 expression in different mouse tissues showed that GCN2 was prominently expressed in pancreatic islets. We analyzed the role of GCN2 in pancreatic β-cells and its involvement in the onset of T2DM.
Materials and methods: We generated generalized GCN2 knockout mice ($GCN2^{-/-}$mice), analyzed their metabolic parameters and histology. We also investigated changes in intracellular signaling by using islets from $GCN2^{-/-}$mice and INS-1 cells. Using isolated islets from wild-type mice fed a normal-chow diet (NCD) and a high-fat diet (HFD), we analyzed amino acid levels and relative charging levels of tRNAs.
Results: $GCN2^{-/-}$ mice fed a NCD did not exhibit any changes in glucose tolerance or pancreatic β-cell mass. However, $GCN2^{-/-}$ mice fed an HFD exhibited significant aggravation in glucose tolerance and reduction in pancreatic β-cell mass. Islets isolated from $GCN2^{-/-}$ mice showed significant increase in mTORC1 activity and decrease in insulin signaling. We also found elevated phosphorylation of TSC2 in islets from $GCN2^{-/-}$ mice, considered that chronic activation of mTORC1 resulted in reduction of insulin signaling through the negative-feedback mechanism, which is consistent with our previous findings on $TSC2^{-/-}$ mice. As $GCN2^{-/-}$ mice displayed reduction in pancreatic β-cell mass only when fed an HFD, we compared the activity of GCN2 in islets from wild-type mice, and found that GCN2 was markedly activated in islets from mice fed an HFD. Next, we investigated the conditions which activate GCN2 in pancreatic β-cells. In INS-1 cells, GCN2 was activated by glucose load, but not by other insulin secretagogues. Because insulin translation was enhanced in islets of mice fed an HFD, we constructed a hypothesis that amino acids were decreased by its consumption and GCN2 was activated when translation of insulin is enhanced. To verify our hypothesis, we compared amino acid levels in islets from wild-type mice fed a NCD with those fed an HFD, and found that a lot of amino acids are decreased in islets from mice fed an HFD. Moreover, we found decreases in charging level of tRNAs in islets from mice fed an HFD.
Conclusion: Our results showed that chronic activation of mTORC1 activity is one of the causes of reduction in pancreatic β-cell mass in $GCN2^{-/-}$ mice. During HFD load, insulin translation is enhanced, amino acids are decreased, and uncharged tRNAs accumulate in islets, which leads to the activation of GCN2. From our data, we consider that activated GCN2 contributes to the maintenance of pancreatic β-cell mass. We expect that $GCN2^{-/-}$ mice could be one of the models of the pathogenesis of T2DM in patients who have a SNP in GCN2.

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Calculation of radiation doses to the islets of Langerhans due to radionuclide imaging with exendin

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Background and aims: Recently, radiolabeled exendin was developed to non-invasively image the beta cells in the islets of Langerhans by Single Photon Emission Computed Tomography (SPECT) or Positron Emission Tomography (PET), aiming at increasing our insight into the pathophysiology of diabetes. The use of radiolabeled exendin could lead to concern about radiation-induced damage to the islets because it is known from literature that high doses of radiation (>10 Gy) can lead to islet death. Estimation of the potential radiation-induced damage requires a method for calculation of the radiation dose to the islets. Commonly used whole organ dosimetry methods do not contain the islets of Langerhans as a separate compartment and therefore do not provide the radiation dose to single islets. Due to high accumulation of the radiolabeled tracer in the islets as compared to the exocrine pancreas, whole pancreas radiation dose estimation will underestimate the actual radiation dose to single islets, while overestimating the dose in the exocrine pancreas. Therefore, the islets of Langerhans need to be treated as a separate compartment. In our work, whole organ dosimetry is combined with the islets of Langerhans as a separate compartment to calculate the islet radiation dose resulting from imaging of beta cells with radiolabeled exendin. Using this model, the islet radiation dose was calculated for different conditions (i.e. different radionuclides, healthy and diabetic patients, different islet sizes).
Materials and methods: The model includes the compartments that contribute to the islet radiation dose. The contribution of the pancreas and kidneys to the islet radiation dose was S-value based, while the self-dose of the islets was calculated using Monte Carlo simulations for a small sphere model (diameter: 50-400 μm). As input for the model both data from a clinical study (organ distribution of the tracer) and data from a preclinical study with Biobreeding Diabetes Prone (BBDP) rats (endocrine-exocrine uptake ratio, intra-pancreatic tracer distribution and beta cell mass), was used.
Results: The islet radiation dose was found to be small with a maximum of 74.3 mGy for the SPECT tracer ^{111}In-exendin (150 MBq injected activity per patient) and a maximum of 2.35 mGy for the PET tracer ^{68}Ga-exendin (75 MBq injected activity per patient). The activity in the islets contributes up to 45% to the total islet radiation dose for ^{111}In-exendin and up to 22% for ^{68}Ga-exendin. Due to the high accumulation of radiolabeled exendin in the kidneys, the kidneys have the largest contribution to the total islet radiation dose (49-81%).
Conclusion: The resulting islet radiation doses suggest that even repeated exendin imaging will not lead to islet death, since the maximum islet radiation dose is smaller by a factor of 130 - 4000 than the one known to cause serious islet damage (>10 Gy). Further reduction of the total islet radiation dose may be achieved by reducing the activity in the kidneys, for example by co-injection with gelofusin or injection of lower amounts of activity.
Clinical Trial Registration Number: NCT01825148
Supported by: IGMD

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Effects of obesity and diabetes on alpha and beta cell mass in surgically removed human pancreas

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Background and aims: The physiological and pathophysiological changes in beta cell mass (BCM) and alpha cell mass (ACM) in humans in the face of obesity and diabetes (DM) remain unclear. We have previously reported that there was no increase in BCM in Japanese obese individuals compared with lean subjects, however, the study was based on autopsy pancreas where we were not able to exclude the possibility of various confounding factors. The aim of this study was that, by using surgically removed pancreas samples, to clarify 1) effect of obesity or duration of obesity on ACM and BCM, 2) relationship between the glycemic markers (HbA1c and glycated albumin (GA)), beta cell function and ACM or BCM, and 3) relationship between postoperative glycemic control and ACM or BCM in humans.
Materials and methods: We obtained human pancreas samples from 69 individuals (41 men, body mass index (BMI) 22.2±2.9 kg/m2, age 66± 12 years (mean±S.D)) who underwent pancreatic surgery between the years 2000 and 2012. Twenty-nine patients were diabetic before operation. Pancreatic sections were stained for insulin or glucagon, and fractional beta (%BCA) or alpha cell area (%ACA) was measured, respectively. In addition, we evaluated the history of obesity by questionnaires to the patients.
Results: 1) In the non-diabetic (NDM) group (N=40), there was no significant correlation between BMI and %BCA or %ACA. Cases in NDM were divided into lean and obese groups (mean BMI 21.6±2.0 vs. 26.4±1.3 kg/m2). Either %BCA or %ACA in obese group was not significantly increased compared with lean group. Cases in NDM were also divided into two groups according to the duration of obesity (less than 10 years and 10 years of more). However, there was no significant difference in %BCA or %ACA between the two groups. 2) In the patients with DM (type 2 or pancreatic DM, N=29, 66±8 years, BMI 21.6± 3.1 kg/m2), %BCA was significantly decreased by 56% compared with age- and BMI-matched NDM group ($P < 0.01$), while there was no change in %ACA. %BCA, but not %ACA was significantly negatively correlated with HbA1c or GA ($r=-0.44$, $P=0.04$ and $r=-0.46$, $P < 0.01$, respectively). Furthermore, there was a significant positive correlation between %BCA and serum C-peptide to plasma glucose ratio before operation ($r=0.37$, $P=0.03$). 3) To clarify the relationship between postoperative glycemic control and %ACA or %BCA, five cases who underwent total pancreatectomy were excluded. There was no significant correlation between HbA1c at 6 months after operation and either %BCA or %ACA. However, %BCA, but not %ACA was significantly negatively correlated with GA at 3 and 6 months after operation ($r=-0.60$, $P < 0.01$ and $r=-0.59$, $P= 0.01$, respectively).
Conclusion: We confirmed that there was no increase in either BCM or ACM in Japanese nondiabetic obese individuals who undertook pancreas surgery, supporting the hypothesis that beta cell regenerative capacity is limited in Japanese population. BCM was decreased by 56% in patients with diabetes, while there was no change in ACM in those individuals. BCM, but not ACM was associated with preoperative and postoperative glycemic control. These findings further support the concept that BCM rather than ACM has a major role in regulating blood glucose level.

PS 030 Islet autoimmunity and inflammation

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The importance of Zinc transporter 8 autoantibody (ZNT8A) in the diagnosis of type 1 diabetes in the Brazilian population

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Background and aims: Recently, the Zinc transporter-8 (ZnT8), an islet cell secretory granule membrane protein, was identified as an autoantigen in T1D. Autoantibodies to ZnT8 (ZnT8A) complement the established antibodies to insulin (IAA), to GAD65 (65 glutamic acid decarboxylase, GAD65A), and to protein tyrosine phosphatase (insulinoma-2 antigen, IA2A) in Caucasians, but there are few data in Latin-America and in the very heterogenous Brazilian population, with diverse patterns of admixture.
Materials and methods: For this purpose, we analyzed 479 T1D patients (12.3±7.7 yrs., diabetes duration 10.9±11.5 yrs.) and 329 controls (27.2 ±10.6 yrs). Serum levels of GAD65A and IA2A were determined by radioimmunoassay (RSR limited, UK; CV <7%). Serum levels of Znt8A were obtained by ELISA (Kronus, USA CV <7). Chi- square test, Fisher exact test and Mann-Whitney test were used in statistical analysis. $P \leq 0.05$ was considered significant.
Results: The Znt8A frequency of all patients analyzed (48.3%) was similar to that of GAD65A (47.7%) and IA2A (45.4%), but GAD65A frequency was slighted greater than that of IA2A (OR=1.25; CI: 1.016-1.559; p=0.04). All three autoantibodies frequencies declined in a similar way following years after diagnosis, being more intense after the first year. The three autoantibody titers also declined with diabetes duration, being much more faster to ZnT8A. There was a negative correlation among ZnT8A titers with age of blood collection (r=-0.369; p<0.0001) and diabetes duration (r=–0.439; p<0.0001), but not with age at diabetes onset (r=-0.006; p=0.91), even when considering only those patients with new onset T1D (less than two years of diagnosis). Titers and frequency of ZnT8A and IA-2A did not differ with respect to gender and ancestry, whereas GAD65A titers were greater in females (12.0±23.9× 6.8±14.9 IU/mL; p=0.003) and in those of European ancestry (10.6± 22.2×6.4±12.6 IU/mL; p=0.01). Among patients with only one islet autoantibody, ZnT8A, GAD65A and IA-2A corresponded to 10.1%, 10.1% and 4.3% respectively. So, the determination of ZnT8A allowed the additional diagnosis of DM1 in 10.1% of patients and, only 3.8% of recent-onset T1 D patients, (until 2 year after diagnosis) remained three autoantibodies negative The CTLA4 +46A/G was slighted associated with greater frequency of ZNT8A (80.4%×66.7; OR 2.048, CI=1.138-3.685; p=0.023). ZnT8A was rare in controls, being the only autoantibody observed in seven control subjects (1,5%) who were GAD65A and IA-2A negative, and its frequency was similar to that GAD65A (1.6%) and IA2-A (1.6%) in controls.
Conclusion: The prevalence of Znt8A in a Brazilian population (Sao Paulo city) was higher than previously reported in Asiatic patients with T1D, but similar to that reported in Caucasians. Therefore, ZnT8A can be considered a marker for T1D autoimmunity even in heterogenous populations.
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In situ detection of components of innate immune response in human insulitis

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Background and aims: Type 1 diabetes (T1D) is an autoimmune disease in which both adaptive and innate immune responses are involved in target organ destruction and dysfunction. The aim of the present study was to analyze pancreatic expression of components of innate immune system (neutrophils; CCL2 and its receptor CCR2; CXCL10 and MICA) in human T1D.
Materials and methods: We analyzed pancreatic specimens obtained from 4 T1D, 4 autoantibody-positive non-diabetic (AAb+) and 6 autoantibody-negative non-diabetic (CTR) organ donors from the nPOD cohort, as well as from pancreatic biopsies obtained from 4 T1D transplanted patients from the nPOD-Transplantation (nPOD-T) cohort. Formalin-fixed and paraffin embedded sections were used in immunohistochemical investigations to analyze the expression of neutrophils, CCR2 and CXCL10. In addition, triple and/or double immunofluorescence with confocal microscopy analysis, was performed to identify islet cells subset(s) expressing CCL2 chemokine and MICA protein (a ligand for NK and CD8 T-cells). Colocalization between CCL2 and MICA with insulin and glucagon was performed by using Las AF software.
Results: Immunohistochemical experiments revealed that neutrophils were detectable in pancreatic exocrine tissue at higher frequency in T1D and in AAb+ vs CTR (p:0,0095 and p:0,038 respectively). Neutrophils were also found in pancreatic exocrine tissue of T1D transplanted patients. CCR2+ cells were scattered in exocrine tissue with the following trend: T1D> AAb+>CTR. The expression of CXCL10 was found in all insulin containing islets of T1D patients and in several islet cells from AAb+ donors, while was virtually undetectable in CTR islets. In nPOD-T donors the expression of CXCL10 was found in several islets. Immunofluorescence analysis revealed that CCL2 was expressed almost exclusively by beta cells in islets of T1D, AAb+ and CTR, as well as of nPOD-T subjects. The same result was detected in islets of transplanted pancreata of nPOD-T subjects. Finally, MICA was expressed in few insulin containing islets with inflammatory infiltrates, exclusively in beta cells in pancreata from transplanted patients of the nPOD-T cohort but not of other nPOD organ donors.
Conclusion: Our findings strengthen the hypothesis that: 1 Innate immunity is indeed involved in T1D pathogenesis. 2 Residual presence of insulin containing cells is associated with ongoing islet inflammation. 3 Immune infiltration affects the whole pancreas and not only pancreatic islets.
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Increased leucocytic infiltration of islets and exocrine tissue in organ donors with prolonged duration of life support

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Background and aims: Leucocytic infiltration of the human endocrine pancreas is considered to be the hall-mark of recent onset type 1 diabetes in young patients. Recently, it has been proposed that a more general infiltration of both endocrine and exocrine pancreas can be found in most type 1 and type 2 diabetic patients. To interpret these findings, it is

important to have insight into the variability of leucocytic infiltration in the normal pancreatic gland in function of the clinical characteristics of the patient. This study assesses the correlation between the duration of life support and the presence of a leucocytic infiltrate in human donor pancreas. It extends our previous observations, using immunophenotyping to identify and quantify the infiltrating leucocytes, study the kinetics of the infiltration in a series of organ donors with increasing duration of life support, and differentiate between infiltration in the exocrine tissue and in the endocrine pancreas.

Materials and methods: Pancreas biopsy specimens from non-diabetic organ donors with different duration of life support (0, 3, 6, 9 and ≥12 days) were used (n=10 per time point). Sections were double-stained for CD68, CD45, CD3, CD8 and CD20 in combination with insulin and digitally imaged to quantify the number of leucocytic cells in total pancreas and islets of Langerhans.

Results: Donor pancreata from patients with a short duration of life support (0-3 days) showed low levels of infiltrating leucocytic cells. Infiltration levels strongly increased from 6 - ≥12 days of life support onwards: CD68+ cells were found to increase 10-fold between day 0 and day 6 ($p<0.0125$), remaining at elevated levels at day 9 and ≥12 ($p<0.0025$), with up to 9% of total cells found to be CD68+ in some donors (Figure 1). CD45+, CD3+, CD8+ and CD20+ cells were less markedly increased, with a significant ($p<0.0125$) 5-fold increase for CD45 and a significant ($p<0.0125$) 2-fold change for CD8 and CD20 between day 0 and day 9. The number of CD68+ cells in the islets showed a significant 2.5-fold increase between 0 and ≥12 days ($p<0.05$), whereas other leucocyte cell types were present in low numbers and did not show significant changes between the two groups.

Conclusion: These results show that increased duration of life support is associated with increased leucocytic infiltration in the endocrine and exocrine human donor pancreas from day 6 of life support onwards, affecting 15-33% of pancreatic organ donors. The effect is strongest for CD68+ cells. Clinical conditions around the time of organ retrieval should therefore be taken into account when studying inflammatory lesions in the diabetic pancreas and necessitate adequate matching of patient samples.

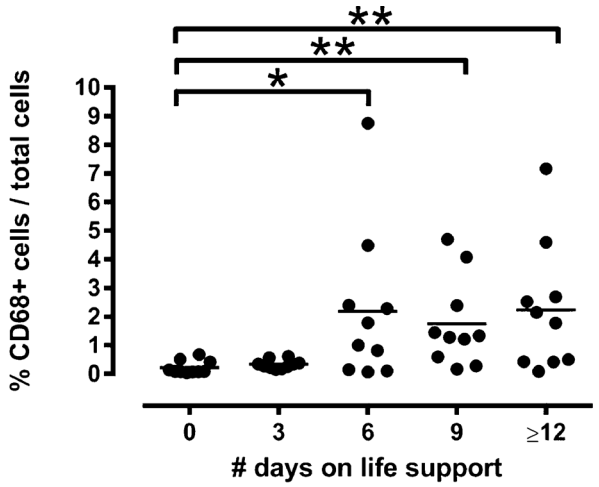

Figure 1. The percentage of infiltrating CD68+ cells in the pancreas, expressed in function of the number of days on life support. (Mann-Whitney U test; * = P<0.05/k and ** = p<0.01/k; with k = # independent comparisons = 4 (Bonferonni correction))

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STZ treatment causes immediate depletion of immune cells in sciatic nerve and dorsal root ganglion

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Background and aims: Streptozotocin (STZ) treatment is common method for the induction of type 1 diabetes in rodent models. However, it has been shown that STZ induces thermal hyperalgesia in mice, independently of hyperglycemia, and is neurotoxic in vitro by induction of oxidative stress. These findings bring into question the limitations of STZ-induced diabetes as a model for diabetic neuropathy. Tissue resident macrophages constitute the first line of response to toxic challenges by clearing debris from apoptotic cells via their scavenger receptors. In this study, the effect STZ treatment on macrophages resident in sciatic nerve (SN) and dorsal root ganglions (DRG) and determine whether the observed thermal hyperalgesia was associated with neuronal inflammation.

Materials and methods: Healthy, wild-type mice were sacrificed 5 days after three consecutive treatments of STZ (50 mg/kg/day; i.p.,n=5) or after 1 week, 3 weeks or 3 months after five consecutive treatments. Following sacrifice, peritoneal cavity lavage was collected, along with the DN and DRGs, which were digested and stained with antibodies against CD45, MHC class II, CD68, CD11b, Ly-6C, mannose receptor CD206 and activated NFkB and analysized by flow cytometry. Thermal hyperalgesia was measured by Hargreaves. Statistical significance was tested with two-tailed student's T-test.

Results: Transient hyperalgesia was detected 4 days after initiation of treatment with STZ (resonse times was decreased by 1.3 secs, $p<0.01$) before significant difference in blood glucose levels had developed. Thermal hyperalgesia was also evident at 6 days (-0.9 secs, $p<0.01$) but had disappeared by 3 weeks. The number of CD68+ macrophages in the DRG was significantly reduced at 5 days after treatment (from 9% of all nucleated cells to 2%, $p<0,001$). The number of CD68+ macrophages were not significantly reduced in the SN at this point, but SN macrophages displayed increased activation of NFkB (1.9 fold increase of flouresence intensity $p<0.01$ and 1.7. fold increase of number of positive cells $p<0.05$). At 1 and 3 weeks, CD68+ cells in SN of STZ-treated mice were reduced to 54% and 39% respectively, as compared to control ($p<0.05$), and in particular macrophages expressing CD206 were depleted (from constituting 70% to 50% of CD68+ macrophages after 1 week, $p<0.01$). None of these changes were evident in macrophages obtained by peritoneal lavage at any of the studied time points, excluding the explanation of direct effects of STZ on macrophages. There were no signs of STZ-induced immediate inflammation in the nerve. MHC class II was at no point up-regulated on macrophages and there was no evidence of inflammatory infiltration of CD45+CD11b+LY6C+ monocytes at 1 week. As a result of the CD68+ macrophage depletion, number of intraneural CD45+ cells was significantly reduced (to 64% and 41% of control at 5 days and 1 week respectively, $p<0.05$ and <0.001). STZ-induced lymphopenia of peripheral nerve gradually normalized over the course of 12 weeks.

Conclusion: Our findings are consistent with a non-inflammatory innate immune response in the peripheral nervous system due to a toxic injury induced by STZ. Depletion of M2 macrophages by STZ in SN may affect the regenerative capacity of the nerve in response to further injury. Symptoms of neuropathy observed during the first three months of STZ-induced diabetes may therefore be indirectly affected by the neurotoxic effects of STZ.

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Functional silencing of CD4+ T-cells by the modulating RIB5/2 antibody prevents islet autoimmunity and systemic prediabetic inflammation in the LEW.1AR1-IDDM rat model

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Background and aims: The LEW.1AR1-*iddm* rat is an animal model of spontaneous autoimmune diabetes. Islet infiltration occurs within a narrow time range between 40 and 60 days after birth with proinflammatory cytokine peaks in blood PBMCs. The rat-specific anti-CD4 antibody RIB5/2 confers temporal internalization of the T-cell receptor complex without depletion of T cells. It was the aim of this study to investigate if RIB5/2 treatment can ameliorate the proinflammatory state in prediabetic LEW.1AR1-*iddm* rats.
Materials and methods: Normoglyceamic LEW.1AR1-*iddm* rats were treated between the age of 40 and 50 days with the monoclonal anti-CD4 antibody RIB5/2 (5×5 mg ab/kg b.w. i.p.). 200 μl blood were collected at day 40 (before antibody application), day 50 and day 60. RNA was purified from peripheral blood mononuclear cells (PBMCs) that were isolated by Ficoll density-gradient centrifugation. The RNA was used for gene expression analysis of proinflammatory (*TNFα, IFNγ, IL-1β*) and antiinflammatory cytokines (*IL-4, IL-10*), T-cell markers (*CD25, CTLA4, NRP1*), *L-Selectin, TGF-β* and *FoxP3* by real-time RT-PCR. Rats were monitored until the age of 120 days. Serial pancreatic sections were stained with Haematoxylin-Eosin (HE) to document the islet infiltration process.
Results: All 12 treated LEW.1AR1-*iddm* rats remained normoglycaemic and developed no diabetes until age of 120 days. In the control cohort the diabetes incidence was 60% after 70 days. In comparison to untreated rats the antibody treated animals revealed by HE- and T-cell staining no lymphocytic infiltrations in pancreatic sections. Treatment with the anti-CD4 antibody RIB5/2 resulted in significantly ($P<0.05$) reduced gene expression levels of *TNFα, INF-γ, IL-4, NRP1* (1.4 - 8-fold) at most 12 days after the last administration of RIB5/2 (age 60 days) compared with infiltrated animals. The treatment led to significantly higher expression of the regulatory genes *TGF-β, FoxP3* and *L-Selectin* (1.2 - 3-fold) but also significant high levels of the T-cell silencer signal *CTLA4* (6-fold) with an age of 50 and 60 days in compared with infiltrated animals.
Conclusion: The anti-CD4 antibody RIB5/2 treatment conferred temporal functional CD4 T-cell silencing without significant immunosuppressive side effects in the LEW.1AR1-*iddm* rats. The treatment induced permanent tolerance against beta cell autoimmunity with downregulation of proinflammatory gene signatures in PBMCs. Moreover, this strategy effectively prevented islet infiltration and beta cell destruction. The data indicate that CD4+ T-cells play a key-role within systemic inflammatory processes of islet autoimmunity. T-cell silencing by modulating antibodies may be an attractive option for prevention of human T1DM.
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The dependence of the insulin-induced cytokine levels production by basic genetic markers in the patients with autoimmune diabetes

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Background and aims: Numerous studies in different countries have been proven association of some single nucleotide polymorphisms (SNP) of the gene CTLA-4 with the development of autoimmune diabetes (T1DM). Its protein product expressed by natural T regulatory cells that control autoimmune inflammation. br />The aim of this study was to investigate the dependence on the insulin-induced cytokine levels production by genotype polymorphisms options rs 231775 and rs 3087243 CTLA-4 gene.
Materials and methods: We examined polymorphisms rs 231775 and rs 3087243 CTLA-4 gene. These two SNPs of CTLA-4 gene were analyzed in 134 patients with T1DM. The control group consisted of 108 blood donors without autoimmune disease and family history on them. Allele identification was performed with Real-Time PCR technique. Association of genetic markers with pathology was considered statistically significant when p-value<0,05. At the same time we examined cytokines' levels in 30 patients with T1DM and 10 healthy people. Mononuclear leukocytes were isolated by centrifugation in the ficoll-verographin density gradient. The cells thus obtained were resuspended in the complete nutritient medium reducing their concentration to 2,0×10^6/ml. Phytohemagglutinin (PHA-P) (10 mcg/ml) and insulin (insulin human) (10 mcg/ml) were added to the samples to stimulate mononuclear leukocytes; cell suspensions were further incubated for 36 hours. Initial, PHA-induced and insulin-induced levels of interleukin-1 (IL-1), IL-2, IL-4, IL-6, IL-7, IL-8, IL-10, IL-12, gamma interferon (IFN-γ) and tumor necrosis factor alpha (TNF-α) in supernatants of cell cultures were measured by solid phase immunoassay at 450 nm. Statistical analysis was performed by analysis of variance adjusted for sex and age. It was calculated the exact two-sided Fisher's test and p-value.
Results: 1) Insulin-induced levels of IL-1, IL-2, IL-4, IL-6, IL-7, IL-12 and IFN-γ were significantly higher in patients with G/G genotype of polymorphism rs 231775 CTLA-4 gene in comparison with genotypes A/A and A/G of the same polymorphism (IL-1 - p=0,0042/p=0,0005; IL-2 - p=0,0025/p=0,0002; IL-4 - p=0,0036/p=0,0036; IL-6 - p=0,0004/p=0,0004; IL-7 - p=0,0176/0,0070; IL-12 - p=0,0573/0,0097 and IFN-γ - p=0,0043/p=0,0004).
2) At the same time, insulin-induced levels of IL-1 and IL-6 were significantly higher in the patients with G/G genotype of polymorphism rs 3087243 CTLA-4 gene in comparison with genotype A/G of the same polymorphism (IL-1 - p=0,0357 and IL-6 - p=0,0080).
3) Insulin-induced levels of IL-6, IL-8, IL-10, IFN-γ and TNF-α were significantly higher in the patients with T1DM in comparison with healthy people (IL-6 - p=0,0215, IL-8 - p=0,0002, IL-10 - p=0,0033, IFN-γ - p=0,0228 and TNF-α - p=0,0034).
Conclusion: The high levels of IL-6, IL-8, IL-10, IFN-γ and TNF-α testify quite aggressive autoimmune inflammation in the patients with T1DM. G/G genotypes of rs 231775 and rs 3087243 CTLA-4 gene polymorphisms are the markers of natural T regulatory cells insufficiency, which is reflected in the levels of insulin-induced cytokine production in the patients with T1DM.

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Betatrophin in newly diagnosed type 1 and 2 patients: preliminary results

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Background and aims: Betatrophin is a factor produced in the liver and white adipose tissue, regulating the metabolism of lipids, especially triglycerides. There is limited data about this recently discovered molecule. It has been shown that in mice, betatrophin significantly improves beta cell function. Preliminary studies in humans suggest that elevated betatrophin concentration may be associated with an increased risk for type 2 diabetes (T2D) and cardiovascular diseases. Elevated concentration of this molecule was also observed in the longitudinal type 1 diabetes (T1D). The aim of the study was to compare the concentration of betatrophin in newly diagnosed patients with autoimmune diabetes (AD - type 1 and LADA), type 2 diabetes and in the control group.

Materials and methods: The study enrolled 80 patients with newly diagnosed diabetes: 28 patients with AD (mean age 36±1.98; mean BMI 23.8±0.94 kg/m2), 26 patients with T2D (mean age 37.7±1.7; mean BMI 29.5±0.93 kg/m2) and 26 healthy controls (mean age 39.5±2.25; mean BMI 25.5±0.94 kg/m2). All subjects were measured also C-peptide concentrations in the glucagon test, the presence of antibodies against beta cells (GADA, IA-2A, IAA), concentration of interleukin 15 (IL15) and lipids profile with free fatty acids (FFA). Concentrations of betatrophin, IL15, FFA, C-peptide, anti-islet antibodies were measured by ELISA and lipid profile-by Cobas analyser.
Results: Serum betatrophin concentrations were higher in the newly diagnosed AD and T2D compared with healthy controls with significant difference between AD and the control group (p=0.02). C-peptide concentration was significantly lower in AD in comparison to the control group (p<0.001). We found significantly lower total cholesterol concentration in AD group vs T2D (p=0.036), HDL-cholesterol concentration in T2D vs control group (p=0.04), LDL cholesterol concentration in AD vs control group (p=0.02), significantly higher triglycerides concentration in T2D vs AD (p=0.003) and in T2D vs control group (p=0.01). Concentration of betatrophin in T2D correlated negatively with the patient's weight (r=-0.4; p=0.04), C-peptide (r=-0.44; p=0.03) and IL15 level (r=-0.47; p=0.01).
Conclusion: Higher betatrophin concentration in newly diagnosed AD and T2D in comparison to the control group, may suggest potential role of betatrophin in the pathogenesis of diabetes, in particular AD. Although Il-15 seems to be important in the pathogenesis of T1D, we found the association between betatrophin and IL15 in T2D. A further research are needed. For that reason, we are going to check the above-mentioned parameters in another 170 subjects soon.

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Liquefied pancreatic ECM: a novel encapsulation platform for diabetes therapy

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Background and aims: Despite the large potential of beta cells encapsulation for diabetic therapy, and the extensive knowledge gained in the field, severe hurdles impede their clinical application. Those include the limited longevity of encapsulated beta cells, and the polymers used, which provide immunological and mechanical protection, but do not mimic the cell biological niche. Extracellular matrix (ECM) plays a vital role in the function of mature tissue. Not only it provides a physical niche for cellular attachments, the ECM is also responsible for transmitting signals, which affect cellular differentiation, maintenance, and biological function. Particularly, ECM based materials were demonstrated to improve beta cells culture and insulin secretion. Therefore, the aim of this study was to develop a unique cell microencapsulation platform, which is based on isolated natural pancreatic ECM hydrogel, and entraps cells that are induced to transdifferentiate into insulin producing cells and to provide a pre-clinical evidence for its applicability for diabetes therapy.
Materials and methods: Insulin-secreting human transduced mesenchymal stem cells and liver cells were encapsulated in gelled ECM—produced by enzymatic decellularization and liquefaction of porcine pancreatic tissue—and injected into diabetic mice.
Results: The newly-developed ECM-microencapsulation system provided the cells with a natural fibrous niche that supports cell viability, and significantly improves differentiation and insulin secretion (up to 5-fold increase comparing to transduced non-encapsulated cells, p 0.001). Moreover, the ECM-encapsulated cells were immunologically silent, as concluded from the negligible levels of pro-inflammatory cytokines in the peripheral blood, the normal values of complete blood count and the viability of the cells within ECM-capsules even four weeks following subcutaneous transplantation to mice. Most importantly, transplantation of approximately 5.5×10^5 ECM-encapsulated transduced cells to streptozotocin induced diabetic mice significantly ameliorated hyperglycemia for up to four weeks. This was demonstrated in reduced blood glucose levels which were maintained less than 300 mg/dl through all the experiment, comparing to the blood glucose levels of the untreated group (approximately 350 mg/dl). Further, fructosamine concentration test and glucose tolerance test were performed two weeks post transplantation. The treatment with encapsulated transduced cells had reduced the fructosamine levels of the diabetic mice to levels of healthy, non-diabetic mice (p 0.005). Similarly, treated mice responded to glucose tolerance test with a clearance rate that paralleled that of healthy mice, whereas in the untreated group no glucose clearance was evident.
Conclusion: Our results indicate that ECM-microcapsules not only provide the transdifferentiated cells with immunological and mechanical protection, but also support cell function by mimicking their natural niche. Thus, transplantation of the ECM-encapsulated cells, even in minimal quantities, to diabetic mice results with a significant amelioration of their condition. Altogether, the advantages of this ECM-encapsulation system, clearly point at our ECM-based microencapsulation platform as a promising prospective cell-based therapy for the treatment of diabetes.

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Bioactive nanocoatings: the integration of active anti-coagulant and anti-inflammatory therapeutics in multilayer nanocoatings to improve survival of pancreatic islets

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Background and aims: There are still many obstacles to successful islet transplantation, the main one being the loss of the functional beta cell mass in the early post-transplantation period, where up to 70% of the islet cells become functionally impaired due to the instant blood mediated inflammatory response (IBMIR). Conformal nanocoating may be one way in which to protect islets in this early time period by providing a nano-thin, protective shield around the islet without changing the islet volume. This would allow transplantation to the clinically relevant intraportal site whilst still providing protection against cell interactions and the hostile microenvironment. Importantly, this coating also has the advantage of being able to incorporate therapeutically active molecules which can target this IBMIR effect. We have developed a novel nanocoating scheme using incorporated anti-IBMIR agents to protect the islets and reduce islet cell destruction in the immediate post-transplantation period.
Materials and methods: Islets were isolated from ICR mouse pancreata by collagenase digestion and Histopaque®-1077 gradient. The nanocoatings were constructed using an anti-coagulant heparin polyaldehyde and glycol chitosan alternating layering scheme and incorporated the anti-inflammatory protein α1-antitrypsin with differing densities of aldehyde groups to alter layering capacity. Anti-coagulant activity, islet viability and function in vitro and in vivo were assessed.
Results: By controlling aldehyde density generated on heparin at below 5%, the cell viability was greater than 99% in both groups and nanocoated islets had similar dynamic insulin secretion to non-coated islets. Mice transplanted with with nanocoated islets had comparable blood glucose concentrations compared to those transplanted with non-coated islets. The nanocoating incorporating α1-antitrypsin exhibited significant anti-coagulant activities in an in vitro clotting assay (strand formation time: 276±6 s vs 235±15 s, control, P<0.05, n=5).
Conclusion: The anti-coagulant and anti-inflammatory activities of our nanocoating have shown that therapeutic molecules can be successfully incorporated. These results demonstrate the potential of this approach for reducing donor cell induced inflammatory responses especially at the crucial early post-transplantation time points, thus improving transplant outcome.
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PS 031 Clinical immunology

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Levels of insulin antibodies negatively associate with insulin sensitivity in autoantibody-positive adults with newly diagnosed diabetes

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Background and aims: Increasing evidence indicates that the pathogenesis of type 1 diabetes (T1D) is not only driven by beta cell-directed autoreactivity but also by insulin resistance (IR). Autoimmunity in T1D is characterized by the presence of antibodies against beta cell-associated molecules, such as islet cell antigen (ICA), glutamic acid decarboxylase (GAD) and insulin, the dominant target autoantigen in T1D. Autoantibodies typically emerge in the prediabetic phase and persist after disease onset. The link between IR and autoimmunity might be relevant for monitoring diabetes progression and adaptation of therapeutic strategies, particularly in the early phase following disease diagnosis. In our current study we therefore hypothesized that IR associates positively with circulating beta cell-directed autoantibodies in patients with newly diagnosed T1D.
Materials and methods: From the German Diabetes Study, 156 patients (97 male, 59 female; mean age 36±11 years; diabetes duration 196± 94 days, A1c 48.6±8.8 mmol/mol) who were positive for at least one antibody against ICA (immuno fluorescence), GAD or insulin (radioimmunoassay) were selected. Insulin sensitivity (M-value) of the patients was determined by hyperinsulinemic-euglycemic clamp tests.
Results: In the patients circulating insulin-directed antibodies (IAA) were found to be correlated with M-values. The levels of IAA had a median of 0.9 U/ml with an interquartile range of 0.2 to 7.4 U/ml. The clamp studies yielded M-values with a median of 8.2 mg * kg-1 * min-1 with an interquartile range of 6.4 to 10.4 mg * kg-1 * min-1. Correlation analyses by applying an unadjusted model revealed that doubling of IAA levels decreased the M-value by 2.6% ($p=0.023$). After adjustment for age, sex and body mass index (BMI), doubling the IAA level resulted in a 3.9% lowering of the M-value ($p<0.001$). Additional adjustment for the patients´ C-peptide concentration and fasting blood glucose levels as well as for their daily insulin dose did not affect the correlation between IAA and M-value ($p=0.009$). In contrast, levels of antibodies against GAD or ICA did not correlate with insulin sensitivity.
Conclusion: From our results we conclude that in patients with newly diagnosed T1D expressing at least one beta cell-directed antibody, insulin sensitivity negatively relates to IAA, but not to other autoantibodies. This finding may be of clinical relevance for monitoring the course of the disease and for optimizing the treatment of T1D in the early phase after disease onset.
Supported by: DZD

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Autoimmune diseases in children and adults with type 1 diabetes from the type 1 diabetes exchange clinic registry

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Background and aims: Type 1 diabetes (T1D) is often associated with other autoimmune diseases (AD), but screening guidelines for these ADs in T1D patients are not well characterized or evidence based. To better understand the factors associated with and the prevalence of AD in people with T1D, we analyzed the frequency of AD and associated factors in all individuals in the T1D Exchange Clinic Registry database.
Materials and methods: Diagnoses of AD, represented as ordinal categories: 1) no AD, 2) 1 AD, or 3) >1 AD based on the list of diseases in the Table, were obtained from medical record review of 25762 T1D Exchange Clinic Registry participants diagnosed with type 1 diabetes (50% female, 82% non-Hispanic white, mean age 23 yrs, mean duration of diabetes 11 yrs). Ordinal logistic regression was used to identify demographic and clinical factors associated with the diagnosis of AD.
Results: Of 25762 people diagnosed with T1D, 6588 (26%) were diagnosed with at least 1 additional AD: 5054 (20%) with 1 AD and 1534 (6%) with>1 AD. The Table displays the percent diagnosed by specific AD. Of the most common ADs, 4871 (19%) were thyroid diseases, with Hashimoto's disease /hypothyroidism being the most common of the thyroid diseases, and 1428 (6%) were celiac disease. When comparing >1 AD with one AD and no AD, subjects with>1 AD were more likely to be slightly older (mean 29 yrs vs 28 yrs vs 21 yrs; $p<0.001$), female (71% vs 62% vs 45%; $p<0.001$), non-Hispanic White (89% vs 88% vs 80%; $p<0.001$), and have a longer T1D duration (mean 15 yrs vs 14 yrs vs 9 yrs; p&lt0.001). Mean HbA1c was similar between AD groups: 8.1% for &gt 1 AD, 8.2% for 1 AD and 8.3% for 0 AD.
Conclusion: In the T1D Exchange Clinic Registry, diagnosis of one or more AD in addition to T1D is common, particularly in women, older participants, and non-Hispanic whites. Autoimmune thyroid disease and celiac disease are the most common associated ADs. Further studies are needed to better understand contributing factors and the prevalence of new diagnosis of AD to aid in the development of AD screening recommendations for patients with T1D.

Table. Frequency of Diagnosis of Autoimmune Disease Overall and by Gender[a]

	Diagnosed with Autoimmune Disease N (%)		
		Gender	
	Overall (N=25762)	Male (N=12857)	Female (N=12905)
Number of Diagnosed Autoimmune Diseases[b]			
0	19174 (74)	10497 (82)	8677 (67)
1	5054 (20)	1911 (15)	3143 (24)
2	1311 (5)	389 (3)	922 (7)
≥3	223 (<1)	60 (<1)	163 (2)
Any thyroid disease	**4871 (19)**	**1625 (13)**	**3246 (25)**
Hashimoto's and/or hypothyroidism	4655 (18)	1566 (12)	3089 (24)
Graves' and/or hyperthyroidism	372 (1)	98 (<1)	274 (2)
Addison's disease	**72 (<1)**	**34 (<1)**	**38 (<1)**
Any GI disease	**1532 (6)**	**659 (5)**	**873 (7)**
Celiac	1428 (6)	609 (5)	819 (6)
Colitis	74 (<1)	31 (<1)	43 (<1)
Crohn's	37 (<1)	21 (<1)	16 (<1)
Any collagen vascular disease	**408 (2)**	**137 (1)**	**271 (2)**
Rheumatoid arthritis	223 (<1)	55 (<1)	168 (1)
Psoriasis	160 (<1)	83 (<1)	77 (<1)
Lupus	37 (<1)	3 (<1)	34 (<1)
Any skin disease	**234 (<1)**	**105 (<1)**	**129 (1)**
Vitiligo	154 (<1)	72 (<1)	82 (<1)
Alopecia	84 (<1)	33 (<1)	51 (<1)

[a] Autoimmune diseases with N<25 participants include: Sjogren's disease, dermatomyositis, multiple sclerosis, and scleroderma
[b] Participants can appear in more than one autoimmune disease category

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The role of IL-7 and IL-7/IL-2 receptor isoforms in the development of the autoimmune T cells of children with type 1 diabetes

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Background and aims: Autoantigen specific CD4+ T cells of a TH1 phenotype support the development of autoimmune diseases and play a

key role in the pathogenesis of type-1 diabetes. Regulatory T cells can prevent development and progress of type 1 diabetes. IL-7 has multifaceted effects on T cells including induction of effector T cells of a TH1 phenotype and suppressing the development of regulatory T cells. The crucial role of IL-7 and its receptor for type 1 diabetes was shown in type 1 diabetes animal models and by functional analyses of IL-7 receptor signalling in patient's material. Association of a polymorphism in the IL-7 receptor with type-1 diabetes indicated a role of genetic components. In contrast to IL-7, IL-2 primarily supports the development of regulatory T cells, which in principle can prevent the development of type 1 diabetes. Notably, both IL-7 and IL-2 receptors are strongly regulated during early T cell activation and polarization. The influence of IL-2 and IL-7 receptor expression pattern on the T cell response to IL-7 and IL-2 and how this effects T cell polarization remains unclear. The aim of this study was to determine cellular and soluble IL-7 and IL-2 receptor expression and to examine the influence of IL-7/IL-2 in vitro on the generation, polarization, and regulation on T cell immunity and to identify possible dysregulation of T cell functions in type 1 diabetes.

Materials and methods: Children with type 1 diabetes were recruited and classified according to the manifestation age (below or above 5 years) and disease duration. T cell activation in the presence or absence of IL-7 dependent T cell response is characterized in vitro. Flow cytometry was applied to determine activation markers, cytokine profile, and IL-7/IL-2 receptor expression. Concomitantly soluble IL-7 and IL-2 receptor isoform expression was quantified in plasma and culture samples using cytometric bead array technology. Immunological results were correlated with clinical data (including autoantibody status and HLA type).

Results: Initial results showed significant differential IL-7 and IL-2 receptor expression during T cell activation with and without IL-7. In addition, we detected increased proportions of IFNγ/TNFα expressing T cells in the presence of IL-7 and remarkably IL-10 expression was also enhanced by IL-7. Notably, IL-7 and IL-2 receptor concentrations in serum samples varied markedly between individual patients with newly diagnosed diabetes.

Conclusion: Functional in vitro analyses of IL-7/IL-2 receptor expression during T cell activation and effector cytokine expression may provide insight into relevant events in the development of type 1 diabetes autoimmunity. Soluble cytokine receptors in serum are biomarker candidates for disease manifestation and severity.

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Relationship between Hashimoto's thyroiditis, autoimmune and metabolic markers in adult onset autoimmune diabetes

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Background and aims: Hashimoto's thyroiditis and juvenile type 1 diabetes mellitus (T1DM) are the most common combination of autoimmune (AI) disorders. It was recently proposed that besides genetic susceptibility central obesity might contribute to unknown mechanisms underlying the thyroid-antibody production. Latent AI diabetes in adults (LADA) is characterized by clinical presentation resembling type 2 diabetes but with T1DM autoimmune markers, primarily glutamic acid autoantibody (GAD Ab). Little is known about the presence of Hashimoto's thyroiditis in LADA and adult onset T1DM. We aimed to investigate the relationship between Hashimoto's disease presence, AI markers and metabolic features in patients with LADA and adult-onset T1DM.

Materials and methods: Four hundred and seven patients diagnosed with AI diabetes over the age of 30 years were divided according to Ab positivity into three groups: GAD single positive group (representing LADA); islet cell Ab (ICA)+GAD positive and ICA+GAD+protein thyrosine phosphatase Ab (IA2) positive group representing adult-onset T1DM. ICA Ab was measured by indirect immunofluorescence. GAD and IA2 Abs were detected by enzyme like immunoadsorbent assay (ELISA). Hashimoto's thyroiditis was diagnosed by thyroid ultrasound guided needle biopsy and serologic tests for thyreoiglobulin and tissue peroxidase Abs by chemiluminescent immunoassay (CLIA). Body mass index (BMI), waist circumference, arterial hypertension (AH), THS, fT3, fT4, triglycerides, HDL cholesterol and statin use were also evaluated.

Results: Patients characteristics are shown in Table 1. LADA group showed significantly higher Hashimoto's thyroiditis presence compared to the groups of adult-onset T1DM. Althought all patients had TSH and peripheral thyroid hormone levels within the normal range, TSH was significantly higher in the LADA group compared to other two groups. Higher BMI, waist circumference, statin use and AH presence were more common in the LADA group. In the regression analysis including Ab status, BMI, waist circumference, the use of statins and the presence of AH adjusted for gender and age at diagnosis, single GAD positivity was significantly associated with Hashimoto's thyroiditis (OR 1.977 (1.174-3.328), p=0.001). The association was also significant for waist circumference and statin use (OR 1.038 (1.003-1.072), p=0.031; OR 1.525 (1.171-1.716), p=0.008).

Conclusion: Hashimoto's thyroiditis is more prevalent in patients with LADA compared to other forms of AI diabetes in adults. The possible association of both GAD positivity and waist circumference with the presence of Hashimoto's thyroiditis in LADA patients was found. These results might indicate the potential contribution of central obesity in Hashimoto's pathogenesis which needs to be clarified in further studies.

Table 1—Patients characteristics and the difference between them

	ICA+GAD+IA2	ICA+GAD	GAD	p for trend
n	111	192	104	
Gender (male:female)	67:44	99:93	52:52	0.354
Age at diagnosis (years)	41(30-31)	42(30-77)	43(30-59)	0.494
BMI (kg/m₂)	23(17-36)	24,5(18-41)	26,5(17-34)	0.037
Waist circumference (cm)	78(63-113)	85(70-122)	92,1(71-145)	0.001
Triglycerides (mmol/L)	1,07 (0,31-18,87)	1,19 (0,26-8,30)	1,08 (0,37-12,43)	0.139
HDL cholesterol (mmol/L)	1,48 (1,23-2,51)	1,49 (1,28-3,87)	1,73 (1,49-2,54)	0.355
Statin use (%)	9,2	15,5	23,4	0.007
Arterial hypertension (%)	11,7	26,2	30,1	0.034
Presence of Hashimoto's disease (%)	38.73	13.02	41.34	0.018
TSH (mU/L)	1.19 (0.51-4.79)	1.97(0.59-4.78)	2.18(0.54-4.49)	<0.001
fT3 (pmol/L)	4.91(3.10-6.10)	5.0(3.25-6.00)	4.85(2.95-5.90)	0.479
fT4 (pmol/L)	15.8(11.80-20.50)	15.7(11.57-21.5)	15.65(11.50-19.20)	0.803

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Islet cell associated autoantibodies in Ethiopians with diabetes mellitus

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Background and aims: Our understanding of the role of autoimmunity in the pathogenesis of diabetes in African population is limited. This study aims to evaluate the prevalence of 4 different islet cell associated antibodies in Ethiopian patients with diabetes as well as non-diabetic controls. In addition, the study intends to assess the utility of a combi-assay for the simultaneous detection of antibodies against glutamic acid decarboxylase (GADA) and the protein tyrosine phosphatase like IA-2 (IA-2A) as a first-line screening test for antibodies.

Materials and methods: A total of 187 subjects from a Diabetic Clinic at an Ethiopian Hospital were evaluated in a cross sectional study. Fifty five patients had type 1 diabetes mellitus (T1DM), 86 patients had type 2 diabetes mellitus (T2DM) and 46 were non-diabetic controls. In addition to clinical information, blood samples well collected. Islet cell associated

antibodies were measured by using 4 different assays for: islet cell antibodies (ICA), GADA, insulin autoantibodies (IAA) and the IA-2A.
Results: The mean age of studied subjects was 29 years in T1DM, 51 years in T2DM and 29 years in controls. The mean duration of diabetes was 7 years in T1DM and 9 years in T2DM. The results of the antibody studies [no. positive/no. tested (% positive)] are shown in the Table below. Testing with individual assays revealed that the combi-assay positivity was entirely due to GADA and none due to IA-2A. The GADA assay had a concordance rate of 95% when compared with ICA assay. In subjects with T2DM, GADA positivity was associated with insulin requirement (P=0.022), lower BMI (P=0.039) and lower basal C-peptide levels (P=0.005).
Conclusion: 1- Ethiopian patients with T1DM have a higher prevalence of islet cell associated autoantibodies than patients with T2DM. 2- GADA seems to be significantly present in Ethiopians whereas IA-2A seems to be absent. 3-Because of its simplicity and its good diagnostic accuracy, GADA should replace the ICA as the assay of choice. 4-Subjects with T2DM, who are GADA+, have some features of T1DM, supporting the notion that a subset of T1DM patients may exist within the group of Ethiopians with T2DM.

	A. Type 1 DM	**B.** Type 2 DM	**C.** Controls
Combi-assay (GADA + IA-2)	16/55 (29%)**	3/86 (3.5%)	0/46 (0%)
ICA+	10/48 (21%)*	1/37(2.7%)	0/46(0%)
GADA+	16/55 (29%) **	3/86 (3.5%)	0/46 (0%)
IA2+	0/55	0/86	0/46
IAA+	15/56 (27%)	15/96 (16%)	1/51 (2%)

** P<0.001, *P<0.05 Type 1 DM versus the other two groups.

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Proinsulin peptide immunotherapy in type 1 diabetes: safety data of a first in new-onset type 1 diabetes phase 1b trial

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Background and aims: In Type 1 Diabetes, nonclinical studies have shown that Antigen Specific Therapy (ASI) strategies have been highly effective in disease prevention, and some of them have worked in the much more stringent setting of close to, or at, disease onset. Furthermore, they have demonstrated that the ASI strategies may be effective when the relevant autoantigen is delivered as a short peptide, representing a key target (termed an epitope) of the pathological T lymphocyte response that is characteristic of the disease. Clinical trials of ASI as applied to Type 1 Diabetes have predominantly focused on insulin as the autoantigen, administered by a variety of routes, in the setting of secondary prevention. Aims and objectives: We aimed to examine the safety of intradermal administration of the naturally processed proinsulin peptide C19-A3 (PPI C19-A3) at a dose of 10 μg every 14 days or every 28 days for a total of 12 doses (Group B) and 6 doses (Group C), respectively, to patients with new-onset Type 1 diabetes.
Materials and methods: The trial was a multi-centre, randomised; double-blind, placebo-controlled, 3-arm study of 10 μg of PPI C19-A3 peptide administered every 14 or every 28 days, with follow-up for 48 weeks. 27 patients aged 18-45 with new-onset Type 1 Diabetes, HLA-DRB1*0401 genotype, antibody positivity and a stimulated c-peptide level >0.2pmol/ml were recruited to the trial within 100 days of diagnosis. TAll subjects received injections at bi-weekly intervals, and in the case of the placebo group (Group A) (n=8) this was normal saline only; for the low frequency dosing group (Group C) (n=10) this was alternate study drug and normal saline only; and for the high frequency dosing group (Group B) (n=9) this was the study drug on every occasion.
Results: The trial injections have been generally well tolerated and all recruited patients completed the trial follow up with no withdrawal. No events of systemic hypersensitivity (type 1) have been reported in the trial, therefore; it has been shown that administration of Proinsulin peptide was safe. Only 5 serious adverse events (SAEs) were reported and classified as mild or moderate in severity (either unrelated or unlikely related to the randomisation drug). Local trial investigators did not report any SUSAR. More than 75% of adverse events (AEs) have been reported as hypoglycaemic episodes unlikely related to the randomisation drug, however; local skin reaction at the injection site has been reported as an AE that is likely related to the trial drug with no systemic complications.
Conclusion: Proinsulin peptide immunotherapy in the dosing regimen used is well tolerated and free from the risk of systemic hypersensitivity and serious adverse reactions. The safety from hypersensitivity reaction was clearly seen in the repeated dosing up to 12 doses in the same patient. This phase 1 trial will pave the route for future phase 2 trials in new-onset Type 1 Diabetes which aim to examine the effectiveness of Proinsulin peptide immunotherapy on T cell autoimmunity and preservation of β cell function.
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Prevalence of latent autoimmune diabetes in young, non obese, adult onset diabetes patient with poor response to oral insulin secretagogues

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Background and aims: Latent autoimmune diabetes in adults (LADA) is defined as adult-onset diabetes with circulating islet antibodies but not requiring insulin initially. Diagnosing LADA has treatment implications because of the high risk of progression to insulin dependency. This study was done to observe the prevalence of LADA in young (25-40 years), adult onset (>20 years) and non obese (BMI<23) diabetic patients having poor glycemic control with Oral insulin secretagogues
Materials and methods: Three hundred young (25-40 years), adult onset (>20 years) and non obese (BMI<23)diabetic with poor glycemic control with optimal dosage of Oral insulin secretagogues (Sulphonylureas) were included in the study. None of the patient had e/o Ketoacidosis. Detailed clinical History, family history and Anthropometric measurements were taken. Ultrasound screening of pancreas was done for every patient. HbA1c, fasting C peptide, 1 hour post meal C peptide, Anti Glutamic acid decarboxylase antibodies (GADA) and Islet Cell Autoantigen 512 Antibodies (AntiIA2) were estimated for every patient.
Results: The mean age of patients was 33 years and they had diabetes for an average of 6 years. On Autoantibody screening, 134 patients came out to be Autoantibody positive. 122 patients (40.67%) were positive for GADA and 21 patients were positive for Anti IA2 antibodies. 9 of the 21 Anti IA2 positive patients were also having Anti GAD Antibodies. Patients who were Antibody positive had lower BMI , more complications and worse glycemic control. Family history of Diabetes was most predictive of absence of Antibodies.
Conclusion: The high prevalence of LADA (44.67%) in our study is among a selected subclass of diabetic with higher pretest probability for LADA. The prevalence in general diabetics will be much lower. However we can say that Antibody screening in this select group of diabetic

(young, non obese, no family history) is warranted and will lead to better standards of care. The low positivity of Anti IA2 Antibody as compared to GADA has also been observed in other Indian studies and may be due to lower prevalence of HLA DR4. Young, non obese diabetes patients with adultonset diabetes, no family history and poor response to oral insulin secretagogues should be screened for Autoimmune Diabetes. Fasting / Stimulated C peptide is a good screening test with a poor response leading to a higher pre test probability for testing positive for LADA. A high C peptide rules out Autoimmune Diabetes.
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Misclassification of outcomes of pancreas transplantation
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Background and aims: Success rates for pancreas transplantation have been steadily improving, with 5-year survival rates over 70% for simultaneous pancreas kidney transplants. Currently, graft failure is defined as the re-introduction of insulin, which has some limitations. Different clinicians have different thresholds to introduce insulin, and some choose to start oral therapies first in some cases. Alternatively, some patients may still have residual C-peptide despite being on insulin therapy, providing important protection against hypoglycaemia. As a result, current graft survival rates may not provide an accurate picture of graft success - i.e. restoration of the patient to the non-diabetic state. Our hypothesis is that a clinically relevant proportion of patients may be misclassified. Our aim was to identify the number of patients with misclassified graft function who had had a pancreas transplant at our transplant unit between 2008 and 2012.
Materials and methods: We were able to obtain latest available HbA1c, fasting glucose [FG], C-peptide, and current therapy for 103/124 pancreas transplant recipients at Guy's hospital between 2008 and 2012, of which 90 were simultaneous pancreas kidney and 13 were solitary pancreas transplantations. We classified pancreas grafts using current definitions of function based on insulin use and compared this to a modified classification based around definitions of diabetes. We categorised grafts using the following criteria: 1] Functioning: HbA1c<6% or FG <6.1 mmol/L, and on no diabetes medication; 2] Partial function: HbA1c≥6% or FG≥ 6.1 mmol/L, and on basal insulin alone/oral therapy/no therapy, or having C-peptide>300pmol/L. 3] Failure: Pancreatectomy or C-peptide< 300pmol/L. If C-peptide was unavailable, HbA1c≥6.5% or FG≥7.0, and full insulinisation was considered graft failure.
Results: Of the 103 transplants, there were 10 early technical failures [<1 month post transplantation]. 71 are currently classified functioning and 22 as late failure, after a mean follow up of 4.0±1.7 years. Of 71 functioning grafts, 7 met our criteria of partial function, with 2 patients on oral therapy and 5 with raised HbA1c on no therapy. Of the 22 with late failure [mean time to failure 1.7±1.4 years], 2 met our criteria of partial function; 1 had residual function with preserved C-peptide, the other on basal insulin only. In the 20 that met our criteria of failure, 45% [n=9] failed after experiencing a period of partial function. In total, 9/103 grafts [8.7%] were misclassified according to our criteria. C-peptide data was incomplete and some patients classified as having late failure may still have some measurable C-peptide.
Conclusion: Our new classification system would reclassify 8.7% of grafts as having partial function. Almost half of the grafts that developed late failure were documented as having a period of partial graft function for some time before complete graft failure, and identifying these patients early may represent an opportunity to understand mechanisms of graft failure and intervene to prolong graft survival.

PS 032 Improving transplantation outcomes

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Protein tyrosine phosphatase 1B is a novel target on the improvement of islet graft's revascularisation
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Background and aims: One of the main limitations encountered in pancreatic islet transplantation, leading to islet loss and graft failure is poor revascularization. Protein tyrosine phosphatase 1B (PTP1B) is known to negatively mediate several signaling pathways by maintaining phosphotyrosine levels in key proteins. Recently PTP1B was described as a negative regulator of angiogenesis through vascular endothelial growth factor (VEGF) signaling. In this sense, our main aim is to investigate if PTP1B ablation contributes to the improvement of graft revascularization and survival.
Materials and methods: Diabetic BALB/c mice (single injection of streptozotocin - 160 mg/Kg) were transplanted, with 200 islets, isolated from $PTP1B^{-/-}$ or $PTP1B^{+/+}$ mice, into the anterior chamber of the eye. Four groups of animals were constitute (n=12 animals/group): Non transplanted; Control - transplanted with $PTP1B^{+/+}$ islets; $PTP1B^{-/-}$ - transplanted with $PTP1B^{-/-}$ islets; and not diabetic group. Animals were followed for 28 days during which weight and glycemic levels were measured. Graft revascularization was evaluated, *in vivo*, at day 7, 15 and 28. *Post-mortem* morphometric analysis was conducted on graft-containing eyes. Gene expression (qRT-PCR), and protein detection (ELISA), were performed in islet samples.
Results: After 28 days the $PTP1B^{-/-}$ group showed normalization of glycemic levels when compared with the not diabetic group ($PTP1B^{-/-}$: 161±32 mg/dL vs not diabetic: 140±22, not significant) and a dramatic decrease when compared with the control (472±52 mg/dL, 66% decrease, $p<0.05$) and non transplanted groups (528±22, 70% decrease, $p<0.01$). Graft functional vascularization was accessed, *in vivo*, after 7, 15 and 28 days post-transplant. In all moments, $PTP1B^{-/-}$ group, showed increased vascular density (day 7: $PTP1B^{-/-}$: 0.0187±0.002% vs Control: 0.0130±0.001%, $p<0.05$/ day 15: $PTP1B^{-/-}$: 0.0290±0.005% vs Control: 0.0188±0.002%, $p<0.05$/ day 28: $PTP1B^{-/-}$: 0.0297±0.004% vs Control: 0.0187±0.005%; $p<0.05$) and increased vascular area (day 7: $PTP1B^{-/-}$: 11.3±3% vs Control: 5.8±2%, $p<0.001$/ day 15: $PTP1B^{-/-}$: 21.4±2% vs Control: 10.8±2%, $p<0.001$/ day 28: $PTP1B^{-/-}$: 23.180±2% vs Control: 10.027±3%; $p<0.001$). Grafts from both groups reached maximum revascularization by day 15. Morphometric analyses of the graft-containing eyes (recovered at day 28) revealed a 5-fold increase on the percentage of ß-cell expressing VEGF on $PTP1B^{-/-}$ grafts when compared with the control ($PTP1B^{-/-}$:89.9±9% vs control: 17.86±3%; $p<0.001$). Moreover, when compared with $PTP1B^{+/+}$ islets, $PTP1B^{-/-}$ islets show a 3 fold increase in VEGF expression ($p<0.05$) and, at the protein level, a 1.8 fold increase in relative VEGF secretion by VEGF islet content ($PTP1B^{-/-}$: 1.95±0.45 vs $PTP1B^{+/+}$: 1.05±0.1, $p<0.05$).
Conclusion: Our results reveal PTP1B as a particular key player on the revascularization of islet grafts, suggesting this as a potential novel target for an improved therapeutic treatment in islet transplantation. Further investigation is being conducted to decipher the signaling mechanism behind these findings.
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The co-transplantation of islets with dental pulp stem cells improved survival and function of transplanted beta cells in streptozotocin-induced diabetic mice

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Background and aims: Pancreatic islet transplantation is an attractive treatment option for Type 1 diabetes mellitus (T1DM). Islet transplantation has some issues, however, such as the required administration of immuno-suppressant drugs and the low rate of insulin withdrawal due to a short survival rate of transplanted islets. Therefore, it is critical how to protect the viability of transplanted islets in recipient. Recent studies reported that co-transplantation of Mesenchymal Stem Cells (MSC) with pancreatic islets improved glycemic control more than that of pancreatic islets alone. Meanwhile, we have reported that secreted factors from stem cells, especially Dental pulp stem cells from human exfoliated deciduous teeth (SHED) rather than MSC, has beneficial effects in paracrine manner on viability of pancreatic islets. SHED have received considerable attention because of requiring a less invasive collection method and their applicability to autologous treatment. Therefore, we examined the effect of co-transplantation of islets with SHED on glycemic control in streptozotosin (STZ) diabetic mice.
Materials and methods: Islets were isolated from 10 week-old male C57Bl/6J. STZ (150 mg/kg body weight) was intraperitoneally injected into 10 week-old male C57Bl/6J mice one time only. Three days after this single STZ injection, the mice were divided into six transplanted groups: Sham (CTL), transplantation of SHED alone (a-SHED: 5×10^5 cells), MSC alone (a-MSC: 5×10^5 cells), islet alone (a-Isl: 75 islets), islets with MSCs (co-MSC: 75 islets and 5×10^5 cells) or islets with SHED (co-SHED: 75 islets and 5×10^5 cells). The cells were transplanted into the kidney capsule of C57Bl/6J. An intraperioneal glucose tolerance test (IPGTT) and insulin content were examined 28 days after the transplantation.
Results: Co- SHED ameliorated glucose intolerance and reduction of body weight in STZ-diabetic mice and the effects continued for 28 days after transplantation, although all other groups did not show any beneficial effect. Only Co- SHED also suppressed the elevated level of blood glucose and enhanced the insulin secretion during IPGTT. These data suggested that the improved viability of transplanted islets possibly contributed to the improvement in glycemic regulation, thus we next investigated the state of insulin preservation in transplanted islets. Co- SHED and co-MSC preserved the insulin content of transplanted islets in the kidney capsule more than a-Isl, and the level of insulin content in co-SHED was much higher than in co-MSC. Interestingly, histological study showed that transplanted SHED survived for 28 days after transplantation, even though any immuno-suppressant drug was not administrated.
Conclusion: This study demonstrated that co-transplantation of islets with SHED had much more beneficial effects on the survival of transplanted islets than transplantation of islet alone and co-transplantation with MSC. Furthermore, the result of their long survival of SHED after transplantation suggested that SHED might modulate the immune reaction. The co-transplantation of islets with SHED has potential as practical strategy for T1DM treatment.

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A newly developed non-haematopoietic erythropoietin analogue prevents damages to transplanted islets and inhibits macrophage activation

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Background and aims: Pancreatic islet transplantation (PITx) efficiency has been hampered by islet damage during isolation and inflammatory reactions at transplantation. Erythropoietin (Epo) exerts anti-inflammatory, anti-apoptotic and cyto-protective effects in addition to its hematopoietic property. We've investigated if a newly developed non-haematopoietic epo analogue, ARA 290, would protect islets and ameliorate inflammatory responses following PITx.
Materials and methods: Effects of ARA 290 on pancreatic islets of C57BL/6J (H-2b) mice and on murine macrophage were investigated in vitro in a culture model. As a marginal PITx, 175 islets were transplanted intraportally to STZ-induced diabetic mice (H-2b). ARA 290 (120 μg/kg) was given intraperitoneally just before and at 0, 6 and 24 hours after PITx. Liver samples were obtained at 12 hours after PITx, and expression levels of pro-inflammatory cytokines were assessed.
Results: ARA 290 protected islets from cytokine-induced damage and apoptosis. Pro-inflammatory cytokine secretions (IL-6, IL-12 and TNF-α) were significantly inhibited by ARA 290. In the transplantation sutdy, ARA 290 treatment significantly improved metabolic control of recipients after marginal mass PITx. Intra peritoneal glucose tolerance test (IPGTT) performed at day 14, showed significantly lower area under the curve (AUC) in the ARA 290 treated group (Figure 1). Up-regulation of MCP-1, MIP-1β, IL-1β and IL-6 mRNA expression within the liver were suppressed by ARA 290 treatment.
Conclusion: ARA 290 protected pancreatic islets from cytokine-induced damage and apoptosis in vitro and ameliorated the inflammatory response following PITx. It appears to be a promising candidate for improvement of PITx.

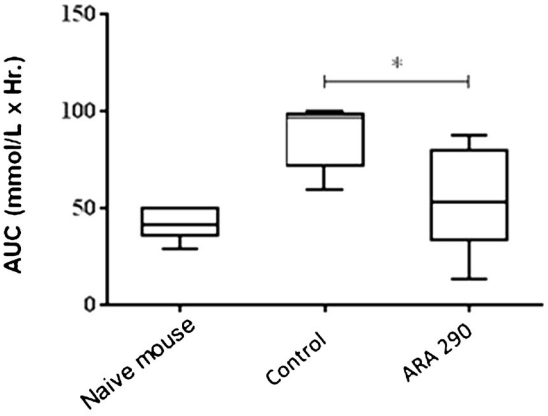

Figure1: AUC in IPGTT (*p < 0.05 vs. control group; values are depicted as lower quartile, median and upper quartile (boxes) with minimum and maximum ranges.).

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Quantitative imaging of intramuscular transplanted islets of Langerhans with SPECT by dopamine 2 receptor targeting

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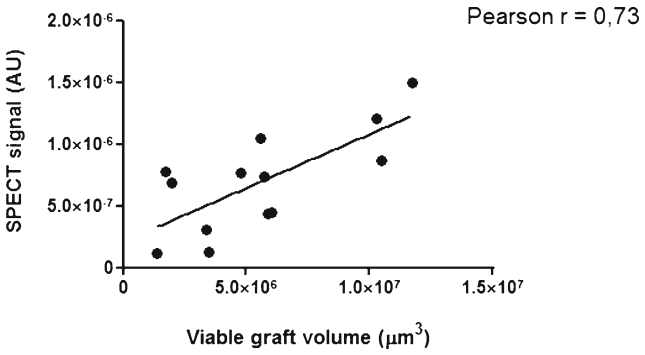
Background and aims: Besides insulin therapy, islet transplantation is considered one of the most promising therapies for type 1 diabetic patients with poor glycemic control. Despite improvements in immunosuppressive therapy, islet transplantation still results in short term insulin independence. A non-invasive imaging method to, longitudinally and quantitatively, monitor the viable transplanted islets might provide insight in the faith of the islets after transplantation. Furthermore, it would offer the possibility to evaluate potential interventions that might improve long term transplantation outcome. Dopamine (D2) receptors are expressed on beta cells and are therefore a suitable target to monitor islet grafts. Here, we investigated the use of the D2 receptor antagonist [^{123}I]Iodobenzamide ([^{123}I]IBZM) for the quantification of viable graft volume.
Materials and methods: Six weeks after rats were transplanted intramuscularly with 1000, 2000 or 3000 islets, they received 50 MBq [^{123}I]IBZM intravenously and one hour post injection SPECT images were acquired. Afterwards, animals used for ex vivo autoradiography were injected with [^{125}I]IBZM, euthanized 1 h post-injection, and the graft containing muscles were dissected for ex vivo SPECT, autoradiography and histological analysis. Viable graft volume was calculated histologically by multiplying insulin-positive area by interslice distance.
Results: Six weeks after transplantation, a clear signal of all grafts was observed by SPECT. Moreover, the intensity of the SPECT signal correlated linearly with the calculated viable graft volume (Pearson r=0,73 ; p=0,005). The SPECT signal observed in the calf muscle ex vivo correlated with the SPECT signal observed in the calf muscle in vivo. Furthermore, ex vivo autoradiography confirmed specific tracer accumulation and co-localization with the grafts location, as determined histologically. These observations confirm that the SPECT signal observed in vivo originates from specific targeting of the D2 receptor, expressed on the graft.
Conclusion: In conclusion, [^{123}I]IBZM can successfully be applied for non-invasive, quantitative graft observation in vivo. Especially in combination with other beta cell specific tracers, such as exendin, it might provide a strong tool to obtain detailed complementary information by non-invasive molecular imaging and to predict islet graft metabolic state and transplantation outcome.



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Comparative biology of porcine and human islets in situ and after xenotransplantation in vivo

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Background and aims: Islet transplantation is a successful experimental clinical approach for the treatment of brittle diabetes. However, its wide application is limited by the shortage of human organ donors. Pig to human xenotransplantation may serve as a potential alternative approach. Sparse previous work on isolated islets and dispersed cells suggested that porcine islet endocrine cell composition and architecture differs from human islets, potentially affecting the regulation of islet cell function. However, detailed information about the physiology of porcine islets as well as the vascular network is still limited. To further increase our knowledge and to circumvent the enzymatic stress during islet isolation, we utilize acute pancreas tissue slices to compare the three-dimensional morphology of porcine and human islets *in situ*. This approach allows the analysis of endocrine cells and intact intra-islet vascular network within a conserved anatomical environment under near physiological conditions. Furthermore, we evaluated the ability of porcine and human islets to rebuild their distinct vascular network after transplantation, by three-dimensional analysis of transplanted islets *in vivo*.
Materials and methods: We here used a combinational approach of pancreas tissue slices and the anterior chamber of the mouse eye platform to assess three-dimensional islet architecture of human and porcine islets in the endogenous pancreas *in situ* and after xenotransplantation *in vivo*.*In situ* analysis of endogenous islet architecture was performed on pancreas tissue slices prepared from slaughterhouse pigs and human tissue obtained after partial pancreatectomy. The tissue was stained for insulin, glucagon for endocrine cell composition and the vascular network was visualized by fluorescently labeled lectin. *In vivo*, intra-islet vascular network was assessed in fully engrafted porcine and human islets transplanted to the anterior chamber of the mouse eye by i.v. injection of a fluorescent tracer.
Results: Three-dimensional analysis of human and porcine pancreatic tissue slices revealed cell compositional differences of the insulin (55% ±6.95 for human vs. 67.4%±6.10 for porcine, p-value <0.05) and glucagon fraction (24.7%±7.73 human vs. 6.2%±2.66 porcine, p-value <0.01). Additionally fractional blood volume displayed significant differences between the species (7.97%±2.65 vs. 10.26%±2.94, p-value <0.05).
After xenotransplantation into the anterior chamber of the mouse eye, the vascular network of porcine donor islets was similar to the *in situ* situation (8.78%±3.63). In contrast, human islets displayed a reduced potential to revascularize under xenogenic conditions (2.32%±1.10).
Conclusion: In conclusion, the three-dimensional *in situ* analysis reveals interspecies differences of islet architecture which are potentially important for islet function. Additionally, our *in vivo* approach could show that porcine islets have the potential to rebuild their endogenous vascular network under xenogenic conditions which might be crucial for therapeutic islet transplantation.

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The effect of transplanted pancreatic islets on recipient liver morphology: rat animal study

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Background and aims: The permanent hyperinsulinemia in Type-2 diabetic patients is considered a likely risk factor for development of primary liver tumor. Pancreatic islets are commonly transplanted into the hepatic portal vein and the liver tissue is subsequently exposed to insulin in locally high concentration. There were published several articles pronouncing the hypothesis of a direct influence of transplanted islets on formation and subsequent progression of morphological changes in liver tissue (tumors, cysts). Therefore our study was focused on monitoring of liver changes during 10 months follow up after transplantation of pancreatic islets in rat animal model.
Materials and methods: Isolated pancreatic islets were transplanted into diabetic (streptozotocin) or healthy recipients in isogeneic manner. Group A (n=5) - healthy Brown Norway recipients, transplantation of 450 islets into the portal vein; Group B (n=17) - STZ (50 mg/kg) diabetic Brown Norway recipients, transplantation of 450 islets into the portal vein; Group C (n=8) - STZ (50 mg/kg) diabetic Brown Norway recipients, transplantation of 1000 islets under the left kidney capsule; Group D (n=8) - STZ (60 mg/kg) diabetic Prague Hereditary Hypertriglyceridemic recipients, transplantation of 1200 islets into the portal vein; Blood glucose levels, weight, IVGTT was monitored in all recipients for 10 months. The morphology of liver was examined by magnetic resonance imaging (MRI) 5 and 8 weeks after islet transplantation. At the end of study all recipients liver were excised, fixed in formalin and examined by histology.
Results: Injection of streptozotocin induced severe diabetes (glycaemia over 25 mmol/l) in all animals. Transplantation of pancreatic islets in suboptimal number normalized fasting blood glucose levels but did not normalize glucose assimilation in IVGTT in all diabetic recipients. In healthy recipients transplanted islets did not modify blood glucose levels. While liver of Group A recipients remained intact, in animals of Group B and C the substantial part of liver tissue was cystically changed. Multiple voluminous cystic complexes with cholangiocyte cubic epithelium lining were detected in these animals irrespective of transplantation site. In insulin resistant animals of Group D, the transplanted islets caused just formation of focal steatosis until the end of study. The total volume of hepatic cysts detected in animals of group B by MRI was 0.42±0.92 ml and 4.68±9.57 ml five and eight months after transplantation, respectively. This represented 4.3% and 29.3% of total liver volume.
Conclusion: The injection of diabetogenic agent streptozotocin and subsequent transplantation of isogeneic pancreatic islets can induce liver morphological changes, which were different in relation to the strain. Further research is needed to identify the differences in Brown Norway and hHTG rats, which are substantial for kind of liver reaction.
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Human IAPP impairs the success of islet transplantation

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Background and aims: ß-cell replacement by islet transplantation has been explored as a potential curative therapy for type 1 diabetes. Despite advancements in islet manipulation and immune suppression that have increased the survival of transplanted islets, the progressive decline of graft function remains a problem, and many recipients return to being insulin dependent within a few years after the transplant. However, the molecular mechanisms underlying the loss of function of grafted islets have not been fully elucidated. Islet amyloid polypeptide (IAPP)-derived amyloid deposits were observed in approximately half of the implanted islets in a recipient with type 1 diabetes. We therefore hypothesized that human IAPP (hIAPP) in transplanted islets contributes to loss of islet function and impairs the success of islet transplantation. The aim of this study was to elucidate the role of hIAPP during islet transplantation, using knock-in mice that express a physiological level of hIAPP.
Materials and methods: Islet donors were 16-week-old hIAPP-KI male mice and C57BL/ 6J (control) male mice. Mice with a plasma glucose level>400 mg/kg 7 days after streptozotocin treatment that were fed a high-fat diet (60% fat) were used as recipients. Recipients were implanted under the kidney capsule with 150 hIAPP- KI or control islets. Following transplantation, blood glucose levels and body weight were measured for 8 weeks. Thereafter, metabolic parameters were measured, and the graft-bearing kidney was subjected to both histological and biochemical analysis. To assess glucose level-dependent cell death, islets were cultured for 4 days in medium containing 200, 300, or 400 mg/dL glucose, and oligonucleosomes released into the cultured cell lysate were quantified.
Results: Plasma glucose levels were significantly higher in mice implanted with hIAPP-KI islets (hIAPP group) than control islets from 3 days after transplantation. Normalized scores, which reached normoglycemic levels (<200 mg/dL) after transplantation, were lower in the hIAPP group than the control group. HbA1c levels were well controlled in the control group (4.8%) but not in the hIAPP group (6.7%) after 8 weeks of transplantation (P<0.005). The insulin content of kidneys from the hIAPP group were significantly lower than the control group. The ratio of cleaved caspase 3 (cell death marker)-positive cells to insulin-positive cells was significantly higher in the hIAPP group than in the control group. Culturing of hIAPP-KI islets for 4 days in 400 mg/dL glucose increased the production of cell death marker, oligonucleosomes was significantly increased by 1.8 times compared with control islets.
Conclusion: In summary, we showed that the physiological level of hIAPP is associated with an increase in ß-cell apoptosis, and inhibits the amelioration of hyperglycemia by islet transplantation.
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5 year safety and effectiveness of islet transplantation in subjects with eGFR <60: retrospective observational study

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Background and aims: Kidney-pancreas transplant is optimal treatment for Type 1 diabetes (T1D) with end-stage renal disease (ESRD) while islet transplantation (ITx) has been reserved for subjects with adequate renal reserve. Since renal impairment increases the risk of severe hypoglycaemia, the short term risks of severe hypoglycemia may justify the potential renal risks of ITx with calcineurin inhibitor (CNI) based immunosuppression in T1D with moderate, stable renal impairment.
Materials and methods: We compared the safety and effectiveness of ITx in T1D subjects with or without renal impairment (eGFR<60 ml / min) in a single centre, 5 year, retrospective study. The primary end points were a) safety: progression to ESRD, doubling of serum creatinine, or death from any cause; and b) effectiveness: proportion maintaining good glycemic control (a1c <7%) and protection from hypoglycemia (HYPO score<423, Clarke score<4 and LI<329) at 5 years.

Results: 118 ITx recipients with at least 5 years of follow up (transplanted between 1999-2009) were included. Maintenance immunosuppression was with sirolimus plus tacrolimus or tacrolimus plus mycophenolate. 29/118 (25%) subjects had eGFR<60 ml/min at baseline. Subjects with eGFR<60 were older (mean ± sd: 52±10 v 44±10 years, p=0.0001), female gender 69 v 52% (p=0.1), median(IQR) eGFR was 81(71-92) v 54 (48-58). The proportion of subjects reaching the safety end point was 3/28 (11%) v 7/86 (8%) (p=0.7). The proportion reaching the efficacy end point was 14/29 (48%) v 37/82 (45%) (p=0.8). Average yearly GFR decline was slightly greater with higher starting gfrs (r=0.15, p<0.0001).
Conclusion: ITx appears to be equally safe and effective to provide good glycemic control and protection from hypoglycaemia over 5 years in carefully selected subjects with moderate renal impairment. These data help guide the risk benefit assessment for T1D subjects with both severe hypoglycemia and renal impairment.
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Efficacy of autologous stem cells transplantation in patients with type 2 diabetes mellitus: a randomised placebo-controlled study
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Background and aims: T2DM is characterized by two major defects: insulin resistance and insulin deficiency. Current therapies in T2DM targeting β-cells are insulin, glitazones and GLP-1 analogs. These therapies have demonstrated glycemic durability over a prolonged period of time but their role in β-cells regeneration is elusive. Therefore, this has led to the emergence of novel β-cells regenerative therapies like hematopoietic stem cell (HSCs), mononucleated stem cells (MNCs) and mesenchymal stem cells (MSCs). This study evaluates the efficacy of autologous bone marrow derived-stem cell transplantation (ABMSCT) and autologous bone marrow derived-mesenchymal stem cell transplantation (ABM-MSCT) in patients with T2DM.
Materials and methods: This prospective, randomized, single-blinded placebo controlled study enrolled 30 patients with triple oral hypoglycemic drug failure and requiring insulin ≥0.4 Units/Kg/day for achieving euglycemia (HbA1c ≤7.5%). They were randomly assigned to MSCs group (n=10), MNCs group (n=10), who received ABM-MSCT and ABMSCT through targeted approach respectively, while control arm (n=10) underwent sham procedure and were followed for 6 months. The efficacy of intervention was assessed by gold standard method "hyperglycemic clamp" to estimate C-peptide response and insulin sensitivity index. The primary end-point was a reduction in insulin requirement by ≥50% from baseline, while maintaining HbA1c ≤7%.
Results: Six out of 10 (60%) patients in the ABM-MSCT and 9 out of 10 (90%) patients in the ABMSCT, while one (10%) in the control group achieved the primary endpoint (p=0.001). There was a modest but not significant decrease in HbA1c in ABM-MSCT from 6.9% (6.6-7.0%) to 6.8% (6.4-7.3%) (p=0.441) , ABMSCT from 6.7% (6.4-7.3%) to 6.9% (6.5-7.0%) (p=0.575) as well as in controls from 6.5% (6.2-6.8%) to 6.2% (6.0-6.2%) (p=0.262). There was a significant increase in C- peptide response in ABMSCT (p=0.046), while improvement in insulin sensitivity index (p=0.04) in ABM-MSCT group during hyperglycemic clamp. There was a significant decrease in HOMA-IR (p=0.028) in ABM-MSCT, while HOMA - β function were remain unaltered (p=0.445) in ABMSCT.
Conclusion: The ABMSCT and ABM-MSCT results in reduction in insulin dose while maintaining target HbA1c. MSCs improves insulin sensitivity index and MNCs improves β cell function. A combined therapy with these two types of stem cells remains to be explored.
Clinical Trial Registration Number: NCT01759823

PS 033 Clinical studies on insulin action

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Effect of pioglitazone, exenatide and combination of pioglitazone and exenatide on plasma alpha-hydroxybutyrate in type 2 diabetes
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Background and aims: Alpha-hydroxybutyrate has been shown to be an early marker of insulin resistance in recent studies. Pioglitazone is known to reduce plasma alpha-hydroxybutyrate in IGT subjects. The aim of this study was to examine the effect of Pioglitazone, Exenatide(Byetta) and a combination of these two on plasma metabolites in subjects with T2DM.
Materials and methods: We randomized thirty T2DM subjects (age=55 ±3 yrs; BMI=34.9±5; FPG=167±10 mg/dl; HbA1c=8.3±0.4%) to receive the following: (i) Exenatide (EXE 10ug bid, n=11), (ii) pioglitazone (PIO, 45 mg/d, n=10), or (iii) PIO+EXE (n=9) for 24 weeks. Subjects also participated in an OGTT and 2-step hyperglycemic (+125 and + 400 mg/dl) clamp followed by IV arginine (5 g) bolus prior to treatment and again at week 24 of treatment. Plasma alpha-hydroxybutyrate, linoleoyl-GPC (LGPC), and oleic acid were measured before and after treatment.
Results: At baseline there was no significant difference in the concentrations of the measured metabolites between subjects. Combined Pioglitazone+Exenatide yielded the greatest reduction in fasting plasma glucose, 2-hr glucose and HbA1c compared to treatment with individual medications (all p<0.05). Combined treatment increased Matsuda Index (MI) of insulin sensitivity from 2.7±0.5 to 5.9±0.6 (p<0.05). Pioglitazone alone improved Matsuda Index of insulin sensitivity (MI) from 4.9±0.8 to 7.9± 1.5 (p<0.05). Pioglitazone treatment alone decreased the fasting FFA (0.643±0.06 to 0.376±0.07 μM, p=0.001) and 2-hour FFA (0.252± 0.09 to 0.09±0.01 μM, p=0.009). Pioglitazone therapy led to a significantly lower alpha-hydroxybutyrate (6.31±0.8 vs 4.8±0.7 μg/mL, p= 0.04) and oleic acid (72±5.4 vs 53.7±6.8 μg/mL, p=0.03), however Pioglitazone did not affect plasma LGPC levels. Alpha-hydroxybutyrate correlated with insulin secretion insulin sensitivity index in Pioglitazone treated subjects (r=0.643, p=0.04). Combined Pioglitazone+Exenatide treatment showed a trend to reduced plasma alpha-hydroxybutyrate (6.31±0.8 vs 4.8±0.7 μg/mL, p=0.058). There were no significant changes in plasma metabolites seen in patients treated with Exenatide alone.
Conclusion: Pioglitazone reduces novel metabolites related to lipid metabolism and oxidative stress. This could represent a novel mechanism by which TZDs improve insulin sensitivity. Plasma alpha-hydroxybutyrate was not affected by Exenatide suggesting that the mechanism of improved glycemic control seen with Exenatide is primarily by improvement in insulin secretion.
Clinical Trial Registration Number: NCT00845182
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Chronic caffeine intake restores insulin sensitivity in high-sucrose diet rats through adaptations in adipose tissue metabolism

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Background and aims: Several epidemiological studies have described beneficial effects of chronic coffee intake on type 2 diabetes and metabolic syndrome. Our group has previously shown that chronic caffeine (caff) intake both prevents and reverses insulin resistance in prediabetes animal models, being the time needed to restore insulin sensibility inversely correlated with the caff concentration administrated. It is known that obesity is associated with insulin resistance and that alterations in adipose tissue (AT) metabolism contribute to the development and maintenance of insulin resistance. Therefore, the aim of this work was to investigate if the enhancement of insulin sensitivity induced by chronic caff intake is due to alterations in AT metabolism, namely in fat deposition, lipid profile and insulin signaling pathway in this tissue.
Materials and methods: All experiments were performed in 8-13 weeks *Wistar* rats of both genders (250-450 g). Two groups of rats were used: the high sucrose (HSu) group and a control group. The HSu model was obtained by submitting animals to a 35% sucrose diet in drinking water during 28 days. After the 28 days, the 2 groups were divided and submitted to different caff concentration in drinking water: 0, 0.5, 0.75 and 1 g/L during 12 weeks. Insulin sensitivity and glucose tolerance were monitored during all the experimental period. Afterwards rats were anaesthetized with pentobarbitone (60 mg/Kg) and blood was collected by heart puncture. Abdominal fat was collected and weighted and plasma cholesterol, HDL, LDL and triglycerides were measured. Western blots of proteins involved in insulin signaling pathway in AT were performed.
Results: HSu diet did not change weight gain in relation to controls, however both visceral and total fat weight increased by 47% and 49% respectively (visceral fat control=5.29±0.86 g; total fat control=23.59± 4.30 g). Chronic caff intake did not modify daily weight gain either in control or in the HSu group. Caff did not promote changes in total or visceral fat in the control group, however in the HSu group, visceral fat deposition was inhibited by caff in all concentrations tested and total fat deposition was inhibited by caff concentrations of 0.75 and 1 g/L. Hsu diet, as well as caff intake, did not significantly modify cholesterol, LDL and HDL plasma levels. Nevertheless, HSu diet increased triglycerides levels to 77.58±5.89 mg/dl (control=42.69±5.17 mg/dl) and caff showed a trend to decrease triglycerides in this group, in a dose dependent manner. Although caff did not change AT insulin receptor (IR), protein kinase B (Akt) and Glut4 total expression, it increased the expression of the phosphorylated forms of the IR and Akt in the Hsu animal model.
Conclusion: Chronic caff administration avoids visceral and total fat accumulation in a concentration dependent manner in HSu animals, without altering animal's weight. Although caff did not directly affect IR expression in the AT, it stimulated insulin signaling cascade, suggesting that this may be one of the mechanisms for caffeine-induced improvement of insulin sensitivity in HSu rats.
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The rapid subcutaneous injection of heparin plus insulin lispro improved dramatically blood glucose of a patient with subcutaneous insulin resistance

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Background and aims: Subcutaneous insulin resistance (SIR) is a syndrome, which is caused by increased insulin inactivation or proteolytic degradation in the dermal tissue and by impaired transport of injected insulin from subcutaneous tissue into the circulation. The blood glucose levels of 48 year-old male with type 2 diabetes, who injected insulin about 200 U per day, were still high. Interestingly, his blood glucose levels were improved near normal after direct infusion of insulin into vein. Thus, he was considered to be a state of SIR. In this report, we evaluate the successful treatment of this SIR case.
Materials and methods: The dimethyl-isopropyl-azulene ointment including 0.1% nafamostat mesilate (NAF) as a protease inhibitor was treated with the case, which is expected to prevent insulin degradation at the injection skin spot. Heparin modulates vascular endothelial growth factor and is expected to accelerate subcutaneous diffusion of insulin into the blood stream. And insulin lispro mixed with heparin (Hepalis) consisted of the concentrations of 500 U heparin and 50 U insulin per one mL respectively. And Hepalis was injected by syringe or continuous subcutaneous infusion (CSII) capillary. The blood glucose level (7 times in a day), serum C-peptide and serum insulin was examined.
Results: The blood glucose levels after lispro insulin injection at the skin spot with NAF contain ointment (310±17 mg/dL) were not significantly changed compared to those without NAF's one (351±22 mg/dL). Next, Hepalis was tried to inject as the continuous basal insulin injection by CSII. The blood glucose levels with Hepalis after replaced similar amounts of lispro insulin were a little decreased (327±16 mg/dL), but not significant ($p=0.05$), compared to those with lispro alone (360± 12 mg/dL). And then, although the Hepalis treatment by CSII was tried as basal-bolus insulin, the blood glucose levels were still not decreased under 300 mg/dL. Finally, we tried to inject as bolus insulin by syringe at the short time. The activity of Hepalis was suspected to impair due to slowly infusion by CSII because an injection time of Hepalis by CSII was took about ten minutes. Basal hepalis infusion was continued by CSII. Drastically, the blood glucose levels after Hepalis infusion by syringe were decreased significantly ($P<0.001$) and reached the levels under 200 mg/dL with gradual Hepalis elevation, followed by the significant increasing serum levels of insulin lispro.
Conclusion: In this SIR case, Injection of Hepalis rapidly into subcutaneous tissue was more effective to circulate the insulin lispro and to improve the blood glucose levels. Though the precise mechanism of this SIR has not been cleared, our repot will be a clue to discover the pathophysiology of the SIR.

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Acetic acid enhances insulin-stimulates glucose uptake by the forearm muscle in patients with type 2 diabetes

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Background and aims: Acetic acid, the main constituent of vinegar, has been shown to have a glucose-lowering effect in patients with glucose abnormalities. However, the mechanisms of this effect are still obscure. The aim of this randomised, cross-over study was to investigate the effect of vinegar on 1) muscle glucose uptake and blood flow rates and 2)

circulating plasma glucose, insulin and lipid levels, in patients with type 2 diabetes, using the arteriovenous difference technique across the forearm muscles.

Materials and methods: Eleven subjects with DM2 (age 53+4 years, BMI 25+1, HbA1c 6.8+0.3%) consumed vinegar (30 ml containing 6% acetic acid, 20 ml water) or placebo (50 ml water), at random order on two separate days (a week apart), before a mixed meal (557 kcal; 75 g carbohydrates, 26 g protein, 17 g fat). Plasma glucose, insulin, triglycerides, non-esterified fatty acids (NEFA) and glycerol were measured preprandially and at 30-60 min for 300 min postprandialy from the radial artery and from a forearm vein. Muscle blood flow was measured with strain-gauge plethysmography. Glucose flux was calculated as the arteriovenous difference of glucose multiplied by the blood flow rates.

Results: Vinegar compared to placebo: 1) increased forearm glucose uptake (765+87 vs 579+63 μmol/100 ml tissue, p=0.0357) 2) decreased plasma glucose (2834+134 vs 3005+149 mM*min, p=0.0279), insulin (16136+3397 vs 20473+4185 mU/L*min, p=0.0457) and triglycerides (371+34 vs 409+38 nmol/L*min, p=0.0439) 4) did not change muscle blood flow, plasma NEFA and glycerol.

Conclusion: In DM2 vinegar reduces postprandial hyperglycaemia, hyperinsulinaemia and hypertriglyceridaemia without affecting lipolysis. Vinegar's effect on carbohydrate metabolism may be partly accounted for by an increase in glucose uptake, demonstrating an improvement in insulin action in skeletal muscle.

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The in vitro pharmacology of LY IGlar (LY2963016): a new insulin glargine product

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Background and aims: Basal insulin analogs, with durations of action sufficient for coverage over the course of 24 hours, are important for the proper management of glycemic control in the patients with diabetes. LY IGlar (LY2963016, insulin glargine), an insulin analog with the same amino acid sequence as insulin glargine (Sanofi-Aventis; IGlar), differs from human insulin due to the addition of two arginine residues to the C-terminus of the B-chain and due to the replacement of asparagine at position A_{21} with a glycine. These changes shift the isoelectric point such that the insulin glargine analog is soluble at an acidic pH but then precipitates after injection at the neutral physiological pH.

Materials and methods: The pharmacological properties of six independent lots of LY IGlar were compared to IGlar using a panel of in vitro biological assays. The receptor binding affinity of LY IGlar for the human insulin receptor isoform A (hIR-A), hIR isoform B (hIR-B), and human IGF-1 receptor (hIGF-1R) was determined by competitive radioligand binding using cell membranes prepared from HEK-293 cells overexpressing the cloned receptors. Functional activation of hIR signaling was determined in cells by quantitation of receptor phosphorylation by ELISA using 293 cells expressing hIR-A or hIR-B, de novo lipogenesis from [^{14}C]-glucose in 3 T3-L1 adipocytes, and cell proliferation by [^{3}H]-thymidine incorporation in human Saos-2 cells. Statistical comparisons were determined for the K_i/EC_{50} responses from each assay using the Sidak and Holm-Bonferroni multiple comparison adjustment methods with the significance level set at α=0.05.

Results: The receptor binding affinity, K_i, of LY IGlar was determined to be 0.41±0.01, 0.45±0.03, and 16.0±0.4 nM for hIR-A, hIR-B, and hIGF-1R, respectively. The affinities were comparable to those observed for IGlar at 0.40±0.02, 0.45±0.04, and 15.5±0.6 nM, respectively. To determine whether LY IGlar and IGlar stimulated similar functional activation of hIR, we used 293 cells expressing hIR-A or hIR-B to determine the half-maximal concentrations, EC_{50}, of each ligand required to stimulate receptor tyrosine phosphorylation. The EC_{50} values for LY IGlar were 3.7±0.2 and 2.1±0.1 nM for hIR-A and hIR-B, respectively. The EC_{50} values for IGlar were 4.5±0.2 and 2.5±0.1 nM for hIR-A and hIR-B, respectively. Using 3 T3-L1 adipocytes to assess ligand stimulation of metabolic activity, we found that LY IGlar and IGlar were maximally efficacious with comparable EC_{50} values for stimulation of de novo lipogenesis from glucose. The EC_{50} of LY IGlar was 0.97±0.09 nM and the EC_{50} of IGlar was 0.87±0.08 for stimulation of de novo lipogenesis. The mitogenic potential of LY IGlar and IGlar was determined by [^{3}H]-thymidine incorporation in the hIGF-1R dominant human cell line, Saos-2. The EC_{50} values for stimulation of cell proliferation by LY IGlar and IGlar were 0.53±0.03 and 0.53±0.03 nM, respectively.

Conclusion: Results showed that the in vitro pharmacological properties of LY IGlar were not different from IGlar, supporting LY IGlar as an alternative therapy for patients with diabetes.

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D-chiro-inositol improves metabolic control in overweight patients with type 1 diabetes

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Background and aims: With the increase of obesity in childhood and adolescence, insulin resistance is now occurring more frequently in patients with type 1 diabetes (T1D). Consequently, insulin doses are often increased causing weight gain and poor glycaemic control in these patients. Therefore, to achieve optimal glycaemic control and to improve insulin sensitivity, insulin sensitizing drugs such as metformin are commonly used in addition to insulin therapy in overweight and obese T1D patients. Similarly to metformin, studies in vitro and in animal models have shown that D-Chiro-Inositol (DCI), as putative mediator of intracellular insulin action can accelerate glucose disposal and act as insulin sensitizer. In recent clinical trials significant reduction of DCI plasma levels and a linear relationship between its decreased urinary excretion and the degree of insulin resistance were observed. In these conditions oral supplementation with DCI seems to improve glucose metabolism reducing insulin resistance status. The aim of this prospective, randomized controlled trial was to evaluate the efficacy of DCI oral supplementation on glycaemic control as assessed by HbA1c in patients with T1D undergoing intensive insulin therapy.

Materials and methods: A total of 25 patients affected by T1D aged 17-50 years (12 males, 13 females) with disease duration>1 year and BMI >25, were enrolled in the study. Patients were randomised to 1 g DCI plus 400 mcg folic acid once daily (treated group) or to 400 mcg folic acid only once daily (control Group). HbA1c and BMI were evaluated at entry into the trial and at 3 and 6 months follow-up. The sample size for the study has been calculated taking into account 80% power and a difference of HbA1c of 0.3% at the end of the study period. Paired t test (two tailed) and analysis of variance were used to evaluate differences in HbA1c and BMI at different time points.

Results: HbA1c at entry was 8.3%±0.14 (SD) in DCI treated group and 7.9%±0.28 (SD) in control patients (p:NS). After 3 months follow-up a statistically significant reduction of HbA1c levels were observed in DCI treated group vs. control group (7.4%±0.8 vs. 7.7%±1.0, respectively, p:<0.05). At the end of the study period HbA1c reduction in DCI treated group vs. control group was statistically confirmed. BMI at entry was 25.6±1.8 (SD) in DCI treated group and 27.1±2.2 (SD) in control patients (p:NS). After 3 and 6 months follow-up there was a tendency for a reduction in BMI in the DCI treated group vs. control group (25.2±1.5 vs. 26.9±1.5, respectively, p:NS)

Conclusion: Insulin therapy is the mainstay of treatment for patients with T1D. This trial demonstrated for the first time that the oral supplementation of DCI to insulin treatment in T1D patients improves glycaemic control as shown by a significant reduction of HbA1c levels.

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The role of a fixed Berberis aristata/Silybum marianum combination in the treatment of type 1 diabetic patients

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Background and aims: Berberis Aristata is available in a fixed dose with Sylibum Marianum, in order to increase its low bioavailability. Even if the effects of Berberis Aristata in type 2 diabetic patients have been already reported, nothing has been published about Berberis Aristata use in type 1 diabetic patients. For this reason, the aim of this study was to evaluate if the addition of Berberis Aristata/Sylibum Marianum leads to a reduction of insulin dose and to an improvement of glycemic control in type 1 diabetic patients.
Materials and methods: We enrolled 85 type 1 diabetic patients and randomized them to add, to their usual insulin therapy, placebo or Berberis Aristata/Silybum Marianum 588/105 mg, 1 tablet during the lunch and 1 tablet during the dinner, for six months. We evaluated if there was a reduction of insulin dose necessary to reach an adequate glycemic control. We also evaluated at baseline, and after 6 months: body mass index (BMI), glycated hemoglobin, fasting plasma glucose (FPG), postprandial glucose (PPG), total cholesterol (TC), low density lipoprotein-cholesterol (LDL-C), high density lipoprotein-cholesterol (HDL-C), triglycerides (Tg). The null hypothesis that the expected total dose of insulin used from randomization would did not differ significantly between placebo, and Berberis Aristata/Silybum Marianum was tested using analysis of variance and analysis of covariance (ANCOVA) models. Similar analyses were applied to the other variables. The statistical significance of the independent effects of treatments on the other variables was determined using ANCOVA. A 1-sample t test was used to compare values obtained before and after treatment administration; 2-sample t tests were used for between-group comparisons. Statistical analysis of data was performed using the Statistical Package for Social Sciences software version 11.0 (SPSS Inc., Chicago, Illinois, USA). For all statistical analyses, $p<0.05$ was considered statistically significant.
Results: We observed a reduction of total insulin dose necessary to achieve an adequate glycemic control in Berberis Aristata/Silybum Marianum, both compared to baseline and to placebo ($p<0.05$ for both). Regarding insulin administration at meals, we recorded that the group treated with Berberis Aristata/Silybum Marianum used less insulin at meals, both compared to baseline and placebo ($p<0.05$ for breakfast, and $p<0.01$ for lunch and dinner). The same can be said for insulin at bedtime ($p<0.05$ for both vs baseline and vs placebo). We observed a decrease of glycated hemoglobin with Berberis Aristata/Sylibum Marianum compared to baseline ($p<0.05$), but not compared to placebo. There was a decrease of FPG, and PPG with Berberis Aristata/Sylibum Marianum both compared to baseline and to placebo ($p<0.05$ for both). We recorded a decrease of TC, Tg and LDL-C and an increase of HDL-C with Berberis Aristata/Sylibum Marianum both compared to baseline and to placebo ($p<0.05$ for both).
Conclusion: The addition of Berberis Aristata/Sylibum Marianum to insulin therapy in type 1 diabetic patients leads to a reduction of the insulin dose necessary to achieve an adequate glycemic control.

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Resveratrol improves glucose homeostasis in diabetic rats: Potential involvement of the hepatic glucose handling

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Background and aims: The polyphenol resveratrol is a powerful activator of the sirtuin 1 (SIRT1), a histone deacetylase that plays an important role in the cellular homeostasis. It has been described that resveratrol improves the metabolic profile of obese subjects; however, the mechanisms by which this effect occurs remain obscure. Glycemic homeostasis involves a balance between glucose uptake and delivery, respectively in skeletal muscle and liver. These glucose fluxes are impaired in diabetes mellitus (DM), and have been related to decreased GLUT4 protein (encoded by the Slc2a4 gene) in muscle and increased GLUT2 protein (encoded by the Slc2a2 gene) and phosphoenolpyruvate carboxykinase (Pepck) expression in liver. Despite evidence that resveratrol improves the metabolic profile, it is not established if it could contribute to improve glycemic homeostasis in DM. We hypothesized that resveratrol could revert the DM-induced alterations in the expression of glucose transporters, thus contributing to improve glycemic homeostasis. Therefore, the present study investigated, in insulin-treated diabetic rats, if resveratrol improves glycemic homeostasis, and regulates gene expression of the glucose transporters in muscle and liver as well.
Materials and methods: Male Wistar rats were rendered diabetic by streptozotocin injection (50 mg/Kg of body weight); citrate buffer-injected rats were used as non-diabetic controls (ND). Twenty days later, the diabetic rats were divided into 3 groups: placebo-treated diabetic (DP), insulin-treated diabetic (DI, 6 U/day insulin) and insulin+resveratrol treated diabetic (DIR) (6 U/day insulin + 10 mg/Kg resveratrol). After 30 days of treatment, glycemia, 24-hour glycosuria and plasma fructosamine were evaluated, and soleus muscle and liver were sampled for analysis of Slc2a4, Slc2a2 and Pepck mRNAs (qPCR), and GLUT4, GLUT2 and p-AMPK (phosphorylated AMP-activated protein kinase) proteins (Western blotting). Results were analyzed by one-way ANOVA, followed by Bonferroni post-test.
Results: As expected, DP rats showed increased glycemia, 24-hour glycosuria and plasma fructosamine. DI and DIR showed similar decrease in glycemia and glycosuria; however, only DIR restored completely the fructosamine concentration ($P<0.01$ vs DP and DI). In soleus, diabetes decreased Slc2a4 (by 60%, $P<0.001$), GLUT4 (by 53%, $P<0.05$) and p-AMPK (by 48%, $P<0.05$); and all these parameters were similarly recovered in DI and DIR rats. On the other hand, in liver, diabetes increased the Slc2a2 (by 3.6 folds, $P<0.001$), GLUT2 (by 30%, $P<0.05$) and Pepck (by 80%, $P<0.01$); and insulin alone was not able to significantly alter these parameters. However, the addition of resveratrol to insulin treatment restored the Slc2a2 ($P<0.001$ vs DP and $P<0.05$ vs DI), GLUT2 ($P<0.05$ vs DP and DI) and Pepck ($P<0.001$ vs DP, $P<0.05$ vs DI) expression to the non-diabetic values.
Conclusion: Addition of resveratrol to insulin treatment of diabetic rats improved long-term glycemic homeostasis, as evinced by plasma fructosamine levels. Resveratrol also decreased Slc2a2 and Pepck in liver, indicating reduction in hepatocyte gluconeogenic activity and glucose efflux as well. No evidence of resveratrol effect in skeletal muscle was observed. In summary, the results reveal that resveratrol, as coadjuvant in the insulintheraphy of diabetic rats, improves glycemic homeostasis, and that involves reduced hepatic glucose production.
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PS 034 Exercise physiology

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Stearoyl-CoA desaturase and its regulation in human skeletal muscle by exercise

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Background and aims: Stearoyl-CoA desaturase 1 (SCD1) catalyzes the rate-limiting step in the synthesis of monounsaturated fatty acids (FA), which are required for *de novo* triacylglycerol synthesis. In this way SCD1 also converts FA with a high lipotoxic potential into FA with lower lipotoxic potential, e.g. palmitic acid (PA) to palmitoleic acid. Recently, a muscle-specific SCD1-overexpressing mouse model was described with elevated glucose uptake, FA oxidation and exercise capacity. Hence, a high SCD1 level in skeletal muscle might be beneficial for its metabolic function. Here, we investigated the regulation of SCD1 in human skeletal muscle.
Materials and methods: Healthy sedentary men categorized either as control (BMI=23.5±2.0 kg/m^2, normal fasting and 2 h serum glucose levels, n=13) or dysglycemic (pT2D; BMI=28.9±2.5 kg/m^2, fasting glucose≥5.6 mmol/L and/or 2 h serum glucose≥7.8 mmol/L, n=11) participated in a combined strength and endurance training for 12 weeks. In addition, an acute endurance test was performed before and after the intervention. Biopsies from *m. vastus lateralis* were taken before, directly after, and 2 h post exercise, before and after intervention. RNA was isolated and analyzed by high throughput mRNA sequencing followed by differential gene expression analysis. Primary human skeletal muscle cells (SkMC) were exposed to electrical pulse stimulation (EPS) to induce contraction, low dose of PA or a combination of both followed by analyses using qRT-PCR.
Results: At baseline SCD1 mRNA expression was 50% lower in skeletal muscle of pT2D compared to control group ($p<0.001$). After the intervention SCD1 expression was enhanced significantly both in pT2D (1.8-fold, $p<0.001$) and control subjects (1.5fold, $p<0.001$). Both groups also had increased VO_2max (13%, $p<0.001$) and insulin sensitivity (pT2D 29%, $p<0.01$; control 36%, $p<0.001$). At baseline acute exercise increased SCD1 mRNA expression in the pT2D group directly after the acute bout (1.3fold, $p<0.05$). After intervention the pT2D group exhibited a significant 1.9-fold increase in SCD1 mRNA expression $p<0.001$) 2 h post exercise, which then reached a similar level as in the controls. During *in vitro* differentiation of SkMC SCD1 mRNA expression increased significantly (3.7-fold, n=5-6) in parallel with up-regulation of myogenin (4-fold) and MHCIIa (10-fold). Stimulation of contraction by EPS (1 Hz, 2 ms, 11.5 V) for 24 h significantly promoted SCD1 mRNA expression (1.5-fold, $p<0.05$, n=7) in parallel with induction of PGC1a (1.8-fold, $p<0.01$) and IL-6 (2.8-fold, $p<0.01$). Incubation of SkMC with PA increased SCD1 mRNA expression significantly at a concentration as low as 20 μM (1.2-fold, $p<0.05$). Maximum induction of 2-fold was observed at 100 μM ($p<0.001$, n=8), and higher concentrations of PA up to 300 μM had no additional effect. The combination of EPS and PA for 24 h increased SCD1 mRNA expression 3.8-fold ($p<0.001$, n=7) compared to control cells.
Conclusion: Given the important role of SCD1 in lipid metabolism its lower expression in skeletal muscle of overweight dysglycemic subjects may be linked to metabolic impairments observed in similar groups of participants. Moreover, the up-regulation of SCD1 by long-term exercise may be associated with increased insulin sensitivity.
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Does the energy cost of walking explain why patients with diabetic peripheral neuropathy walk more slowly?

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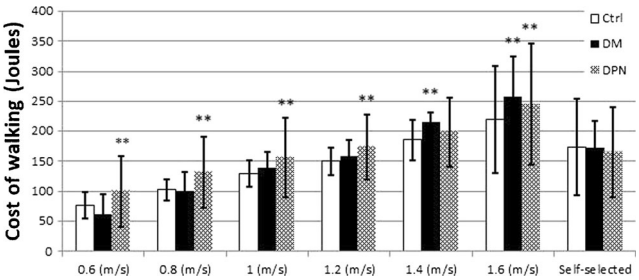
Background and aims: It is has previously been shown that patients with diabetes and especially those with diabetic peripheral neuropathy (DPN) choose to walk more slowly (self-selected speed) and display a number of other gait alterations compared to age-matched healthy controls. It has been suggested that patients with diabetes and DPN have a lower self-selected walking speed to lower the physical demands of walking. However, there are a number of physiological alterations with diabetes that would increase the energy cost of walking (CoW). We therefore hypothesise that the CoW will be higher in patients with diabetes and DPN compared to controls when walking at the same speed, and will dictate the choice of a slower self-selected walking speed in patients with diabetes and DPN. The aim of this study was to investigate the CoW in patients with diabetes and neuropathy at different speeds.
Materials and methods: Participants were allocated into one of the three groups: healthy controls (Ctrl; mean age: 56, n=31), patients with diabetes but no neuropathy (DM; mean age: 51, n=22) and patients with diabetes and moderate-severe peripheral neuropathy (DPN; mean age: 66, n=14). Participants walked at a range of control speeds on a treadmill while expired air was measured to determine oxygen uptake and the CoW calculated. Participant's self-selected walking speed was measured in a gait laboratory using motion analysis. Group differences were tested using an ANOVA and LSD post-hoc test.
Results: Self-selected walking speed was lower in DM and DPN patients compared to controls, albeit not reaching significance (Ctrl: 1.45; DM: 1.38; DPN: 1.35 m·s−1; $P>0.05$) but the CoW was not different between groups. At standardised speeds, patients with DPN walked with a higher CoW compared to the Ctrl and DM group and this was mostly evident at the speeds of 0.6, 0.8, 1.0, 1.2 and 1.6 m·s−1 (Figure 1).
Conclusion: When walking at the same controlled speeds, the CoW was significantly higher in people with DPN compared to controls, people with DPN chose to walk more slowly than controls, but there were no differences in the CoW between groups at this self-selected speed. There are a number of physiological mechanisms such as stiffer tendons that cause a higher CoW in people with DPN when they are 'forced' to match the walking speed of controls. We suggest that the CoW is a major factor in determining why people with DPN choose to walk more slowly, since they seek to minimise the energy CoW. This will likely have implications for the level physical activity and amount of walking people with DPN choose to perform, which might impact upon glycaemic control.



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Energy expenditure from daily physical activity and risk of developing diabetes and change in cardiovascular risk factors

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Background and aims: Prior studies have shown that leisure time physical activity decreased the risk of incidence of diabetes and hypertension, and increasing daily steps ameliorated lipid metabolism in Japanese. However, very few studies used the information of energy expenditure (EE) estimated from time and intensity during daily physical activity. Here, we examined the association between daily EE estimated by validated questionnaires and risk of developing diabetes and change in cardiovascular risk factors.
Materials and methods: We registered 1,532 Japanese health checkup examinees at SSK hospital who were neither previously diagnosed diabetes, or medicated hypertension or dyslipidemia. They completed the Japan Arteriosclerosis Longitudinal Study Physical Activity Questionnaire, which examined usual patterns of physical activity during the previous month. Of those, 1,096 (746 men, average age: 55±8 years) were followed for 5 years. Incidence of diabetes was defined when HbA1c ≥6.5% or fasting plasma glucose (FPG) ≥126 mg/dl was observed at least once during the follow-up period. The relation between total (tertile) or leisure time (0 or >0) EE at baseline and 5-year change in waist circumference (Wc), body mass index (BMI), FPG, blood pressures, lipids, and high-sensitivity C-reactive protein (CRP) were analyzed. Cox's proportional hazard model was used to calculate hazard ratios (HRs) and their 95% confidence intervals (CIs) for incidence of diabetes from total or leisure time EE at baseline.
Results: In men, total EE had negative relations with FPG and triglyceride (TG) at baseline, and leisure time EE had a positive relation with HDL-cholesterol (HDL-c) and negative relations with TG and CRP at baseline (all $p<0.05$). In women, total EE had negative relations with Wc and BMI at baseline and a positive relation with HDL-c, and leisure time EE had no relation with cardiovascular risk factors at baseline. Increasing total EE (tertile1 →3) increased 5-year change of HDL-c (3.0→5.0%, $p=0.037$) in men. Men with leisure time EE›0 showed lower 5-year change in TG and higher 5-year change in HDL-c compared with men with leisure time EE=0 (25 vs 14% and 1.5 vs 6.3% respectively, all $p<0.05$). In comparison with the 1st tertile of total EE, HRs (95% CI) for the risk of developing diabetes in the 2nd and 3rd tertiles were 0.71 (0.32-1.61) and 0.73 (0.33-1.65) for men, and 1.33 (0.30-5.96) and 0.32 (0.03-3.04) for women, respectively. The HRs (95%CI) for the risk of developing diabetes in leisure time EE›0 in comparison to leisure time EE=0 were 0.78 (0.34-1.78) for men and 0.54 (0.13-2.27) for women.
Conclusion: Increasing total EE, especially leisure time EE, ameliorated lipid metabolism during five years in Japanese men. The non-significant effect of EE on the development of diabetes may in part reflect the heterogeneity of pathophysiology of diabetes.
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Reduced and adverse metabolic response to supervised 8-week endurance exercise in a group at high risk for type 2 diabetes

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Background and aims: Increase in physical activity is essential for the prevention of type 2 diabetes. However, there is evidence that people differ in their individual response to exercise and there is little information available about potential pathomechanisms involved.
Materials and methods: In our study center in Tübingen, a group of 20 middle-aged individuals (13 women, 7 men), at high risk for type 2 diabetes, completed an 8-week supervised endurance training (3×1h/week) on sub-maximal intensity (80% VO_2max). Individual anaerobic threshold (IAT) was determined by lactate measurements during incremental exercise, peak aerobic capacity (VO_2max) by spiroergometer at exhaustion. Pre and post intervention, insulin sensitivity was measured by a 75 g frequently sampled oral glucose tolerance test. Body fat mass and distribution were determined by magnetic resonance imaging, liver fat content by ^{1}H magnetic spectroscopy.
Results: Despite the relative short time of the intervention, an increase in VO_2max (from 22.9±5.1 to 25.4±5.8; $p=0.0442$) and IAT (from 1.1±0.3 to 1.3±0.3; $p<0.0001$), a decrease in BMI (from, 32.5±4.7 to 32.2±4.8, $p=0.0309$), percent body fat (from 39.0±10.7 to 36.9±10.7; $p=0.0320$), total adipose tissue mass (from 35.4±10.4 to 34.6±10.6; $p=0.0234$), $RR_{diastolic}$(from 92±10 to 88±9; $p=0.0324$), heart ratio (from 76±13 to 70±9; $p=0.0200$), triglycerides (from 114±36 to 98±38, $p=0.0176$), and LDL cholesterol (from 117±28 to 111±25; $p=0.0268$) was observed. Unexpectedly, insulin sensitivity, did not change significantly (8.3±6.4 vs. 9.5±7.8; $p=0.1$). We found a high variability in the change of insulin sensitivity, ranging from 0.6 - to 2.7-fold during the intervention. Insulin sensitivity did not improve in 20 percent of the participants; in 40 percent of the subjects a negative effect on insulin sensitivity was seen. Between the two groups with the largest changes of insulin sensitivity (8 responder versus 8 non-responder) there were no differences in baseline parameters. However, after adjustment for sex, age and BMI, transaminases (GOT: 28±6 vs. 24±8, $p_{adj}=0.0082$; GPT: 32±13 vs. 30±16; $p_{adj}=0.0080$; γ-GT: 38±14 vs. 21±15; $p_{adj}=0.05$) and intrahepatic lipids (8.4±6.6 vs. 7.8±8.5, $p_{adj}=0.03$) were higher in the responders, pointing to a possible impact of intrahepatic lipids on the increase of insulin sensitivity during a supervised exercise training.
Conclusion: We found that, although mean body fat mass and cardiorespiratory fitness improved during a supervised exercise intervention, insulin sensitivity did not increase in 60 percent of these individuals. High liver fat content and elevated liver enzymes may predict a larger increase in insulin sensitivity during exercise training.
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IL6 mediates the reduction of insulin secretion in exercise-trained mice

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Background and aims: Exercise training reduces glucose-induced insulin secretion (GIIS) in lean and obese rodents. Despite higher insulin sensitivity and blood glucose clearance capacity, some intracellular mechanisms have been proposed to explain the lowered insulin secretion. However, it is still unknown how this signal reaches pancreatic beta cells

activating the intracellular pathways. IL6 is highly released by skeletal muscle cells and it was reported to mediate the crosstalk between contracting muscles and pancreatic beta cells. Thus, our aim was investigating the role of IL6 on GIIS modulation of exercise-trained mice.
Materials and methods: After weaning, 21 days old male C57BL-6 mice were randomly assigned into the control group (C) remaining untrained, limited only to cage movement and the trained group (TRE). Mice were submitted to an endurance training 5× per week, during 1 h, at 70% VO2 max for 8 weeks. After training, pancreatic islets were isolated and the blood collected. GIIS was measured in islets from trained mice. In addition, islets from non-trained mice were incubated with serum from trained animals with or without IL6 inhibitor. It was also quantified UCP2 expression and ATP production. Insulin content was measured by radioimmunoassay.
Results: Isolated islets from TRE mice displayed 50% lower GIIS under stimulatory glucose concentration. CTL islets incubated with serum from TRE animals showed the same magnitude of insulin secretion decrease, reported in the islets from trained mice. When the islets from CTL animals were pre-incubated with IL6 inhibitor, before incubating with serum from TRE mice, the effect of exercise-induced reduction on GIIS was lost. Although UCP2 expression was increased in TRE, ATP production did not differ between the groups.
Conclusion: Exercise-induced GIIS modulation seems to be an endocrine signaling through plasma factors, being IL6 among them. Higher islets UCP2 content was associated with reduced GIIS. However, ATP production was not altered.
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Synergistic mtDNA point mutations of OXPHOS complexes resulted in reduced fat mass, improved genetic fitness in a conplastic mouse strain

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Background and aims: Mutations in complexes of the respiratory chain, which are encoded in the mitochondrial genome (mtDNA), can alter cellular energy metabolism during aging. Due to mitochondrial dysfunction mtDNA mutations may result in a reduced physiological fitness and metabolic diseases such as adipositas and type 2 diabetes mellitus. Previous studies indicate that multiple mutations in the OXPHOS system predispose to hepatosteatosis. It was the aim of this study to investigate the fitness, muscle and fat mass from conplastic mice carrying a single mtDNA point mutation in the cytochrome c oxidase (complex IV) or a combined mutation in the cytochrome c oxidase (complex IV) and the NADH dehydrogenase (complex I).
Materials and methods: The genetic fitness was quantified by a computer-controlled treadmill in conplastic mouse strains C57BL/6NTac-mtBPL/1J (NADH dehydrogenase mutation und cytochrome c oxidase mutation, mtBPL), C57BL/6NTac-mtNOD/LtJ (cytochrome c oxidase mutation, mtNOD) and C57BL/6NTac-mtAKR/J (control; mtAKR) at the age of 6 months. In addition, we analyzed blood glucose levels. Muscle and fat mass was quantified by weighting femoral muscle, gonadal and brown adipose tissue, respectively. Data are expressed as percentage of total body weight.
Results: At the age of 6 months mtBPL mice (7.2 mmol/l) showed significantly higher blood glucose levels compared to mtNOD mice (6.1 mmol/l) and mtAKR control mice (6.2 mmol/l). mtBPL (70 m) mice moved over longer distances on a treadmill than mtAKR control mice (28 m). Furthermore, mtBPL mice showed a significantly higher muscle mass (1.3% vs. 1.0%) and a significantly lower fat mass for both gonadal fat (2.9% vs. 4.2%) and brown fat (0.2% vs. 0.3%) compared to mtAKR control mice.
Conclusion: Double mtDNA point mutations in the NADH dehydrogenase and cytochrome c oxidase resulted in higher blood glucose levels in 6 month old mtBPL mice without manifestation of diabetes. However, mtBPL mice showed a better physiological aerobic fitness, higher muscle mass and lower fat deposition. Apparently, OXPHOS mutations induce tissue-specific adaptations of mitochondrial dynamics and energy metabolism. The reduced fat stores in adipose tissue may favor hepatic steatosis and subsequent insulin insensitivity as observed in older mtBPL mice. Thus, mtDNA mutations in humans may also play a causative role for disproportional fat distribution between fat tissue and liver in adipose patients.

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Moderate intensity exercise training rapidly increases insulin stimulated intestinal glucose uptake in sedentary individuals

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Background and aims: Recently it has been shown that insulin is a potent stimulator of glucose uptake (GU) in intestine and that intestinal insulin resistance manifests in obesity and type 2 diabetes. Exercise training improves whole body glycaemia and insulin stimulated skeletal muscle GU. Thus, we aimed to study the tissue specific effects of exercise training on insulin-stimulated intestinal GU.
Materials and methods: Healthy individuals (n=26, aged=48 [SD 5] yrs, BMI=26.1 [SD 2.4] $kg \cdot m^{-2}$, VO_2peak=34.2 [SD 4.1] $ml \cdot kg^{-1} \cdot min^{-1}$) and patients with IFG/IGT/T2D (n=20, aged=49 [SD 4] yrs, BMI= 30.1[SD 2.7] $kg \cdot m^{-2}$, VO_2peak=28.3[SD 4.6] $ml \cdot kg^{-1} \cdot min^{-1}$) were randomized into high intensity interval training (HIT) and moderate intensity training (MIT) groups. The groups were studied before and after two weeks and six sessions of HIT (4-6×30 s all out sprints on cycle ergometer with 4 minutes of recovery) or MIT training (40-60 min cycling with ergometer at 60% of VO_2max). Intestinal GU was measured during euglycemic hyperinsulinemic clamp using positron emission tomography and ^{18}F-FDG.
Results: In healthy individuals, VO_2peak and whole body insulin sensitivity improved and visceral fat decreased similarly in both groups (all $p < 0.05$), following intervention. Training increased GU in colon and tended to increase in small intestine in MIT group, whereas opposite was observed in HIT group [colon: MIT 30%, HIT - 2% ($p=0.02$) and small intestine: MIT 10%, HIT - 9% ($p=0.08$), respectively]. The GU in the small intestine correlated positively with the VO_2peak [Pre: $r=0.46$ $p=0.03$; Post: $r=0.45$ $p=0.03$] and negatively with visceral fat mass [Pre: $r=-0.42$ $p=0.05$; Post: $r=-0.45$ $p=0.03$]. The results for IFG/IGT/T2D group are under analysis and will be presented in the congress.
Conclusion: This study suggests that MIT rapidly enhances insulin stimulated intestinal GU already after two weeks of training in sedentary individuals.
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Effect of individualised physical activity treatment on drug naïve type 2 diabetic patients

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Background and aims: Exercise is considered a corner stone of diabetes management, along with diet and medication since long. The benefits of exercise are determined by its type, duration, frequency and intensity. The aim of this study was to evaluate the health effect of physical activity on prescription in newly diagnosed type 2 diabetes (T2D) patients in Vietnam.
Materials and methods: Twenty-three newly diagnosed T2D patients were randomized in two groups: 12 patients received a 12 week - program of individualized physical activity (IPA group) (at least 30 minutes/day of moderate-to-vigorous intensity activity, face-to-face education each week, guided to use pedometer Yamax 500, physical activity diaries); 11 patients received standard care (SC group) according to WHO guidelines. Fasting plasma glucose (FPG) was measured before, every second week and after twelve weeks. HbA1c, insulinemia, liver enzymes, serum creatinine, BUN, lipid profile, oral glucose tolerance test, blood pressure, weight, waist and hip circumference were measured before and after 12 weeks.
Results: During the treatment period, all patients in IPA group kept walking: mean 5.3±0.9 days/week, 8317±1962 steps/day (67.3±25.9 minutes/day). In IPA group, FPG decreased and achieved the treatment goal after 4 weeks (from 8.8±1.0 to 7.1±0.8 mmol/L, $p<0.001$), which was sustained during the treatment course. Over the twelve weeks, the FPG level decreased by 1.6±0.9 mmol/L in the IPA group and by 0.4±1.2 mmol/L in the SC group (from 8.7±1.9 to 8.3±1.0 mmol/L) ($p<0.05$). HbA1c in the IPA group was reduced by 16.3±7.3 mmol/mol (from 66.8±5.1 to 50.5±5.5 mmol/mol, $p<0.001$) and by 1.3±6.8 mmol/mol in the SC group. In addition, triglyceride levels decreased more ($p<0.05$) in the IPA group (1.1±1.6 mmol/L) between baseline and twelfth week than in the SC group (0.6±1.1 mmol/L). There was no difference between the two groups in homeostasis model assessment of insulin resistance (HOMA - IR), blood pressure, weight, waist and hip circumference.
Conclusion: Individualized physical activity of sufficient frequency, duration and intensity shows a promising improvement of glycemia and lipid profile in drug naïve type 2diabetes.
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PS 035 New insights on old therapies: experimental studies

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Insulin increases the expression of the genes associated with glycoprotein 130 signalling in human skeletal muscle and this effect is reversed by NEFAs

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Background and aims: Glycoprotein 130 (gp130) cytokines, including interleukin 6 (IL-6), act through plasma membrane receptors consisting of 2 glycoproteins: a cytokine binding subunit (like IL-6R) and gp130, responsible for signal transduction. IL-6 may induce insulin resistance, however, in some conditions it may exert insulin-sensitizing effect in skeletal muscle. The aim of the present study was to assess the effect of insulin and NEFAs on the expression of the genes associated with gp130 signaling in skeletal muscle of healthy humans and in cultured human myotubes.
Materials and methods: In experiment 1, we examined 20 healthy male subjects with normal glucose tolerance (mean age 25.2±3.2 years, mean BMI 26.5±4.6 kg/m2). The biopsy of vastus lateralis muscle was performed before and after 6-hour clamps without or with Intralipid/heparin infusion. In experiment 2, we performed muscle biopsy in 8 healthy male subjects with similar characteristics and then satellite cells were differentiated into myotubes. We treated myotubes with insulin and palmitate separately and in combination. In both experiments, the expression of IL-6R, gp130 and associated genes was analyzed with Real Time PCR.
Results: In experiment 1, serum IL-6 increased and soluble forms of IL-6R and gp130 decreased similarly during both clamps (all $p<0.05$). Insulin infusion resulted in an increase in muscle expression of IL-6R ($p<0.001$) and JAK2 ($p=0.041$) in the entire group and in gp130 ($p=0.043$) in normal-weight individuals. Concurrent Intralipid heparin infusion, which reduced insulin sensitivity by approx. 40%, abolished all these insulin's effects. In experiment 2, incubation with insulin increased the expression of IL-6R, gp130 and SOCS3 (all $p<0.05$). Incubation with palmitate reversed all these changes and decreased JAK1 ($p=0.041$).
Conclusion: Our data show that the regulation of genes associated with gp130 signaling in skeletal muscle may be important for lipid-induced insulin resistance.
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Metformin action on cell cycle arrest and insulin resistance condition in hepatocarcinoma cells

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Background and aims: Nutrient overload is associated with the development of obesity, insulin resistance and type 2 diabetes (T2DM). Insulin resistance is a characteristic feature of T2DM and is characterized by defects in insulin signaling. However, the underlying mechanisms for developing insulin resistance in the presence of excess nutrients are

incompletely understood. In recent decades, global changes in dietary habits have led to an increase in added sugar consumption, which has been linked to increased trends in obesity and type 2 diabetes (T2DM). T2DM is associated with a higher risk of liver diseases frequently degenerating in hepatocellular carcinoma (HCC). Fructose (FR) has been reported to be responsible for the metabolic disturbances, liver fat deposition and HCC. Unfortunately, most HCC seem to be resistant to conventional chemotherapy and radiotherapy. The poor efficacy of antitumor agents is also due, at least in part, to the inefficient drug delivery and metabolism exerted by the steatotic/cirrhotic liver that host the tumor. Excessive IGFs signaling is a characteristic feature of liver tumors. Recent studies suggest that downregulation of IGFI together with upregulation of IGFII and overactivation of IGFI receptor and insulin receptor isoform A are important events in HCC development. These alterations cause a higher cell proliferation. Metformin (METF), the most commonly prescribed drug for T2DM that inhibits hepatic gluconeogenesis and decreases glycogenolysis, has been found to lower HCC risk. We investigated molecular effects of METF on in vitro human hepatoma cell lines (HepG2), an useful insulin resistance cell model.

Materials and methods: HepG2 cells were treated with METF (400 μM) in association or not with 5 mM FR for 24 or 48 hours. METF action on cell cycle progression and signaling pathways involved in metabolism regulation was evaluated by Immunofluorescence and Western Blot assay.

Results: Since assessing HepG2 cell viability, METF seemed to decrease growth cell capacity, we performed western blot and immunofluorescence studies to investigate cell cycle regulators. METF showed to induce expression of cyclin D3 and p21 protein. In addition, METF increased ratio retinoblastoma (Rb)/pRb. These data suggest that METF could arrest the cell cycle at G0/G1 phase without inducing apoptosis as supported by p53 unmodified levels. Furthermore, studying PPARs modulation, target signaling of FR action on liver fatty acid metabolism, we observed that METF seemed to increase PPAR γ levels, confirming that METF could reduce cellular lipid drops accumulation caused by FR, as showed by Red Oil staining. Besides, METF showed to improve insulin resistance increasing pIRS1tyr/pIRSser ratio, principal molecular marker of IR condition, and GSK activation.

Conclusion: In conclusion, our data shows that METF could suppress HepG2 proliferation, through induction of cell cycle arrest at G0/G1 phase. Also, METF effect on fatty acid metabolism and on insulin resistance leads to the development of new METF therapeutic use in liver diseases associated with insulin resistance.

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Bone/Body Morphogenetic Proteins (BMPS) act as insulin-sensitisers via upregulation of PPARgamma expression in mature 3T3-L1 adipocytes

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Background and aims: Antidiabetic drugs like Metformin and thiazolidinediones improve insulin sensitivity of muscle and adipose tissue, but can cause severe side effects in type-2-diabetes patients. Therefore, the identification of new insulin sensitizers and a detailed understanding of their functionality is of great interest. Bone/Body morphogenetic proteins are secreted growth factors clinically used because of their bone healing properties. Beside their classical role in development, BMPs are now seen as metabologens. They have been found to regulate adipogenesis, iron metabolism in liver and the formation and thermoregulatory activity of BAT, but their metabolic functions in adult tissues need to be further investigated.

Materials and methods: Fully differentiated 3 T3-L1 adipocytes were stimulated with insulin, BMP-2, BMP-6 or both. To generate insulin-resistant adipocytes, the cells were kept in medium containing insulin for 8 h prior to stimulation. Adipocytes stably expressing a GLUT-4_GFP_7myc-construct were stimulated as described, stained for myc-tag-translocation and analyzed via flow cytometry. Uptake of 3H-2-deoxyglucose was measured after short or long-term stimulations. Protein expression levels were analyzed by qRT-PCR and Western Blot or via flow cytometry.

Results: Previous work showed that overnight stimulation with BMPs in combination with a short-term insulin stimulation significantly increases glucose uptake rates compared to controls ($F=25.998$, $p<0.05$). Furthermore in vitro generated insulin resistant adipocytes show elevated glucose uptake upon 18 h of BMP2 treatment independent of an additional insulin stimulus ($F=9.365$, $p<0.05$). The magnitude of this effect is comparable to the known insulin sensitizer Rosiglitazone that served as positive control. The BMP-mediated effect is not caused by enhanced translocation of GLUT-4 vesicles as shown with a GLUT-4_GFP_7myc-translocation assay and time resolved glucose uptake assays. The insulin sensitizing effect of BMP-2 and BMP-6 was observed earliest 4 h after stimulation. Canonlical Smad 1/5/8 as well as MAP kinase signaling was triggered by BMPs in mature adipocytes. Sequential alteration of PPARgamma, RXRalpha, GLUT-4 and GLUT-1 mRNA expression indicates a transcriptional effect of BMPs on glucose uptake.

Conclusion: Long-term BMP stimulation has an insulin sensitizing effect on mature adipocytes. We suggest that this effect is mediated by activation of PPARgamma expression and not via direct glucose transporter translocation. Phospho-Smads activating the PPARgamma promoter via known Smad-binding elements might mediate this effect on glucose metabolism. The impact of MAP kinase signaling on this effect will be further investigated using selective inhibitors. PPARgamma is a nuclear receptor and its upregulation via BMP signaling could lead to a higher sensitivity of the nuclear surface for natural ligands like polyunsaturated fatty acids. Therefore, PPARgamma together with RXRalpha can act as a transcription factor and initiate transcription of a defined set of downstream targets like GLUT-1 and GLUT-4. To investigate metabolic changes in BMP stimulated adipocytes extracellular flux measurements will be performed. Further work needs to determine if BMPs could serve as a new antidiabetic treatment strategy with reduced side effects, especially beneficial for osteoporosis patients.

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Effect of the long-acting insulin analogues glargine and degludec on cardiac cell function

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Background and aims: Long acting insulin analogues are designed to mimic the physiological pattern of endogenous insulin secretion resulting in a better glycaemic control while reducing the risk of hypoglycaemia. One important aspect for their long-term use is cardiovascular safety data: Insulin glargine (IGla) has proven to be safe in the cardiovascular outcome ORIGIN trial; for Insulin degludec (IDeg), the outcome trial DEVOTE is ongoing. The aim of this study was to investigate IGla, its active metabolite M1 (IGla-M1) and IDeg on signaling and contractility in three pre-clinical cardiovascular cellular models (mouse atrial cell line HL-1, primary rat cardiomyocytes (ARVM) and iPS-cell derived human cardiomoycytes (Cor.4U)).

Materials and methods: Adult rat ventricular cardiomyocytes were isolated from Lewis rats and incubated with 100 nM regular insulin (Ins), IGla, IGla-M1 or IDeg to compare the positive inotropic effect of each

insulin. To examine the role of Akt, we preincubated ARVM for 30 min with the Akt inhibitor triciribine. Additionally, ARVM and HL-1 cells were used to investigate the insulin signaling pathway by Western Blot analysis. To determine the impact of insulins on cardiotoxicity and efficacy in human Cor.4U cells, beating rate was monitored with an impedance-based device (xCelligence Cardio) for up to 24 h.

Results: In HL-1 cells, IDeg stimulation results in a significantly lower Akt (Ser473) phosphorylation compared with Ins, IGla and IGla-M1 after 5- and 10-min incubation. Nonetheless, after 60-min treatment the phosphorylation was comparable with Ins and IGla M1. In ARVM we observed similar increases in pAkt (Ser473) phosphorylation with Ins, IGla-M1 and IDeg (7.4-, 8.2- and 7.8-fold, respectively) after a single timepoint (10 min). Incubation of electrically paced ARVM with the different analogues resulted for all insulins in a significantly increased sarcomeric shortening (2.5-2.8 fold) and similarly increased contractility (2.1-2.4 fold) and relaxation velocities (2.6-3.5 fold). The positive inotropic effect of Ins and long-acting analogues could be totally abrogated by the specific Akt inhibitor triciribine. In human Cor.4U cells, all insulins displayed no obvious cardiotoxicity and slightly increased the rate of spontaneous beating 1.1-1.2-fold.

Conclusion: In conclusion, we demonstrated similar efficacy under steady-state-conditions in regard to insulin signaling, positive inotropy and beating rate for the insulin analogues tested compared with regular insulin. In HL-1 cells, IDeg exhibited a slower on-set of action on AKT phosphorylation. Whether the observed kinetic difference plays a role in more complex tissue models may need to be investigated.

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Insulin glargine and (A21Gly,DiD-Arg) insulin do not promote breast cancer growth in a mouse model

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Background and aims: Since it was found that the insulin analog AspB10 led to mammary tumors in rats, there have been concerns over the potential mitogenicity of insulin analogs. As insulin analogs and delivery systems continue to be developed, there is a need to understand the mitogenic mechanisms of certain analogs.Previous studies have reported that insulin analogs that activate the insulin-like growth factor receptor (IGF-1R) may be more mitogenic, while others have suggested that prolonged activation of the insulin receptor (IR) may be responsible for the tumor promoting effects. Insulin glargine has greater affinity for the IGF-1R than human insulin (HI) in vitro, but in vivo it is metabolized to an M1 metabolite that has similar affinity to the IR and IGF-1R as HI. (A21Gly,DiD-Arg) Insulin is a modified form of insulin glargine that is resistant to in vivo metabolism and has higher affinity for the IGF-1R than HI.The aim of our study was to determine if insulin glargine and the non-metabolizable insulin (NMI) glargine analog (A21Gly,DiD-Arg) promote the growth and metastasis of breast cancer in a mouse model.

Materials and methods: We used female MKR mice on an FVB/n background for these studies.The MKR mouse is a model of insulin resistance, due to muscle specific (M) overexpression of a mutant (K to R mutation) kinase dead IGF-1R. At 8 weeks of age, the 4th mammary fat pad of MKR mice was injected with murine Mvt-1 breast cancer cells, derived from a c-myc/vegf overexpressing model. Tumor bearing mice were divided into 3 groups (n=8 per group) and injected with insulin glargine (12.5 IU/kg twice daily), NMI (12.5 IU/kg twice daily) or an equal volume of vehicle for 3 weeks. Tumors were measured twice weekly for 3 weeks. At the end of the study tumors were weighed, and pulmonary macrometastases were quantified.

Results: There was no difference in tumor size (vehicle 234.3± 39.5 mm^3, glargine 237.4±33.8 mm^3, NMI 265.5±21.8 mm^3) at the end of the study. Similarly, tumor weight and the number of metastases were not significantly different between the insulin glargine, NMI and control groups at the end of the study.

Conclusion: These data demonstrate the lack of mitogenic effect of insulin glargine or the non-metabolizable form of insulin glargine in a mouse model of breast cancer.

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GLP-1 agonist induces transdifferentiation of pancreatic alpha cells dedifferentiated by FOXO signalling

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Background and aims: Pancreatic alpha cell has been more emphasized in pathophysiology of diabetes by glucagoncentric explanation and transdifferentiation to beta cell. One recent study reported that GLP-1 directly increased intrinsic GLP synthesis in alpha cell line and changed alpha cell specific gene transcription. FOXO signaling under glucotoxic injury is related to dedifferentiation of pancreatic beta cell and delta cell. We investigated FOXO signaling in pancreatic alpha cell under hyperglycemia and GLP-1 effect on transdifferentiation of alpha cell.

Materials and methods: α-TC1-6 cell line was cultured in hyperglycemia or FOXO inhibitor. And then GLP-1 agonist, Exendin-4 was applied to them. We measured FOXO transcription factor, Pax6 levels and MafB gene, glucagon gene (Gcg) and protein expression after hyperglycemia or FOXO inhibitor treatment. PDX1, Pax4 levels, Pax6 levels, MafA and MafB gene, glucagon gene (Gcg) and protein expression were checked after Exendin-4 treatment.

Results: Hyperglycemia induced FOXO nuclear localization in pancreatic alpha cell. Hyperglycemia and FOXO inhibitor decreased Maf B, glucagon gene and Pax 6. Exendin-4 increased Maf A, Pax 4 and PDX1 in FOXO inhibitor affected alpha cells

Conclusion: GLP-1 agonist treatment can induce transdifferentiation of pancreatic alpha cell dedifferentiated by FOXO signaling and hyperglycemia

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Effects of glucose concentration on AMPK activation and activity of metformin and berberine

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Background and aims: Both metformin and berberine are well-known hypoglycemic agents for treatment of type 2 diabetes. Underlying mechanisms involves activation of AMP-activated protein kinase (AMPK) pathway. However, theoretically excess nutrient such as hyperglycemia may inactivate AMPK. This study aimed to examine whether metformin and berberine were able to exert hypoglycemic effects under high glucose condition via AMPK activation.

Materials and methods: Glucose consumption was used to evaluate the glucose-lowering effect of metformin and berberine on HepG2 hepatocytes and C2C12 myotubes. AMPK phosphorylation and ACC phosphorylation were measured with western blot to assess the activity of AMPK pathway. Anaerobic glycolysis was evaluated through determining lactate concentration in the medium.

Results: In this study, we found that in HepG2 hepatocyte and C2C12 myotubes, metformin and berberine significantly increased glucose consumption in a dose-dependent manner. In different glucose concentrations, e.g. 5.6 mmol/L, 15 mmol/L and 30 mmol/L, metformin and berberine exerted similar glucose-lowering effects. On the other hand, the lactate production was largely enhanced by metformin or berberine in the cells in the same way as they affect glucose consumption. Meanwhile, AMPK and ACC phosphorylation was stimulated by 5 mmol/L metformin or 10 μmol/L berberine in the presence of 5.6 mmol/L glucose concentration. Nevertheless, in 15 mmol/L glucose concentration, metformin and berberine failed to activate AMPK pathway. In 30 mmol/L glucose concentration, metformin and berberine even reduced phosphorylation of AMPK and ACC. Surprisingly, more phosphorylation of AMPK and ACC was observed in high glucose concentration compared with low glucose concentration.
Conclusion: These results suggest: 1) Metformin and berberine promote glucose metabolism by stimulation of glycolysis, which is independent of glucose concentration. 2) Metformin and berberine activate AMPK pathway in low glucose concentration other than in high glucose concentration. 3) High glucose concentration is able to activate AMPK pathway by itself.
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Glucagon receptor siRNA is not as effective at normalising glycaemia and lipid metabolism as leptin treatment in streptozotocin-diabetic mice

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Background and aims: The hormone leptin can reverse hyperglycemia in rodent models of type 1 diabetes. Supraphysiological levels of leptin dramatically reduce the counter-regulatory hormone glucagon which may be one mechanism by which leptin acts to reduce glycemia. However, leptin therapy can also improve hyperglycemia prior to normalization of glucagon and can reverse hyperglucagonemia without restoring normoglycemia. Therefore, the contribution of glucagon suppression to the metabolic actions of leptin remains unclear. To investigate this we compared the metabolic effects of leptin with hepatic glucagon receptor (GCGR) knockdown using GCGR siRNA encapsulated in liposomal nanoparticles (LNPs) in streptozotocin (STZ)-treated mice.
Materials and methods: To find an effective GCGR siRNA, 3 GCGR siRNAs, and factor VII (FVII) siRNA as a control, were encapsulated into LNPs and delivered via the tail-vein at a dose of 5 mg/kg to wildtype mice and subsequent glucose homeostasis was assessed for 7 days. To compare the effect of glucagon suppression in type 1 diabetes to that of leptin treatment, STZ diabetic mice were administered either 5 mg/kg GCGR siRNA or FVII siRNA as a control, then half of the animals from each group were implanted with a pump delivering 20 μg/day leptin resulting in mice that received FVII siRNA, GCGR siRNA, leptin+FVII siRNA, or both GCGR siRNA and leptin treatments. Various metabolic parameters were assessed over 7 days to compare the therapies.
Results: Two of three GCGR siRNAs appeared to work in wildtype mice; the most effective GCGR siRNA was capable of reducing fasting blood glucose (5.6±0.1 mM vs 8.1±0.3 mM, $p<0.05$), enhancing oral glucose tolerance (10.8±0.6 mM vs 19.2±0.8 mM, 20 min post gavage, $p<0.05$), and elevating plasma glucagon levels (140±30 pg/mL vs 14±3 pg/mL, $p<0.05$) compared to controls given FVII siRNA. This GCGR siRNA was then used in mice with STZ-diabetes to compare with leptin treatment. As expected, glucagon levels were suppressed by leptin treatment, and increased by GCGR siRNA treatment. In addition, this dose of leptin induced supraphysiological levels of leptin that were unaltered by GCGR siRNA treatment. Blood glucose levels were significantly reduced by GCGR siRNA treatment, however, not as dramatically as with leptin treatment (29.5±0.6 mM FVII siRNA, 19.4±1.2 mM GCGR siRNA, 9.9±0.7 mM leptin+FVII siRNA, $p<0.05$). Plasma β-hydroxybutyrate was similarly normalized by both treatments compared to FVII siRNA treatment (2.67±0.22 mM FVII siRNA, 0.56±0.04 mM GCGR siRNA, 0.38±0.03 mM leptin+FVII siRNA, $p<0.05$). Conversely, plasma cholesterol, triglycerides, fatty acids, and glycerol were all significantly reduced by leptin treatment, but were unaffected by GCGR siRNA treatment. For all parameters, the addition of GCGR siRNA to leptin treatment did not further improve metabolism compared to leptin treatment alone.
Conclusion: Compared to leptin treatment, GCGR siRNA was equally effective at reducing plasma ketones, partially effective at reducing glycemia, and ineffective at reducing cholesterol, triglycerides, fatty acids, and glycerol. Therefore our results suggest that the glucagon suppression by leptin only accounts for a portion of the metabolic actions of leptin.
Supported by: CIHR

PS 036 Insulin signalling and metabolism in muscle

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Fibroblast growth factor-21 is secreted by skeletal muscle in vivo and correlates with muscle glucose uptake in humans with type 2 diabetes

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Background and aims: Fibroblast growth factor-21 (FGF-21) belongs to the fibroblast growth factor family, and serves as an endocrine hormone, which was originally identified to increase glucose uptake in fat cells. In rodents, FGF-21 is highly expressed in the liver and regulates hepatic glucose production. The physiological role of circulating FGF-21 in the regulation of carbohydrate metabolism in-vivo in skeletal muscle which is the most important tissue for insulin-stimulated glucose uptake, still remains to be clarified. The aim of this study was to investigate the association of circulating FGF-21 levels with glucose uptake by the skeletal muscle in humans with type 2 diabetes (DM2), using the arteriovenous difference technique across the forearm muscle.
Materials and methods: A mixed meal (557 kcal; 75 g carbohydrates, 26 g protein, 17 g fat) was given to 11 newly diagnosed subjects with DM2 (age 53±4 years, BMI 25±1, HbA1c 6.8±0.3%) and 10 control subjects (age 48±4 yrs, BMI 24±1 kg/m2). Plasma FGF-21, glucose [G] and insulin were measured preprandially and at 60 min intervals for 300 min postprandialy from the radial artery (A) and from a deep forearm vein (V). Muscle blood flow [BF] was measured with strain-gauge plethysmography. Calculations: Glucose flux: [G(A-V)]x[BF]; Glucose clearance:[G(A-V)]x[BF]/[G(A)].
Results: 1) FGF-21 concentrations were increased in the forearm vein compared to the artery throughout the whole 5-h period in both DM2 (poverall=0.0028) and control (poverall=0.0146) subjects 2) FGF-21 levels were changed after the mixed meal in both DM2 (poverall= 0.0008) and control (poverall=0.0092) subjects, reaching a peak at 120 min (following the postprandial glucose and insulin increase) 3) Fasting FGF-21 was increased in patients with DM2 compared to controls (157±30 vs 82±12 pg/ml respectively, p=0.0146) (3) Fasting FGF-21 was positively correlated with muscle glucose flux (r=0.87, p=0.0004) and glucose clearance (r=0.07, p=0.0127) in patients with DM2.
Conclusion: FGF-21 is secreted by human skeletal muscle in-vivo. In type 2 diabetes FGF-21 is increased and positively correlated with glucose flux and glucose clearance by the forearm muscle. Therefore, FGF-21 may be considered as a beneficial myokine for improving muscle glucose metabolism in type 2 diabetes.

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AS160 vs Tbc1d1: cooperative determination of insulin-responsive GLUT4 trafficking activity after exercise-mimetic stimuli

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Background and aims: AS160 and Tbc1d1, proteins of the TBC1D Rab GTPase-activating (RabGAPs) family, emerged as the key nexus deciphering biochemical phosphorylation signals into physical processes of GLUT4 translocation. Unlike adipocytes which express only AS160, skeletal muscle cells express both RabGAPs and their relative abundances vary among different skeletal muscles. With our novel GLUT4 nanometry combined with cell-based reconstitution model, we clearly determined the respective functional roles of the RabGAPs in GLUT4 behavior (MBoC 24: 806, 2013), *i.e.* AS160 mediates insulin-responsive GLUT4 release from its static storage sites and, in contrast, Tbc1d1 temporally acquires insulin-responsiveness which triggers GLUT4 release only after exercise-mimetic stimuli (AICAR-pretreatment and transient cytosolic Ca^{2+} increases). We herein analyzed their combinatorial regulator y mechanisms employing our methodology with varied relative abundances of the two RabGAPs.
Materials and methods: All experiments were performed in 3T3L1 fibroblasts exogenously expressing myc-GLUT4, HA-sortilin, AS160-enhanced yellow fluorescent protein (EYFP) and HaloTag-Tbc1d1, and relative abundances of AS160 and Tbc1d1 were estimated by relative fluorescence intensities of EYFP to those of HaloTag TMR ligand. Intracellular myc-GLUT4 was labeled with Quantum dot-conjugated anti-myc antibodies, and the movement of individual Quantum dot signals was tracked with positional precision at 6 nm.
Results: First, Tbc1d1 inhibited AS160-mediated insulin-dependent GLUT4 release in a relative abundance-dependent manner, indicating that Tbc1d1 functionally dominates AS160. Detailed functional assessments with varied relative abundances of AS160 and Tbc1d1 revealed that AS160 modulates sensitivity to external stimuli in Tbc1d1-mediated activation of GLUT4 trafficking. For example, GLUT4 release induced by transient cytosolic Ca^{2+} increases in the presence of insulin with abundant AS160 occurred much faster (~30 s) than that in cells with little or no AS160 (~10 min). In addition, acquired insulin responsiveness after AICAR-pretreatment is more obvious in cells with both Tbc1d1 and AS160 even after acute (5 min) stimulation than in cells expressing only Tbc1d1. Importantly, this modulatory action of AS160 was completely abolished by an obesity-related Tbc1d1 mutant (R125W) with an impaired phosphotyrosine-binding 1 (PTB1) domain.
Conclusion: Our GLUT4 nanometry combined with cell-based reconstitution model revealed that AS160 potentiates insulin sensitivity in Tbc1d1-mediated GLUT4 releasing activity after exercise-mimetic stimuli, apparently mediated through functional interaction with the PTB1 domain of Tbc1d1. This modulatory action could be involved in the differences in insulin sensitivity among skeletal muscles and beneficial effects of exercise on muscle insulin sensitivity. To further verify this notion, we currently examine GLUT4 behavior in isolated skeletal muscle and *in vivo* using several imaging techniques.

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Alpha-lipoic acid increases "exercise-mimetic" effects of the PPARbeta/delta pathway in skeletal muscle

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Background and aims: The quality of the acute and adaptive response to physical exercise depends on exercise-induced molecular signals and signaling pathways, the activation of which varies with redox status. α-lipoic acid (α-LA), a powerful antioxidant, is independently involved in some exercise-signaling pathway activation driven by PPARβ/δ, a transcription factor known to mimic the phenotypic adaptation to aerobic exercise training. We validated the hypothesis that α-LA interacts with PPARβ/δ genic effects in skeletal muscle, identified the molecular mechanisms involved in those effects, and determined their potential physiological implications.
Materials and methods: Differentiated C2C12 myotubes and C2C12 expressing a negative-dominant form of PPARβ/δ, were treated during 48 h with various concentrations of α-LA (50 to 250 μmol/l) and PPARβ/δ agonist GW0742 (0.3 to 300 nmol/l). Different inhibitors were used (eg. the non-competitive inhibitor of PPARβ/δ GSK3787 (1 μmol/l) and the inhibitor of the c-Jun N-terminal (JNK) pathway, SP600125 (30 μmol/l)).

The agonist activity of α-LA on PPARβ/δ was measured in a reporter assay. In vivo studies with 3-month C57Bl6 mice (minimum 6 per group) were conducted. Mice performed a one bout of stressful treadmill exercise (35 cm/s, 1 h) and/or one night or 7-weeks voluntary wheel running training. Analyses were done by qPCR and Western-Blot. Statistical analyses were performed using parametric or non-parametric tests depending on cases.

Results: Treatment of C2C12 with α-LA (250 μmol/l) induces ($p<0.05$) a pattern of gene expression (PDK4, CPT1a, Catalase, Prkag3) similar to those observed in response to GW0742 in C2C12 myotubes. In the reporter assay, treatment with α-LA did not lead to an increase in luciferase activity, suggesting that α-LA was not an agonist of PPARβ/δ. However, the inhibition of PPARβ/δ activity, in C2C12 PPARβ/δ-DN or in C2C12 treated with GSK3787, led to a loss of α-LA effect on PPARβ/δ target gene expressions, suggesting that this pathway had to be active to allow α-LA effects. Both PPARβ/δ mRNA levels and protein expression were increased in response to α-LA treatment (2-fold; $p<0.05$). Moreover, co-treating cells with α-LA and GW0742, maximized the effect of GW0742 on PDK4 expression for lower concentration (3 nmol/l) than those usually used (300 nmol/l) to induce this effect. Under physiological conditions, PPARβ/δ expression was decreased ($p<0.05$) in vastus lateralis in response to one bout of stressful treadmill exercise whereas it was increased ($p<0.05$) when lowering oxidative stress exposition in response to voluntary exercise training (2-fold). We evidenced in C2C12 that the JNK pathway activity was decreased with α-LA treatment. The inhibition of the JNK pathway with SP600125 induced a 2-fold ($p<0.05$) increase in PPARβ/δ mRNA level and protein expression and a significant response on its target-genes, similar to α-LA treatment.

Conclusion: We have evidenced a novel potential mechanism of regulation of the adaptation to stress involving the JNK pathway on the level of expression of PPARβ/δ in skeletal muscle. This mechanism could be relevant when the JNK pathway is chronically active in skeletal muscle such in diabetic or obese patients and/or in conditions where PPARβ/δ expression in skeletal muscle is known to be particularly low, such as with aging and/or physical inactivity. Furthermore, treatment with α-LA might be beneficial in these conditions.

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Fractalkine (CX3CL1) a novel myokine protecting skeletal muscle from insulin resistance in humans

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Background and aims: Fractalkine (CX3CL1) is a chemokine produced as a membrane-bound protein shed to the circulation through enzymatic cleavage by Adam 10 and 17 and regulated by exercise. Recently it has been shown to be associated with Type 2 Diabetes and we showed that its mRNA expression is increased in human primary skeletal muscle cells with TNF-α induced insulin resistance. However, the impact of CX3CL1 on primary human skeletal muscle cells exposed to TNF-α remains to be explored.

Materials and methods: CX3CL1 mRNA and protein levels were measured in plasma and muscle biopsies from healthy volunteers performing either 3 h of bicycling exercise or 2 h of one legged knee extensor exercise. Plasma CX3CL1 levels were also measured in healthy males infused for 4 h with TNF-α or placebo. Human skeletal muscle cells were obtained from abdominal muscle and cultured with CX3CL1 for 48 h alone or with TNF-α 20 ng/ml for the last 24 h to induce insulin resistance. We investigated insulin induced-deoxyglucose uptake, insulin and NFkB signaling pathways using western blot and ER and mitochondrial alterations by immunostaining. Deeper investigation of CX3CL1 effects on myotube gene expression was conducted by RNAseq.

Results: Plasma CX3CL1 increased rapidly after the beginning of exercise, reaching a maximum by 3 h (1.4±0.4 μg/l; $p<0.01$ vs starting condition). In the one-legged knee extensor model, mRNA content of CX3CL1 in the exercising leg (EL) was higher compared to the resting leg (RL). During exercise CX3CL1 plasma levels increased in all vessels (arterial, RL femoral vein and EL femoral vein), even if its release did not increase significantly from the EL compared to RL. 24 h post exercise, CX3CL1 protein content in the EL was decreased (1.08±0.07 vs 0.31±0.04; $p<0.05$), while ADAM10 mRNA expression was not increased. Human volunteers infused with TNF-α showed an increase of plasma CX3CL1 with a maximum after 4 h (8.2±0.8 μg/l; $p<0.001$ vs placebo). In vitro, myotubes treated with CX3CL1 showed an increase of basal glucose uptake (190±8 vs. 120±18 cpm/mg protein/min; $p<0.05$) and were protected from the impact of TNF-α on insulin stimulated-glucose uptake (TNF-α+CX3CL1: 245±9 vs. TNF-α: 146±25 cpm/mg protein/min; $p<0.05$). CX3CL1 moreover prevented the negative effect of TNF-α on Akt and AS160 phosphorylation and IRS1 expression, restoring control levels. Phosphorylation of ERK, NFkB and p38 induced by TNF-α was blunted in the presence of CX3CL1. In addition, confocal imaging highlighted that CX3CL1 in the face of TNF-α reorganized a proper structure of ER and increased considerably the number of mitochondria measured by cytochrome C staining. Although TNF-α modulated the expression of 5276 genes (CTR vs TNF-α), CX3CL1 failed to reverse the vast majority of these changes (TNF-α vs TNF-α+CX3CL1).

Conclusion: Our study demonstrates that CX3CL1 is expressed by human skeletal muscle and regulated by exercise and TNF-α infusion. We also show that this novel myokine protects human myotubes from the negative impact of TNF-α. This suggests that its autocrine role might have important implications in modulating the effects of insulin resistance on skeletal muscle function including glucose uptake. Ongoing studies are underway to determine more precisely any possible impact of CX3CL1 on human muscle gene expression.

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Regulation of skeletal muscle energy metabolism and insulin action by perilipin 5

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Background and aims: Intramuscular triglycerides lipid droplets can fuel muscle contraction during exercise but also promote insulin resistance when in excess. Recent work indicates that perilipin 5 (PLIN5) may play a gatekeeper role at the lipid droplet surface. The aim of the present work was to characterize the role of PLIN5 in the regulation of skeletal muscle lipid and glucose metabolism.

Materials and methods: We measured PLIN5 protein content in different mouse skeletal muscles and in lean sedentary, endurance trained and obese glucose intolerant (IGT) subjects. We also overexpressed PLIN5 using an adenoviral approach in human skeletal muscle cells primary culture to characterize its functional role in the regulation of energy metabolism and insulin action.

Results: PLIN5 is highly expressed in oxidative compared to glycolytic muscles. PLIN5 protein content strongly correlates with insulin sensitivity ($r2=0.42$, $p<0.0001$), is elevated in endurance-trained subjects, and reduced in obese IGT. PLIN5 overexpression in human skeletal muscle cells slows down triglyceride depletion (-53%, $p=0.0025$), as well as lipolysis-derived fatty acids mobilization and oxidation (-46%, p=

0.0035). This is accompanied by an increase in glucose oxidation and glycogen synthesis, concomitant with a decrease of *pyruvate dehydrogenase kinase 4* gene expression. Finally, PLIN5 overexpressing cells are partly protected from palmitate-induced lipotoxicity and insulin resistance.
Conclusion: Collectively, our data indicate that PLIN5 plays a key role in the regulation of skeletal muscle energy metabolism. PLIN5 could contribute to maintain insulin sensitivity by modulating fatty acid trafficking in and out of the lipid droplet in skeletal muscle.
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p300 is essential for the full effect of calorie restriction to enhance skeletal muscle insulin sensitivity
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Background and aims: The E1A binding protein, p300, is a transcriptional co-activator and acetyltransferase (KAT), that along with its paralog, CREB binding protein (CBP), interacts physically and functionally with 2000 or more transcriptional regulators and other proteins, many of which play an integral role in regulating metabolism. Surprisingly, however, very little is known regarding the contribution of p300, and KATs in general, to skeletal muscle metabolism. Our objective was to determine the contribution of p300 to skeletal muscle insulin action, including calorie restriction (CR)-induced enhancement of skeletal muscle insulin sensitivity.
Materials and methods: Mice with knockout of p300 (mKO) in skeletal muscle were generated using Cre Recombinase methodology. Beginning at 10 weeks old, individually housed male, mKO and floxed/wildtype (WT) littermates continued on an ad libitum (AL) diet, or were switched to a CR (20 d at 60% of AL) diet. At 12 weeks old, an oral glucose tolerance test (OGTT; 2 g/kg) was performed, and at 13 weeks old, basal and insulin-stimulated 2-deoxyglucose uptake (2DOGU) was measured in isolated extensor digitorum longus (EDL) and soleus muscles using a physiological insulin concentration (60 μU/mL). Before the 2DOG experiments, body composition was assessed in all mice by magnetic resonance imaging.
Results: As expected, CR decreased fat, lean and total body mass compared to AL fed mice, with no genotype differences observed. Interestingly, while CR significantly reduced the glucose response to an OGTT by ~60% in WT mice, this improvement was ~25% lower in mKO vs. WT mice, suggesting that p300, in skeletal muscle, is required for the full effect of CR to improve glucose tolerance. Supporting this, insulin-stimulated 2DOGU (insulin 2DOG basal 2DOGU) in the EDL and soleus of WT-CR mice was ~3-5-fold higher as compared to WT-AL mice, but this CR-induced improvement was ~40-60% lower in mKO mice.
Conclusion: These results demonstrate while loss of p300 does not alter skeletal muscle insulin sensitivity in young AL-fed mice, p300 is essential for the full effect of CR to enhance skeletal muscle insulin sensitivity.
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Dysregulated iron metabolism in human skeletal muscle cells are associated with free fatty acid-induced insulin resistance
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Background and aims: Although iron is an important component of the respiratory system in mitochondria, the relationship between iron metabolism and insulin resistance (IR) in skeletal muscles has not been well studied.
Materials and methods: In this study, we investigated the relationship between iron metabolism and palmitate-induced insulin resistance in human skeletal muscle cells. We measured cellular iron levels in palmitate-induced insulin resistant skeletal muscle cells using calcein-AM. In addition, we tested the effects of a variety of chemical iron chelators such as deferoxamine (DFO), deferasirox (DS), $FeCl_3$, and $FeSO_4$ in palmitate-induced insulin resistant skeletal muscle cells using the glucose uptake method. Because iron is a critical factor for respiratory metabolism, the current study was initiated to determine whether abnormal iron metabolism is involved in palmitate-induced insulin resistance.
Results: Palmitate treatment reduced insulin-induced Akt phosphorylation and glucose uptake but increased the intracellular iron content in skeletal muscle cells compared with control cells. When palmitate-treated skeletal muscle cells were treated with iron chelators, insulin-stimulated phosphorylated Akt levels and glucose uptake were recovered. However, iron supplementation by treatment with $FeCl_3$ and $FeSO_4$ augmented palmitate-induced insulin resistance. Interestingly, iron supplementation in normal skeletal muscle cells reduced insulin-stimulated phosphorylated Akt and glucose uptake and evoked insulin resistance. Similarly, treating skeletal muscle cells with TNF-α elicited similar effects.
Conclusion: The current study revealed that iron overload in skeletal muscle cells induced insulin resistance, whereas reduced intracellular iron levels protected palmitate-induced insulin resistance. Therefore, these data provide a therapeutic strategy for the treatment of insulin resistance.

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Muscle glucose uptake and insulin signalling do not correlate over the feeding/fasting cycle in high fat fed rats
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Background and aims: Many studies investigating mechanisms of diet-induced insulin resistance impose controlled experimental conditions (constant insulin stimulation, constant glucose and/or fatty acid availability) designed to make interpretation of results simpler. These models may alter important parameters such as insulin or glucose to levels that are not ever experienced physiologically by the animal during normal feeding or fasting. We examined glucose uptake and markers of insulin action in muscle of high fat diet-fed (HFD) rats during the normal diurnal cycle.
Materials and methods: Male Wistar rats were fed a high fat diet (45% calories as fat) for 4 weeks. After 3 weeks of fat feeding rats were anaesthetised, implanted with a single chronic jugular cannula and allowed to recover. One week after surgery the cannula was used to sample blood and deliver 14C-glucose and 3H-2-deoxyglucose to monitor in vivo glucose uptake at several timepoints along the feedings/

fasting axis (4 pm, 9 pm, 12 am and 8 am). Tissues were collected for further analysis.
Results: Plasma insulin levels were the same in Chow and HFD rats in the basal state and after feeding. However glucose uptake into skeletal muscle was impaired in the tibialis anterior (TA) muscle of HFD rats at 12 am and 8 am (12 am Chow=19.3±2.8, HFD=7.7±0.9, 8 am Chow=10.4±1.2, HFD=5.6±0.6, umol/min/100 g, $p<0.05$). There was no difference in the phosphorylation state of canonical signalling proteins AKT, GSK3ß and TBC1D4 in TA muscle from Chow and HFD rats at any of the time points studied. In contrast, pyruvate dehydrogenase (PDH) activity in the TA was dramatically lower in HFD rats at both 12 am and 8 am (12 am Chow=4.26±1.73 HFD=0.96±0.17, 8 am Chow=6.46±0.97 HFD±1.05±0.18, mU/g, $p<0.01$).
Conclusion: These data suggest that reduced glucose uptake in muscle of HFD rats assessed during the normal diurnal cycle of energy metabolism relates more to changes in glucose metabolism than altered canonical insulin signalling.
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PS 037 Glucagon regulation

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Effect of glucagon-like peptide-1 on glucagon secretion, endogenous glucose production and whole body lipolysis in patients with non-alcoholic liver disease

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Background and aims: We evaluated the glucagon-suppressive effect of glucagon-like peptide-1 (GLP-1) and its potential effects on endogenous glucose production and whole body lipolysis in non-diabetic patients with non-alcoholic fatty liver disease (NAFLD).
Materials and methods: Ten non-diabetic patients with biopsy-verified NAFLD (NAFLD activity score 2.5±1.0) and 10 matched controls underwent a 2-hour intravenous GLP-1 (0.8 pmol×kg-1×min-1) and placebo infusion on separate days. Since GLP-1-mediated glucagon suppression is glucose-dependent, plasma glucose was clamped at fasting level during the first hour, then raised and clamped at 'postprandial level' (fasting plasma glucose level plus 3 mmol/l) for the remaining hour. We evaluated plasma levels of glucagon, endogenous glucose production and whole body lipolysis rates with stable isotopes, and respiratory quotient using indirect calorimety. Data are presented as median±interquartile range and groups are compared using non-parametric analysis of variance.
Results: Compared to controls, patients with NAFLD were more insulin resistant (HOMA-IR: 3.8±2.2 vs 1.6±1.5, $p=0.003$) and had higher fasting glucagon (7.5±5.3 vs 5.8±1.5 mmol/l, $p=0.045$). Similar relative glucagon suppression was seen in both groups during GLP-1 infusion at fasting (-97±75 vs -93±41 pmol/l×min-1 $p=0.566$) and 'postprandial' plasma glucose levels (-108±101 vs -97±53 pmol/l×min-1, $p=0.196$). Patients with NAFLD had impaired GLP-1-induced suppression of endogenous glucose production at fasting and 'postprandial' glucose levels and impaired elevation of respiratory quotient during 'postprandial' glucose levels.
Conclusion: Patients with NAFLD are insulin resitant and exhibit fasting hyperglucagonaemia, but intact GLP-1-mediated glucagon suppression independently of plasma glucose concentrations. Preserved glucagonostatic effect of GLP-1 in patients with NAFLD may be important to sustain normoglycaemia.

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Glucagon suppression is, besides hyperinsulinaemia, an adaptation to reduced insulin sensitivity in individuals with normal and impaired glucose regulation

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Background and aims: Hyperinsulinaemia is an important adaptive mechanism to insulin resistance to maintain normoglycaemia. The aim of this study was to assess whether also glucagon is involved in the islet adaptation to insulin resistance.
Materials and methods: We evaluated 1,433 individuals (762 men/671 women) with normal or impaired glucose regulation from the Danish ADDITION-PRO study. All underwent 75 g oral glucose tolerance tests

with sampling of glucose, insulin and glucagon at 0, 30 and 120 min. Mean (SD) age was 66.1 (7.1) years, BMI 27.1 (4.5) kg/m2 and HbA1c 5.7 (0.4) % or 38 (4) mmol/mol. Total areas under the curves (AUCs) for glucose, insulin and glucagon were calculated. Glucagon suppression (%) was calculated as (1 - (AUCglucagon/(fasting concentration × 120 min)) × 100). The insulin sensitivity index (ISI0-120) was calculated using fasting and 120 min glucose and insulin concentrations as well as information on body weight. The study participants were divided into two groups depending on glucagon suppression: 1) No suppression (≤0%; n=287); 2) Suppression (>0%; n=1146).

Results: Median (IQR) of glucagon suppression was 22% (6-34%). While AUC for glucose did not differ between the groups, AUC for insulin was higher and insulin sensitivity was lower in individuals with glucagon suppression (Table 1). When quantifying the effect of insulin sensitivity on glucagon suppression, adjusting for age, sex, BMI, AUC insulin and AUC glucose, we found that a doubling in ISI0-120 resulted in 13.0% less glucagon suppression (95%-CI: 8.1-18.2%, P<0.001). Glucagon suppression was lower in individuals with screen-detected diabetes (median (IQR): 17% (3-30%)) than in individuals with pre-diabetes (13% (7-34%)) or normal glucose tolerance (13% (5-36%); P<0.001). However, glucose tolerance status (P=0.745) or obesity (P=0.505) did not modify the association between glucagon suppression and ISI0-120.

Conclusion: The results suggest that glucagon suppression, together with increased insulin secretion, is an adaptation to reduced insulin sensitivity in individuals with normal or impaired glucose regulation. Therefore, preservation of glucose tolerance in the presence of insulin resistance may depend not only on an individual's ability to secrete more insulin but also on their ability to suppress glucagon.

Table 1: AUCs of glucose and insulin as well as insulin sensitivity by glucagon suppression group

Glucagon suppression	Number	AUC glucose (mmol/l · 2hr)	AUC insulin (nmol/l · 2hr)	Insulin sensitivity (ISI_{0120})
No	287	946 (195)	21.2 (15.0-31.2)	39.6 (28.8-54.0)
Yes	1146	947 (184)	23.5 (16.1-34.2)	36.1 (25.3-47.1)
P-value		*0.938*	*0.007*	*<0.001*

Means (SD) or medians (IQR). P-value is the unadjusted t-test of difference between groups according to glucagon suppression. AUC insulin and ISI_{0-120} were log-transformed before performing the statistical test

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Distinct roles of somatostatin in the control of glucagon secretion by glucose and K_{ATP} channel blockers from the perfused mouse pancreas

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Background and aims: The mechanisms by which glucose controls glucagon release are hotly debated. In particular, it is unclear whether it acts directly on α-cells or indirectly through a paracrine factor released from non-α-cells within the islet. One of the reasons for the controversies might be linked to the different experimental models used in which paracrine and endocrine interactions might be altered (fresh or cultured tissue, single cells or isolated islets). To be as close as possible to the physiological situation, we used the *in situ* perfused mouse pancreas which preserves endocrine and paracrine interactions, and we investigated the role of somatostatin (SST) in the control of glucagon secretion by glucose and K_{ATP} channel modulators.

Materials and methods: The effects of glucose and K_{ATP} channel modulators were tested on glucagon secretion of the *in situ* perfused pancreas of $Sst^{+/+}$ and $Sst^{-/-}$ (to address the role of SST) and of C57BL/6 and $Sur1^{-/-}$ or $Kir6.2^{-/-}$ mice (to address the role of K_{ATP} channels, the last two mouse models lacking a major functional subunit of the K_{ATP} channels). The effluent from the portal vein was collected every 4 min. The experiments were performed in the presence of a 6 mmol/l mixture of amino acids or a 2 mmol/l mixture of various amino acids present at physiological concentrations.

Results: Glucagon release of C57BL/6 or $Sst^{+/+}$ mice was inhibited by a rise of the glucose concentration of the medium from 1 to 7 mmol/l, or by the addition of the K_{ATP} channel blocker tolbutamide (100 or 500 μM) or the K_{ATP} channel opener diazoxide (100 μM) to a medium containing 1 mmol/l glucose. Glucagon release of $Sur1^{-/-}$ or $Kir6.2^{-/-}$ mice was also inhibited by 7 mmol/l glucose but it was, as expected, unaffected by tolbutamide or diazoxide. This suggests that glucose can inhibit glucagon release independently of K_{ATP} channels. In $Sst^{-/-}$ mice, glucagon secretion was inhibited by a rise of the glucose concentration of the medium from 1 to 7 mmol/l, but it was stimulated by tolbutamide. Because it has been suggested that tolbutamide might stimulate exocytosis by activating Epac2, we tested the effect of another sulfonylurea, gliclazide, which is ineffective on Epac2. Gliclazide (10 μM) mimicked the glucagonostatic effect of tolbutamide in $Sst^{+/+}$ mice and the glucagonotropic effect of tolbutamide in $Sst^{-/-}$ mice.

Conclusion: Using the preparation of the *in situ* perfused mouse pancreas, we confirmed key observations previously obtained on perifused mouse islets. Glucose can inhibit glucagon release independently of K_{ATP} channels. SST is responsible for the glucagonostatic effect of sulfonylureas since ablation of SST transformed their glucagonostatic effect into a glucagonotropic effect. This suggests that sulfonylureas modulate glucagon secretion by two distinct mechanisms: a direct stimulatory effect of α-cells (independent of Epac2 activation and observed in the absence of SST influence) and an indirect inhibition via SST released from δ-cells which counteracts the direct stimulation.

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Paradoxical Ca^{2+} kinetics in islet-located glucagon-releasing alpha cells

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Background and aims: Insulin and glucagon are the principle hormones that maintain blood glucose homeostasis. Previous studies have shown that insulin and glucagon are secreted in pulses with opposite phase from glucose-stimulated islets. Also secretion of somatostatin is pulsatile in opposite phase to glucagon. Since Ca^{2+} is regarded as the main trigger of both insulin and glucagon release, we compared glucose-induced cytoplasmic Ca^{2+} dynamics in α- and β-cells within pancreatic islets.

Materials and methods: Mouse islets were loaded with the fluorescent Ca^{2+} indicator Fluo-4 and the Ca^{2+} concentration in the sub-plasma membrane space ($[Ca^{2+}]_{pm}$) was monitored with total internal reflection fluorescence microscopy. Discrimination between α- and β-cells was based on transgenic α-cell expression of red fluorescent protein and cell-characteristic Ca^{2+} responses. Glucagon and insulin secretion was measured from batch-incubated islets with immunoassays.

Results: At 3 mM glucose, RFP-positive α-cells showed non-synchronized irregular spikes of $[Ca^{2+}]_{pm}$, whereas RFP-negative β-cells showed low and stable $[Ca^{2+}]_{pm}$. Introduction of 20 mM glucose induced pronounced $[Ca^{2+}]_{pm}$ elevation with oscillations that were perfectly synchronized among β-cells whereas there was often temporary interruption of $[Ca^{2+}]_{pm}$ signaling in the α-cells. However, the recurring $[Ca^{2+}]_{pm}$ oscillations tended to synchronize among the islet α-cells, and cross-correlation analysis revealed unexpected synchronization between the $[Ca^{2+}]_{pm}$ oscillations in α- and β-cells. This synchronization was neither affected by addition of 400 nM somatostatin, nor by 100 nM insulin. The somatostatin receptor SSTR2 antagonist PRL 2903 (5 μM) had only

subtle effects on $[Ca^{2+}]_{pm}$ signaling in α-cells despite potently stimulating glucagon secretion at both 3 and 20 mM glucose without preventing the inhibitory effect of glucose. Moreover, the $[Ca^{2+}]_{pm}$ synchronization between α-cells and β-cells exposed to 20 mM glucose remained after 18 h treatment of the islets with pertussis toxin, which prevents somatostatin receptor signaling via Gαi. The insulin receptor antagonist S961 (20 nM) lacked significant effects on $[Ca^{2+}]_{pm}$ in α- and β-cells, as well as on insulin and glucagon release at 20 mM glucose.

Conclusion: Synchronization of $[Ca^{2+}]_{pm}$ oscillations between α-cells and β-cells at 20 mM glucose surprisingly indicates that pulsatile glucagon release is not generated by α-cell oscillations of $[Ca^{2+}]_{pm}$. Since somatostatin receptor antagonism potently stimulated glucagon release with little effects on $[Ca^{2+}]_{pm}$, pulsatile glucagon release is probably generated by inhibitory somatostatin pulses overriding the stimulatory effect of Ca^{2+}.

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Calcium activated potassium channels modulate pancreatic alpha cell electrical excitability and glucagon secretion

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Background and aims: Ca^{2+} influx into pancreatic alpha-cells through voltage-dependent Ca^{2+} channels (VDCCs) is required for glucagon secretion and regulatory mechanisms of this process become defective during the pathogenesis of diabetes. Potassium channels are the primary regulators of membrane potential, and thus, regulate the activity of VDCCs. However, the roles of Ca^{2+} activated potassium channels (K_{Ca}^{2+}) during alpha-cell Ca^{2+} entry and glucagon secretion have not been determined. The aim of this study was to determine the influence of alpha-cell K_{Ca}^{2+} channels on electrical excitability, Ca^{2+} entry and glucagon secretion.

Materials and methods: Whole cell current clamp and voltage clamp electrophysiological recordings were used to measure K_{Ca}^{2+} currents and electrical excitability from human and mouse alpha-cells. Cells were confirmed as alpha-cells with either glucagon staining or via expression a red fluorescent protein in glucagon positive islet cells. Ca^{2+} imaging was performed on mouse and human pancreatic islets and single cells with and without inhibitors of small conductance (SK) or intermediate conductance (IK) K_{Ca}^{2+} channels (apamin and TRAM34 respectively); glucagon secretion was assayed under similar conditions. Immunofluorescent imaging was used to detect IK expression in mouse and human pancreatic sections.

Results: Mouse pancreatic alpha-cells have K_{Ca}^{2+} currents (13.3 +/- 1.78 pA, n=19), which were elicited by Ca^{2+} entry following 26 voltage ramps that resembled action potentials. The alpha-cell K_{Ca}^{2+} currents were partially inhibited by SK channel blockade with apamin (37.87 +/- 5.7%, n= 8) or IK channel blockade with TRAM34 (25.75 +/- 4.4%, n=11).The membrane potential of alpha-cells was depolarized in response to SK or IK channel inhibition (1.61 +/- 0.49 mV with apamin, n=5; 1.94 +/- 0.32 mV with TRAM34, n=6) under elevated glucose (11 mM) conditions. Inhibition of SK channels also enhanced alpha-cell Ca^{2+} influx under both low and high glucose conditions, increasing Ca^{2+} levels by 36.18% ($p<.01$) and 17.15% ($p<.0001$) respectively. Moreover, SK channels limited glucose induced Ca^{2+} influx into alpha-cells within clusters of islet-cells and when inhibited increased Ca^{2+} influx by 23.72% ($p<.05$). IK channels are also expressed in pancreatic islets and we find that mouse and human pancreatic sections show IK channel expression in glucagon positive alpha-cells. Inhibition of IK channels caused a 41.76% ($p<.001$) increase of mouse alpha-cell Ca^{2+} influx in low glucose and a 28.76% ($p<.05$) increase of Ca^{2+} influx in high glucose. Human alpha-cells mirrored this behavior in response to IK channel inhibition, showing a 31.66% ($p<.05$) increase in Ca^{2+} influx in low glucose and a 33.28% (p .05 vs. apamin treated islets); whereas IK channel blockade with TRAM34 caused an insignificant trend toward increased glucagon secretion.

Conclusion: In conclusion, this study suggests that SK channels play a role limiting glucagon secretion under high glucose conditions. SK and IK channels hyperpolarize the alpha-cell membrane potential, decreasing excitability and Ca^{2+} influx during glucose stimulation, which reduces glucagon secretion. Thus, K_{Ca}^{2+} channel activity helps to modulate glucose-dependent inhibition of alpha-cell glucagon secretion.

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Direct effects of anti-diabetic drugs on glucagon secretion in rat pancreatic islets

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Background and aims: Glucagon secreted by pancreatic α-cells plays an important role in maintaining glucose homeostasis via its ability to increase hepatic glucose production. Patients with type 2 diabetes exhibit fasting and paradoxical postprandial hyperglucagonemia, suggesting dysfunction of the pancreatic α-cell may contribute to hyperglycemia. Thus, suppression of inappropriate glucagon secretion is potentially an effective treatment strategy for patients with type 2 diabetes. We have previously shown that Na+ channel blockers directly inhibit glucagon secretion from pancreatic islets and have glucose lowering properties, without effecting hypoglycemia-stimulated glucagon release. Various glucose lowering therapeutics have been shown to modulate glucagon levels and action in both clinical and non-clinical studies, but it is unclear which of these therapies have a direct effect on glucagon secretion at the level of the pancreatic islet. The present study was carried-out to determine the direct effects of different classes of glucose lowering medications on glucagon secretion from rat pancreatic islets.

Materials and methods: Pancreatic islets were isolated from SD rats. Glucagon secretion was measured in equal sized isolated islets incubated with agents in Kreb-ringer buffer with 0.1% BSA, in 3 or 11 mM glucose for 1 h. Glucagon levels were measured by an ELISA kit.

Results: In the presence of 3 mM glucose, insulin at 10 or 100 nM significantly reduced glucagon secretion by 48±8 or 36±11% compared to vehicle control, respectively. Exendin-4 at 100 nM also significantly decreased glucagon secretion in the presence of 3 or 11 mM glucose, by 41±11 or 62±5% compared to control, respectively. In addition, exendin-4 significantly increased (154±24%) insulin secretion in the presence of 11 mM glucose compared to control. Tolbutamide (10-100 μM), sitagliptin (0.003-3 μM), canagliflozin (0.1-30 μM) and metformin (1-100 μM) had no significant effect on glucagon secretion. Veratridine, a Na+ channel activator, significantly increased glucagon secretion with 8-fold induction at 30 μM, suggesting a direct role of Na+ channels in glucagon secretion. Accordingly, glucagon secretion was significantly and concentration-dependently reduced by Na+ channel blockers TTX or GS-458967, with a 47±6 or 53±8% reduction at 100 nM or 3 μM, respectively.

Conclusion: Data show that insulin at high concentrations directly inhibits glucagon secretion in rat islets. GLP-1R agonist exendin-4 directly inhibits glucagon secretion from α-cells as well as through paracrine effect on β-cells by stimulating insulin secretion. The sulfonylurea tolbutamide, DPP-IV inhibitor sitagliptin, SGLT2 inhibitor canagliflozin and metformin do not directly regulate glucagon secretion in rat islets. Na+ channel blockers directly inhibit glucagon secretion in isolated pancreatic islets and present a potential new strategy for the treatment of diabetes.

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Sodium-glucose transporter 1 and 2 are involved in the GLP-1 release from pancreatic alpha cells

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Background and aims: It has been recently reported that human alpha cells express sodium-glucose co-transporters (SGLT) and that the diabetic condition is associated with reduced expression of SGLT2 and a concomitant increase in the expression of SGLT1 and glucagon genes. Moreover, SGLT2 silencing or functional inhibition with dapagliflozin was associated with increased expression of the glucagon gene. We have tested whether this system also is involved in the recently reported regulation of GLP-1 secretion by the alpha cell. Moreover, since glucagon/GLP-1 secretion by the alpha cell has been claimed to be under the control of the *TCF7L2* signaling pathway, we have also evaluated the interaction of the two systems in the alpha cell.
Materials and methods: We have studied a murine alpha cell line (TC1/6) in the presence of low (LG, 5.5 mM) or high (HG, 16.7 mM) glucose concentrations. Experiments were repeated with and without phloridzin (50 μM), a non-selective SGLT inhibitor. SGLT1, SGLT2 and *TF7L2* mRNA and protein expression were determined by RT-PCR and Western blot. Glucagon and total GLP-1 secretion in culture medium was measured by ELISA. Cell viability was determined by MTT method, Reactive Oxigen Species (ROS) by H2DCFDA fluorescence and apoptosis by Annexin staining and Propidium Iodine (PI) fluorescence.
Results: At LG, mRNA and protein expressions of SGLT1 and 2, and *TCF7L2* were all detectable. HG incubation was associated with an increase of both mRNA and protein expression of *TCF7L2* (protein: +46±10%, $p<0.001$; mRNA 2.79±0.47 folds, $p<0.001$). Concomitantly, SGLT1 mRNA expression increased (2.31±0.49 folds, $p<0.001$) while SGLT2 decreased (0.48±0.09, $p<0.001$). Changes in mRNA expressions were associated with -34±11% ($p<0.01$) reduction in SGLT1 protein expression with no significant changes for SGLT2 protein. GLP-1 concentration in the medium increased by 25±3% ($p<0.001$) as compared to LG. SGLT1/2 inhibition by phloridzin did not affect GLP-1 release at LG (-10±6%, p=NS), while it was associated with a 30±3% reduction ($p<0.001$) as compared to LG, and also with an increment in glucagon secretion as compared to HG (HG: -16±5% vs. LG, $p<0.03$; HG+Phl: 1±5% vs. LG, $p<0.05$ vs. HG) and PC2 expression (HG+Phl: 1.40±0.12, $p<0.02$ vs. LG). The presence of phloridzin did not modify TCF7L2 expression, at both low and high glucose concentrations. The presence of the SGLT inhibitor also modulates cellular state. When added to LG, phloridzin produced an increment in cell viability (+13±2%, $p<0.001$ *vs.* LG), a decrement in ROS production (-11±3%, $p<0.001$ *vs.* LG) with no modification of apoptosis rate. On the contrary, addition of phloridzin to cell incubated at HG was associated with reduced cell viability (-23±5%, $p<0.001$) and increased apoptosis (+19±2%, $p<0.001$ *vs.* LG).
Conclusion: These data suggest that GLP-1 and glucagon release from alpha cells in response to high glucose could be mediated by SGLT expression independently of *TCF7L2* activation.

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Enteroendocrine secretion after meal challenge is preserved across glucose tolerance spectrum: lack of correlation with beta cell function

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Background and aims: Although it is known that meal ingestion stimulates gut enteroendocrine secretion (ES) and beta cell function (BCF), the relationship between ES and BCF is not known across GT populations.
Materials and methods: In studies examining BCF in subjects with normal glucose tolerance (NGT), prediabetes (PDM), and type 2 DM (T2DM), total and active GLP-1, GIP, PYY, and glucagon (glgn) were measured before and after a mixed meal (MTT). We hypothesized that these responses were associated with BCF. ES was assessed in overnight fasted subjects before and 30, 60 and 120 min after 470 kCal MTT.
Results: Table summarizes responses pre- and 30-min post MTT. Parameters of insulin secretion (Φtot) and sensitivity (SI) and disposition index (DItot) were estimated using minimal model. BCF: ES association was conducted using Spearman's Rank correlations. Before the MTT, ES was generally higher for T2DM versus NGT and PDM. MTT increased total and active GLP-1, GIP and PYY at 30 min. Changes were generally comparable across GT populations except glgn which showed increases across the GT Populations NGT<PDM<T2DM (ANOVA $p<0.001$). Corr analysis revealed no strong relationships between ES at baseline or 30 min post-MTT, with SI, Φtot or DItot (to be shown in poster).
Conclusion: ES is not impaired after MTT in PDM and T2DM, yet there is no apparent correlation between ES and BCF, suggesting a defect in BCF rather than the ES in PDM and T2DM.

		Total GLP-1 (pg/ml)	Active GLP-1 (pg/ml)	GIP (pg/ml)	PYY (pg/ml)	Glucagon (pg/ml)
NGT (n=25)	Pre-MTT	15.5 (13.0,18.4)***	2.1 (1.5,2.8)***	84 (67,104)***	34.2 (30.2,38.6)***	31.5 (26.3,37.9)
	30 min post- MTT	38.6 (31.0,48.1)	10.8 (7.6,15.3)	607 (519,711)*##	53.6 (45.0,63.9)***	54.6 (46.3,64.4)***#
PDM (n=16)	Pre-MTT	13.0 (9.1,18.6)***	1.8 (1.3,2.6)***	91 (72,115)***	40.1 (32.5,49.5)**	35.0 (26.9,45.4)
	30 min post- MTT	30.5 (23.8,38.9)**	9.7 (7.6,12.5)*	925 (791,1081)	59.3 (50.4,69.7)*	75.7 (59.5,96.3)
T2DM (n=23)	Pre-MTT	27.1 (23.1,31.9)	4.9 (3.7,6.6)	184 (150,225)	56.3 (48.5,65.4)	41.2 (34.7,48.9)
	30 min post- MTT	54.3 (41.4 71.1)	16.7 (13.8,20.2)	793 (660,953)	85.2 (71.7,101.2)	93.4 (80.1,109.0)

Values are geometric means (95% CI). Within each glucose tolerance group, the top cell represents the baseline (pre-MTT) value. The values below represent 30 min post MTT.

*** p <0.001 v T2DM ; ** p < 0.01 v T2DM; * p < 0.05 v T2DM ; ## p<0.01 v PDM; # p < 0.05 v PDM

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PS 038 GLP-1 physiology

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Renal extraction and acute effects of glucagon-like peptide-1 on central and renal haemodynamics in patients with type 2 diabetes

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Background and aims: During acute administration of native GLP-1, we previously demonstrated central hemodynamic effects in healthy males, whereas renal hemodynamics, despite renal extraction of GLP-1, were unaffected. In the present study, we elucidated hemodynamic effects of GLP-1 in type 2 diabetic subjects (n=8) under fixed sodium intake.
Materials and methods: During a 3-hour infusion of GLP-1 (1.5 pmol kg-1 min-1) or saline, cardiac output was continuously estimated by pulse contour analysis, concomitantly with intra-arterial blood pressure and heart rate. Renal plasma flow, glomerular filtration rate, and uptake/release of hormones and ions were measured using Fick's Principle after catheterization of a renal vein. Urine collection was conducted throughout the experiments at voluntary voiding, and subjects remained supine.
Results: During GLP-1 infusion, systolic and diastolic blood pressure remained unchanged. Heart rate increased significantly, whereas cardiac output remained unchanged. GLP-1 was extracted significantly in the kidneys, however, renal plasma flow and glomerular filtration rate as well as renal sodium and lithium excretion were not affected.
Conclusion: In conclusion, like in healthy subjects, acute administration of GLP-1 in type 2 diabetic subjects led to a positive chronotropic effect, but in contrast to healthy subjects, cardiac output did not increase in type 2 diabetic subjects. Renal hemodynamics and sodium excretion were not affected by GLP-1 infusion.
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Preserving expression of GLP-1 receptor in beta cells of db/db mice increases insulin secretion

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Background and aims: GLP-1 signaling has been shown to play important roles in maintaining β-cell functions, such as insulin secretion and β-cell proliferation. While expression levels of GLP-1 receptor (Glp1r) and GIP receptor (Gipr) are compromised in the islets of diabetic rodent models, it remains unclear at which stage of glucose intolerance the incretin receptors are downregulated and whether preserving expression of Glp1r and/or Gipr affects the phenotypes of diabetic model mice.
Materials and methods: To address the first question, we investigated expression levels of Glp1r, Gipr, and other β-cell specific genes in the islets of db/db mice at 4, 8, and 12 weeks of age. Then, we generated a transgenic mouse model "CAG-CAT-Glp1r -IRES-eGFP" that conditionally and specifically expressed Glp1r in β cells, and evaluated their glucose tolerance.
Results: The expression levels of Glp1r and Gipr in db/db islets were comparable to those in control (db/misty) mice at the age of 4 weeks, and significantly decreased at the age of 8 and 12 weeks. When CAG-CAT-Glp1r -IRES-eGFP mice were crossed with Pdx1-CreER mice, eGFP expression was detected exclusively in β cells of Pdx1-CreER; CAG-CAT-Glp1r -IRES-eGFP (βGlp1r). Oral glucose tolerance test (OGTT) after 4-week treatment of Exendin-4 (0.1 mg/kgBW/day) revealed that the glucose profiles were comparable between βGlp1r; db/db and control db/db mice, whereas serum insulin levels during OGTT were significantly higher in βGlp1r; db/db than control db/db mice.
Conclusion: The expression levels of Glp1r and Gipr in db/db mice were significantly downregulated with aging. Activation of Glp1r signaling in β cells of db/db mice increased insulin secretion.

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Changes in postprandial glucagon-like peptide-1 secretion during the development of diet-induced obesity in rats

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Background and aims: Diet-induced obesity is a major cause of metabolic syndrome such as diabetes. Incretins including glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP) are secreted from entoroendocrine cells in response to meal ingestion, and contribute to preventing postprandial hyperglycemia by enhancing insulin secretion. It is unclear whether postprandial GLP-1 secretory response is affected (increased or decreased) during the development of diet-induced obesity. In the present study, meal tolerance test (MTT) was conducted in rats fed normal or high-fat/high-sucrose diet, and postprandial GLP-1, insulin and glycemic response were monitored.
Materials and methods: Male Sprague Dawley rats (5 weeks age) were fed control diet (AIN-93G, n=8) or high-fat/high-sucrose diet (HF/HS, n=8) containing 30% fat and 40% sucrose for 5 weeks. MTT was conducted every week for 4 weeks. In MTT, all rats were fed the control diet (5 or 10 g/kg) for 15 min after overnight fasting. Tail vein blood was collected before and after the meal feeding until 120 min, and plasma glucose, insulin and GLP-1 levels were measured. After 5 weeks feeding period, rats were killed and intestinal tissue samples (jejunum, ileum, cecum and colon) were collected for measurement of mRNA expressions by real-time PCR.
Results: Body weigh was significantly higher in HF/HS rats than control rats (423.4 g vs 380.8 g, $P<0.05$) at the end of experiment (5 week). Fasting glucose levels were higher in HF/HS rats (94.7 mg/dL vs 75.0 mg/dL, $P<0.05$) after week 3 until the end of experiment. However, postprandial glycemia did not differ between control and HF/HS rats throughout the experimental period, suggesting some protective adaptations occurred in HF/HS rats. Postprandial GLP-1 secretion was significantly larger ($P<0.05$) in HF/HS rats than control rats at week 2 and 4, and this was reflected by increased insulin response in HF/HS rats. In the intestinal mucosa, among various nutrient sensing receptors and transporters evaluated, one of fatty acid receptors, FFAR1 had higher mRNA expression (150% increment against control rats, $P<0.05$) in the upper small intestine of HF/HS rats compared to that of control rats.
Conclusion: The present study demonstrates that postprandial GLP-1 secretion is increased in the early period of developing diet-induced obesity. These results suggest that increased postprandial GLP-1 response contributes to normalize postprandial glycemic response by enhancing insulin secretion. Increased expression of a fatty acid receptor may be involved in the protective adaptation against induction of glucose intolerance.
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Farnesoid X receptor inhibits glucagon-like peptide-1 production by enteroendocrine L-cells

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Background and aims: Bile acids (BA) are signalling molecules which activate the transmembrane receptor TGR5 and the nuclear receptor FXR. BA sequestrants (BAS) complex BA in the intestinal lumen and decrease intestinal FXR activity. The BAS-BA complex also induces Glucagon-Like Peptide-1 (GLP-1) production by L-cells which potentiates beta-cell glucose-induced insulin secretion. Whether FXR is expressed in L-cells and controls GLP-1 production is unknown.
Materials and methods: Glucose homeostasis and Proglucagon/GLP-1 production were studied in different in vitro-ex vivo models (GLUTag cell line and human jejunal biopsies) and in different mouse strains (Wild-type mice, mice invalidated for FXR or TGR5, conventional-raised and germ-free mice). FXR was activated by a synthetic ligand.
Results: We show that FXR activation in L-cells decreases proglucagon expression by interfering with the glucose-responsive factor Carbohydrate-Responsive Element Binding Protein (ChREBP) and GLP-1 secretion by inhibiting glycolysis. In vivo, FXR-deficiency increases GLP-1 gene expression and secretion in response to glucose hence improving glucose metabolism. Moreover, treatment of ob/ob mice with the BAS colesevelam increases intestinal proglucagon gene expression and improves glycemia in a FXR-dependent manner.
Conclusion: These findings identify the FXR/GLP-1 pathway as a new mechanism of BA control of glucose metabolism and a pharmacological target for type 2 diabetes.
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The glucose lowering effect of small molecule GPR120 agonists is driven by glucagon-like peptide

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Background and aims: GPR120 is a fatty acid receptor suggested to have anti-diabetic effects through multiple effects on different organs. It is also known that GPR120 is expressed in many tissues, including enteroendocrine cells in the gut, where it has been suggested to be involved in mediating the fatty acid effect on GLP-1 secretion. Whether GLP-1 plays a role in the glucose lowering effect observed after GPR120 activation by small molecule compounds in vivo is not completely understood. Here, we examine the the acute mechanism of action of GPR120 agonism in lean mice.
Materials and methods: Insulin secretion was studied in lean female mice, following oral administration with a specific GPR120 agonist prior to an intravenous glucose tolerance test (IVGTT, 0.35 g/kg), with and without addition of a GLP-1 receptor antagonist, exendin 9-39 (30 nmol/kg). In a second experiment, the combination of a GPR120 agonist and a DPP-4 inhibitor, sitagliptin (15 mg/kg), to reduce the degradation of GLP-1, was examined. Insulin and glucose were measured after 0, 1, 5, 10, 20 and 40 min following the intravenous glucose administration. In a separate experiment, total GLP-1 levels were measured in plasma following oral administration with the GPR120 agonist.
Results: The GPR120 agonist significantly increased insulin secretion in the IVGTT in lean mice, where the acute insulin response (AIR) was 0.9±0.09 ng/ml versus 1.4±0.21 ng/ml after GPR120 agonist administration ($p=0.001$). This increase in insulin secretion resulted in improved glucose elimination ($K_{G1\text{-}20min}$ 2.5±0.24%/min versus 3.3±0.28%/min after GPR120 agonist administration ($p=0.004$). Exendin 9-39, a GLP-1 receptor antagonist, completely blocked this effect, while the DPP-4 inhibitor sitagliptin, induced a significantly increased insulin response compared to GPR120 agonist (AIR 2.0±0.22 ng/ml, $p<0.05$ compared to GPR120 agonist alone) or sitagliptin alone. Circulating total GLP-1 levels were measured and found to be increased after administration of the GPR120 agonist comapred to vehicle (58±6 pM versus 69±6 pM, $p=0.02$.
Conclusion: This study clearly demonstrates that administration of a GPR120 agonist to lean mice resulted in increased insulin secretion, and it is suggested to be the main mechanism behind the acute glucose lowering effect of GPR120 agonists. The increased insulin secretion was mediated through increased circulating GLP-1 levels. The combination of GPR120 agonists and DPP-4 inhibition may be an attractive combination to improve glycemic control in diabetic individuals.

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CART regulates GIP and GLP-1 expression and secretion in vitro and in vivo

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Background and aims: Cocaine- and amphetamine-regulated transcript (CART) is a brain-gut peptide with several functions including regulation of islet hormone secretion and beta-cell survival. We have recently reported that CART is a constituent of human incretin-producing L- and K-cells in the duodenum and jejunum. The aim of the present study was to examine whether CART regulates secretion and expression of glucose-dependent insulinotropic peptide (GIP) and glucagon-like peptide 1 (GLP-1) in vitro and in vivo.
Materials and methods: To test the effect of CART on GIP and GLP-1 secretion in vivo in mice, CART was administered i.v. during an oral glucose-tolerance test (OGTT). Effects of siRNA-mediated CART silencing on incretin expression and secretion in vitro were studied in GLUTag and STC-1 cells, used as L- and K-cell models respectively. Gene expression was assessed with qPCR, protein content and secretion with ELISA
Results: In both GLUTag and STC-1 cells silencing of CART augmented incretin gene and protein expression. In GLUTag cells GLP-1 mRNA and protein (active) levels were increased by 133.7±12.5% and 230±80% respectively ($p<0.001$ and $p<0.05$). In STC-1 cells silencing of CART increased mRNA levels of both GLP-1 and GIP by 147±7.1% and 125±8% respectively ($p<0.001$). There were also trends towards increased GLP-1 and GIP protein content (128±10%, $P=0.06$ and 117±8%, $P=0.06$). Moreover, silencing of CART provoked robustly increased GLP-1 (active) secretion in GLUTag cells stimulated with 2.8 mM glucose, 16.7 mM glucose and10 μM IBMX and 16.7 mM glucose (3.79±0.15, 3.78±0.32 and 4.45±0.32-fold respectively, $p<0.001$). CART silencing in STC-1 provoked 1.5-fold increase in GLP-1 secretion in cells stimulated with 16.7 mM glucose and 10 μM IBMX ($p<0.05$).
Intravenous administration of CART 54-102 during an OGTT in mice provoked elevated glucose-stimulated GIP secretion at 10 and 20 min ($P<0.01$ and $P<0.001$), greater AUC for GIP and GLP-1 at 0-60 min ($P<0.05$) and higher acute GIP release ($P<0.01$).
Conclusion: We conclude that CART in L- and K-cells acts as an endogenous inhibitor of GIP and GLP-1 expression and secretion. On the other hand, in vivo in mice CART stimulates GIP and GLP-1 secretion.

Whether this latter effect is direct or a consequence of altered insulin and glucagon secretion needs further investigation.
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Intestinal GLUT2 invalidation leads to glucose malabsorption and reduces enteroendocrine cell density

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Background and aims: In western countries, diet has been considerably enriched in readily absorbable sugars. The absorption of sugars in the small intestine is mediated by several transporters. GLUT2, a very efficient glucose and fructose facilitative transporter, is located at the apical membrane of enterocytes, transiently during sugar-rich meals but constitutively in insulin resistant states. At the basal membrane of enterocytes, GLUT2 mediates glucose and fructose exit to the bloodstream. Our team have shown that GLUT2 is also present in another intestinal cell type: the enteroendocrine cells. Those cells produce Glucagon-Like Peptide-1 (GLP-1) and Glucose-dependent Insulinotropic Polypeptide (GIP), strong activators of glucose-induced insulin secretion. This study aims to elucidate the role of intestinal GLUT2 in enterocytes and enteroendocrine cells on glucose homeostasis in mice.
Materials and methods: To address this question, we generated an inducible GLUT2 deficient mouse model specifically in intestinal epithelial cells (GLUT2ΔIEC) by CreLox strategy. Body weight gain, oral glucose tolerance tests and plasma insulin measurements were performed at different time points after intestinal GLUT2 invalidation. GLP-1 and GIP positive cells were quantified in jejunum and colon by immunostaining.
Results: GLUT2ΔIEC mice have a lower body weight gain compared to control mice (7.5 g±0.2 vs. 5.2±0.3 g, $P<0.01$, 11 weeks post invalidation). This result could be explained by a slower glucose absorption in invalidated mice compared to their littermate controls from 4 weeks post invalidation (12.7±2.9 vs. 4.3±1.9 mg of glucose/dL/min, $P<0.05$) up to 12 weeks post invalidation (18.7±1.1 vs. 13.5±1.3 mg of glucose/dL/min, $P<0.01$). Nevertheless, glucose malabsorption is affecting neither oral glucose tolerance nor plasma insulin levels. Interestingly, there is a 6-fold decrease of GLP-1-producing cell density in jejunum of GLUT2ΔIEC compared to control mice (9.3±2.2 vs. 1.4±0.4 GLP1+ cells/mm2, $P<0.01$). Intestinal GLUT2 invalidation reduces to a lesser extent (by 2-fold) GIP-producing cell abundance in this small intestine segment (19.2±6.3 vs. 8.9±1.2 GIP+ cells/mm2, $P=0,06$), suggesting a preferential effect of GLUT2 on GLP-1-producing cells. Moreover, enteroendocrine cell density is not affected by intestinal GLUT2 invalidation in the colon (16.7±5.8 vs. 8.2±1.7 GLP1+ cells/mm2, $P=0.1135$ and 12.2±5.3 vs. 12.8±1.8 GIP+ cells/mm2, $P=0,8747$), probably because GLUT2 expression is low in the large intestine.
Conclusion: Our data highlight crucial roles of intestinal GLUT2 in glucose absorption and enteroendocrine cell density in the absorptive segment of the intestine. The impact of reduced enteroendocrine cell density on diabetes remains to be challenged by metabolic perturbations. The reduction of enteroendocrine cell number could be a consequence of the absence of GLUT2 either in enteroendocrine cells or in neighbouring enterocytes. To discriminate which GLUT2 expressing cell is involved in enteroendocrine cell plasticity, we are studying a new mouse model with specific GLUT2 invalidation in enteroendocrine cells.
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Mechanisms regulating gastrointestinal somatostatin secretion

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Background and aims: Beginning with the control of acid secretion and gastric emptying, entero-endocrine cells (EECs) in the gastrointestinal tract shape post-prandial glucose excursions and nutrient delivery to peripheral tissues. Somatostatin (Sst)-producing D-cells are of particular interest because they exert profound inhibitory control over other EECs, particularly in the stomach where they regulate gastric acid secretion. The objective of this study was to identify molecular pathways underlying Sst release from D-cells.
Materials and methods: We generated transgenic mice expressing Cre driven by the Sst promoter. Upon crossing with Cre reporter strains, mice expressed red fluorescent protein (tdRFP), enhanced yellow fluorescent protein (EYFP), or a genetically encoded calcium indicator, GCaMP3, in Sst-expressing cells. RNA sequencing of gastric D-cells purified by fluorescence activated cell sorting identified the D-cell transcriptome. Molecular targets were verified by quantitative RT-PCR (qPCR). Primary gastric cultures were used in Sst secretion assays and to monitor calcium responses to test stimuli in D-cells by fluorescence imaging.
Results: By qPCR, sst message was enriched 690-fold ($p<0.001$) in the EYFP positive population, confirming D-cell specificity of transgene expression. RNA sequencing showed D-cells expressed mRNA encoding peptideYY (pyy), albeit at 14-fold lower levels than sst. RNAseq and qPCR found G-protein coupled receptors enriched in D-cells included glucose-dependent insulinotropic peptide (GIP) receptor (gipr), muscarinic receptor-4 (chrm4), calcitonin gene related polypeptide (CGRP) receptor (calcrl/ramp1), trace amine associated receptor (taar1) and calcium sensing receptor (casr) (fold-enrichment vs neighbouring cells of 1300, 98, 110, 42, 13, and 78, respectively; $p<0.01$ each). D-cells also expressed receptors for glucagon-like peptide-1 (GLP-1: glp1r) and cholecystokinin (CCK: cckar but not cckbr). In gastric cultures Sst secretion was stimulated 1.9-fold by GLP-1, 1.5-fold by GIP and 1.8-fold by CCK ($p<0.01$ each). Acetylcholine decreased secretion 2-fold ($p<0.001$), whereas vasoactive intestinal polypeptide (VIP) and CGRP increased Sst release 2.3-fold and 4.6-fold, respectively ($p<0.001$ each). Oligopeptides and the TAAR1 agonist Ro5166017 stimulated Sst secretion 1.9-fold and 4.5-fold ($p<0.001$ each) suggesting that D-cells can sense ingested luminal factors. CCK and oligopeptides elicited increases in intracellular calcium in imaging experiments, consistent with the coupling of CCK-A receptors and CaSR to Gq signalling pathways.
Conclusion: Sst secretion from gastric D-cells is directly inhibited by the vagus nerve via muscarinic M4 receptors, and stimulated by the enteric neurotransmitters CGRP and VIP. Luminal factors like oligopeptides and trace amines stimulate Sst release via CaSR and TAAR1. Feedback stimulation of Sst by small intestinal hormones including CCK, GIP and GLP-1 likely provides a mechanism to tune the termination of acid secretion according to the nutrient content of a meal. Our data provide a first transcriptomic analysis and functional characterization of gastric D-cells, and indentify regulatory pathways that underlie the direct detection of stimuli by this cell type which is intricately linked to rates of food intake and absorption, and the coordination of post-prandial digestion.
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PS 039 Regulation of insulin secretion

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Impaired beta cell function and insulin secretion in non-diabetic patients with extrahepatic cholestasis

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Background and aims: Several studies have shown the inverse correlation between serum bilirubin levels and the risk of diabetes, suggesting that bilirubin acting as antioxidant and cytoprotectant could antagonize oxidative stress-induced beta cell damage. Whereas, in both human and animal models, cholestasis is associated with altered glucose tolerance. However, the relationship between cholestasis and beta cell function has not been fully evaluated in the clinical setting. To investigate whether cholestasis, as evidenced by hyperbilirubinemia, affects β cell function and insulin secretory response, we performed oral glucose tolerance tests and hyperglycemic clamps (HC) followed by arginine stimulation in 44 patients (27 F/17 M, 51±15 yrs) scheduled for pancreatoduodenectomy for periampullary diseases, all without known history of diabetes.
Materials and methods: Based on bilirubin levels, subjects were divided into 2 groups: with resolved cholestasis and/or normal bilirubin levels (NChol, n=21, Bil: 0.29±0.03 mg/dl) and with active extrahepatic cholestasis (Chol, n=23, Bil: 4.30±0.69 mg/dl). Amylasemia was similar between the two groups (76.6±15.8 vs. 86.9±10.3 UI/L). To evaluate β cell function, β cell glucose sensitivity (GS) during hyperglicemic clamp was calculated as the ratio of insulin secretion (IS) and glucose increments.
Results: Cholestatic subjects displayed a significantly lower Insulinogenic index (II) (0.46±0.06 vs. 0.76±0.11, p=0.01), while no differences were detected in the Matsuda index (5.89±0.88 vs. 4.66± 0.67, p=NS). The incremental first phase (p=0.01), second phase insulin secretion (p=0.02) and GS (57.5±10.5 vs. 92.3±11.3 pmol·min-1·m-2· mM-1, p=0.03) were significantly lower in Cholestatic subjects. Analysis of the entire group revealed an inverse correlation between bilirubin levels and Insulinogenic index (r=-0.40; p<0.05), and Arginine-stimulated Insulin Secretion (AIS) (r=-0.51; p<0.01).
Conclusion: Our data indicate that cholestasis associates with impaired β cell function and possibly reduced β cell mass (as estimated by AIS). We speculate that, in subjects with extrahepatic cholestasis, impaired β cell glucose sensitivity could represent a major determinant of the observed insulin secretory defect in response to both glucose and arginine stimulus. Further investigation of this mechanism might improve our understanding of the pathogenic events leading to altered insulin secretion in type 2 diabetes.

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No effect of high-dose vitamin D treatment on beta cell function, insulin sensitivity or glucose homeostasis in subjects with abnormal glucose tolerance: a RCT

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Background and aims: There has been conflicting evidence regarding the potential role of vitamin D in glucose homeostasis. This study was designed to investigate the effect of high-dose vitamin D treatment on beta cell function, insulin sensitivity and glucose tolerance in middle-aged subjects with pre-diabetes or diet treated type 2 diabetes.
Materials and methods: Subjects were randomised to 30 000 IU vitamin D3 once weekly or placebo for eight weeks. Hyperglycaemic clamp assessed first- (0-12 min) and second-phase (12-120 min) insulin response, insulin sensitivity and disposition index. Oral glucose tolerance test and HbA_{1c} assessed glycaemic control. Data are presented as medians (interquartile range) and non-parametric tests were used for the outcome assessments.
Results: 21 (vitamin D) and 22 (placebo) subjects completed the study, respectively. Baseline characteristics did not differ significantly between the two groups. Season-adjusted 25-OH-vitamin D level was doubled in the vitamin D group (+42 (32-50) nmol/l) and remained unchanged in the placebo group (0 (-7-11) nmol/l). No effect of vitamin D treatment, compared to placebo, was seen on first-phase insulin secretion, second-phase insulin secretion, insulin sensitivity or disposition index. There was a small reduction in median HbA_{1c} in the vitamin D group of 1 (-3-1) mmol/mol (P=0.06), but with no significant difference versus placebo (P=0.84). No other measurements of glycaemia showed any changes. Sub-group analyses of those with the lowest basal vitamin D values did not change these results. No hypercalcemia or other adverse effects of vitamin D treatment were seen compared to placebo.
Conclusion: This study gives no support for any substantial effect of high-dose vitamin D treatment for eight weeks in pre-diabetes or diet treated type 2 diabetes on beta cell function, insulin sensitivity or glycaemic control.
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Early insulin secretion defect characterises males with type 2 diabetes of Yemenite origin

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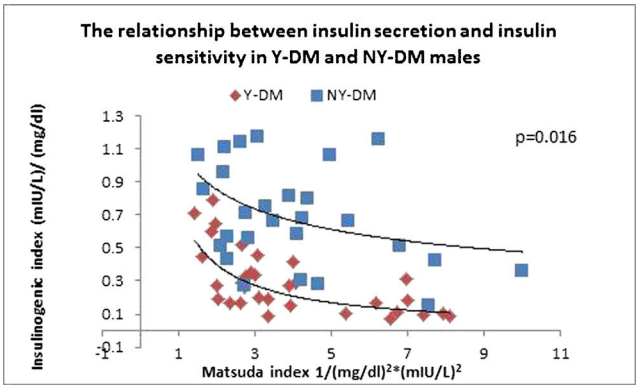
Background and aims: Yemenites who immigrated to Israel demonstrated within 6 decades a dramatic 20-fold increase in the prevalence of diabetes but the underlying mechanisms remain obscure. The aim of the current study was to compare parameters of β-cell function and insulin sensitivity in patients with new-onset Type 2 diabetes of Yemenite (Y-DM) and non-Yemenite origin (NY-DM) treated by diet with or without oral antihyperglycemic monotherapy.
Materials and methods: A 180-minutes meal tolerance test (MTT) was performed in 121 GAD negative patients: 59 Y-DM (57% males) and 62 NY-DM (48% males). Based on the MMT we calculated indexes of insulin resistance (Matsuda index, HOMA2IR) indexes of insulin secretion including the insulinogenic index [Δ insulin (30-0 min)/ Δ glucose (30-0)], and the disposition index that indicates the hyperbolic relationship between insulin sensitivity and insulin response.
Results: Age, sex, DM duration, BMI, HbA1c and lipid profile were with no significant differences. A significant difference was found in family history: 63% of Y-DM had maternal inheritance compared with 35% in NY-DM (p<0.001), but no difference was found in paternal inheritance. Y-DM had increased hyperglycemia manifested by a significantly higher area under the curve (AUC) of glucose during MTT compared with NY-DM (p=0.005) but there was no difference in insulin and C-peptide AUC. There were no difference in insulin sensitivity but both the insulinogenic index and the disposition index were significantly lower in Y-DM compared with NY-DM (0.68±0.5 vs. 0.95±0.8, p=0.04; 2.25 vs. 3.3, p= 0.03, respectively). When females and males were analyzed separately the difference in maternal inheritance remained significant in both (p<

0.005). The difference in β-cell function however remained significant only in males, with Y-DM having a lower insulinogenic index (0.56±0.4 vs.1.02±1.12 p=0.02) and a shift to the left and downward of the curve describing the hyperbolic relationship between insulin sensitivity and insulin secretion (Figure).

Conclusion: Y-DM have a significantly higher maternal inheritance compared with NY-DM. Males of Yemenite origin have a significant reduction of β-cell function and reduced ability to compensate for insulin resistance compared with NY-DM. These data suggest different underlying mechanisms leading to early loss of β-cell function in Y-DM observed in males.



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Exploring the role of secreted frizzled related protein 4 as a novel biomarker of beta cell dysfunction, insulin resistance and type 2 diabetes

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Background and aims: There is emerging data to indicate that secreted frizzled-related protein 4 (SFRP4) is highly expressed in islets. SFRP4 is associated with increased risk of type 2 diabetes mellitus [T2DM] but the physiological role of SFRP4 remains poorly understood. We report on the systemic levels of SFRP4 and its association with insulin resistance, beta cell dysfunction, prediabetes and type 2 diabetes in Asian Indians.

Materials and methods: Individuals with normal glucose tolerance [NGT; n=90], impaired glucose tolerance [IGT; n=50] and T2DM [n=90] were recruited from a large tertiary diabetes center and a medical college hospital in southern India. Insulin resistance was calculated using the homeostasis assessment model (HOMA-IR) and beta cell function by oral disposition index (DIo). SFRP4 levels were measured by the enzyme-linked immunosorbent assay.

Results: Circulatory age adjusted SFRP4 levels were highest in T2DM (246±99 ng/ml) followed by IGT (167±62 ng/ml) and NGT (101±45 ng/ml; p<0.001). Patients with older age onset T2DM had higher levels of SFRP4 compared to youth onset T2DM (286 vs 165 ng/ml, p <0.001]. However, no differences in SFRP4 levels were seen between males and females (158±60 vs. 169±64 ng/mL, respectively; p>0.05). SFRP4 was positively correlated with HOMA-IR (p<0.01), C- Peptide (p <0.01) and inversely with DIo (p<0.01). In linear regression analysis, SFRP4 was significantly associated with insulin resistance (β=1.592; p< 0.001) and DIo (β=- 0.353; p<0.05) after adjusting for age, gender and waist circumference. In standardized polytomous regression models, higher levels of SFRP4 were independently associated with T2DM [odds ratio (OR) per standard deviation: 2.867, 95% CI 1.189 - 4.343; p< 0.001]. Adjustment for age, gender, waist circumference and insulin resistance, did not substantially change the association between SFRP4 and T2DM [OR per standard deviation: 2.185, 95% CI: 1.309 - 3.649, p= 0.003].

Conclusion: In Asian Indians, the circulatory levels of SFRP4 showed a significant association with glucose intolerance, insulin resistance, beta cell dysfunction and T2DM and could serve as a biomarker for early beta cell dysfunction and glucose intolerance.

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The impact of family history of young-onset type 2 diabetes on metabolic profile and beta cell function in euglycaemic young-to-middle age Chinese individuals in Hong Kong

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Background and aims: The prevalence of young-onset type 2 diabetes (YOD) is increasing globally. Family history of type 2 diabetes is associated with defect in insulin sensitivity and/or beta-cell function. We investigated the impact of family history of YOD (defined as age at diagnosis <40 years) on the metabolic traits, insulin sensitivity and beta-cell function in young-to-middle aged Chinese individuals with euglycemia in Hong Kong.

Materials and methods: 415 participants with normal glucose tolerance (fasting plasma glucose [PG]<5.6 mmol/L and 2-hour PG <7.8 mmol/L during the 75-gram OGTT test) were studied. Among them, 220 individuals did not have family history of diabetes (FH-), 122 individuals had family history of late-onset type 2 diabetes (age at diagnosis ≥40 years, FH+LOD) and 73 had family history of YOD (FH+YOD). Insulin sensitivity was measured by Matsuda insulin sensitivity index (ISI) and early-phase insulin response was measured by insulinogenic index (IGI). Disposition index (DI) was used to measure beta-cell function. Metabolic data were compared between groups with adjustment for age, sex, body mass index and waist-to-hip ratio.

Results: The mean age was 35.5±7.2 [SD] years in FH+YOD group, lower than those with FH+LOD (43.0±7.2 years) and without family history of diabetes (42.2±7.6 years, p trend <0.001). Individuals with FH+YOD and FH+LOD had higher BMI, waist circumference (in men), LDL-cholesterol, and lower HDL-cholesterol compared to those without family history (all p trend <0.05, table). The FH+YOD group had highest 1-hour PG (p trend=0.001, table), while fasting and 2-hour PG during OGTT were similar between the 3 groups. Subjects with positive family history had lower DI than the FH- group (p trend=0.005), although DI did not differ between FH+LOD and FH+YOD groups. ISI was similar between all 3 groups. There was a tendency for IGI to be lower in the FH+YOD group with borderline significance between FH+YOD and FH- groups (post-hoc p=0.078, table).

Conclusion: Normal glucose-tolerant first-degree relatives of patients with type 2 diabetes, especially those with family history of YOD, exhibit reduced beta-cell function and increased obesity. The disease burden of YOD calls for early detection and intervention program in subjects with family history of diabetes, especially YOD.

Table. Metablic profile, insulin sensitivity and bete-cell function stratified by family history of diabetes.

	FH$^-$ (n=220)	FH$^+$LOD (n=122)	FH$^+$YOD (n=73)	p trend
Age (years)	42.2±7.6	43.0±7.2	35.5±7.2[b,c]	**<0.001**
Male, number (%)	99 (45.0%)	54 (44.3%)	28 (38.4%)	0.372
Systolic blood pressure (mmHg)	113.9±16.3	115.6±16.2	116.5±15.3	0.219
Body mass index (kg/m^2)	23.0±3.0	24.0±3.7[a]	24.3±3.9[b]	**0.004**
Waist circumference (men,cm)	82.3±7.0	83.9±8.6	87.0±9.5[b]	**0.006**
Waist circumference (women,cm)	73.1±7.6	75.4±9.2	74.4±7.7	0.350
Waist-to-hip ratio	0.83±0.07	0.84±0.08	0.81±0.07	0.140
Total cholesterol (mmol/L)*	5.12±0.92	5.22±0.93	5.06±0.95	0.195
HDL-cholesterol (mmol/L)*	1.65±0.46	1.58±0.46	1.37±0.37[b,c]	**0.001**
LDL-cholesterol (mmol/L)*	2.96±0.83	3.13±0.88	3.14±0.86[b]	**0.005**
Triglyceride (mmol/L)*	0.87 (0.62, 1.49)	0.95 (0.76, 1.40)	1.02 (0.72, 1.45)	0.133
Fasting plasma glucose(mmol/L)*	4.77±0.37	4.84±0.36	4.83±0.37	0.282
1-hour plasma glucose (mmol/L)*	7.36±1.97	7.57±1.92	8.23±2.24[b,c]	**0.001**
2-hour plasma glucose (mmol/L)*	5.47±1.20	5.50±1.11	5.61±1.22	0.404
Matsuda Insulin sensitivity index *	5.68 (3.94, 8.28)	4.77 (3.20, 6.77)	4.84 (4.00, 7.52)	0.275
Insulogenic index (IGI)*	0.84 (0.51, 1.29)	0.91 (0.42, 1.23)	0.80 (0.45, 1.31)	0.060
Disposition index*	4.44 (3.01, 7.02)	3.79 (2.41, 6.55)	3.65 (2.40, 5.08)[b]	**0.005**

Data are expressed as mean±SD, number (%) or median (interquartile range).

a, post-hoc p-value (FH$^+$LOD vs. FH$^-$) <0.05; b, p (FH$^+$YOD vs. FH$^-$)<0.05; c, p (FH$^+$YOD vs. FH$^+$LOD) <0.05.

*The comparison was adjusted for age, sex, body mass index and wait-to-hip ratio

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Active GLP-1 but not insulin, glicentin or glucagon predicts the 2-hour OGTT glucose value in obese children

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Background and aims: Proglucagon is post-translationally processed to glucagon, glucagon-like peptide 1 (GLP-1) and glicentin, among other peptides. Despite the fact that glucagon and GLP-1 are well-known regulators of glucose metabolism, little is known of them in the context of childhood obesity and early stages of impaired glucose metabolism. Glicentin is less studied than other proglucagon peptides but functions described to date are inhibition of gastric acid secretion and gastrointestinal motility. The aim of this study was to investigate the levels of GLP-1, glicentin and glucagon in childhood obesity and their importance for the development of impaired glucose tolerance in this patient group.
Materials and methods: Obese (n=50) and lean (n=19) children, 10-18 years of age, were recruited from the Uppsala Longitudinal Study of Childhood Obesity cohort between June 2012 and October 2014. After an overnight fast, subjects underwent a standard OGTT. Classification of normal glucose tolerance (NGT), impaired glucose tolerance (IGT) and/or impaired fasting glucose (IFG) and type 2 diabetes (T2DM) was done according to WHO criteria. Fasting plasma active GLP-1 was quantified by electrochemi-luminescent immunoassay (Meso Scale Discovery, MD, USA). Fasting plasma insulin, glicentin and glucagon levels were quantified by ELISA (Mercodia AB, Uppsala, Sweden). Differences among groups were analysed by one-way ANOVA with Bonferroni post hoc test.
Results: Of the obese children, 25 had NGT, 21 had IGT (including 9 children with IFG) and 4 met the criteria for T2DM. All lean children had NGT. As the number of subjects with T2DM was limited, these were excluded from the data analysis. Lean controls had lower fasting GLP-1 levels than obese children with NGT (0.37±0.05 vs 0.58±0.07 pmol/l) and lower fasting glucagon levels than obese children with IGT (8.1±0.5 vs 14.1±1.4 pmol/l). Fasting GLP-1 levels were lower in obese children with IGT than in those with NGT (0.34±0.03 vs 0.58±0.07 pmol/l) and the same was true for glicentin levels (16.1±1.6 vs 26.5±2.4 pmol/l). Fasting glucagon levels were higher in obese children with IGT than in those with NGT (14.1±1.4 vs 10.8±0.7 pmol/l). In addition, multiple regression analysis was performed with 2-hour glucose as the dependent parameter and age, sex, age-adjusted BMI, fasting insulin, GLP-1, glicentin and glucagon as independent parameters. Only fasting GLP-1 levels were significantly correlated to the two-hour blood glucose values (parameter estimate -1.91; SE 0.9; $p<0.05$).
Conclusion: Obese children with IGT are characterized by increased fasting glucagon and decreased fasting glicentin and GLP-1 levels. In obese children, fasting plasma active GLP-1 is correlated to the 2-hour OGTT glucose value independent of age, sex, BMI, and fasting levels of insulin, glicentin and glucagon.
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Combined N-terminal and C-terminal truncations of Glucose dependent Insulinotropic Polypeptide (GIP) are potent and efficient GIP receptor antagonists

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Background and aims: The intestinal hormone glucose-dependent insulinotropic polypeptide (GIP) exhibits several functions within lipid, bone, and glucose homeostasis. The GIP receptor (GIP R) is a family B 7-transmembrane receptor with the most established signaling pathway being $G\alpha_S$. It is expressed in the pancreas, brain, bone, cardiovascular system, and gastrointestinal tract. High affinity ligands are needed to explore the role of GIP in adiposity, osteogenesis, and glucose homeostasis in vivo. hGIP(1-30) is naturally occurring in humans. Here we report a pharmacological characterization of eigth N-truncated GIP analogs of hGIP(1-30): hGIP(2- to 9-30).
Materials and methods: COS-7 cells were transiently transfected with the human GIP R for all assays. We used radiolabeled ^{125}I-hGIP(1-42), ^{125}I-hGIP(1-30), ^{125}I-hGIP(2-30) and ^{125}I-hGIP(3-30) for competitive binding analyses and an enzyme fragment-based cyclic AMP-accummulation assay. Pancreas perfusions in rats were performed with a single-pass system (10 mmol/l glucose) through arteries and collected effluent from the portal vein.
Results: hGIP(1-30) displaced ^{125}I-GIP(1-42) with similar affinity as native GIP(1-42) (Ki of 0.75 nmol/l). The eight truncated analogs displaced ^{125}I-GIP(1-42) with lower affinities (Ki from 2.3 to 347 nmol/l) following the truncation lengths with the highest affinities identified for GIP(3-30) and GIP(5-30) (Ki of 2.6 and 6.1 nmol/l, respectively). GIP(1-30) acted as a full agonist (EC_{50} 9.8 pmol/l), whereas GIP(2-30), as the only one of the truncations, displayed potent partial agonistic properties (20% efficacy of hGIP(1-30) and an EC_{50} of 1.8 nmol/l). GIP(2- to 9-30) displayed length-dependent antagonistic properties (IC_{50} from 12 to 450 nmol/l) and resulted in right-shifts of the hGIP(1-42) dose-response curve in a dose-dependent manner. Schild plot analysis concluded that GIP(3-30) and GIP(5-30) were competitive antagonists with equal pA2 values of 15 nmol/l and Hill-slopes of 0.93±0.02 and 1.1±0.04 respectively. Homologous binding studies revealed different binding confirmations that corresponded to the functional properties of the agonist GIP(1-30), the partial agonist GIP(2-30) and competitive antagonist GIP(3-30). In pancreas perfusion studies 100 nmol/l GIP(3-30) significantly reduced somatostatin secretion induced by 1 nmol/l GIP(1-42), and stimulated de novo and potentiated 1 nmol/l GIP(1-42)-induced insulin secretion. GIP(3-30) did not affect GIP(1-42) induced glucagon secretion nor stimulated de novo secretion.
Conclusion: Human GIP(3-30) and GIP(5-30) were highly potent competitive antagonists of the human GIP R. GIP(3-30), a presumed naturally

occurring degradation product, showed diverse effects on somatostatin and insulin release in vivo (rat). These antagonists could serve as tool compounds for further elucidation of the human GIP physiology and thereby in turn for future pharmacological interventions.

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Translation of predictive in vitro GIP receptor assays to in vivo acute efficacy

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Background and aims: Glucose-dependent insulinotropic polypeptide (GIP) and glucagon-like peptide 1 (GLP-1), both Gs-coupled GPCRs, are incretin hormones whose primary role is to potentiate glucose-stimulated insulin secretion from pancreatic beta-cells. Dual agonism of the GIP and GLP-1 receptors has recently been speculated as being advantageous in treating type 2 diabetes. Drug discovery projects tasked with identifying agonists at these incretin receptors require high throughput *in vitro* functional assays to drive structure activity relationship hypothesis testing. It is imperative that these assays translate to biological effect assays in disease relevant cell lines and are predictive of the effects seen in preclinical *in vivo* models. In this study we show that potency and efficacy translates from transfected receptor cell line second messenger assays to endogenous receptor second messenger and insulin secretion assays, and also to acute efficacy *in vivo* in glucose tolerance tests.
Materials and methods: A panel of GIP peptides palmitoylated at different positions along the length of the peptide were tested *in vitro* for potency and intrinsic activity in transfected CHO-human GIPR, clonal rat beta cell (INS-1E) and mouse adipocyte (3T3L1) cell line cAMP accumulation assays. Cells were incubated with peptides for 30 min prior to lysis and detection of cAMP levels. A subset of potent peptides was then tested for glucose-stimulated insulin secretion in glucose-responsive INS 832/3 cells. Cells were cultured in the presence of low glucose (2.8 mM) for 1 hr at 37°C prior to incubation of peptides in the presence of high glucose (8.3 mM) for 1 h. Insulin secretion was quantified using Mesoscale Discovery ECL detection. Key peptides were then further profiled *in vivo* using glucose tolerance tests in C57Bl/6J mice. Fasted C57BL/6J male mice were dosed SC with either Na^{2+}phosphate buffer or palmitoylated GIP ligands 2 h prior to intraperitoneal 2 g/kg glucose (ipGTT) challenge. Blood glucose was measured from tail vein samples at -120, 0, 15, 30, 45, 60 and 90 mins.
Results: The palmitoylated GIP molecules were all full agonists in CHO-human GIPR transfected receptor cAMP assays with potencies spread over a 1000-fold range. The peptide EC_{50} from the transfected human GIPR correlated well with EC_{50} determinations from both rat INS-1E and mouse 3T3L1 cAMP assays. Six peptides with potencies spanning 40 pM to 60 nM in the INS1E cAMP assay were shown to have robust activity in glucose stimulated insulin secretion (GSIS) assay in INS 832/3. There was also positive correlation for peptide potency in the INS 832/3 between cAMP and GSIS assays (slope =0.92, r^2=0.94). Key molecules with a broad range of potencies in the INS GSIS assay were profiled in a lean mouse ipGTT study. There was consistent correlation between INS GSIS pEC50 and AUC for ipGTT and peptide rank order was maintained with a good correlation (r^2=0.91).
Conclusion: Here we show correlation of potency from transfected receptor *in vitro* assays to endogenous receptor assays at the level of cAMP accumulation. These potencies directly correlate with biological effect readouts of insulin secretion in rat beta cells. Furthermore we showed translation of these *in vitro* potencies and rank order to *in vivo* efficacy through glucose disposal in ipGTT studies. Based on these results we have the tools to successfully interrogate GIPR potency in a drug discovery screening cascade.

PS 040 New experimental models of insulin secretion

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Enhancement of beta cell function by combination treatment with stromal derived factor 1 alpha (SDF-1) and the DPP-4 inhibitor vildagliptin in streptozotocin diabetic mice

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Background and aims: In both type 1 and type 2 diabetes, there is a decline in the mass and function of beta cells resulting in insulin insufficiency and hyperglycemia. In type 1 diabetes this is relatively rapid whereas in type 2 diabetes this occurs over a longer period of time. Recently it has been reported that the pancreatic islet alpha cells of type 2 diabetic humans and mouse models produce elevated amounts of the incretin hormone glucose dependent insulinotropic polypeptide 1 (GLP-1). In genetic mouse models of type 2 diabetes, alpha cell GLP-1 production appears to increase as diabetes develops. Additionally, it has been demonstrated that alpha cell production of GLP-1 is enhanced by the chemokine stromal derived factor 1 (SDF-1). We hypothesized that chemically induced beta cell loss would lead to a compensatory increase in alpha cell GLP-1 which could be enhanced by intervention with SDF-1 leading to preserved beta cell function. We therefore treated streptozotocin diabetic mice with a combination of recombinant stromal derived factor 1 and the DPP-4 inhibitor Vildagliptin to determine whether it could preserve and enhance beta cell function at an early stage of diabetes development.
Materials and methods: Four month old female C57BL6JBomTac mice were given interperitoneal injections of streptozotocin (50 mg/kg) or vehicle daily for 5 days. Three days after the final injection mice were subjected to a baseline oral glucose tolerance test (OGTT) and immediately placed on a regimen of daily injections with recombinant mouse SDF-1 alpha (1 μg/mouse) or vehicle for 5 days. Mice were concurrently treated with either a background of Vildagliptin (3 umol/mouse per day) or water. Mice remained on a background of Vildagliptin or water for an additional 3 weeks and OGTTs were performed mid-study and at termination (4 weeks). Bodyweight was monitored weekly. Plasma glucose and insulin were measured at multiple time points during the OGTTs and beta cell function was calculated using the insulinogenic index. Beta cell mass was determined from histological sections of whole pancreas stained with insulin specific antibodies.
Results: After an initial decline the first week of the intervention, bodyweight was maintained throughout the final 3 weeks of the study. Diabetic mice that did not receive treatment saw a decline in beta cell function (insulinogenic index) by the end of the four week study period. Diabetic mice that were treated exclusively with a background of Vildagliptin saw a 2 fold improvement in insulinogenic index after four weeks compared to baseline (57±14 vs. 29±6 pmol/mmol, p=0.043). Diabetic mice that were treated with SDF-1 injections in week 1 combined with background Vildagliptin saw a 4-fold improvement in beta cell function after four weeks compared to baseline (53±6 vs. 14±4 pmol/mmol, p=0.0003). In addition, the SDF-1-vildagliptin treated group displayed significantly greater glucose elimination rates than untreated diabetic controls (1.02±0.07 vs. 0.79±0.07%/min, p=0.028). Immunohistochemical analysis of pancreata after the study revealed that all diabetic groups have significant reductions in beta cell mass with no significant differences in beta cell mass between treated and untreated diabetic mice.
Conclusion: The combination of SDF-1 alpha and vildagliptin represents a novel treatment concept which improves beta cell function and glucose elimination in an insulin deficient mouse model of diabetes.
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Calcium independent functional compensation of beta cells predominates islet mass adaptation in diet induced insulin resistance

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Background and aims: Pancreatic islets have been suggested to compensate in both mass and function to meet the increased insulin demand during obesity and insulin resistance (IR). However, the dynamics and relative contribution of these two forms of compensation are not well understood. In addition, the mechanisms underlying compensation in vivo remain to be elucidated. Here, we longitudinally in vivo assessed the adaptation of islet cell mass and function in correlation to glucose homeostasis during diet-induced obesity and IR in mice.
Materials and methods: Mice were transplanted with MIP-GFP or Pdx1CreER-GCaMP islets into the anterior chamber of the mouse eye. After engraftment period, the recipient mice were fed with HFD (60%) for 17 weeks before switching back to normal chow. Several parameters of glucose homeostasis were assessed and in parallel transplanted islets repetitively monitored noninvasively in vivo. Islet morphology and dynamics of cytosolic calcium were visualized by GFP and GCaMP expression.
Results: HFD feeding induced obesity, IR and impaired glucose tolerance. Total islet mass (1.92±0.50 fold increase, $p<0.001$; 16wk) and beta cell volume (1.91±0.39 fold increase, $p<0.001$; 16wk) continuously increased over the entire experimental time course. Intra-islet vessels adapted by dilation in response to HFD feeding. Glucose stimulated insulin secretion during an intraperitoneal glucose tolerance test (IPGTT) increased until 10 weeks HFD and reached a plateau thereafter. The functional efficacy of islets in relation to islet mass was initially increased (3.17±1.78 fold increase, $p<0.05$; 8wk) but slowly declined, indicating an initial functional compensation, followed by decompensation. To evaluate the mechanisms of functional beta cell compensation we measured beta cell calcium dynamics ([Ca2+]i) in vivo., Basal [Ca2+]i increased shortly after HFD onset in association with elevated fasting plasma insulin. In contrast glucose-stimulated [Ca2+]i decreased throughout the time course of HFD feeding (388±256 vs 853±230, $p<0.01$, 16wk), while insulin release remained increased. This indicates an early calcium independent compensatory mechanism, followed by a calcium dependent dysfunction of beta cells in response to long-term HFD. Interestingly, even after 17 weeks HFD feeding the detected changes were rapidly reversible after by 2 weeks normal diet feeding.
Conclusion: Our results demonstrate that the beta cell functional response outweighs mass adaptation in the compensation for insulin resistance. In addition, our data indicate that the observed functional compensation and dysfunction of beta cells are based on diverse mechanisms.
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Regulation of GPR40 gene expression in pancreatic islets of non-obese and obese glucose-intolerant rat models, Goto-Kakizaki rat and Koletsky rat

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Background and aims: G protein-coupled receptor 40 (GPR40; FFAR1) is a Gq-coupled receptor for middle- to long-chain fatty acids. GPR40 is highly expressed in pancreatic beta cells in rodents and we have reported that GPR40 is probably highly expressed in pancreatic beta cells also in humans. GPR40 is reportedly involved in the regulation insulin secretion and is a potential therapeutic target in diabetes mellitus. However, little is known about the regulation of its gene expression. This study was designed to elucidate the regulation of GPR40 gene expression in glucose intolerance and to explore the pathophysiological implication of GPR40.
Materials and methods: As a non-obese glucose-intolerant model, we used Goto-Kakizaki (GK) rat, a Wistar substrain that develops Type 2 diabetes. As an obese glucose-intolerant model, we used Koletsky rat, a genetically obese model whose nonsense mutation in the Ob-R gene was reported by us. Six-week-old GK rat and 14-week-old Koletsky rat with established glucose intolerance were subjected to intraperitoneal glucose-tolerance tests (IPGTT, 2 g/kg), and compared with age-matched control rats (6-week-old Wistar rat and 14-week-old lean littermate, respectively). Blood glucose levels were determined by the glucose oxidase method. Plasma insulin levels were measured by EIA. Pancreatic islets were isolated from these rats and GPR40 mRNA expression was measured by quantitative PCR.
Results: In 6-week-old GK rats, blood glucose levels were markedly elevated at all time points during IPGTT. Serum insulin levels at 15 and 30 min after glucose load were significantly lower in GK rats compared to those in Wistar rats, though there was no significant difference at fasting, and at 60 and 120 min after glucose load. GPR40 mRNA levels in pancreatic islets of GK rats were markedly decreased compared to those of Wistar rats (43% *vs* Wistar rats, $p<0.05$). In 14-week-old Koletsky rats, blood glucose levels were significantly elevated after glucose load compared to lean littermates, while there was no significant difference at fasting between two groups. Serum insulin levels in Koletsky rats were approximately ~7 times higher than those in lean littermates at all time points during IPGTT, however, at 15 minutes after glucose load it was rather decreased *vs* before glucose load in Koletsky rats. GPR40 mRNA levels in pancreatic islets of Koletsky rats were markedly decreased compared to those of lean littermates (39% *vs* lean littermates, $p<0.05$).
Conclusion: We demonstrate that GPR40 mRNA levels in pancreatic islets are markedly decreased in both non-obese and obese model rats, suggesting decreased GPR40 gene expression in pancreatic beta cells in glucose intolerance. In both models, increment of insulin secretion after glucose load was attenuated, suggesting impaired early insulin response. GPR40 is implicated in the augmentation of glucose stimulated insulin secretion (GSIS), so the possible link between decreased GPR40 gene expression in pancreatic beta cells and impaired early insulin response *in vivo* seems plausible. Further investigations are now in progress.

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Limited impact on glucose homeostasis of highly beta or alpha cell-selective disruption of leptin signalling

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Background and aims: The adipose tissue-derived hormone leptin plays an important role in maintenance of body weight and glucose homeostasis. Leptin mediates its effects by interaction with leptin receptors (LepR), which are highly expressed in the hypothalamus and reportedly at low levels in pancreas. Previous studies have used relatively promiscuous Cre deleter strains to explore the role of LepR in pancreatic beta and alpha cells, with conflicting results. Here, we investigate the role of LepR in these cells using more selective Cre deleter strains.
Materials and methods: Mice lacking leptin signalling specifically in beta and alpha cells respectively were generated by Ins1-Cre or iGlu[proglucagon promoter]-Cre-mediated recombination of LoxP sites within exon 17 of the leptin receptor gene. Highly purified beta and alpha cells were prepared by fluorescence-activated cell sorting (FACS) of islets from mice expressing YFP selectively in the latter. LepR expression was quantified by massive parallel sequencing (RNASeq) of purified islet

cells. In vivo glucose homeostasis, pancreatic histology and hormone secretion were monitored by standard techniques. Intracellular Ca2+ dynamics were assessed by confocal microscopy of the entrapped fluorescent probe, Fluo-2, in intact islets.

Results: Expression of LepR mRNA was undetectable or vanishingly low (0.2 reads per kilobase per million mapped reads, RPKM) in purified mouse pancreatic alpha and beta cells, respectively. Whereas male mice lacking the leptin receptor in beta cells mice exhibited no evident abnormalities in glucose tolerance up to 16 weeks of age, females displayed slightly improved glucose tolerance at 8 weeks (AUC=865.1±31.21 for WT, 937.7±32.38, Ins1Cre::LepRF/F; n=9-13 per genotype; mice, P=0.0292), but not at 12 weeks. No differences were seen between genotypes in body weight, fasting glucose or beta/alpha cell ratio. No differences were observed in glucose- (16.7. vs 3 mM) induced Ca2+ changes between WT and Ins1Cre::LepRF/F (AUC=1191±105.4 for WT, AUC=1024±148.3, Ins1Cre::LepRF/F, NS) mouse islets. Deletion of LepR from alpha-cells, a minority of beta cells, and a subset of proglucagon-expressing cells in the brain, exerted no effects on body weight, glucose or insulin tolerance, nor on pancreatic hormone secretion assessed in vivo and in vitro.

Conclusion: In contrast to earlier reports, the use here of a highly cell type-selective Cre recombinase indicates that leptin signalling plays a relatively minor, age- and sex-dependent role in the control of beta cell function in the mouse, consistent with low levels of LepR on these cells. No in vivo role for leptin receptors on alpha cells, nor in other proglucagon-expressing cells, was detected in this study. These studies suggest that the roles of leptin signalling in the endocrine pancreas may need to be re-evaluated.

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Role of TGF-beta superfamily receptor ALK7 in the regulation of glucose-induced insulin secretion

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Background and aims: Activin ligands of the TGF-β superfamily are important regulators of adult pancreatic endocrine function. We have previously shown that mice lacking globally activin B or its receptor ALK7 (activin receptor-like kinase 7) display fasting hyperinsulinemia in early stages and increased glucose-stimulated insulin secretion (GSIS). The aim of our study is to determine whether ALK7 functions cell-autonomously in islets to negatively regulate insulin secretion.

Materials and methods: To address this issue, we generated the mouse line Alk7fx/fx::Ptf1aCre/+ that displays a conditional invalidation of Akl7 gene in pancreatic progenitors. The Cre recombinase activity was checked by immunohistochemistry using the Cre reporter strain, ROSA26-stop-YFP. ALK7 expression was analysed by q-PCR. Metabolic parameters were assassed in 8-10 week-old mice. Fasting intraperitoneal glucose tolerance test (IPGTT, 2 g/kg) and in vivo GSIS were assassed in mutant mice and their control littermates fed a standard chow diet or a 60% calorie high-fat diet for 2 weeks. Ex vivo GSIS was also assassed in isolated islets from conditional knock-out mice and their controls fed a standard chow diet.

Results: Using the Cre reporter strain ROSA26-stop-YFP, we first confirmed the Cre mediated-recombination occurred in different islet cell types such as alpha and beta cells in Alk7fx/fx::Ptf1aCre/+ mice. In addition, ALK7 deletion in islets has been confirmed by q-PCR with a 70% decrease (P<0.001) in ALK7 mRNA levels. In Alk7fx/fx::Ptf1aCre/+ mice, we showed a 60% significant increase (P<0.01) in fasting insulinemia compared to controls (Alk7fx/fx). Islets derived from Alk7 mutants fed a standard chow diet significatively (P<0.05) increase in vitro GSIS by 36%. in vivo GSIS and IPGTT performed in mice fed a chow diet showed that Alk7 mutant mice tended to secrete more insulin in response to glucose (N.S) and displayed a slightly impaired glucose tolerance. However, when mice were fed a high-fat diet for 2 weeks, in vivo GSIS was highly enhanced (P<0.05) in Alk7fx/fx::Ptf1aCre/+ mice although their glucose tolerance is not improved compared to controls. Interestingly, the conditional loss of ALK7 expression is associated to a significant (P<0.01) increase in mRNA level of the ATGL (adipose triglyceride lipase) lipase in islets of Alk7fx/fx::Ptf1aCre/+ mice.

Conclusion: Our study suggests that ALK7 may regulate glucose-induced insulin secretion in a cell autonomous manner in islets. Moreover we have recently shown that ALK7 inhibition in adipocytes enhances lipolysis. Thus, ALK7 deletion could promote lipolysis in islets through lipases upregulation, thereby potentiating insulin secretion in response to glucose.

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CART is a novel glucose-dependent peptide with antidiabetic actions in humans

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Background and aims: Cocaine- and amphetamine-regulated transcript (CART) is necessary for islet function in mice, but information from humans is lacking. We examined CART expression and function in human healthy and T2D islets, as well as the effect of genetic variations in the *CARTPT* gene. We also aimed to dissect the mechanisms by which CART affects islet hormone secretion using rodent models.

Materials and methods: Human islets from healthy and T2D cadaver donors and rodent islets were used to study CART expression by immunohistochemistry, qPCR and western blot. Islets from 64 human donors were subjected to Affymetrix GeneChip Human Gene 1.0ST array. Insulin and glucagon secretion was studied *in vivo* in mice and in human and mouse islets. Ca^{2+} oscillations and islet single cell exocytosis was studied in mouse islets. 22132 non-diabetic individuals were selected from 2 population-based cohorts (Malmö Preventive Project and the Prevalence, Prediction and Prevention of diabetes Botnia study) and genotyped for *CARTPT* gene variants.

Results: CART was expressed in human beta cells, alpha cells, and in cholinergic pancreatic neurons. CART mRNA was higher in hyperglycemic donors (p<0.015) and CART mRNA (p<0.009) and protein expression (p<0.008) were 3-times higher in islets of T2D patients compared to controls. Islet CART expression was several-fold increased in 4 diabetic rodent models and up regulated by glucose *in vivo* in rats (p<0.03). CART increased insulin secretion by 25% in human control- and T2D islets (p<0.02). Administration of CART i.v. and beta cell specific overexpression of CART provoked a 2-fold increased first phase insulin release (p<0.01), as well as improved glucose elimination *in vivo* in mice (p<0.02). The effect of CART on first phase insulin secretion was comparable to that of GLP-1 and notably, CART caused a 30% further increase in insulin secretion on top of that achieved by GLP-1 alone (p<0.02). Furthermore, CART augmented beta cell exocytosis 2-fold (p<0.05), triggered slow wave Ca^{2+} oscillations and synchronized oscillations within the islets. In addition, glucagon secretion was reduced by CART *in vivo* in mice (p<0.001) and CART diminished alpha cell exocytosis (p<0.05). Genetic variants in the *CARTPT* gene were associated with increased glucose levels (p<0.03), reduced insulin (p<0.04) and elevated glucagon secretion (p<0.008), and a BMI-dependent 1.5-1.7 fold increased T2D risk (p=0.001).

Conclusion: We conclude that CART is a novel glucose-dependent peptide with potential as a future anti-diabetic therapy. This is based upon its properties to stimulate insulin- and inhibit glucagon secretion, as well as the demonstration that genetic variants in the *CARTPT* gene increase risk of T2D in obese individuals.
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Involvement of Rab3B in the regulation of beta cell insulin secretion
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Background and aims: Trafficking and exocytosis of insulin granules in pancreatic beta cells is a sophisticated process in which the coordinated interplay of many different proteins is essential. Rab proteins which belong to the Ras superfamily are crucial factors within this scene. Research in the past 2 decades mainly focused on Rab3A and Rab27A and revealed both as key regulators of late stages of exocytosis. Recent studies proposed that another member of the Rab family, Rab3B, has a pivotal function in coordination of beta cell insulin secretion. Therefore the aim of this study was to explore the role of Rab3B in beta cells.
Materials and methods: Mouse islets, murine tissues and MIN6 beta cells were analyzed for Rab3 expression by RT-PCR, Western Blot and immunofluorescence analyses. The influence of different culture conditions on Rab3 expression in MIN6 cells and isolated islets was followed by RT-PCR. For overexpression of Rab3A and Rab3B, cells were transiently transfected. Down regulation of Rab proteins was evoked by lentiviral shRNA transduction. Glucose-induced insulin secretion was measured by ELISA.
Results: Expression of both, Rab3A and Rab3B was detected in mouse islets, brain, liver and pancreas as well as in MIN6 beta cells. RT-PCR analysis showed that the two other isoforms Rab3C and Rab3D were only expressed at a low level in beta cells. But equal amounts of Rab3A and Rab3B were detectable in isolated islets, indicating the pivotal role of Rab3B in endocrine tissue. Immunofluorescence analyses of Rab3A and Rab3B in MIN6 cells and in primary beta cells revealed a vesicle-like distribution with, surprisingly, only little colocalization with each other. Rab3A displayed a higher degree of colocalization with insulin granules than Rab3B. Endogenous Rab3B protein expression was additionally detected in glucagon containing alpha cells. Investigating the effects of glucose and palmitic acid on Rab3 gene expression we observed a decrease in Rab3A and B expression in MIN6 cells but a significant increase in the expression levels of both isoforms in islets. To explore the functional impact of Rab3B on insulin excocytosis glucose-induced insulin secretion was studied. Neither Rab3A nor Rab3B overexpression in MIN6 beta cells modified insulin secretion. In contrast, knocking down Rab3A or Rab3B protein expression in MIN6 cells resulted in a significant reduction of glucose-induced insulin secretion. This result was also obtained after downregulation of Rab3A in mouse islets.
Conclusion: Besides the well known Rab3A we could determine Rab3B as an additional crucial factor of the insulin exocytosis machinery: 1) Rab3B is expressed in primary murine beta cells; 2) expression is altered upon exposure to high glucose and palmitic acid and 3) reduced protein expression impaired insulin secretion. Thus, both Rab3B and Rab3A are essential factors in beta cell function and a balanced expression might be a prerequisite to prevent the development of type 2 diabetes mellitus.
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Knock-down of the alpha subunit (PDHA1) of pyruvate dehydrogenase E1 in insulin-secreting cells reduces glucose-induced respiration
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Background and aims: It has been reported that tight coupling between cytosolic and mitochondrial metabolism increases glucose-stimulated insulin secretion (GSIS). In β-cells, metabolism of pyruvate is critical for metabolic coupling, and hence the mitochondrial pyruvate dehydrogenase complex (PDC) may play an important role in β-cells in control of GSIS and regulation of blood glucose levels. We have previously reported that GSIS is regulated by miR-130a/b and miR-152 via PDHA1, which is a component of PDC enzyme complex. We showed that PDHA1 knock-down by siRNA reduced GSIS by 40%. Here, we aim to investigate the bioenergetics underlying the actions of PDHA1 in insulin-secreting cells.
Materials and methods: We silenced PDHA1 by siRNA in INS-1 832/13 cells .We measured the knock-down efficiency at both mRNA and protein levels by RT-qPCR and western blot, respectively. Basal respiration, glucose-induced respiration, non-mitochondrial respiration, maximal mitochondrial respiration capacity and maximal respiration were measured by the Seahorse Extracellular Flux Analyzer, XF24.
Results: We observed an approximately 70% knock-down of PDHA1 at both mRNA and protein levels ($n=3$; $p<0.01$). Glucose-induced respiration decreased by ~25% ($n=3$, $p<0.05$), non-mitochondrial respiration decreased by ~20% ($n=3$, $p<0.05$) and maximal respiration reduced by ~15% ($n=3$; $p<0.01$) in insulin-secreting cells upon PDHA1 knock-down. We found no significant difference in the proton leak, but there was a trend of decreased basal respiration and a decreased maximal mitochondrial respiration capacity.
Conclusion: PDHA1, a component of pyruvate dehydrogenase complex potentially involved in metabolic coupling in insulin-secreting beta cells, may contribute to beta cell dysfunction.
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PS 041 Liver metabolism: in vivo studies

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Larger glucagon response to OGTT compared to isoglycaemic i.v. glucose infusion translates into increased endogenous glucose production in patients with type 2 diabetes

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Background and aims: OGTT induces less suppression of glucagon than isoglycaemic i.v. glucose infusion (IIGI) in healthy subjects and this difference is exaggerated in patients with type 2 diabetes (T2D). The impact of this difference on plasma glucose levels is not clear. We evaluated glucose clearance and endogenous glucose production (EGP) during OGTT and IIGIs with and without simulated lack of glucagon suppression in patients with T2D and healthy control subjects (CTRLs).
Materials and methods: Ten patients with T2D (age: 57±2 years [mean ±SEM]; BMI: 29.0±1.4 kg/m2; HbA1c: 53.8±3.5 mmol/mol) and 10 matched CTRLs (age: 56±3 years; BMI: 29.8±0.9 kg/m2; HbA1c: 33.8±1.7 mmol/mol) underwent three investigations, day A: 75 g-OGTT labelled with [U-13C]glucose, day B: [6,6D-2]-labelled IIGI corresponding to day A, and day C: IIGI as day B with glucagon infusion (0.8 ng/kg/min from 0-25 min).
Results: Isoglycaemia was obtained during all three study days in both groups. As expected, larger glucagon responses were observed in the initial phase of the OGTT (0-90 min) compared to the IIGI in both groups (Table 1). The IIGI with concomitant glucagon infusion resulted in similar glucagon AUCs (0-90 min) compared to the T2D OGTT response (Table 1). In both groups more glucose was infused during day B compared to day C ($P=0.01$ for healthy and $P=0.14$ for T2D; Table 1). In the T2D group, EGP was higher during day A compared to day B and C, whereas no difference in EGP between days were seen in the CTRL group. Peripheral glucose disappearance was higher during day A compared to day B and C among CTRLs, but similar on all days in the T2D group.
Conclusion: These findings suggest that glucagon release arising from glucose stimulation of the gastrointestinal tract may stimulate EGP and that exaggerated glucagon release during OGTT in patients with T2D may contribute to reduced glucose tolerance characterising these patients.
Table 1. Glucose and glucagon following OGTT and isoglycaemic i.v. glucose infusions (IIGIs) in patients with type 2 diabetes (T2D) and healthy control subjects (CTRL). Data are mean±SEM values. P values are from paired sample t test or repeated-measurement ANOVA. Significant differences ($P\leq0.05$) between days (derived from post hoc analysis) are indicated by asterisks (*) followed by the letter of the day compared with.

Glucose and glucagon

	CTRL				T2D			
	OGTT (Day A)	IIGI (Day B)	IIGI + glucagon (Day C)	*P*	OGTT (Day A)	IIGI (Day B)	IIGI + glucagon (Day C)	*P*
Glucose								
OGTT (g)	75.0±0.0				75.0±0.0			
Absorption (%)	71.6±1.4				72.1±1.4			
Appearance 4h								
OGTT (g)	53.6±1.1				54.0±1.1			
IIGI (g)		38.4±2.5	33.2±2.7	0.01		62.9±4.2	59.5±4.0	0.14
Endogenous (g)	24.2±1.9	21.9±2.4	25.4±2.2	0.09	32.6±1.7^{*B,S}	20.6±1.5^{*A}	23.1±2.0^{*A}	0.01
Disappearance 4h								
Urine (g)	0.0±0.0	0.0±0.0	0.0±0.0	0.17	5.0±1.4	3.8±1.2	3.3±0.9	0.09
Peripheral (g)	78.9±1.7^{*BS}	58.5±2.2^{*A}	58.2±2.0^{*A}	<0.0001	79.7±1.7	71.7±3.2	74.3±2.7	0.06
Glucagon								
AUC, pmol/l × 90 min	551.0±72.2^{*BS}	447.7±69.0^{*AS}	780.3±109.0^{*A,B}	0.001	946.2±101.0^{*B}	739.8±57.4^{*AS}	1,062.7±89.2^{*B}	0.004

Clinical Trial Registration Number: NCT02010827

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Identification of secreted factors linking hepatic steatosis to insulin resistance

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Background and aims: Non-alcoholic fatty liver disease (NAFLD) is a common health problem that is associated with obesity. Liver steatosis is an early manifestation in the aetiology of NAFLD, which usually precedes the development type 2 diabetes. We tested the hypothesis that protein and lipid signals originating from the fatty hepatocyte induce 'cross-talk' with other tissues to modulate metabolic phenotypes.
Materials and methods: Male C57Bl/6J mice were fed a chow diet (Chow) or high-fat diet (HFD) for 6-8 weeks to induce simple steatosis. Hepatocytes were isolated and cultured, and the secreted products were collected in protein and lipid free media for analysis (=conditioned media, CM).
Results: HFD CM but not Chow CM induced insulin resistance and inflammation in cultured cells. Quantitative analysis of the CM detected 17 lipid classes and >200 lipid species, and showed increased secretion of 11 lipid classes in HFD CM compared with Chow CM (including cholesterol esters, ceramides, diacylglycerols, phosphatidylcholine and triacylglycerol). Isobaric tag for relative and absolute (iTRAQ) labelling and mass spectrometry quantification of the CM detected 538 proteins. Of these proteins, 115 were identified as 'classically secreted', 31 of which were upregulated, and three that were downregulated in HFD CM. Fetuin B was increased in the CM of HFD hepatocytes and subsequent studies showed that fetuin B was elevated in patients with liver steatosis compared with weight-matched patients without steatosis, and fetuin B correlated with insulin resistance. Further studies in lean mice showed that acute injection of fetuin B decreased glucose tolerance, whereas adeno-associated virus administration of fetuin B shRNA in obese mice reduced plasma fetuin B by 33% and improved glucose tolerance.
Conclusion: Hepatic steatosis invokes changes in the protein and lipid secretory profile that in turn causes inflammation and insulin resistance, and targeted profiling identified fetuin B as a steatosis responsive protein that was shown to cause glucose intolerance. Further interrogation of the secreted proteins may be a useful strategy for understanding the development of impaired glucose metabolism.
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Metabolic assessment of cirrhotic patients before liver transplantation: relation between impaired glucose homeostasis, liver function and HCC

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Background and aims: Impaired glucose tolerance (IGT) and Diabetes Mellitus (DM) are common in patients with liver cirrhosis. DM development has been associated to advanced liver disease (LD) and HCV infection, but the relative roles played by these factors are still somewhat unclear. Moreover, it has been demonstrated that Insulin Resistance (IR), is related to increased risk of developing Hepatocellular Carcinoma (HCC), the first cause of death in patients with cirrhosis. Aim of the study is to assess, in cirrhotic patients candidate to organ transplantation with no

history of glucose abnormalities: a) prevalence of altered glucose homeostasis and its relation to liver function; b) IR and its relationship with HCC.

Materials and methods: 204 patients, aged 54.3±8.4, with liver cirrhosis and candidate to organ transplantation underwent an anthropometric and metabolic evaluation and an OGTT. IR was assessed with HOMA-IR (a cut off of 3 was utilized to identify insulin-resistant patients) and OGIS.

Results: 35 patients had normal glucose tolerance (NGT), 68 had IGT, 101 had DM. Diabetic patients were older than patients showing IGT or NGT (age 56.2±6.9 vs 53.3±8.4 vs 50.9±11.1, respectively, P<0.01). Glucose tolerance worsened with worsening of liver function: DM was diagnosed in 24.6% of patients in stage A and 65.4% of those in stage B or C, according to Child Pugh classification. Moreover, diabetes was related to lower levels of total cholesterol (128.3±44.9 vs 132.2±45.1 vs 160.0±73.5, P<0.01), LDL-cholesterol (65.1±35.1 vs 68.9±35.5 vs 96.2±65.4, P<0.01), albumin (3.4±0.5 vs 3.5±0.6 vs 3.7±0.5, P=0.01), pseudocholinesterase (2683.2±1673.2 vs 3720.3±2472.9 vs 3534.5±2169.8, P<0.01) and platelets count (73.1±35.9 vs 102.8±67.9 vs 124.8±105.3, P<0.01) if compared to patients with IGT and NGT, showing worse hepatic synthetic capacity and liver function in patients with altered glucose homeostasis. HOMA-IR steadily increased with declining of glucose tolerance (2.85±0.34 vs 4.66±0.59 vs 5.9±0.70 from NGT to DM, P<0.05), and it was higher in presence of HCV: 68% of HCV+ patients showed IR vs 44.8% of HCV- patients (P<0.01). OGIS level decreased from NGT to IGT to DM (425.5±78.0 vs 372.5±82.2 vs 352.9±80.2, P<0.01). Finally, prevalence of HCC was significantly higher in patients with IR vs cirrhotic patients without IR (45.1% vs 27.9%, P<0.01).

Conclusion: In cirrhotic patients, DM is related to the severity of LD. HCV infection might play a role in deteriorating glucose homeostasis via IR. Moreover HOMA, a reliable index to identify insulin-resistant patients, allows to characterize a class of patients at highest risk of developing HCC.

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Selenoprotein-P as marker of insulin sensitivity in prediabetes and diabetes

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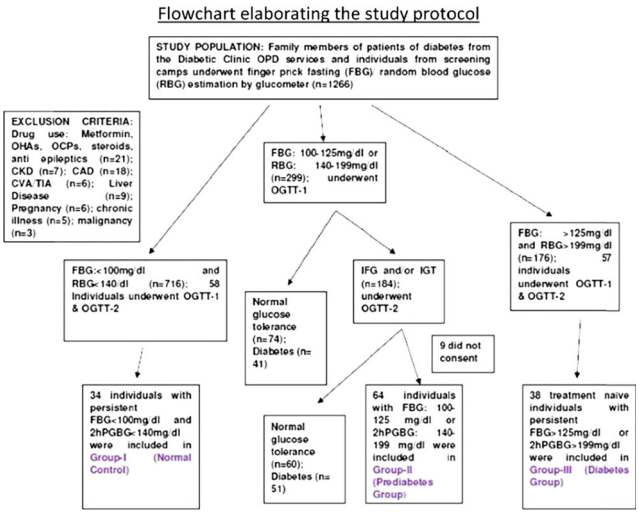
Background and aims: Initial reports have suggested selenprotein-P (SeP), a novel hepatokine, to modulate insulin resistance (IR) and energy metabolism in animals and humans. This study evaluated the relation between SeP, anthropometric parameters, IR, systemic inflammation and vitamin-D across the spectrum of glycemia, viz. normoglycemia (NGT), prediabetes (preDM) and newly diagnosed treatment naïve type-2 diabetes (T2DM) patients.

Materials and methods: Anthropometric parameters (waist circumference, hip circumference, neck circumference (NC)], Serum selenoprotein, fasting blood glucose (FBG), 2 h post 75gram glucose blood glucose (2hPGBG), HbA1c, fasting insulin, lipids, inflammatory cytokines [interleukin (IL)-6, IL1β, soluble tumor necrosis factor receptor (sTNFR)-1, sTNFR2] and 25-hydroxy-vitamin-D (25OHD) were evaluated in 34 NGT, 64 preDM and 38 T2DM patients, selected from the eastern India vitamin-D in prediabetes study. Serum insulin was estimated using CLIA (Immunite-1000, Gwynedd, UK). Human SeP was estimated using sandwich ELISA, (Cat No. MBS2515309, MyBioSource, USA), detection range 0.156-10 ng/ml, sensitivity 0.094 ng/ml with intra and interassay coefficient of variation of <10%. 25OHD was measured using 125I radioimmunoassay kit (REF 68100E, Diasorin, USA). IL6, IL1b, sTNFR1 and sTNFR2 were estimated using sELISA. Insulin sensitivity was estimated using QUICKI (Quantitative Insulin Sensitivity Check Index).

Results: NGT (age: 50.29±13.2 years), prediabetes (age: 46.9±12.3 years) and T2DM (age: 49.73±13.4 years) had significantly different BMI (24.7±2.75, 24.6±4.28, 27.29±5.08 kg/m2, P=0.035), waist-height ratio(0.55±0.04, 0.55±0.06, 0.59±0.07, P=0.009), NC (34.72±3.19, 35.33±2.9, 37.3±2.68 cm, P=0.003),triglycerides (126.7±60.1, 115.9±63, 200.9±62.6 mg/dl, P=0.031), IL6 (4.93±0.44, 4.74±2.65, 5.7±1.6 pg/ml, P=0.08), sTNFR1 (2863.77±1106.72, 3371.89±1102.4, 3830.41±1529.65 pg/ml, P=0.021), 25OHD (30.50±18.37, 31.95±23.25, 42.07±20.23 ng/ml, P=0.075) and SeP (848.35±220.21, 1002.93±279.50, 826±240.59 ng/ml, P=0.011). SeP had very strong inverse correlation with insulin sensitivity (QUICKI) in NGT (r=-0.94, P<0.001), prediabetes (r=-0.92, P<0.001) and diabetes (r=-0.96, P<0.001). SeP was persistently the strongest predictor of QUICKI in NGT, prediabetes and T2DM at baseline (β=-1.124, P<0.001; β=-1.003, P<0.001; β=-0.957, P<0.001, respectively) and after adjusting for age, BMI and waist circumference (Model-1) (β=-1.11, P<0.001; β=-1.006; P<0.001; β=-0.942, P<0.001, respectively).

Conclusion: SeP is elevated across the spectrum of dysglycemia with lowest in normoglycemia. SeP is a very good predictor of insulin sensitivity and may be considered as a biomarker of insulin sensitivity.



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Phosphorylated serum IGFBP-1 as a non-invasive predictor of liver fat in NAFLD

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Background and aims: Non-alcoholic fatty liver disease (NAFLD) is currently the most common chronic liver disease and it strongly associates with metabolic syndrome, insulin resistance, and type 2 diabetes. There is a need to develop non-invasive tools for diagnosis of NAFLD. Insulin is the major regulator of the hepatospecific insulin-like growth factor binding protein 1 (IGFBP-1). The majority of serum IGFBP-1 is phosphorylated. IGFBP-1 inversely correlates with liver fat content. Of all subjects 30-50% carry an I148M variant allele in PNPLA3, which increases the risk of NAFLD but not insulin resistance. The aim of the study is to determine whether measurement of fasting serum

phosphorylated IGFBP-1 (fS-pIGFBP-1) helps in prediction of liver fat compared to routinely available clinical parameters and PNPLA3 genotype at rs739409.

Materials and methods: A cross-sectional study comprised 378 subjects (62% women, age 43 (30-54) years, BMI 32.7 (28.1-39.7) kg/m2, 46% with NAFLD) of whom liver fat content was determined by proton magnetic resonance spectroscopy (1H-MRS). fS-pIGFBP-1 was determined with an immunoenzymometric assay with an in-house monoclonal antibody as the detecting antibody. Subjects were randomly divided into discovery (2/3) and validation (1/3) groups that matched with respect to clinical and biochemical parameters and PNPLA3 genotype. Univariate analysis was used to evaluate correlations between liver fat content and measured parameters. To create a model for prediction of liver fat, variables identified by univariate analysis in the discovery group were selected for multiple linear regression analysis using backward stepwise regression based on Akaike Information Criteria. Random Forest modeling was used to evaluate relationships between liver fat predictors.

Results: fS-pIGFBP-1 inversely correlated with liver fat content (r=-0.21, p=0.0009). The final model, the '% Liver Fat Equation', included age, fS-pIGFBP-1, an interaction term (age times fS-pIGFBP-1), S-ALT, waist-to-hip ratio, fP-Glucose and fS-Insulin, adjusted R2=0.44, p< 0.0001 (adjusted R2=0.49 in the validation group and 0.47 in all subjects) (Table 1). The model was significantly better than S-ALT (adjusted R2= 0.25) or S-AST (adjusted R2=0.15) (p<0.0001). Exclusion of fS-pIGFBP-1 significantly reduced the variation explained (p=0.03). Random Forest modeling identified the same predictors (adjusted R2=0.39, p <0.0001). Correlation coefficient between measured liver fat% (1H-MRS) and estimated liver fat% with '%Liver fat equation' was r=0.62, p<0.0001.

Conclusion: fS-pIGFBP-1 is significantly inversely correlates with liver fat content in NAFLD and independently contributes to prediction of liver fat even when its known associates are considered.

Table 1. Multiple linear regression analysis

Liver fat (*log*, %) $R^2 = 0.44$ [a], $P < 0.0001$	Beta	Standard error	*P*-value
Age (*years, log*)	-2.707	1.376	0.05
fS-pIGFBP-1 (*µg/l, log*)	-2.635	1.177	0.026
fS-pIGFBP-1 x Age (*log*)	1.644	0.735	0.026
S-ALT (*U/l, log*)	0.571	0.127	<0.0001
Waist-to-hip ratio *(log)*	2.813	0.957	0.0037
fP-Glucose (*mmol/l, log*)	1.064	0.350	0.0027
fS-Insulin (*mU/l, log*)	0.393	0.125	0.0019
Constant	0.960	0.735	<0.0001

ALT, alanine aminotransferase; fS, fasting serum; fP, fasting plasma; pIGFBP-1, phosphorylated insulin-like growth factor binding protein-1; S, serum. Beta, coefficient.

log variables used after logarithmic transformation

[a] Adjusted

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Metabolomics profiling identifies potential pathways involved in the interaction of iron homeostasis with insulin resistance

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Background and aims: Elevated serum ferritin has been linked to type 2 diabetes and adverse outcomes in subjects with the Metabolic Syndrome (MetS). As the mechanisms underlying the negative impact of excess iron have so far remained elusive, we aimed to identify potential metabolic pathways using a metabolomics analysis.

Materials and methods: Metabolomics profiling was performed in patients with MetS with (n=56) and without iron overload (n=54) and a lean, healthy control group (n=53) in a targeted metabolomics approach utilizing the AbsoluteIDQ™ p180 kit (BIOCRATES Life Sciences AG).

Results: Clinically, subjects with MetS and high ferritin had higher indices of impaired glucose homeostasis as assessed by fasting glucose and HOMA-IR compared to subjects matched for components of the MetS without iron overload. Several differences between MetS and healthy controls confirmed previous studies, i.e. branched-chain amino acids, kynurenin, and alpha AA. However, significant differences between MetS with high and low ferritin were detected in the serum concentrations of sarcosine and methionine, citrulline and particularly long-chain phosphatidylcholines.

Conclusion: Our data confirm that high serum ferritin concentrations are linked to impaired glucose homeostasis. Additionally, our study identifies novel associations of iron excess in MetS subjects with distinct subsets of phosphatidylcholines as well as a pathway involving methionine, sarcosine and citrulline. These metabolic pathways may be involved in iron-induced augmentation of IR.

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Gender specific association of serum leptin and insulinaemic indices with nonalcoholic fatty liver disease in prediabetic subjects

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Background and aims: Adipose tissue-derived hormone leptin play a functional role in glucose tolerance through its effects on insulin secretion and insulin sensitivity which also the risk factors for nonalcoholic fatty liver disease (NAFLD). The present study explored the gender specific association of serum leptin with insulinemic indices in Bangladeshi prediabetic subjects having NAFLD.

Materials and methods: Under a cross-sectional analytical design a total of 110 prediabetic subjects diagnosed by WHO Group Study criteria, aged 35-68 years consisting of 57.3% male (55.6% non NAFLD and 44.4% NAFLD) and 42.7% female (57.4% non NAFLD and 42.6% NAFLD), were investigated. NAFLD was diagnosed by liver ultrasound to assess serum (S) glucose by glucose-oxidase method, S lipid profile by enzyme colorimetric method and glycated hemoglobin (HbA_{1c}) by high performance liquid chromatography (HPLC) method. S insulin and and leptin were measured by enzyme-linked immunosorbent assay (ELISA). Insulin secretory function (HOMA%B) and insulin sensitivity (HOMA%S) were calculated from homeostasis model assessment (HOMA).

Results: On Pearson's correlation analysis, S leptin showed significant positive correlation with fasting insulin (r=0.530, p=0.004), postprandial insulin (r=0.384, p=0.042) and HOMA-IR (r=0.541, p=0.003) as well as significant negative correlation with HOMA%S (r=-0.388, p=0.046)

and HOMA%B (r=-0.356, p=0.039) in male prediabetic subjects having NAFLD. On multiple regression analysis, S leptin showed significant positive association with HOMA-IR (β=0.706, p<0.001) after adjusting the effects of major confounders of body mass index (BMI), triglyceride (TG) and HOMA%B in male subjects having NAFLD. On binary logistic regression analysis, only S leptin [odds ratio (OR)=1.296] in male subjects as well as HOMA%B (OR=0.942), HOMA-IR (OR=3.305) and leptin (OR=1.106) in female subjects were found to be independent determinants of NAFLD after adjusting the effects of potential confounders of BMI and TG.

Conclusion: Elevated levels of serum leptin seems to have an association with NAFLD both in male and female prediabetic subjects and this association in turn, is mediated by insulin secretory dysfunction and insulin resistance among these subjects.

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Insulin pulse amplitude and frequency do not determine rates of endogenous glucose production in nondiabetic subjects

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Background and aims: Diabetes, obesity and aging cause changes in insulin pulse amplitude and frequency, which affects the pulsatile secretion of insulin into the portal circulation. Insulin concentrations in the hepatic sinusoids in turn affect glucose production. In dogs, partial pancreatectomy decreases inter-digestive insulin pulsatility and results in decreased hepatic insulin action. Whether this mechanism is extant in humans remain unknown.

Materials and methods: 30 non diabetic subjects (Age 43±2 years, BMI 29.0±0.8 Kg/M2) were studied on 2 occasions. Endogenous glucose production (EGP) was measured on one occasion using an intravenous infusion of [3-H3] glucose after an overnight fast prior to a euglycemic clamp. On a separate study day a hepatic vein catheter was placed to allow sampling of hepatic venous insulin concentrations at 2 minute intervals. Samples were obtained during fasting conditions and after peripheral glucose concentrations were raised to 8.5 mmol/L. Indocyanine green was used to measure splanchnic blood flow.

Results: Under fasting conditions, Peak and Nadir insulin concentrations, (53±9 and 21±4, respectively) and mean pulse amplitude and pulse frequency (30±6pmol/L and 11±1 pulses/hr respectively) were measured. The relationship of insulin pulse frequency and amplitude to fasting EGP (14.3±0.6 μmol/kg/min) was also examined. Under fasting basal conditions EGP did not correlate with peak (R2<0.01, p=0.80), and Nadir (R2=0.01, p=0.72) insulin, pulse frequency (R2=0.08, p=0.30) or pulse amplitude (R2=0.04, p=0.52). The mean rates of insulin appearance calculated by multiplying mean insulin concentrations in the hepatic vein (35±6 pmol/L) with splanchnic blood flow (1439±95 ml/min) however correlated inversely with EGP (R2=0.36, p=0.03).

Conclusion: The current experiment demonstrates that in in nondiabetic individuals, it is mean insulin concentrations that determine EGP during fasting, rather than pulse frequency or pulse amplitude.

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PS 042 Type 1 diabetes: new insights into therapy

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Presence of remission after onset of type 1 diabetes predicts higher insulin sensitivity at 7 years

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Background and aims: After onset of type 1 diabetes some patients experience remission of the disease. This phenomenon is associated with regeneration of beta cells and preserved insulin secretion, but also with increased insulin sensitivity. It was not evaluated whether presence of remission may be of value in predicting the degree of insulin sensitivity in the later course of disease. Aim of the study was to determine the association between presence or absence of remission after onset of type 1 diabetes and insulin resistance after 7 years from diagnosis of disease.

Materials and methods: We followed prospectively 240 consecutive adult patients (77 women and 143 men) hospitalized with new onset type 1 diabetes (NOT1DM) in the Department of Internal Medicine and Diabetology in the years 2004-2007. The presented analysis is a part of POZREM Study. Inclusion criteria were: new diagnosis of autoimmune type 1 diabetes (specific autoantibody-positive), age 18-35 years and informed consent to participate in the study. Adult patients with NOT1DM from the catchment area (the majority of Great Poland region) are referred to our department on a routine basis, independently from the severity of presentation. In the follow-up time of onset and duration of diabetes remission were registered. Presence of remission was determined in the third month after diagnosis of diabetes. Remission was defined as the time when the patients met all of the the following criteria: HbA1c< 6.5%, daily dose of exogenous insulin <0.3 U/kg body weight and serum C-peptide concentration >0.5 ng/ml. Patients were divided into groups with and without remission. Insulin sensitivity was assessed using normoglycemic-hyperinsulinemic clamp with measurement of glucose disposal rate (GDR [mg/kg/min]). The test was performed after 7 years from diagnosis of diabetes. We excluded seven patients still fulfilling remission criteria.

Finally 74 patients [24 women and 50 men, median age at diagnosis 26 years (IQR 22-31)], in whom insulin sensitivity at 7 years was measured, were included into analysis. Patients were divided into groups with GDR<4 (lower insulin sensitivity) and GDR≥4 (higher insulin sensitivity). In the statistical analysis Mann-Whiney U test, Spearman correlation and logistic regression were used. The calculations were performed using Statistica 10.

Results: In the study group GDR value <4 mg/kg/min was observed in 23 (31%) patients. Comparison of GDR between groups with and without remission showed higher insulin sensitivity in the remission group [GDR 6.2 (IQR 4.2-7.0) vs 3.8 (IQR 3.0-4.8); p=0.01]. Furthermore, in the group with GDR≥4 duration of remission was longer than in the group with lower insulin sensitivity: [351 days (IQR 206-561) vs 70 days (0-289); p=0.002]. Duration of remission correlated positively with GDR value (Rs 0.42; p=0.002). In multivariate logistic regression analysis presence of remission was associated with higher odds of GDR≥4 at 7 years (OR 4.14; 95%CI: 1.08-15.88; p=0.03), independently from age, sex and BMI at 7 years.

Conclusion: Patients with type 1 diabetes who entered remission, despite its completion, have higher insulin sensitivity at seven years after diagnosis than nonremitters.

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Inverse association of HbA_{1c} with faecal elastase 1 in people without diabetes

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Background and aims: Faecal elastase 1 (FE1) concentrations, a marker of pancreatic exocrine function, are lower in type 2 diabetes than in non-diabetic controls. FE1 is also inversely correlated with glycated hemoglobin and diabetes duration in type 2 diabetes. The association of FE1 and HbA1c has not been investigated in people without diabetes.
Materials and methods: Patients with type 2 diabetes (oral antidiabetic drugs or insulin: n=391; medically untreated: n=145) and matched (age, sex, practice) controls without diabetes (n=529) from general practices were included. FE1 measurements (μg/g stool) were performed centrally. Linear mixed regression models were fitted using FE1 as dependent variable and HbA1c, diabetes diagnosis (no, yes-untreated, yes-treated) and interactions as independent variables. Potential confounders were age, sex, body mass index, alcohol consumption, smoking, triglycerides, and amylase. Univariate regression models were stratified by diabetes groups, multivariate models were fitted on the whole study population.
Results: In univariate linear regression models, HbA1c was significantly inversely related to FE1 in non-diabetic controls (ß-coefficient: -108.74, p <0.0001), whereas no significant associations were found for the diabetes groups. The inverse relationship of HbA1c with FE1 concentrations in patients without diabetes persisted after adjusting for potential confounders in multivariate regression (ß-coefficient of HbA1c: -109.18, p <0.0001). In people without diabetes, there were lower FE1 concentrations among those with increased diabetes risk (HbA1c 5.7%-6.4% [39-46 mmol/mol]: 395±204 μg/g vs. HbA1c ≤5.6% [≤38 mmol/mol]: 476± 219 μg/g (p<0.0001). The prevalence of FE1<100 μg/g was significantly increased among non-diabetic controls with an HbA1c of 5.7%-6.4% (39-46 mmol/mol) compared with those with a normal HbA1c ≤5.6% (≤38 mmol/mol) (6.1% vs. 1.4%; p=0.004).
Conclusion: The present study suggests that pancreatic exocrine dysfunction might be an early disturbance in prediabetes that develops in parallel with hyperglycemia. Whether it is a consequence or a determinant of hyperglycaemia requires further investigation.
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A parsimonious model of a mixed-meal test in patients with type 1 diabetes in insulin pump therapy

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Background and aims: Currently available closed-loop insulin delivery systems stem from sophisticated models of the glucose-insulin (G/I) system mostly based on complex studies employing glucose tracer technology. We asked the question whether simpler studies based on the minimal modeling approach (Bergman, 1981; Cobelli, 2007) could likewise reliably describe the G/I system during a mixed meal test (MMT) in patients with type 1 diabetes (T1D). If effective, this approach would expedite the creation of large bio-banks of virtual patients to develop robust models "to close the loop". To this end, we tested the performance of a minimal model of the G/I system to characterize the glucose (G) and insulin (I) dynamics during MMT in T1D patients on insulin pump therapy (CSII).
Materials and methods: In six T1D patients on CSII enrolled in the MMT-T1D Pilot Study we assessed on three separate days: (1) insulin sensitivity, by the hyperinsulinemic euglycemic clamp (HEC); (2) the G/I time-courses during a standardized 5 h-MMT (MMT1: 292 Kcal; 38.9 g complex CHO, 8.9 g lipids, 14 g proteins); (3) the G/I time-courses during a second identical (3 patients) or double-sized (3 patients) MMT (MMT2). The parameters estimated by modeling of the HEC were used to cast a comprehensive MMT model (GLUKINSLOOP.2), including the insulin delivery system and the metabolic G/I system of each patient. GLUKINSLOOP.2 was implemented and run in the SAAM 2.1 software.
Results: The GLUKINSLOOP.2 model identified the G/I system parameters (among others: insulin sensitivity, SI; glucose effectiveness, SG; glucose distribution volume, Vd; time of oral carbohydrate appearance in the peripheral circulation, expressed as ICMTT, Intestinal Carbohydrate Mean Transit Time) and provided a good fit of the G/I time-courses in all studies, as proved by the analysis of mean weighted residuals (WR, mean ± SD; MMT1: WR(G)=-0.03±0.71; WR(I)=0.33± 1.04; MMT2: WR(G)=0.09±0.86; WR(I)=0.01±1.37). Both the identical and double-sized repeated MMT2s showed good reproducibility of the G/I system parameters (mean±SEM; MMT1: SI=0.57±0.12 (mL˙min-1)/(pmol˙L-1); SG=15.6±10.1 mL˙min-1; Vd=11,482±1, 131 mL; ICMTT=105±14 min; MMT2: SI=0.57±0.13; SG=38.2± 13.6; Vd=11,609±1,031; ICMTT=101±11).
Conclusion: The GLUKINSLOOP.2 model herein presented can provide a fairly good and reproducible description of the G/I system in T1D patients on CSII and it may be used to build a bank of "virtual patients". Our results might be relevant to strategies directed to improve the architecture of upcoming closed-loop CSII systems.
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Partial recovery of insulin secretion in type 1 diabetes?

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Background and aims: Even long-term Type 1 diabetes (T1D) can be associated with low level insulin production. Residual C-peptide may reduce risk of vascular complications and hypoglycaemia.
Materials and methods: Plasma C-peptide levels were measured in a cross-sectional study of 249 healthy controls (CON) and 365 T1D patients (F/M=198/167), aged 10-80 y, (mean ± SD) 30±16 y, with T1D duration of 16±12 y. Of the T1D patients 129 had microvascular complications (CX+). HbA1c was 8.4±1.6% and 5.1±0.4% in T1D and CON respectively (p=0.00001). C-peptide levels were measured by ultrasensitive ELISA (Mercodia, Sweden), with a low detection limit of 1.25 pmol/L (0.0038 ng/mL). C-peptide levels below OD of the lowest calibrator were expressed as ¼ of the concentration of the assay's lowest calibrator as per manufacturer's instructions (Technical Note 34-0144).
Results: C-peptide in CON and T1D were 547.1±1.2 pmol/L and 3.6± 1.2 pmol/L respectively; p=0.0001. Undetectable C-peptide level frequency did not differ between CX+ and CX- groups. For T1D subjects with detectable levels C-peptide levels were lower in those with vs. without complications: 2.7±0.9 pmol/L and 4.4±0.6 pmol/L respectively, p= 0.04. Subjects were divided into cohorts by age of T1D diagnosis (≤10, 10 to ≤20 and >20 y) and T1D duration (≤10, 10 to ≤20 and >20 y). C-peptide levels are shown in the table. Young age diagnosis (≤10 y.o.) was associated with higher rates of undetectable C-peptide, p=0.0001 vs.

combined age T1D diagnosis 10 to ≤20y.o. and >20y.o. For T1D diagnosed in early childhood C-peptide levels are higher in those with longer T1D duration, whilst for those diagnosed >20 y longer T1D duration is associated with lower C-peptide.

Conclusion: Although cross-sectional, our results are supportive of more complete beta cell loss in younger onset T1D, and potential beta cell regeneration. Residual C-peptide levels are higher in T1D subjects without vs with complications. Longitudinal studies are merited.

C-peptide levels (geometrical mean±SEM, age and gender adjusted)

Age T1D diagnosis (yrs)	n	T1D duration (yrs)	% with undetectable C-peptide	C-peptide (pmol/L)
≤10	62	≤10	82	2.2±1.2
≤10	61	10 to ≤20	67	3.5±1.2
≤10	34	>20	56	5.1±1.2 ‡
10 to ≤20	50	≤10	44	2.5±1.2
10 to ≤20	26	10 to ≤20	23	5.2±1.2 ‡
10 to ≤20	38	>20	21	3.1±1.2
>20	39	≤10	10	7.2±1.2
>20	17	10 to ≤20	29	2.2±1.2 ‡
>20	26	>20	31	2.5±1.2 ‡
‡ p<0.05 vs. duration ≤10 in respective age of diagnosis group.				

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Endogenous C-peptide secretion in patients with long-duration type 1 diabetes

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Background and aims: Classically, Type 1 diabetes is thought to proceed to absolute insulin deficiency; however, recent reports suggest that some Type 1 patients secrete low levels of endogenous insulin. It is thought that even small amounts of residual β-cell function may result in fewer complications including lower rates of hypoglycaemia and lower incidences of retinopathy and nephropathy. We aimed to assess amounts of detectable C-peptide in the Golden Years cohort who have had Type 1 diabetes for over 50 years, and to assess whether this had a bearing on the presence/absence of complications.

Materials and methods: Random serum C-peptide was measured in 334 samples from the Golden Years cohort, using a commercially available immunometric C-peptide assay. Subjects were divided into 'no secretor' and 'secretor' groups based on the detection limit of the C-peptide assay (5.0 pmol/L).

Results: A substantial proportion (15.9%) of the Golden Years patients continued to secrete detectable levels of C-peptide (5.1 - 245.2 pmol/L) despite having been diagnosed with Type 1 diabetes for ≥50 years. Both 'no secretor' and 'secretor' groups were well matched, with no significant difference in age, age at diagnosis, duration of diabetes, BMI, HbA1c or lipid levels (Table 1). There was however, a significant difference in insulin dose ('no secretor' vs 'secretor': 34.4 v 26.7 U/24 hr; p=0.002) between the groups, which appeared to be clinically significant. With respect to complications, there was no difference in UACR, or prevalence of diabetic retinopathy, IHD or hypertension.

Conclusion: In common with recent reports, our results confirm that some level of endogenous C-peptide production is evident in Type 1 diabetes and this persists for decades after disease onset, allowing a possible route for therapeutic intervention to preserve β-cell function, even in a population of patients with long-duration diabetes, such as the Golden Years cohort. However, the presence of detectable C-peptide did not appear to confer protection against diabetes related complications such as retinopathy, IHD or nephropathy.

Table 1: Clinical and biochemical measurements for Golden Years C-peptide samples

Measurement	'No secretor'	'Secretor'	P
n	281	53	
Age (yrs)	69.0 (9.0)	69.2 (9.5)	0.879
Males % (n)	54.8 (154)	43.4 (23)	0.127
BMI (kg/m^2)	24.8 (3.7)	24.4 (2.7)	0.599
Age at diagnosis (yrs)	13.8 (7.0)	14.1 (6.7)	0.805
Duration (yrs)#	54.0 [52-58]	55.5 [52-61]	0.301
Insulin dose (U/24hr)*	34.4 (5.9)	26.7 (4.8)	**0.002**
HbA_{1c} (%)	7.6 (1.4)	7.3 (1.1)	0.162
Cholesterol (mmol/L)	5.8 (1.1)	5.8 (1.0)	0.975
LDL (mmol/L)*	3.2 (0.4)	3.2 (0.4)	0.971
HDL (mmol/L)	1.9 (0.6)	1.8 (0.5)	0.599
Triglyceride (mmol/L)*	1.3 (0.3)	1.4 (0.3)	0.253
Creatinine (μmol/L)#	96.0 [86-110]	100.0 [81-117]	0.499
UACR (mg/mmol)*	4.16 (2.3)	5.92 (4.0)	0.177
Retinopathy % (n)	51.7 (91)	42.9 (12)	0.384
IHD % (n)	36.4 (64)	35.7 (10)	0.947
Hypertension % (n)	31.8 (56)	28.6 (8)	0.731
Smoking % (n)	16.9 (30)	3.6 (1)	0.156

Mean and standard deviation shown for normally distributed data. *Log transformed data shown by geometric mean and approximate standard deviation. #Median and interquartile range shown for data that was not normally distributed even after log transformation.

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Glucose metabolism under differing exercise conditions in type 1 diabetes

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Background and aims: The combination of aerobic exercise with interspersed high-intensity activity (intermittent high intensity exercise IHE) may be a strategy to attenuate exercise-related glucose fluctuations in diabetic patients not willing or unable to reduce insulin in the context of exercise. The present study comprehensively investigated glucose metabolism during IHE compared to isoenergetic continuous exercise (CONT) in individuals with T1DM.

Materials and methods: In a prospective, randomized cross-over study, 12 male, well controlled, complication-free patients with T1DM (mean ± SD age 26±4 y, HbA1C 7.0±0.6%, diabetes duration 14±6 y) injected their usual morning insulin and had a standardized breakfast. In the postabsorptive phase they performed 90 min of cycling at 50% VO2peak with (IHE) and without (CONT) 10 s maximal sprints every 10 min. Blood glucose, counter-regulatory hormones, lactate and acid-base parameters were measured at regular intervals. Euglycemia was maintained using a 10% oral glucose solution labelled with [U-13C] glucose following a pre-specified algorithm. Analysis of glucose kinetics was based on dual labelling stable isotope technique ([6,6-2H2]glucose and [U-13C] glucose). Intramyocellular glycogen was measured using MR spectroscopy.

Results: Mean±SE glucose was 6.8±0.1 and 7.2±0.2 mM in IHE and CONT (n.s.). Insulin levels were 21.0±0.5 and 20.7±0.2 mU/l (n.s.). Exogenous glucose requirements in IHE were significantly lower compared with CONT (13.4±5.2 g vs 39.8±7.6 g, p<0.004). Catecholamine and growth hormone (GH) levels were significantly higher in IHE when compared to CONT (p<0.05) while cortisol and glucagon levels did not differ between groups. Lactate was significantly higher in IHE compared with CONT (7.3±0.4 vs 2.6±0.3 mM, p<0.01) and was paralleled by

significantly lower pH ($p<0.001$) and bicarbonate ($p<0.001$). Analysis of glucose fluxes revealed higher total Ra in CONT compared with IHE ($p<0.01$) but similar hepatic glucose output (Ra from liver) and a trend towards lower Rd (glucose utilization) in IHE ($p=0.054$). Relative changes in intramyocellular glycogen were -41.0±3.7 and -38.6±5.3% for IHE and CONT (n.s.).

Conclusion: The present findings suggest that IHE is associated with lower requirements of exogenous glucose for maintenance of euglycemia in patients with T1DM compared with isoenergetic CONT over 90 min if insulin is not reduced. IHE elicits a strong metabolic response with higher levels of catecholamines, GH and lactate. Kinetic analyses suggest that hepatic glucose production is similar in IHE and CONT. Consumption of intramyocellular glycogen was also comparable between conditions. These findings implicate that the metabolic difference between IHE and CONT mainly relates to higher lactate levels in IHE, potentially providing an alternative carbohydrate source to the working muscle.

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Regional cerebral haemodynamic response to incremental exercise is blunted in poorly controlled patients with uncomplicated type 1 diabetes

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Background and aims: Cerebral vasoreactivity to pharmacologically induced hypercapnia is impaired in poorly controlled patients with type 1 diabetes but otherwise free from microangiopathy. However, whether this response is also compromised during exercise, a daily-life physiological condition challenging regional cerebral hemodynamics, is unknown. We aimed to investigate prefrontal cortex hemodynamics incremental maximal exercise in patients with uncomplicated type 1 diabetes, taking into account long-term glycemic control as well as exercise- and diabetes-influenced vasoactive stimuli.

Materials and methods: Two groups of patients (type 1 diabetes with adequate glycemic control [T1D-A], n=8, HbA1c 6.8±0.7% [51±7.7 mmol/mol]; type 1 diabetes with inadequate glycemic control [T1D-I], n=10, HbA1c 9.0±0.7% [75±7.7 mmol/mol]) were compared with 18 healthy control subjects (CON-A and CON-I) matched for physical activity and body composition. Throughout exercise, near-infrared spectroscopy allowed investigation of changes in oxyhemoglobin (O2Hb), deoxyhemoglobin (HHb), and total hemoglobin (THb) in the prefrontal cortex. Venous and arterialized capillary blood was sampled during exercise to assess for factors that may alter prefrontal cortex hemodynamics and oxygenation.

Results: No differences were observed between T1D-A and CON-A, but VO2max was impaired ($P<0.05$) and cerebral blood volume (THb) increase blunted ($P<0.05$) in T1D-I compared with CON-I. Nonetheless, O2Hb appeared unaltered in T1D-I probably partly due to blunting of simultaneous neuronal oxygen extraction (i.e., a lower HHb increase; $P<0.05$). There were no intergroup differences in arterial oxygen content, PACO2, pH, [K+], and free insulin levels.

Conclusion: Maximal exercise highlights subtle disorders of both hemodynamics and neuronal oxygenation in the prefrontal cortex of poorly controlled patients with type 1 diabetes. These findings may warn clinicians of brain endothelial dysfunction occurring even before overt microangiopathy during exercise.

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Imbalance of muscular carnosine and fatty acids assessed by 1H MRS in subjects with type 1 diabetes

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Background and aims: Skeletal muscle represents one of the most active organs in glucose metabolism and is directly involved in insulin resistance and sensitivity. In subjects with type 1 diabetes (T1D), there is little information about muscle particularities and specific metabolism. Carnosine is a dipeptide with antioxidant activity that reflects the content of muscular type II fibers (glycolitic). Imbalances in muscular lipid metabolism may be estimated by intramyocellular lipids (IMCL), representing saturated fatty acids (FA), but also some unsaturated FA (UFA) components. The aim of this study was to analyze muscle alterations in T1D patients by estimating muscle carnosine, IMCL, UFA and unsaturation index (UI) by proton magnetic resonance spectroscopy (1H MRS), and to investigate the influence of physical activity and clinical variables on these parameters.

Materials and methods: 18 men with T1D (10 athletes and 8 sedentary) with 41.3±7.2 years of age, 18.8±9.4 years of T1D evolution, were matched with 14 control (CT) men (9 athletes and 5 sedentary). Clinical data were registered, body fat was obtained by iDXA, VO2peak was estimated by ergoespirometry test, basic biochemical parameters were analysed and 1H MRS were performed in the tibialis anterior, soleus and vastus intermedius in a 3 T unit. The spectra were processed in jMRUI for obtaining the concentrations of carnosine (C2-H peak at 8 ppm), IMCL (methylene protons at 1.2 ppm) and UFA (olefinic protons at 5.2 ppm) content, and UI was obtained from UFA/IMCL ratio. Differences between groups (Kruskal-Wallis and U-Mann Whithey), influence of exercise and diabetes (Anova with covariates) and the pearson's correlation for clinical and muscle lipid variables were analysed ($p<0.05$).

Results: T1D athletes presented a VO2peak of 41.3±10.4 mL/kg/min, and sedentary T1D subjects 20.2±6.7, confirming the differences between groups ($p=0.01$). T1D patients presented an increase in carnosine ($p=0.045$) in the soleus muscle after adjustments for age, physical activity and the presence of dyslipidemia, when compared to the CT group. Soleus carnosine was positively correlated with total body fat by iDXA ($R=0.577$) and serum triglycerides ($R=0.501$) and negatively correlated with VO2peak ($R=-0.579$) and UI UFA/IMCL in soleus ($R=-0.473$). In the CT group, carnosine of soleus muscle presented a positive correlation with waist ($R=0.848$), waist/hip ratio ($R=0.836$), total body fat ($R=0.699$), abdominal body fat ($R=0.578$), BMI ($R=0.674$) and soleus IMCL ($R=0.795$). There was no difference in carnosine estimations between athletes and sedentary subjects, in both T1D and CT groups.

Conclusion: Carnosine (marker of glycolytic fibers) is increased in muscles with a predominance of oxidative fibers (soleus) in T1D patients. These findings could indicate a dysfunction in oxidative muscle fibers, thus requiring the increased function of glycolytic fibers, a circumstance also associated with poorer clinical parameters.

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PS 043 Liver metabolism: experimental models

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Effect of renal sympathetic denervation on hepatic glucose metabolism and blood pressure in a rat model of insulin resistance

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Background and aims: Hypertension and diabetes are associated with impaired glucose metabolism and insulin resistance. Chronic activation of the sympathetic nervous system may contribute to either condition. However , the effects of RD on glucose metabolism and insulin sensitivity have also not been systematically examined.The purpose of this study was to investigate the effect of renal sympathetic denervation (RD) on glucose kinetics and insulin signal pathways in high fat diet (HFD) fed rats.
Materials and methods: Mainly,we used 12-week-old male Sprague-Dawley rats as research object .We examined the effects of RD on glucose kinetics and insulin sensitivity in HFD-fed rats with the hyperinsulinemic-euglycemic clamp technique combined with [3-3H]glucose and [U-14C]-lactate as a tracer. We also analyzed the in vivo flux through glucose-6-phosphatase (G6Pase) and the relative contribution of gluconeogenesis and glycogenolysis in RD rats. In addition, Western blotting was used to identify the activities of insulin signaling proteins.
Results: RD in HFD-fed rats markedly decreased blood pressure and hepatic glucose production (HGP). HGP reduction in RD-treated rats was due to an 18.2% decrease for left RD and a 31.9% decrease for bilateral RD in glycogenolysis and a 16.3% decrease for left RD and a 42.8% decrease for bilateral RD in gluconeogenesis. These changes were accompanied by decreased hepatic expression of glucose-6-phosphatase (G6Pase) and phosphoenolpyruvate carboxykinase (PEPCK). Importantly, RD increased phosphorylation of insulin receptors, insulin receptor substrate-1 (IRS-1) and Akt kinase in HFD-fed rats.
Conclusion: These data provide evidence for a mechanism of the RD role and corroborate the notion that RD potentiates hepatic insulin sensitivity.

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Distinct contributions of hepatic insulin and IGF-1 signalling in metabolic alterations induced by liver-specific PTEN deletion in mice

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Background and aims: PTEN is a lipid/protein phosphatase that counteracts insulin and IGF1 signalling in hepatocytes by antagonizing the activity of PI3K. Hepatic Pten deletion in mice triggers the development of steatosis and liver to muscles and adipose tissue crosstalks resulting in increased muscle insulin sensitivity, a reduced fat mass and browning of white adipose tissue. Here, we investigated the specific contributions of hepatic insulin and IGF1 signalling in the deregulated liver metabolism and inter-organ communication triggered by liver-specific Pten deletion in mice.
Materials and methods: Mice with liver-specific deletions of the insulin receptor, the IGF1 receptor, or both, in addition to deletion of Pten were generated and metabolically phenotyped. Histological and molecular analyses were performed on explanted tissues.
Results: Suppression of both insulin receptor (IR) and IGF1 receptor (IGF1R) signalling was required in liver-specific PTEN knockout mice to completely prevent hepatomegaly and steatosis development. Surprisingly, the improved systemic glucose tolerance and impaired hepatic gluconeogenesis induced by PTEN deficiency in hepatocytes were reverted by suppressing hepatic IGF1R signalling. In contrast, the decreased adiposity observed in liver-specific PTEN KO mice was prevented in mice lacking hepatic expression of the IR.
Conclusion: Although they share important similarities, hepatic insulin and IGF-1 signaling exacerbated by PTEN deficiency in hepatocytes distinctly affect liver metabolism and communication with peripheral tissues including muscles and white adipose tissues.
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Induction of hepatic de novo lipogenesis impairs hepatic but not muscle insulin sensitivity and associates with increased adiposity

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Background and aims: Non-alcoholic fatty liver (NAFL) has been associated with insulin resistance, however, the causal relationships between NAFL, hepatic and muscle insulin sensitivity as well as underlying mechanisms are yet unclear. The aim of this study was to determine the effects of NAFL on the tissue-specific insulin sensitivity in mice with genetically induced hepatic *de novo* lipogenesis.
Materials and methods: We examined 36-weeks-old female mice with primary NAFL due to liver-specific overexpression of the sterol regulatory-element binding protein-1c (Alb-SREBP-1c: PRIM; n=7-8) and compared to wild-type C57BL/6 controls (CON; n=6-7). All mice were maintained on chow diet. Intracellular lipids and body fat distribution were assessed with proton magnetic resonance spectroscopy. Fatty acid composition of ceramides and diacylglycerols (DAG) was analyzed with HPLC-MS. Insulin sensitivity was measured with hyperinsulinemic-euglycemic clamp. Protein expression was assessed by western blot and gene expression by qRT-PCR. Redox potential in serum and hydrogen peroxide production in tissues were assessed to estimate oxidative stress. Data were statistically analysed by one-way ANOVA with Tukey post-hoc.
Results: Body weight (BW) was unchanged in PRIM vs. CON mice, while visceral and subcutaneous fat depots were enlarged by 225% ($p<0.001$) and 275% ($p<0.001$), respectively. Liver weight to BW ratio was unchanged, however, hepatic lipid content was increased by 87% ($p<0.01$) and expression of fatty acid synthase (fas) by 217% ($p<0.001$) in PRIM mice. While total ceramides and DAG were unchanged, the cytosolic DAG containing two oleic fatty acids (C 18:1) were increased by 61% ($p<0.05$) in the liver of PRIM mice. Insulin suppression of hepatic glucose production was decreased by 43% in PRIM mice ($p<0.01$). These results were in accordance with 63% lower tyrosine phosphorylation of IRS (pIRS2-Tyr; $p<0.001$) and lack of insulin stimulation of protein kinase B (Akt) in the liver of PRIM mice. No differences in serum redox potential and hydrogen peroxide production from the liver were observed in the PRIM mice. Intramyocellular lipid content of the soleus and tibialis muscles as well as ceramide and DAG levels were similar to CON mice. Furthermore, insulin-stimulated glucose disposal (Rd) as well as translocation of GLUT4 remained unimpaired in PRIM mice. No changes in muscle hydrogen peroxide production were observed.

Conclusion: Increased hepatic *de novo* lipogenesis *per se* leads to hepatic insulin resistance, even in the absence of obesity. This associates with increased accumulation of specific DAG species containing two oleic fatty acids. Furthermore, *de novo* lipogenesis does not necessarily result in impaired muscle insulin sensitivity, potentially due to increased storage of inert lipids in adipose tissue.
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Up-regulation of selenoprotein P, a diabetes-associated hepatokine, and HIP/PAP, a mitogenic factor for the liver, in hepatocytes by intermittent hypoxia

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Background and aims: Sleep apnea syndrome (SAS) is characterized by recurrent episodes of oxygen desaturation during sleep, the development of daytime sleepiness, and deterioration in the quality of life. Accumulating evidence suggests that SAS is associated with glucose intolerance and insulin resistance independently age, gender, smoking status, body mass index, and waist circumference. The progression to type 2 diabetes is dependent on the development of glucose intolerance and insulin resistance. However the direct effects of intermittent hypoxia (IH), a hallmark of SAS, on insulin resistance have obscured. Recently, several proteins that are exclusively or predominantly secreted from the liver are known to directly affect glucose and lipid metabolism and are named as hepatokines. In the present study, using rat and human hepatocytes, we investigated the direct effect of IH on gene expression of hepatokines.
Materials and methods: Human JHH5, JHH7, and rat H4IIE hepatocytes were exposed either to 64 cycles/24 h of IH (5 min hypoxia (1% O_2)/10 min normoxia (21% O_2)), mimicking hepatocytes of SAS patients, or normoxia for 24 h. After the treatment, mRNA levels of *selenoprotein P*, *fibroblast growth factor (FGF) 21*, *alpha2-HS glycoprotein (AHSG)*, *angiopoietin-like protein (ANGPTL) 6*, *sex horomone-binding globulin (SHBG)*, *leukocyte cell-derived chemotaxin (LECT) 2*, *Lipasin*, which are diabetes-associated hepatokines, and *regenerating gene (Reg)* family genes, were measured by real-time RT-PCR. The concentrations of selenoprotein P in the culture medium were also measured by an ELISA kit.
Results: The mRNA levels of *selenoprotein P* were significantly increased in IH-treated H4IIE (1.31 fold vs normoxia, P=0.0057), JHH5 (2.25 fold, P=0.0477), and JHH7 (2.17 fold, P=0.0027) cells whereas mRNA levels of *FGF21*, *AHSG*, *ANGPTL6*, *SHBG*, *LECT2* and *Lipasin* were not increased by IH. The concentrations of selenoprotein P in the culture medium were significantly increased in all IH-treated hepatocytes (H4IIE (3.33±1.24 vs 6.15±0.545 ng/mL, P=0.048), JHH5 (1.57±0.464 vs 11.20±3.09 ng/mL, P=0.037), and JHH7 cells (2.24±1.65 vs 8.68± 1.92 ng/mL, P=0.043)). In addition, mRNA levels of *hepatocarcinoma-intestine-pancreas (HIP)/pancreatitis-associated protein (PAP)* were significantly increased in IH-treated JHH5 (P=0.0022) and JHH7 cells (P= 0.0016) whereas mRNA levels of the other *REG family* members (*REG I alpha*, *REG I beta*, *REG III* and *REG IV*) were not increased by IH.
Conclusion: These results indicate that IH stress up-regulates expression of *selenoprotein P* as well as *HIP/PAP* in hepatocytes. It is quite possible that the cyclic changes of hypoxia-reoxygenation, which occurs in SAS patients, up-regulate HIP/PAP to proliferate hepatocytes and selenoprotein P, accelerating insulin resistance in SAS patients.

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The novel TRPVI antagonist, AZV1, improves insulin sensitivity in ob/ob mice

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Background and aims: Transient receptor potential cation channel subfamily V member 1 (TRPV1) is a ligand gated non-selective cation channel and the receptor for capsaicin on primary sensory nerves. Capsaicin treatment, which ablates these sensory nerves, has been shown to increase insulin secretion and improve insulin sensitivity in animal models. Functional TRPV1 antagonism might have an equivalent effect. AZV1 is a novel TRPV1 antagonist originally developed as an approach to suppress nociceptive pain (IC50 of ~100 nM in cells overexpressing rodent TRPV1). The aim of this study was to test the ability of AZV1 to ameliorate aspects of the metabolic syndrome (diabetes, insulin resistance and dyslipidemia) in the ob/ob mouse.
Materials and methods: Nine week old male ob/ob mice on a C57BlJ/6 background (B6.V-Lepob/OlaHsd) were orally gavaged daily for 12 days with either vehicle or the TRPV1 antagonist at 30 mg/kg/day. Body weight and food intake were recorded. At termination, blood was collected under anaesthesia, 3 h following final dosing for the measurement of AZV1 content, plasma glucose, insulin, fructosamine and triglycerides (TG). In addition, liver TG content was determined.
Results: AZV1 was well tolerated with no apparent reductions of food intake or body weight gain over the dosing period. The AZV1 plasma concentration achieved 3 h following the final dosing was approximately 6-fold the in vitro potency consistent with robust TRPV1 antagonism. AZV1 treatment lowered plasma glucose and fructosamine levels compared to controls (20.7±2.1 to 13.0±0.6 and 95.0±2.5 to 76.0±6.8, $p<$ 0.01, t-test), consistent with a substantial treatment induced reduction in average diurnal blood glucose levels. This effect was achieved without any significant alteration in the 3 h fasted plasma insulin levels, indicating a treatment induced increase in glucose metabolic insulin sensitivity. AZV1 had no apparent effect on fasting plasma FFA, TG or liver TG content.
Conclusion: In conclusion, AZV1 was well tolerated and exerted a substantial antidiabetic action in the ob/ob mouse. Our data suggests that the improvement in glucose control was at least partially mediated by an enhancement in insulin sensitivity.

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Insulin resistance impairs human liver progenitor cell function in vitro: Consequences for liver injury and tumorigenesis in metabolic disease and type 2 diabetes?

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Background and aims: Insulin promotes liver growth and the proliferation of hepatocytes following injury, whereas insulin resistance is associated with chronic liver damage and the increased risk of hepatocellular carcinoma. However, the precise effects of aberrant insulin signalling on regenerative processes in the liver remain unclear. In this study, we set out to explore the role of insulin signalling in de novo human hepatogenesis by modelling insulin resistance during the differentiation of bipotent liver progenitor cells using; (1) hepatoma derived HepaRG progenitor cells and (2) hepatoblast-like cells derived from pluripotent human embryonic stem cells (hESCs).
Materials and methods: Insulin resistance was modelled by stable knockdown of the insulin receptor (IR-beta), IRS1 and IRS2 using shRNA-lentivirus. Enhanced insulin sensitivity was conferred by

overexpression of lentiviral mouse (m)IRS2, whereas the direct effects of insulin were assessed by inclusion/exclusion of supplemented insulin (0.88 uM) in the differentiation medium. High-content "IN Cell" cytometry was used to quantify relevant aspects of hepatocyte differentiation.
Results: Insulin was required for the differentiation of bipotent human liver progenitor cells to hepatocytes as well as for the cell-specific proliferation of hepatocyte precursors expressing albumin and ApoA2 within the cultures, whereas insulin resistance, induced by knockdown of either IR-beta, IRS1 or IRS2, significantly blocked differentiation without diminishing the proliferation of undifferentiated cells. Conversely, overexpression of mIRS2, which mimicked hyperinsuliaemia by increasing insulin/IRS signalling, significantly enhanced the number and percentage of progenitors that differentiated to hepatocyte-like cells, whilst in long-term experiments excessive signalling blocked maturation and promoted steatosis in the differentiating fraction. Finally, we demonstrate that insulin sensitivity is dynamically regulated by IRS expression in a time dependent manner such that dampening of insulin/IRS signalling in immature hepatocytes leads to cell cycle exit, maturation and CYP3A4 expression. Insulin/IRS signalling therefore plays a dual role in promoting de novo hepatogenesis and transient amplification of immature hepatocytes whilst impairing normal maturation and promoting steatosis.
Conclusion: Our results demonstrate that insulin/IRS signalling has pro-hepatogenic actions in both tumour derived HepaRG, as well as non-transformed hESC derived hepatic progenitor cells, thus highlighting a potential role for IRS proteins in both regenerative and tumorigenic mechanisms in the liver. Chronic activation of this pathway, by sustained exogenous IRS expression in the presence of insulin had pathogenic effects in differentiating cells including steatosis, whereas, by limiting differentiation, insulin resistance maintained cells in a progenitor-like state - which if observed in vivo could have adverse consequences for regenerative pathways whilst simultaneously perpetuating a more aggressive undifferentiated state in liver cancer stem cells in insulin resistant patients.
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Liver glutamate dehydrogenase knockout mice display impaired gluconeogenesis and altered feeding behaviour

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Background and aims: Glutamate dehydrogenase (GDH, encoded by *Glud1* gene) is mainly expressed in the liver, pancreas, kidney and the brain. In mammals, this enzyme is a homohexamer located in the mitochondrial matrix where it catalyzes the reversible oxidative deamination of glutamate to alpha-ketoglutarate and ammonia. GDH catalyzes the same reaction in every tissue, although direction of the predominant flux might be tissue specific. Moreover, physiological function of GDH varies greatly according to specific organs. GDH is of major importance for ammonia detoxification through nitrogen metabolism and urea synthesis in the liver and kidney. Hepatic GDH is also involved in the metabolism of most amino acids, in particular glutamine and alanine being transported from skeletal muscle during periods of active gluconeogenesis. The importance of GDH activity is witnessed by the severity of disorders where GDH function is inappropriate, such as hyperinsulinemia/hyperammonemia syndrome. Here, we have generated inducible liver-specific GDH knockout mice (Hep*Glud1*$^{-/-}$) to investigate consequences of the lack of hepatic GDH on metabolic homeostasis in basal and energy challenging conditions.
Materials and methods: The *in vivo* deletion of GDH in hepatocytes was induced at 8 weeks of age by subcutaneous implantation of tamoxifen pellets in *Glud1*$^{lox/lox}$ mice (generated in our laboratory) carrying the Albumin-Cre-ER^{T2} construct (provided by P Chambon). Metabolic parameters were investigated by *ip* challenges (glucose, pyruvate, glutamine, alanine), monitoring in metabolic cages, EchoMRI and commercially available kits.
Results: Three weeks after *in vivo* induction of recombination by tamoxifen, deletion was successful and specific for the liver in Hep*Glud1*$^{-/-}$ mice (<5% remaining of immunoreactive GDH). We also observed abrogation of GDH enzymatic activity in liver homogenates (-67%, $p<0.01$). In isolated primary hepatocytes, we measured the half-life of GDH lasting for about 2-3 days. In non-hepatic tissues of Hep*Glud1*$^{-/-}$ mice, GDH expression was fully preserved as revealed by immunoblotting. Immunohistochemical analyses of Hep*Glud1*$^{-/-}$ livers did not show morphological abnormalities. Intra-peritoneal glucose tolerance tests revealed normal glucose homeostasis in Hep*Glud1*$^{-/-}$ mice upon standard conditions and both body weight and fat/lean mass ratio were similar between control and Hep*Glud1*$^{-/-}$ mice. Remarkably, the food intake profile was changed by the absence of liver GDH, knockout animals exhibiting a shift in the circadian rhythm of feeding. Moreover, lipids were favored over carbohydrates as energy fuel in Hep*Glud1*$^{-/-}$ mice. Finally, we tested the gluconeogenic capacity of Hep*Glud1*$^{-/-}$ mice in response to substrates specific for situation of energy imbalance, such as fasting, i.e. glutamine and alanine. Control mice could increase glycemia in response to both glutamine and alanine challenge. However, the alanine response was abrogated in Hep*Glud1*$^{-/-}$ mice, while its deaminated product pyruvate could induce gluconeogenesis.
Conclusion: Liver-specific GDH deletion induced substrate-specific impairment of liver gluconeogenesis, higher lipid consumption, and a shift in the circadian rhythm of feeding. These results highlight the central role of hepatic GDH in energy metabolism.
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Phenotyping of the mitochondrial phosphoenolpyruvate carboxykinase (PEPCK) knockout mouse

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Background and aims: The GTP-dependent enzyme phosphoenolpyruvate carboxykinase (PEPCK) is the key enzyme that provides phosphoenolpyruvate (PEP) for gluconeogenesis (de novo glucose production) and glyceroneogenesis (de novo glycerol production to store free fatty acids in form of triglycerides). Although, there are two isoforms, a cytosolic (PEPCK-C) and a mitochondrial (PEPCK-M) form, most research focused on the function and regulation of PEPCK-C, probably due to its hormonal regulation and the suggested absence of PEPCK-M in rodent metabolism. In contrast to PEPCK-C, in which transcription is strongly inhibited by insulin, PEPCK-M is constitutively expressed and therefore PEP is synthesized according to fuel supply and mitochondrial GTP (mtGTP). Although, previous reports suggest that rodents do not have significant PEPCK-M activity, we aimed to confirm the existence and importance of PEPCK-M in rodent metabolism.
Materials and methods: To assess the role of PEPCK-M in rodent metabolism we created PEPCK-M knockout mice on a C57Bl6 background (PCK2-/-). At the age of 6 weeks we fed mice either a normal chow diet or a high fed diet up to 15 weeks long and performed metabolic tests to assess glucose homeostasis, including glucose tolerance test, insulin tolerance test and measurement of energy expenditure using a controlled animal monitoring system (CLAMS).
Results: There was no difference in body weight gain for PCK2-/- on a normal chow diets and high fat diet compared to wild-type animals (wt). PCK2-/- mice on a normal chow diet had higher fasting blood glucose levels (9.33±0.29 mmol/l versus 7.97±0.31 mmol/l; P=0.0031) but showed normal glucose tolerance. After 4 weeks on a high fat diet PCK2-/- mice showed glucose intolerance, measured by glucose and insulin tolerance test (GTT-area under the curve 1796±80.14 versus

1444.46±82.64; ITT-area under the curve 913.35±163.97 versus 607.91 ±61.8). The difference in glucose intolerance disappeared with increase of age. Thus, we targeted further experiments to investigate why knock-out mice are prone to become glucose intolerant. Interestingly, we noticed higher heat production and activity with PCK2-/- mice on a normal chow diet using CLAMS. However, PCK2-/- mice on high fat diet showed no difference in heat production or activity but had significant lower energy expenditure.

Conclusion: Despite previous reports that deletion of PCK2 would be insignificant, we confirm that PCK2 does have a role in rodent glucose metabolism and PCK2-/- mice on a high fat diet are prone to develop glucose intolerance.

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PS 044 Mouse models of type 2 diabetes

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The role of TBC1D1 and TBC1D4 in contraction-induced glucose uptake in mouse skeletal muscle

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Background and aims: The RabGTPase-activating proteins TBC1D1 and TBC1D4 (AS160) are key players in AKT- and AMP-dependent protein kinase (AMPK) regulated glucose metabolism. The aim of this study is to analyze the contribution of these two homologous proteins to the contraction-mediated glucose uptake and metabolism in skeletal muscle.

Materials and methods: Tbc1d1-deficient mice (RCS.B6.SJL-Nob1.10) were crossbred with conventional Tbc1d4 knockout mice to yield double-deficient mice (D1/4KO) on a C57BL/6J background. *Ex vivo* muscle contraction of male 12-16 wk old knockout mice and wildtype controls (WT) was conducted using isolated *Extensor digitorum longus* (*EDL*) and *Soleus* muscles in a myograph chamber. Contraction force and time for half-capacity were measured. Subsequently, contraction- and insulin-induced ^{3}H-2-deoxyglucose uptake and glycogen levels were determined.

Results: We could previously demonstrate that D1/4 knockout muscles show substantially reduced GLUT4 protein abundance in *EDL* and *Soleus* muscle (~50% of WT). Consistently, contraction-induced glucose uptake was similarly reduced in both, glycolytic *EDL* and oxidative *Soleus* muscle from D1/4KO mice (WT vs. D1/4KO; *EDL*: 4.45±0.25 vs. 2.00±0.14; $p<0.001$ and *Soleus*: 4.14±0.32 vs. 2.86±0.26 nmol/mg/20 min; $p<0.05$). Combined stimulation of the muscles with *ex vivo* contraction and insulin further increased glucose uptake in wildtype controls. In skeletal muscle from D1/4KO mice this effect was blunted (Contr vs. Ins+Contr; *EDL*, WT: 11.24±0.81 vs. 14.45±0.731, $p<0.05$; D1/4KO: 8.02±0.66 vs. 8.48±1.08 and *Soleus*, WT: 16.83±1.05 vs. 21.76 ±1.45, $p<0.05$; D1/4KO: 12.71±1.40 vs. 13.57±2.01 nmol/mg/20 min). Moreover, contraction force and time for half-capacity were unchanged between the genotypes. Glycogen levels were increased in skeletal muscle of D1/4KO mice (WT vs. D1/4KO: 1.53±0.30 vs. 2.74±0.31 μg/mg; $p<0.05$) whereas liver showed decreased glycogen content (WT vs. D1/4KO: 9.35±1.50 vs. 3.07±0.44 μg/mg; $p<0.001$).

Conclusion: Contraction-induced glucose uptake is severely reduced but not completely eliminated in *EDL* and *Soleus* muscle from D1/4KO mice, suggesting that a TBC1D-independent pathway also contributes to contraction-mediated GLUT4 translocation. Elevated glycogen levels may enable muscles of D1/4KO mice to develop the same contraction forces and times for half-capacity as wildtype controls.

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Uncoupling protein 1 is required for fibroblast growth factor 21 mediated improvements in glucose tolerance but is dispensable for body weight loss in diet-induced obese mice

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Background and aims: Fibroblast growth factor 21 (FGF21) has emerged as a metabolic regulator with therapeutic potential for the treatment of diabetes. Administration of FGF21 to rodent models of type 2

diabetes reduces body weight and improves lipid and glucose homeostasis. FGF21 treatment also increases uncoupling protein 1 (*Ucp1*) mRNA and protein levels in brown and white adipose tissues. We sought to determine whether the anti-diabetic and anti-obesity effects of FGF21 therapy require UCP1.

Materials and methods: Mice with a genetic deletion of *Ucp1* ($Ucp1^{-/-}$) and wildtype controls ($Ucp1^{+/+}$) were fed either a low-fat diet or rendered obese with high-fat diet feeding. Next, we administered a vehicle (controls) or plasmid encoding the human *FGF21* gene by hydrodynamic delivery to high-fat fed mice and body weight and fasting blood glucose levels were tracked for 4 months. In addition, we performed glucose tolerance tests (days 6 and 22) and an insulin sensitivity test (day 14).

Results: High-fat diet resulted in excess body weight gain in both $Ucp1^{+/+}$ and $Ucp1^{-/-}$ mice. Hydrodynamic delivery of the human *FGF21* gene increased plasma human FGF21 to supraphysiological levels by day 3 compared to vehicle controls (42.6±7.8 ng/ml $Ucp1^{+/+}$, 51.2±12.7 ng/ml $Ucp1^{-/-}$, <0.05 ng/ml in both vehicle groups). On day 30, FGF21 treated $Ucp1^{+/+}$ and $Ucp1^{-/-}$ mice lost comparable body weight (-19.6±8.7% and -19.5±5.2%) whereas control $Ucp1^{+/+}$ and $Ucp1^{-/-}$ mice gained weight (9.4±1.4% and 4.8±1.1%). Despite maintenance of supraphysiological plasma human FGF21 levels, body weight reducing effects of FGF21 therapy waned in both $Ucp1^{+/+}$ and $Ucp1^{-/-}$ mice between days 22 and 30, and body weights returned to near pre-treatment values in most mice by day 95. FGF21 treated $Ucp1^{+/+}$ mice had improved glucose tolerance at day 6 compared to vehicle (AUC 446 ±63 mM and 1253±31 mM, $p<0.05$), even beyond that of low-fat diet-fed $Ucp1^{+/+}$ mice (AUC 713.4±61 mM, $p<0.05$). In contrast, FGF21 treatment did not improve glucose tolerance relative to vehicle in $Ucp1^{-/-}$ mice (AUC 2174±37 mM vs 1892±174 mM). Similarly, glucose tolerance was also improved at day 22 with FGF21 treatment relative to vehicle in $Ucp1^{+/+}$ mice (AUC 550±54 vs 1574±123, $p<0.05$) but not in $Ucp1^{-/-}$ mice (AUC 1246±108 vs 1329±86). Glucose stimulated insulin secretion at 15 minutes post-glucose delivery in FGF21 treated $Ucp1^{+/+}$ mice tended to be higher than vehicle on days 6 (2.8±0.8 fold vs 1.7±0.05 fold, $p=0.1$) and 22 (2.9±0.9 fold vs 1.9±0.3 fold, $p=0.4$). Similarly, FGF21 treated $Ucp1^{-/-}$ mice had improved glucose stimulated insulin secretion at 15 minutes post-glucose delivery compared to vehicle on days 6 (5.2±1.9 fold vs 1.9±0.1 fold, $p<0.05$) and 22 (3.6±0.4 fold vs 1.9±0.2 fold, $p<0.05$). FGF21 treated $Ucp1^{+/+}$ and $Ucp1^{-/-}$ mice had improved insulin sensitivity compared to vehicle, and the degree of blood glucose lowering by insulin was similar between FGF21 treated $Ucp1^{+/+}$ and $Ucp1^{-/-}$ mice (AUC 3778±132 and 4006±581).

Conclusion: Genetic ablation of UCP1 does not prevent FGF21 mediated lowering of body weight but prevents improvements in glucose tolerance despite improvements in glucose stimulated insulin secretion and insulin sensitivity by FGF21 therapy. These data suggest that UCP1 dependent thermogenesis in brown or white adipose tissue is not required for body weight reduction but required for improvements in glucose tolerance by FGF21 therapy.

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Validation of the intraperitoneal insulin tolerance test for the measurement of insulin sensitivity in mice

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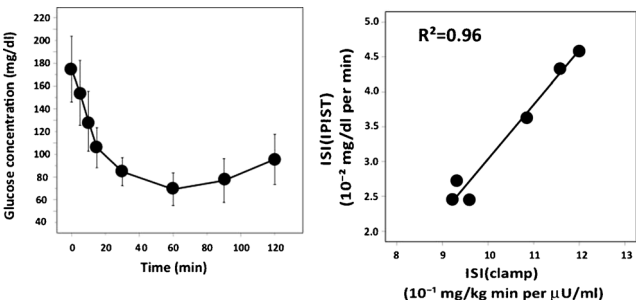
Background and aims: Mouse models are helpful in clarifying the pathophysiological mechanisms by which insulin resistance contributes to the development of obesity and diabetes. Thus, the ability to accurately and easily quantify insulin resistance in mice is of interest. The reference standard for measuring insulin sensitivity is the glucose clamp, but simpler methods previously developed in humans are frequently adopted in mouse studies. Here, we focused on the intraperitoneal insulin sensitivity test (IPIST). The domain of validity of the insulin sensitivity index derived from IPIST is unknown since no direct validation against the clamp is available in mice. Aim of this study was to compare the indeces of insulin sensitivity derived from IPIST and the clamp in a group of wild-type mice.

Materials and methods: Six CB57BL6 12-week old mice underwent the IPIST first, and the euglycemic clamp two weeks later. In the IPIST, after a 30-min stabilization period, a bolus of human insulin (0.5 U/kg) was administered by an intraperitoneal injection at t=0. Blood samples (~5 μl) for the determination of glucose concentration were collected at 0, 5, 10, 15, 30, 60, 90, 120 min. The IPIST-based index of insulin sensitivity, SI(IPIST), was calculated as the slope of the regression line between the logarithm of glucose concentration *vs*. time in the interval 0-15 min (i.e., when the linearity hypothesis was tenable). In the hyperinsulinemic-euglycemic clamp, after a 30-min stabilization period, a primed-continuos infusion of human insulin (2.5 mU/kg/min) was begun at t=0 and maintained constant for 120 min. Concurrently, 20% glucose was infused at a variable rate to maintain glucose at basal concentration. The clamp-based index of insulin sensitivity, ISI(clamp), was calculated as the ratio between the levels of the glucose infusion rate and insulin concentration measured at the end of the clamp. The relationship ISI(IPIST) *vs*. ISI(clamp) was investigated by linear regression and the coefficient of determination, R^2, was evaluated to assess the goodness of the fit. Results were expressed as Mean ± SD and the level of statistical significance was 0.05.

Results: During IPIST, the glucose decay curve achieved a nadir (69.2± 14.4 mg/dl) at 60 min (Figure, left panel). At the end of the clamp, insulin concentration was 50.3±2.0 μU/ml. ISI(IPIST) was 3.36±0.95 10^{-2} mg/dl per min. ISI(clamp) was 10.41±1.20 10^{-1} mg/kg min per μU/ml. The association between the two indices was excellent and R^2 was 0.96, $p=0.001$ (Figure, right panel).

Conclusion: IPIST is simple and calculation of ISI(IPIST) from glucose data collected during the initial 15 min of the test is straightforward. The findings of this preliminary report are encouraging since ISI(IPIST) resulted extremely well correlated with ISI(clamp). If confirmed in a larger sample of mice, IPIST may constitute a cost-effective and validated approach to measure insulin sensitivity in mice.



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Acyl-carnitines induce insulin resistance in high fat diet-fed and diabetic db/db mice

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Background and aims: The important pathological consequences of diabetes arise from the detrimental effects induced by fatty acids and their

metabolites. The aim of this study was to test the role of long chain acylcarnitines in the development of insulin resistance. In addition, we tested whether pharmacological decreasing of the content of long chain acylcarnitines represents an effective strategy to improve insulin sensitivity in diabetes.

Materials and methods: In this study acylcarnitine content was measured in plasma and muscles of high fat diet-fed animals, as well as diabetic db/db and control db/L mice in fasted and fed states. Palmitoylcarnitine administration was used to study the detrimental effects of long chain acylcarnitines. To test whether the decreased content of acylcarnitines in skeletal muscle improves insulin sensitivity, we administered a novel compound, 4-[ethyl(dimethyl)ammonio]butanoate (methyl-GBB), which effectively decreases contents acylcarnitines in plasma and muscle.

Results: Pronounced accumulation of long chain acylcarnitines was detected in the fed state of high fat diet-fed and diabetic db/db mice (Fig.A). In addition, even single administration of palmitoylcarnitine induced decrease in 2-deoxy-glucose uptake in skeletal muscles by 47%. Long term administration of palmitoylcarnitine resulted in the insulin resistance equal to high fat diet-induced changes in insulin sensitivity. Methyl-GBB administration both improved glucose utilisation in tissues and insulin sensitivity, and thus significantly reduced blood glucose level in fed and fasted db/db mice (Fig.B).

Conclusion: The reduction of long chain acylcarnitine content represents an effective strategy to improve insulin sensitivity. Methyl-GBB decreases contents of carnitine and acylcarnitine, significantly reduces blood glucose concentration and improves insulin and glucose tolerance in db/db mice.

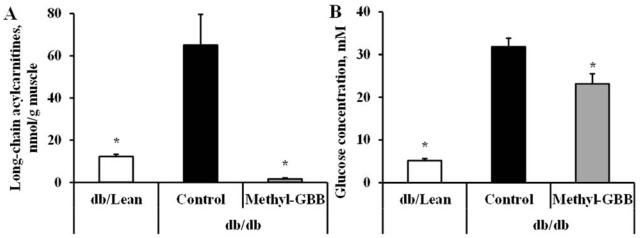

Figure. Methyl-GBB decreases content of acylcarnitines in muscles (A) and significantly reduces blood glucose concentration (B) in db/db mice. Each value represents the mean ± SEM of 5-10 animals. *Significantly different from db/db control group (Tukey's test $P < 0.05$).

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The roles of heat shock protein 72 in hepatic glucose metabolism in mice model of type 2 diabetes

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Background and aims: Cell stress, such as endoplasmic reticulum stress or oxidative stress is one of the key mediators in pathophysiology of type 2 diabetes. Molecular chaperone, which modulate protein folding and/or assembly and protect cells from those stresses, may be a favorable target for diabetic treatment. Heat shock protein (HSP) 72 is a major inducible heat shock protein against heat, ultraviolet, heavy metals or infection, and works as a molecular chaperone to protect cells from inflammatory stresses or apoptotic signals. Induction of HSP72 by pharmacologic agent or mild electrical stimulation with heat shock improves glucose intolerance in diabetic model mice. In this study, we investigated glucose metabolism in whole body HSP72 deficient (KO) mice to explore the roles of HSP72 in diabetes.

Materials and methods: Male HSP72 KO mice or control (C) mice were subjected to a high-fat diet (HFD) regimen for 16 weeks. Several metabolic parameters and cellular stress markers were evaluated.

Results: KO mice showed significantly higher body weight after 10 weeks of HFD (KO: 36.9 g v.s. C: 33.4 g). Fasting blood glucose was significantly elevated after 11 weeks of HFD (KO: 143.3 mg/dL v.s. C: 115.5 mg/dL). Random fed blood glucose and food intake were comparable. Upon glucose challenge, blood glucose levels at any time points measured were higher in KO mice. On insulin tolerance test, KO mice exhibited insulin resistant phenotype. Epididymal, mesenteric and retroperitoneal fat masses were all increased in KO. Hepatic steatosis was obvious in KO liver. Upon insulin stimulation from inferior vena cava, phosphorylation of Akt was decreased by approximately 50% in KO liver extracts, with increased activation of c-jun N-terminal kinase. Hepatic gluconeogenesis was not suppressed in KO mice.

Conclusion: In summary, deficiency of HSP72 leads to increased visceral adiposity, hepatic insulin resistance, glucose intolerance and fatty liver. As induction of HSP72 is beneficial to treat diabetes, our observations strongly indicate the abundance of HSP72 is critical in diabetic pathophysiology.

Supported by: MEXT KAKENHI JAPAN

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Western diet intervention modulates regulation of IGFBP-1 protein in C57/Bl6 mice

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Background and aims: High calorie diet has a negative impact on glucose homeostasis, insulin responsiveness and liver function, especially during the development of adolescence. Growing evidence supports the concept that diet-induced metabolic disturbances lead to impairments both in the peripheral and brain insulin responsiveness, glucose utilization and energy metabolism. However, the mechanism by which diet interventions provokes tissue-specific insulin resistance is unclear. Insulin-like growth factor binding protein-1 (IGFBP-1), produced in the liver, modulates the distribution, bioactivity and bioavailability of the IGF-I and IGF-II proteins. Insulin is the predominant inhibitor if IGFBP-1 secretion under normal conditions. IGFBP-1 is regulated by acute changes in nutrition and cellular stress. Under stress and/or intensive exercise this negative feedback between insulin and IGFBP-1 is lost possibly due to hepatic insulin resistance or due to factors that directly stimulate IGFBP-1. In the current study we explored in mice the effect of short-term intervention with high-fat/high-sucrose diet (Western Diet, WD) during adolescence and its effect in the regulation on IGFBP-1.

Materials and methods: We subjected adolescent 5-week old male and female C57Bl/6CR mice to a 7-week diet intervention with high fat / high sucrose (WD) or regular chow diet (CD) and studied body weight during the diet intervention. After 7 weeks on diet we analyzed the glucagon, insulin, glucose and IGFBP-1 plasma levels during different metabolic conditions (starvation, after sleep phase 7 PM and wake phase 7 AM) and performed intraperitoneal insulin tolerance tests (ipITT) and morphological studies of the liver.

Results: After the diet intervention, we observed that the WD mice had developed overweight, dyslipidemia, hyperglycemia and presented significantly higher serum insulin and glucagon levels as compared to the CD mice. The insulin levels were increased at all the metabolic conditions. The IGFBP-1 fasting levels were decreased in the males but not in the females in the WD mice. Insulin and glucose homeostasis impairments detected in the WD mice were concomitant with a significant increase in IGFBP-1 levels in spite of increased insulin levels both in the sleep phase and wake phase. Furthermore, animals on WD

experienced insulin intolerance in the ipITT together with an evident deterioration of liver morphology.
Conclusion: Altogether these results suggest that high calorie diet can increase the levels of IGFBP-1 independently of circulating insulin. Hepatic insulin resistance as well as increased glucagon may explain the IGFBP-1 induction. These factors may also explain the increase in glucose levels. It is not excluded that the increase in IGFBP-1 secretion is secondary to WD activation of the central nervous system.
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Aonlteratis in insulin signalling in insulin-sensitive tissues contribute to insulin resistance induced by chronic intermittent hypoxia in rats
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Background and aims: Chronic intermittent hypoxia (CIH) and sleep fragmentation are consequences of obstructive sleep apnea (OSA) that is characterized by recurrent obstruction of the upper airways. In last year's several clinical studies have demonstrated that OSA is associated with an increased prevalence of type 2 diabetes and it was recently shown to be a risk for incident in diabetes. In fact, evidence from animal models of CIH, that mimics OSA, shows its association with insulin resistance and dyslipidemia, however, the mechanisms underlying metabolic effects of CIH are still unknown. The aim of the present work was to investigate the effect of CIH on insulin sensitivity, exploring the time frame necessary to induce insulin resistance and the alterations in the insulin signaling pathways in skeletal muscle and adipose tissue.
Materials and methods: Experiments were performed in male Wistar rats (280-440 g). Control animals were maintained in room air atmosphere and CIH rats were submitted for 28 and 35 days during their sleeping period to a CIH paradigm: 5.6 hypoxic (5%O2) cycles/h, 10.5 h/day. At the end of CIH paradigm the animals were anaesthetized (pentobarbitone 60 mg/Kg) and insulin sensitivity was evaluated by an insulin tolerance test. Mean arterial pressure (MAP), plasma catecholamines (norepinephrine plus epinephrine) levels and adrenal catecholamines content were evaluated. Skeletal muscle and adipose tissue expression were collected to quantify, by western blot, alterations in the expression of proteins involved in insulin signaling pathways.
Results: CIH during 28 and 35 significantly increased MAP. CIH Insulin sensitivity decreased by 20.84 and 35.92% ($p<0.01$) with 28 and 35 days of CIH, respectively, but not modify fasting glycemia. Plasma catecholamines doubled at 28 days of CIH and more than triple after 35 days of CIH. 35 days of CIH, but not 28, decreased insulin receptor phosphorilation by 54.94% ($p<0.01$) in skeletal muscle, without altering Glut4 expression. Also, in adipose tissue, 35 days of IH, but not 28, decreased: Akt expression by 36.25 ($p<0.01$), AKt phosphorilation by 36.09% ($p<0.05$) and insulin receptor expression by 32.49% ($p<0.001$).
Conclusion: We have shown that insulin resistance induced by CIH is positively correlated with the period of time of CIH exposure. Also, our results demonstrated that insulin resistance induced by CIH is mediated by alterations in insulin signaling pathways in skeletal muscle and adipose tissue as well as in plasma catecholamines.
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PS 045 Insulin action and glucose transport

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Changes in the adenosine / nitric oxide signalling pathways are associated skeletal muscle insulin resistance
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Background and aims: Endothelial nitric oxide (NO) production is known to be positively co-related with insulin sensitivity. It is well established that adenosine induces NO release via A1, A2A and A2B receptors and our group has recently described that adenosine increases insulin sensitivity in vivo through an NO-mediated effect. The aim of the present work was to investigate if adenosine modulates NO production in insulin-sensitive tissues, like the skeletal muscle, the liver and adipose tissue and if an altered interaction between adenosine and NO is present in insulin-resistant states. Additionally, adenosine receptors responsible for NO production in insulin-sensitive tissues were also characterized.
Materials and methods: Experiments were performed in 10-20 weeks Wistar rats (250-450 g). Two groups of rats were used: a high-sucrose diet-group (Hsu, 35% sucrose in drinking water for 28 days) and a control group. The liver, soleus muscle and visceral adipose tissue were dissected and incubated in Tyrode-Bicarbonate solution during 10, 30 and 60 minutes in the absence or in the presence of different concentrations of the following adenosine agonists: Bay-60-6583 (A2B agonist), CPA (A1 agonist) and CGS-21680 (A2A agonist). Tissues were weighted and homogenized and NO in the homogenate was determined by chemiluminescence.
Results: NO in soleus muscle was 22.86±2.54 nmol/g tissue, 20.06± 1.76 nmol/g tissue and 25.79±4.41 nmol/g tissue after 10, 30 and 60 minutes of incubation in the absence of adenosine agonists, respectively. The HSu diet did not change NO levels either in the liver or in adipose tissue, but in soleus muscle the NO levels were significantly increased by 35.89%, 50.42% and 41.37% when incubated during 10, 30 and 60 minutes, respectively. In control animals: CPA (3 nM) significantly increase NO production in liver by 54,2% and 58.75% after 30 and 60 min of incubation and in soleus muscle by 5.11%, 34.74% and 67.81% after 10, 30 and 60 minutes of incubation; CGS (30 nM) increased by 93.87%, 18.91% and 71.84% in soleus 100.64%, 68.17% and 6.81% and in adipose tissue after 10, 30 and 60 min of incubation respectively, without affecting the liver; and BAY (1 μM) did not modify NO production in soleus muscle but decreased NO production in the liver by 53.2%, 32.0% and 55.9% after 10, 30 and 60 min of incubation. In HSu insulin-resistant animals: CPA (30 nM) did not alter soleus muscle NO production; BAY (1 μM) decreased NO production by 43.6%, 53.6% and 15.2% in the liver and by 47.0%, 37.9% and 46.8% in the soleus muscle after 10,30 and 60 min of incubation.
Conclusion: In a physiological state adenosine modulates NO production in insulin sensitive tissues via an action on A1 receptors in soleus muscle, A2B in the liver and A2A in adipose tissue. In HSu insulin resistant animals, NO production increases only in the soleus muscle and there is a switch between A1 and A2B receptor in the control of NO production in this tissue. We conclude that an altered interaction between adenosine and NO in soleus muscle is associated with insulin resistant states.
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Role of circular dorsal ruffles in the internalisation of the insulin receptor

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Background and aims: Upon insulin binding, the insulin receptor is rapidly internalized in clathrin-coated pits. After being endocytosed, the clathrin coat disassembles and the activated receptors are concentrated in endosomes to further stimulate pathways that regulate metabolism and mitogenesis. The uncoupling of the insulin-insulin receptor complex leads to signal termination. The receptors can then be degraded, recycled back to the surface or translocated to the nucleus. However, the number of receptors undergoing each pathway, how the cell decides the pathway that receptors take and how many rounds of recycling undergo each receptor before degradation, is currently unknown. The insulin receptor's fate influences peripheral insulin levels, as 50-70% of portal insulin is cleared during the first pass in the liver. Since insulin receptor trafficking is critical for normal development and maintenance of homeostasis, controlling the magnitude and specificity of the cell's response, it is crucial to determine the mechanisms involved in this process, both in physiology and pathophysiology. Our hypothesis is that alterations in the internalization and trafficking of the insulin receptor in hepatocytes impair insulin clearance.
Materials and methods: We used Hepa 1-6 and HUH-7 mouse and human hepatoma cell lines, respectively, to characterize the trafficking of the insulin receptor, in physiological conditions. For this, cells were stimulated with various insulin concentrations (50, 75 and 100 nM), during different time points, and processed for immunofluorescence, using phalloidin to label the actin cytoskeleton and antibodies against the receptor.
Results: Our results suggest that after insulin stimulation, hepatocytes form ring-shaped actin-rich structures known as Circular Dorsal Ruffles (CDRs). CDRs are dynamic and transient, forming exclusively on the dorsal surface of the cell, upon growth factor stimulation. The main function of these membrane-bound structures is the rapid internalization by macropinocytosis of tyrosine kinase receptors, like the insulin receptor. After this step, the receptors can be recycled back to the surface, following the recycling pathway or degraded in lysosomes. We observed that CDRs form as soon as 1 min after insulin stimulation. Furthermore, we found that the insulin receptor localizes to CDRs, suggesting it is internalized via this pathway.
Conclusion: Thus, our results suggest that the insulin receptors are rapidly internalized and recycled back to the plasma membrane through CDRs. This fast recycling might be crucial for the liver to fulfill its functions, especially the insulin clearance.
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Lack of CD2AP disrupts Glut4 trafficking and attenuates glucose uptake

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Background and aims: Glucose transporter 4 (Glut4) is the primary insulin responsive glucose transporter. In the absence of insulin, Glut4 resides in intracellular vesicles, and undergoes exocytosis upon insulin stimulation. CD2-assosiated protein (CD2AP) is a ubiquitously expressed cytoplasmic protein, which is necessary for the formation of slit diaphragms and glomerular ultrafiltration. Interestingly, CD2AP gene variants associate with end-stage renal disease in patients with type 1 diabetes. CD2AP functions as a scaffolding protein in different signaling and vesicle trafficking pathways, but it is not known whether it regulates glucose uptake into cells. In this study we investigated the role of CD2AP in insulin-dependent Glut4 trafficking and glucose uptake.
Materials and methods: Glucose uptake was measured in immortalized wildtype (WT) and CD2AP-/- podocytes, and in HIRC and L6 cells transfected with CD2AP and control siRNAs, using tritium-labeled 2-deoxyglucose in basal, serum starved and insulin stimulated conditions. CD2AP-/- and WT podocytes stably expressing HA-Glut4-GFP were surface-labelled with an antibody against HA to quantify the amount of Glut4 on the plasma membrane. HA-Glut4-GFP expressing podocytes were also used to study the trafficking of Glut4 by live cell imaging, and to quantify the circulation of Glut4 between the intracellular compartment and the plasma membrane in a time-dependent manner.
Results: In the basal state lack of CD2AP attenuated glucose uptake into podocytes by 20% compared to WT cells ($p<0.05$). Also knockdown of CD2AP in HIRC and L6 cells by siRNA reduced glucose uptake by 25% ($p<0.05$). Insulin stimulation induced glucose uptake into WT podocytes by 20% ($p<0.05$), whereas CD2AP-/- podocytes failed to respond. In line with this, insulin stimulation increased the amount of HA-Glut4-GFP on the plasma membrane by 50% in WT podocytes ($p<0.01$), whereas no difference was observed in CD2AP-/- podocytes. In WT podocytes, live cell imaging revealed dynamic trafficking of HA-Glut4-GFP in response to insulin, whereas in CD2AP-/- podocytes HA-Glut4-GFP formed dense insulin unresponsive clusters. Quantification of HA-Glut4-GFP on the plasma membrane in a time-dependent manner after insulin stimulation revealed that in WT podocytes the amount of Glut4 on the plasma membrane increased by 30% after 5 minutes ($p<0.01$), and by 270% after 30 minutes ($p<0.001$). In CD2AP-/- podocytes, however, there was no difference in the amount of HA-Glut4-GFP on the plasma membrane after insulin stimulation for 5 minutes. After 15 minutes, there was a 50% increase ($p<0.001$), which did not significantly increase by 30 minutes ($p<0.22$).
Conclusion: Our results indicate that CD2AP is essential for regulating glucose uptake into podocytes and other insulin responsive cells. CD2AP appears to be vital for sorting of endocytosed Glut4 to correct insulin sensitive compartments and insulin-responsive trafficking of Glut4 to the plasma membrane in podocytes.
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MicroRNAs potentially regulators of GLUT4

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Background and aims: Diabetes is a metabolic disease characterized by hyperglycemia associated with impaired glucose uptake, in which reduced GLUT4 protein expression (encoded by the SLC2A4 gene) plays an important role. The recently described microRNAs (miRNAs), which are small RNAs involved in gene regulation at the post-transcription level, usually by affecting the stability and degradation of mRNAs, have been described as involved in the pathophysiology of diabetes. However, the miRNAs participation in the reduction of GLUT4 expression, consequently in impaired glucose uptake, especially in skeletal muscle, remains unclear. Thus, the objective of this study was to investigate the expression of miRNAs potentially involved in the regulation of Slc2a4/GLUT4 expression in skeletal muscle of diabetic rats.
Materials and methods: Male Wistar rats (70-day old) were rendered diabetic by receiving streptozotocin (50 mg/kg, i.v.), After 13 days, 3 groups were formed: non-diabetic (ND), and diabetic treated with placebo (DP) or insulin (DI) (NPH insulin, 6U/day). Treatment was conducted for 7 days, totalizing 21 days of diabetes. At the end of the experimental

period, the animals were anesthetized, blood was collected and the soleus muscle was removed for evaluation of mRNA and protein expression. In silico analysis was used to select miRNAs predicted as potential regulators of Slc2a4/GLUT4 in rat. After that, based on previous description of diabetes-induced modulation of the miRNAs, and/or their participation in the skeletal muscle cell homeostasis, some miRNAs were selected for validation. The Slc2a4 mRNA and the miRNAs were analyzed by qPCR. The GLUT4 protein was assessed by Western blotting. The comparison among groups was performed by analysis of variance (ANOVA) followed by Bonferroni post-test.
Results: Diabetic rats developed hyperglycemia, glycosuria and increased plasma fructosamine; insulin treatment improved these parameters. Diabetes reduced ($P<0.01$) by 60% and 40% the Slc2a4 mRNA and GLUT4 protein; insulin treatment was able to restore these parameters completely. In silico analysis scored 32 miRNAs, predicted by at least 3 algorithms, as potential regulators of Slc2a4/GLUT4 expression. From these, 10 miRNAs were selected for validation: miR-1, miR-29a, miR-29b, miR-31, miR-93, miR-106b, miR-133a, miR-133b, miR-199a and miR-206. Four of these miRNAs were modulated by diabetes in skeletal muscle: MiR-1 and miR-29b were upregulated by 28% and 119%, respectively ($P<0.05$ and $P<0.01$), and miR-93 and miR-199a were downregulated by 39% and 30%, respectively ($P<0.05$). Insulin treatment was able to fully restore the expression of the miR-29b, miR-93 and mir-199, but had no effect upon miR-1.
Conclusion: This study shows that diabetes represses the Slc2a4/GLUT4 expression in soleus muscle, and that may be related to enhanced miR-1 and miR-29b and repressed miR-93 and miR-199 activity. Except by miR-1 overexpression, insulin treatment was able to reverse all the diabetes-induced regulations, which can be fundamental to improve the skeletal muscle glucose uptake in diabetes.
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APPL1 promotes mechanical stretch induced glucose uptake through PKCζ-Myosin IIa pathway in C2C12 myotubes

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Background and aims: Adaptor protein, phosphotyrosine interaction, PH domain and leucine zipper containing 1 (APPL1) promotes insulin or adiponectin induced glucose uptake via protein kinase B (PKB/Akt) or 5' adenosine monophosphate-activated protein kinase (AMPK) dependent pathway. We have reported that APPL1 also promotes mechanical stretch induced glucose uptake through PKCζ dependent pathway in C2C12 myotubes, but the mechanism was unknown. Therefore, this study aimed to investigate the mechanism of PKCζ dependent pathway involved in glucose uptake stimulated by mechanical stretch.
Materials and methods: C2C12 myoblasts were grown on an elastic silicone chamber and induced differentiation. Differentiated C2C12 myotubes were stimulated by cyclic uniaxial stretch (10% of initial length, 10 cycle/min) for 5 hours. Plasmid DNA was transfected into C2C12 myoblasts by electroporation. Glucose uptake was measured by enzymatic assay and the localization of PKCζ was examined by immunofluorescence. To find the binding partner of PKCζ, PKCζ was immunoprecipitated from cell lysates and analyzed by MS/MS.
Results: Mechanical stretch statistically increased PKCζ phosphorylation by 30% and translocation to plasma membrane in APPL1 over-expressed myotubes compared to control cells. To investigate PKCζ pathway, we have tried to find PKCζ binding partner. Mass spectrometry analysis revealed non-muscle myosin IIa binds phosphorylated PKCζ by immunoprecipitation in APPL1 over-expressed cells. This interaction was confirmed by western blotting. Furthermore, blebbistain, myosin IIa ATPase inhibitor, suppressed PKCζ phosphorylation, translocation and glucose uptake to the basal level.
Conclusion: These results suggest that APPL1 promotes stretch induced glucose uptake through PKCζ-myosin IIa pathway.
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Favorable actions of Insulin-like Growth Factor Binding Protein (IGFBP)-1 and its RGD domain in insulin sensitivity and glucose regulation

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Background and aims: In prospective studies, low circulating levels of IGFBP1 predict the development of type 2 diabetes. We have previously shown that *in vivo* over expression of IGFBP1 improves insulin sensitivity and protects against atherosclerosis. IGFBP1 can impact on cellular functions via an RGD ($\alpha_5\beta_1$ integrin binding) motif independent of IGF binding. However, whether IGFBP1 is causally implicated in insulin sensitivity and glucose regulation and could be exploited therapeutically remains unexplored.
Materials and methods: C2C12 myotubes, 3 T3 adipocytes, HepG2 hepatocytes and INS823/13 pancreatic β-cells, were used to investigate the effects of acute treatment of rIGFBP1 and the synthetic hexapeptide GRGDTP (which binds $\alpha_5\beta_1$-integrin) on the insulin sensitivity and glucose regulation, through several complimentary *in vitro* assays. To examine whether the RGD motif of IGFBP1 is mechanistically implicated in glucose regulation, we administered GRGDTP peptide to both C57BL/6 mice with diet-induced obesity and KKAy diabetic Mice.
Results: Treatment of C2C12 myotubes with rIGFBP1 or GRGDTP, enhanced insulin stimulated IRS1, AKT phosphorylation and insulin stimulated glucose uptake ($P\leq0.05$, ≤0.02 and $P\leq0.01$ respectively). Enhanced insulin sensitivity is mediated through integrin engagement and focal adhesion kinase. Co-incubation with glucose and IGFBP1 or GRGDTP, increased glucose stimulated insulin secretion in INS823/13 pancreatic β-cells ($P\leq0.01$ and ≤0.05 respectively) and is dependent upon integrin linked kinase. Acute administration of GRGDTP significantly improved glucose clearance and insulin sensitivity in mice with diet-induced obesity and KKAy diabetic mice ($P\leq0.05$). Long term administration of GRGDTP had no detrimental effect.
Conclusion: We have shown that the RGD integrin-binding domain of IGFBP1 may play an important role in insulin sensitivity and glucose regulation and representsa potential novel therapeutic agent in the field of type 2 diabetes that requires further investigation.
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PS 046 Novel biomarkers

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Transcriptome profiling in lympho-monocytes of healthy subjects identifies a biomarker of early insulin resistance with potential implications in chronic heart failure

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Background and aims: Gene expression profile (GEP) of peripheral blood mononuclear cells (PBMCs) is significantly affected by various diseases and may serve as biomarker of pathological changes occurring in other tissues. The aim of our study was to investigate whether insulin resistance (IR) per se is characterized by a specific pattern of GEP.
Materials and methods: 150 healthy young adults, were recruited in a cardiovascular risk assessment study (Multi-Knowledge project).Two groups of 10 subjects with extreme HOMAIR indices (High HOMA:4.0±2.8; Low HOMA:0.5±0.1), but with normal, similar BMI (22.2±2.6 vs 22.5±2.2 kg/m^2), and comparable age (33.9±6.2 vs 40.2± 11.3 years) and gender (7 M and 3 F in both groups) were selected. Standard anthropometric, hemodynamic, and metabolic variables were assessed. GEP of PBMCs from all subjects was evaluated using Agilent whole genome oligonucleotide mRNA microarrays. Data were analyzed with a novel ad-hoc rank-based classification method, optimized to extract a gene expression signature with highest power and statistical significance (p at least<0.005) to discriminate the two groups. Gene enrichment analysis was applied on the significant differentially expressed list of genes. All analyses were performed with R and Matlab.
Results: We identified a set of 512 probes corresponding to 321 annotated genes which summarize the characteristics of each sample and allow distinguishing Low HOMA and High HOMA subjects with an accuracy of 100%, according to a 5-fold cross-validation approach. Gene enrichment analysis of the 321 genes showed significant enrichment for the KEGG pathway "Adrenergic signaling in cardiomyocytes", in which differentially expressed genes were compatible with a cluster of alterations, including increased intracellular cAMP and Ca2+ and accelerated apoptosis in the High HOMA group. We hypothesized that this pathway should biomark chronic heart failure (CHF), of which IR is a known risk factor. In two publicly available gene expression data sets (GSE21125 and GSE9128) we investigated whether the genes in the pathway of interest could differentiate between patients with CHF and controls. Two biomarkers (length 41 and 22, respectively) were found with high discriminant accuracy (95%), at a significant p value (p<0.02), overlapping with the "adrenergic signaling in cardiomyocytes" pathway.
Conclusion: GEP of the "adrenergic signaling pathway in cardiomyocytes" in lymphomocytes is a fingerprint of IR and is implicated also as a biomarker of patients with CHF. This may be a major molecular platform linking IR to CHF.
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Mitochondrial respiratory capacity of peripheral blood mononuclear cells associates with insulin resistance

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Background and aims: Subclinical inflammation contributes to the development of insulin resistance. In adipose tissue, infiltration by pro-inflammatory M1 macrophages has been linked to insulin resistance through release of tumor necrosis factor alpha and enhanced reactive oxygen and nitrogen intermediates. Pro-inflammatory M1 macrophages have higher non-oxidative respiration at resting stage, coupled with higher generation of reactive oxygen species. We aimed to study the association between whole-body insulin resistance and mitochondrial respiration in monocytes in circulation and systemic oxidative stress.
Materials and methods: Recently diagnosed type 2 diabetes patients (T2D) and lean individuals (CON) were recruited from the German Diabetes Center Study (GDS), and underwent thorough metabolic and blood analysis, including hyperinsulinemic-euglycemic clamps to assess whole-body insulin sensitivity (M-value). Leukocytes were isolated from fasting blood samples using density gradient centrifugation. Mitochondrial respiration was measured in isolated leukocytes using multiple substrate protocols and high-resolution respirometry. Tricarboxylic acid cycle activity was assessed by using saturating concentrations of malate, glutamate, succinate and adenosine diphosphate. Beta-oxidation was measured in response to octanoyl carnitine. Finally, maximal respiration capacity in the uncoupled state u was investigated using the uncoupler carbonyl cyanide-4-trifluoromethoxy phenylhydrazone (FCCP). Leak respiration was measured in response to ATPase inhibitor, oligomycin. The coupling efficiency of the mitochondrial complexes was assessed as the ratio of leak respiration to maximal respiration, known as the leak control ratio. Mitochondrial content was assessed by citrate synthase activity (CSA) in the leukocytes. Heparinized blood was used for the quantification of the ratio of CD11 positive M1 and CD 206 positive M2 macrophage subsets. Lipid peroxidation measured by thiobarbituric acid reactive substances assay (TBARS) was performed to assess systemic oxidative stress.
Results: Rates of oxygen consumption by leukocytes were markedly lower in patients with T2D than in CON (State u: 4.0±1 vs 8.3±1.3 pmol/s/mg protein, P<0.05). Maximal beta-oxidation was also lower in leukocytes of T2D (3.9±0.7 vs 6.2±0.6 pmol/s/mg protein, P<0.05). CSA did not differ between groups. The leak control ratio was higher in T2D indicating lower mitochondrial coupling efficiency and thus a source of reactive oxygen species (0.5±0.07 vs 0.2±0.04, P<0.05). The ratio of M1:M2 expression was also higher in leukocytes of T2D (0.2±0.01 vs 0.1±0.01, P<0.05) in line with a pro-inflammatory phenotype. TBARS in plasma was higher in T2D compared to CON (2±0.2 vs. 1±0.2 μM). Leukocyte state u respiration associated positively to whole-body insulin sensitivity (M-value: R=0.45, P<0.05) and negatively with TBARS (R=-0.49, P<0.005). Beta-oxidation also related positively to M-value (R=0.57, P<0.05) and negatively to total blood cholesterol (R=-0.47, P< 0.05).
Conclusion: Lower mitochondrial respiration in circulating leukocytes may influence pro-inflammatory polarization of leukocytes and contribute to oxidative stress, which have been related insulin resistance.
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Sclerostin and insulin resistance in prediabetes. Evidence of a cross-talk between bone and glucose metabolism

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Background and aims: Sclerostin is a peptide inhibiting Wnt/β-catenin signaling. Impaired glucose regulation (IGR), which includes impaired fasting glucose (IFG) and/or impaired glucose tolerance (IGT), is characterized by insulin resistance and altered insulin secretion, two key pathophysiological features preceding the development of type 2 diabetes. Our aims were to evaluate serum sclerostin levels in IGR subjects and to analyze its relationship with insulin-resistance and β-cell function

Materials and methods: In a cross-sectional study, 43 healthy normal glucose tolerant (NGT) individuals (12 male and 31 women; age=44.0±1.9 years; BMI=31.1±1.1 Kg/m2) and 79 individuals with impaired glucose regulation (IGR) (22 male and 57 women; age=46.0±1.4 years; BMI=31.9±1.2 Kg/m2) which included subjects with IFG (n=27), IGT (n=18) and combined IFG/IGT (n=34), underwent OGTT and DXA. Serum sclerostin levels were measured at fasting. β-cell function parameters were calculated using C-peptide deconvolution. Dynamic indexes of insulin sensitivity were calculated from OGTT. A subgroup (n=18 with NGT and n=30 with IGR) underwent to an euglycemic hyperinsulinemic clamp with 3-3H-glucose to estimate the endogenous glucose production (EGP) and the rate of insulin-mediated total body glucose disposal (TGD/SSPI)

Results: Sclerostin levels were higher in IGR as compared to NGT (50.8±2.4 vs. 38.7±2.3 pmol/l; p=0.01). IFG and combined IFG-IGT manifested higher sclerostin levels compared to NGT (55.8±2.9 and 50.8±5.1 vs. 38.7±2.3 pmol/l; p=0.02, respectively) while no difference was found between isolated IGT and NGT. In whole population serum sclerostin was correlated with HOMA-IR (r=0.62), OGIS (r=-0.38), Matsuda Index (r=-0.42), adipose tissue insulin resistance index (ATIRI) (r=0.61) and TGD/SSPI (r=-0.40) (all p<0.001) although subjects with isolated IFG were mostly accountable for the correlations with insulin sensitivity in skeletal muscle (r=0.63 HOMA-IR; r=- 0.55; Matsuda Index; r=- 0.64 OGIS; r=- 0.57 TGD/SSPI), liver (r=0.61; fasting EGP) and adipose tissue (r=0.71 ATIRI) (all p<0.05). EGP, hepatic insulin resistance and ATIRI were correlated with sclerostin levels (r=0.48 and r=0.62; r=0.61; p<0.001). Fasting and OGTT insulin clearance were correlated with sclerostin serum levels (r=-0.52 and r=-0.44; both p<0.001) but no correlations were found with β-cell function parameters. In multiple linear regression analysis, sclerostin levels improved the r2 associated to HOMA-IR (r2=0.192 to 0.247) to TGD/SSPI (r2=0.518 to 0.577). Sclerostin levels were positively correlated with fasting plasma glucose (r=0.22; p=0.01), fasting and 2-hours plasma insulin (r=0.63 and r=0.45 respectively; p<0.001) and HbA1c (r=0.18; p=0.04). IFG and combined IFG-IGT were accountable for the correlation between sclerostin levels and fasting plasma insulin (r=0.64 and r=0.52; p<0.01) and 2-hours plasma insulin (r=0.72; p<0.01 and r=0.29; p=0.08)

Conclusion: Sclerostin levels are increased in IGR individuals and correlated with insulin-resistance in skeletal muscle, liver and adipose tissue. These data suggest that sclerostin might play a role in determining insulin resistance possibly acting on insulin clearance and degradation

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Elevated circulating microRNA-143-3p is associated with insulin resistance in the metabolic syndrome

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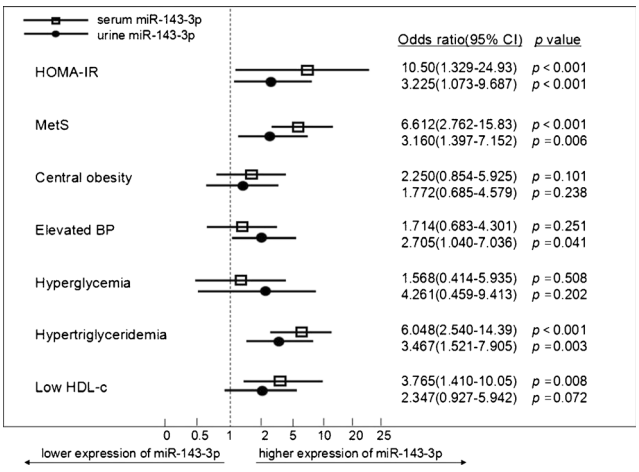
Background and aims: Metabolic syndrome (MetS) is a cluster of cardiovascular risk factors that includes obesity, diabetes, and dyslipidemia.Insulin resistance(IR) is the most accepted unifying theory explaining the pathophysiology of the metabolic syndrome.Accumulating evidence implies that circulating microRNAs (miRNAs) are involved in the regulation of MetS,but existing studies have yielded inconclusive results. Examining the associations of circulating miRNAs levels with MetS and insulin sensitivity in human may lead to improved insight.

Materials and methods: The genome-wide circulating miRNA profiles were detected via microarray in serum samples from 4 patients with MetS and 4 control without MetS. Elevated miR-143-3p was selected and validated in individual serum and urine samples from 50 MetS patients(52.0% men) and 50 control (48.0% men) subjects. Associations between circulating miR-143-3p levels and parameters related to adiposity, insulin resistance, lipid profiles and hepatic enzymes were further assessed.

Results: Eleven miRNAs were found to be expressed differently in the sera of MetS patients compared to control subjects (p<0.001). Further qRT-PCR analyses confirmed that circulating miR-143-3p was increased significantly in MetS compared with controls (p<0.001) both in the serum and urine samples. Serum and urine miR-143-3p levels were correlated with homeostasis model assessment of insulin resistance (HOMA-IR) (r=0.726,0.624), high density lipoprotein-cholesterol (r=-0.321,-0.473) and percentage of body fat(Fat%)(r=0.307,0.392,all p<0.001). After controlling for confounding factors, miR-143-3p remained an independent risk factor for insulin resistance (OR=10.500, 95% CI=1.329 - 24.93 for surem, OR=3.225, 95% CI=1.073 - 9.687 for urine,both p< 0.001).

Conclusion: Elevated circulating miR-143-3p is positively associated with MetS and insulin resistance. These findings provide a better understanding regarding the role of circulating miRNAs in insulin sensitivity.



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Bone turnover markers and prediabetes in humans

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Background and aims: Recent studies have suggested a link between bone remodeling and glucose metabolism. The aim of the study was to examine the relationship between hormones involved in bone remodeling and glucose metabolism alterations in subjects with impaired glucose regulation (IGR), which is known to lead to type 2 diabetes mellitus
Materials and methods: We performed a cross-sectional study including 43 healthy normal glucose tolerant (NGT) individuals (12 male and 31 women; age=44.0±1.9 years; BMI=31.1±1.1 Kg/m2) and 79 individuals with impaired glucose regulation (IGR) (22 male and 57 women; age=46.0±1.4 years; BMI=31.9±1.2 Kg/m2) which included subjects with IFG (n=27), IGT (n=18) and combined IFG/IGT (n=34), undergoing OGTT and DXA. Osteopontin, Osteocalcin, Osteoprotegerin and PTH were measured at fasting. β-cell function parameters were calculated using C-peptide deconvolution. Dynamic indexes of insulin sensitivity were calculated from OGTT (SI, OGIS and Stumvoll). A subgroup (n=18 with NGT and n=30 with IGR) underwent in addition to an euglycemic hyperinsulinemic clamp with 3-3H-glucose to estimate the endogenous glucose production (EGP) and the rate of insulin-mediated total body glucose disposal (TGD/SSPI)
Results: Osteopontin levels were higher in IGR as compared to NGT (5.3 ±0.5 vs. 3.3±0.2 μg/ml; p=0.008) while osteocalcin, osteoprotegerin and PTH were similar in both groups. Osteopontin levels were higher in isolated IGT as compared to isolated IFG and IFG/IGT (6.3±0.5 vs. 4.5 ±0.3 and 5.4±0.5 μg/ml; p=0.02). Osteocalcin levels were similar between IFG and NGT (8.0±0.5 vs. 8.3±0.3 ng/ml; p=0.74) but lower in isolated IGT and IFG/IGT as compared to NGT (7.2±0.3 and 5.4±0.2 vs. 8.3±0.3 ng/ml; p<0.01). Osteoprotegerin and PTH levels were similar between glucose tolerance subgroups in IGR subjects. Osteopontin levels positively correlated with HbA1c, fasting and 2 h plasma glucose and PTH (r=0.20; r=0.21; r=0.26 and r=0.26; all p<0.05). Conversely, osteocalcin levels were negatively correlated with body fat, 2 h plasma glucose, insulin (r=- 0.25; r=- 0.18; r=- 0.21, all p<0.05) and positively correlated with Stumvoll index (r=0.21; p<0.05). Osteoprotegerin levels correlated with TGD/SSPI (r=-0.29; p<0.05) and with EGP and Hepatic Insulin Resistance Index in IGR subjects (r=0.51 and r=0.43; p<0.01). PTH did not correlate with insulin sensitivity and β-cell function parameters
Conclusion: Osteopontin and osteocalcin were inversely correlated with glucose metabolism parameters. Interestingly, in IGR subjects osteoprotegerin levels were strongly associated with increased EGP and increased insulin resistance in skeletal muscle and liver. In prediabetes, hormone known to be involved in bone remodeling may directly affect glucose metabolism before overt type 2 diabetes occurs

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The adipokine Zinc-α2-Glycoprotein (ZAG) is downregulated with insulin sensitivity in polycystic ovary syndrome

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Background and aims: Zinc-alpha2-glycoprotein(ZAG) is a novel adipokine,which can act locally to influence adipocyte metabolism. Recent studies have shown that ZAG decreases in type 2 diabetes mellitus, which suggest that ZAG is likely to associate with insulin resistance(IR).Meanwhile,polycystic ovary syndrome (PCOS) characterized by androgen eccess is also linked to IR. Currently, no report has demonstrated any relationships between ZAG and IR in a relative large population of PCOS. The aim of the current study is to investigate whether ZAG correlates with IR in PCOS women.
Materials and methods: 99 PCOS women and 100 healthy age-matched control subjects were recruited. Euglycemic- hyperinsulinemic clamps (EHCs) were performed to assess their insulin sensitivity, which was expressed as M-value. Circulating ZAG was measured with ELISA kit. Plasma glucose and HbA1c were measured by the glucose oxidase method and HPLC,respectively. Plasma insulin was detected by radioimmunoassay. Plasma TC, HDL, LDL, and TG were analyzed enzymatically using an autoanalyzer. The percentage of body fat (FAT%) was determined by bioelectrical impedance. HOMA-IR=fasting insulin (FIns, mU/L)×fasting blood glucose (FBG, mmol)/22.5. The area under the curve for glucose (AUC glucose) and insulin (AUC insulin) during the OGTT was calculated geometrically using the trapezoidal rule.The ROC curves of ZAG was analyzed to investigate the predictive value of ZAG for PCOS.
Results: Circulating ZAG and M-value were much lower in PCOS than in controls (35.3±18.3 vs. 53.9±15.3 mg/L for ZAG; 5.9±2.9 vs. 10.1± 2.6 mg/kg/min for M-value, both P <0.01). Pearson correlations showed that in PCOS , ZAG correlated positively with M-value and negatively with BMI, FAT%, TG, FIns, HbA1c, HOMA-IR, AUCglucose and AUCinsulin. Multiple linear regression analysis revealed that only M-value and AUCglucose were independently related factors to ZAG in PCOS. Multivariate logistic regression analysis showed that ZAG was associated significantly with PCOS even after controlling for anthropometric variables, blood pressure, lipid profile and hormones. The ROC curve analyses revealed that the best cutoff value for ZAG to diagnose PCOS was 42.6 mg/L(sensitivity 84.0%, specificity 77.8%).
Conclusion: Circulating ZAG is reduced in PCOS and is associated with IR,which suggests that ZAG may play a role in the pathogenesis of IR in PCOS ,and even become a marker of insulin resistance that predicts outcomes and therapeutic responses to assist clinical management of PCOS.
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PS 047 In vivo metabolic studies

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Acute exercise impacts kynurenine metabolism in type 2 diabetic and healthy people

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Background and aims: Tryptophan (Trp) is an essential amino acid and a primary source for the synthesis of neurotransmitters and signalling peptides such as serotonin and kynurenine (KYN). KYN is the substrate for two different neuro-active metabolites with opposing effects: kynurenic acid (KYNA) or quinolinic acid (QUIN). Kynurenine aminotransferase (KATs) enzymes catalyse the conversion of KYN into KYNA. In contrast to KYN, KYNA does not cross the brain blood barrier. KYN entry into to the brain is associated with psychiatric disorders such as schizophrenia or depression. Reductions in circulating KYN coupled to resistance to depression. Exercise and PGC1α overexpression in skeletal muscle increases skeletal muscle KATs mRNA expression, thus enhancing conversion of KYN into KYNA in the periphery. Given that type 2 diabetic (T2D) patients are prone to depression, we determined whether resting and exercise-induced KATs expression and circulating KYN and KYNA concentration are altered compared to people with normal glucose tolerance (NGT).
Materials and methods: We recruited sedentary T2D age- and BMI-matched NGT volunteers. Subjects underwent clinical phenotyping and VO2max testing. Subjects performed a 30 minute exercise bout on a cycle ergometer at 80% of VO2max. A vastus lateralis muscle biopsies and plasma sample was collected at rest, after exercise and during recovery (3 hours post-exercise). Skeletal muscle mRNA expression of KATs and metabolism-related genes, as well as plasma measurement of Trp, KYN and KYNA were determined. Statistical analysis was performed using repeated measures two-way ANOVA with Bonferroni post-tests.
Results: Exercise increased PGC1α mRNA expression in skeletal muscle as expected. Skeletal muscle mRNA expression of KAT1 and KAT2 was reduced in T2D, while acute exercise altered KAT4 expression in both NGT and T2D subjects. Plasma concentrations of Trp and KYN were decreased and KYNA increased in response to exercise.
Conclusion: mRNA expression of KATs is reduced in skeletal muscle from T2D patients. However, KATs mRNA was not correlated with acute variations in plasma levels of KYN or KYNA. Acute exercise (30 min) reduces plasma level of Trp and KYN and increases KYNA over 3 hours, indicating that exercise directly alters KYN/KYNA balance in the circulation.
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Analysis of anti-insulin receptor antibody in two patients with rheumatoid arthritis and systemic lupus erythematosus

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Background and aims: Type B insulin resistance syndrome (IRS) proposed by Flier et al in 1976, is a rare autoimmune disease characterized by the presence of autoantibodies against the insulin receptor. The majority of the patients present marked hyperglycemia and compensated hyperinsulinemia, but some patients may present with hypoglycemia caused by an insulin-like effect of the receptor autoantibodies. A common feature of type B IRS is the co-occurrence of autoimmune disorders, such as systemic lupus erythematosus (SLE) and rheumatoid arthritis(RA). We have reported here 2 cases of novel type B IRS presented with hypoglycemia and unstable diabetes control in a patient with SLE and RA, respectively.
Materials and methods: Two Japanese woman were examined. One women have RA and the another woman have SLE. We examined an insulin receptor antibody by immunoprecipitation method.
Results: Case1. 17 years old Japanese woman had both shoulders and cubital arthralgia, butterfly rash, stomatitis, photoallergy, and proteinuria were presented, suggesting SLE with lupus nephritis (WHO classII) diagnosed by renal biopsy. Moreover, hypoglycemia (less than 60 mg/dl) were appeared with hyperinsulinemia (IRI90.7 μl/ml and CPR11.5 ng/ml). We examined an insulin receptor antibody by immunoprecipitation method. We identified the 95 kDa band which showed anti-insulin receptor β subunit antibody using patient's serum. When we treated with mycophenolate mofetil and predonisolone (30 mg/day), the hypoglycemia and pyrexia had been disappeared. Case2. 52 years old Japanese woman have been suffered with rheumatoid arthritis treated with salazosulfapyridine, methotrexate and tacrolimus. General fatigue and hyperglycemia (300 mg/dl) appeared. When intensive insulin therapy had been started, her glycemic control was poor with hyperglycemia (>400 mg/dl) and hypoglycemia (<40 mg/dl). We identified the 135 kDa band which showed anti-insulin receptor α subunit antibody. Etanercept treatment improves rheumatoid arthritis and glucose control in accodance with amelioration of insulin resistance and vanishing of hypoglycemia.
Conclusion: When hypoglycemia and unstable control of diabetes occurred in autoimmune disease such as SLE and rheumatoid arthritis, we should be suspicious of type B insulin resistance syndrome.
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Association of faecal elastase 1 with non-fasting triglycerides in type 2 diabetes

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Background and aims: Morphological alterations and moderate impairment of exocrine pancreatic function are present in patients with type 2 diabetes. Despite the fact that decreased pancreas lipase activity is a hallmark for pancreatic exocrine insufficiency, little is known about the impact of exocrine pancreatic function on plasma triglycerides. Moreover, studies specifically investigating the metabolic importance of a mild impairment of pancreatic exocrine function demonstrated as a low fecal elastase-1 (FE1) levels in patients with diabetes are lacking. We investigated the association between FE1 and non-fasting triglycerides in patients with type 2 diabetes and controls.
Materials and methods: Data from 544 patients with type 2 diabetes (age: 63±8 years) randomly selected from diabetes registers, and 544 matched controls (age, sex, practice) without diabetes were retrospectively analysed. FE1 (μg/g stool) measurements were performed centrally. Non-fasting plasma triglycerides were centrally determined. Linear regression models were fitted using FE1 as dependent and log-triglycerides as independent variable adjusting for sex, age, body mass index, alcohol consumption, serum lipase, HbA1c, and smoking.
Results: FE1 concentrations were lower (mean ± sd: 337±204 vs. 437± 216 μg/g, $p<0.05$) and plasma triglycerides were higher (geometric mean */: standard deviation factor: 2.2*/:1.9 vs. 1.6*/:1.8 mmol/l, $p<0.05$) in

patients with type 2 diabetes vs. controls, respectively. In type 2 diabetes, a 10% increase in plasma triglycerides was associated with 4.5 μg/g higher FE1 concentrations (p=0.001) after adjusting for confounders. The model in controls comprised triglycerides (+10% increase: + 4.5 μg/g FE1, p=0.005), age, heavy alcohol drinking, HbA1c, serum lipase, and current and ex-smoking. Triglyceride levels and the confounders explained about 10% of the variance in FE1 in both groups. In contrast, patients with diabetes and controls with pathological FE1 (<100 μg/g) showed an inverse relation: low FE1 levels were associated with high plasma triglycerides, but the association was statistically significant only in controls (p=0.012 for controls, n=20, and p=0.135 for patients with type 2 diabetes, n=65).

Conclusion: Non-fasting triglycerides were positively related to FE1 in both patients with type 2 diabetes and control subjects, suggesting that impairment of exocrine pancreatic function is influencing plasma triglycerides. Marked loss of exocrine pancreatic function is associated with an opposite effect, resulting in higher plasma levels of plasma triglycerides.

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Balance between innate immunity pathways (MYD88/TRIF) is associated with insulin sensitivity in muscle and adipose tissue of obese post-menopausal women

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Background and aims: During obesity, innate immune Toll like receptors (TLRs) activation in insulin dependent tissues such as adipose tissue (AT) and skeletal muscle (SM) is one of the key mechanisms involved in insulin resistance (IR) development. In fact, in obese subjects, the excess of free fatty acids (FFA) and lipopolysaccharides (LPS) at systemic level induced TLRs activation such as TLR4. TLR4 activation occurs via two pathways: - The myeloid differentiation primary response gene 88 "MYD88 pathway" which induces IR by activating nuclear factor κB (NFκB) transcription factor, inflammatory cytokines (TNFα, IL6) and inflammatory kinases. - The toll/interleukin-1-receptor-domain-containing adapter-inducing interferon β "TRIF pathways" which is less studied than "MYD88 pathway", and involved interferon β (IFNβ) production, and proteins such as oligoadenylate synthetase 2 (OAS2), the latent endoribonuclease (RNase L) and TLR3. The aim of this study was to identify the role of "TRIF pathway" in early mechanisms leading from obesity to IR in AT and SM of grade I obese women.

Materials and methods: We recruited 30 post-menopausal women (50-65 years old), with no personal or familial history of diabetes and any treatment that could interfere with IS. Among these subjects 10 were normal-weight control (CT) and 20 were grade I obese subjects. IS/IR was first assessed by HOMAIR index in order to classify grade I obese volunteers as obese insulin-sensitive OIS (n=11) or obese insulin-resistant OIR (n=9), and the accuracy of these method was then confirmed by glucose infusion rate (GIR) measurement determined by a hyperinsulinemic euglycemic clamp test. SM and AT of all volunteers were obtained respectively from biopsies in the vastus lateralis and abdominal subcutaneous adipose tissue.We analyzed tissues insulin response, inflammatory state at systemic level and innate immune MYD88 and TRIF pathways at tissues level (SM and AT).

Results: Our results show that at systemic level, there is no difference in cytokines levels between OIS and OIR. In AT, IS (measured by Phospho Akt/Akt ratio) was comparable in CT, OIS and OIR while it was significantly decreased in OIR's SM compare to CT (P=0.002) and OIS (P=0.002). The preservation of IS in AT is associated with activation of "TRIF pathway" (up- regulation of OAS2, RNase L and TLR3 mRNA level and increase in IRF3 phosphorylation). On the other hand, alteration of IS in OIR's SM is associated with "MYD88 pathway" activation. In grade I obese subjects, IR occurs only in OIR's SM. This muscular IR appears without any systemic inflammation but is associated with "MYD88 pathways activation". On the other hand, preservation of IS in AT is associated with "TRIF pathway" activation. These results show that "TRIF pathways" activation seems to have positive effects on IS.

Conclusion: Further, the balance between MYD88 and TRIF pathways seems to be important for IS regulation.

Clinical Trial Registration Number: N°:2011.01.04, ANSM: B110204-20
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The prevalence of pre-diabetes, insulin sensitivity indices and glucose levels are increased in patients with autoimmune thyroiditis

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Background and aims: The prevalence of thyroid autoimmunity in general population (positive tests for thyroglobulin antibodies (TgAb) and/ or thyroperoxidase antibodies (TPOAb) is increased up to 14.8% in men and 23.4% in women without palpable goiter. The prevalence of pre-diabetes is also increased and it is estimated up to 14.6%.The aim of the study was to investigate whether there is an association between thyroid autoimmunity and β-cell secretion in patients with impaired fasting glucose (IFG) and/ or impaired glucose tolerance (IGT).

Materials and methods: 609 patients (457 females) 46.8±14.5 years studied for pre-diabetes (defined as IGT either IFG or HbA1c≥5.7%) and autoimmune thyroiditis (AIT, TgAb and/or TPOAb positive) were included in the study. Characteristics recorded were age, gender, waist circumference (cm), BMI (Kg/m2). Fasting glucose (mg/dl) and insulin levels (μIU/ml), glucose 120 min post-oral glucose tolerance test (OGTT), HbA1c (%), HOMA and QUICKI insulin-resistance (IR) indices, and IR status as HOMA >2.16 and QUICKI <0.34 were also assessed. In 291 patients who had an oral glucose tolerance test (OGTT) with glucose and insulin measurement in five different time points, first, second phase insulin sensitivity index (ISI) and ISI were also assessed. Patients with hypothyroidism and DM2 were excluded from the study.

Results: Patients (n=311) who had AIT compared to non-AIT patients (n=293) were older [49.2±13.9 vs. 44.2±14.7 (p<0.001)], had larger waist [96±15.3 vs. 91.7±15.3 (p=0.002)], higher glucose levels [99.6±12 vs. 95.6±12.2 (p<0.001)], and higher pre-diabetes rates (71.2%) (chi-square=21.5, p<0.001) but similar rates of IR (44.6%). Pre-diabetic (n=221) patients with AIT had first phase ISI (1470.9±458.2), and ISI (0.1±0.03) higher to non-AIT (first phase ISI:1262.8±596.4, p<0.001; ISI:0.09±0.05, p<0.001).

Conclusion: Patients with AIT and impaired β-cell secretion had higher HbA1c levels with similar rate of insulin resistance and higher prevalence of pre-diabetes compared to non-AIT. Thyroid autoimmunity could be eventually a possible factor modifying β-cell secretion. Nevertheless further studies are needed to confirm these findings.

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Insulin sensitivity with reactive hypoglycaemia: a metabolic subtype of polycystic ovary syndrome with reduced risks of type 2 diabetes and cardiovascular disease

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Background and aims: Polycystic Ovary Syndrome (PCOS) is associated with insulin resistance (IR) and is a risk factor for type 2 diabetes (T2D) and cardiovascular disease (CVD). However, Reactive Hypoglycaemia (RH) in PCOS has received little attention. This study aims to identify the frequency of RH in PCOS, its potential mechanisms and clinical significance.
Materials and methods: 48 women with PCOS (Rotterdam criteria) and 53 BMI-matched controls underwent a 6-point, 2-hour 75 g oral glucose tolerance test to identify beta cell function (computer modelling from glucose, insulin and C-peptide data) and Insulin Sensitivity Index (ISI) (Matsuda method). Central adiposity was measured by bioimpedance and anthropometry. Biochemical RH was defined as 2-hour glucose less than fasting.
Results: (1) There were no signjificant differences between PCOS and controls in beta cell glucose sensitivity (BCGS) (103.8 [69.1] vs 105.1 [54.9]pmol/min/mL/mmol; p=0.91) or ISI (0.94 [0.57] vs 1.17 [0.77]; p=0.11) but ISI was strongly associated with adiposity (BMI categories <24.9, 25-29.9, 30-34.9, >35 kg/m^2; PCOS and controls, ANOVA both p< 0.001). (2) RH was common in both PCOS (37%) and controls (52%) (Chi-square; p=0.48). Compared to women without RH, those with RH had lower increases in insulin levels from 0-120 mins (PCOS 171.6 [28.5-243.8] vs 340.6 [228.8-429.5] pmol/l; p=0.005: Controls 66.7 [8.7-122.1] vs 250.8 [165.8-504.4] pmol/l; p<0.001), and controls with RH also had higher ISI (1.68 [0.98] vs 0.89 [0.46]); (p<0.001). (3) In women with PCOS, glucose at 120 mins correlated with waist circumference (r=0.40, p=0.008), triglycerides (r=0.33, p=0.03), systolic (r=0.32, p=0.03) and diastolic blood pressure (r=0.35, p=0.02) and C-reactive protein (r=0.31, p=0.04), but these relationships were not present in controls. (4) Women with PCOS and poor insulin secretion (lowest quartile of BCGS), low waist circumference (r=0.81, p=0.001), low visceral fat (r=0.82, p=0.001) and high ISI (r=-0.60, p=0.01) were associated with lower 2 hour glucose levels. Women with PCOS in the lowest waist tertile (<87 cm) also had significantly lower 2 hour glucose (5.29 [1.07] mmol/l) vs) than those in the middle and upper tertiles (6.12 [1.85] mmol/l) (p=0.005). Finally, (5) women with PCOS who self-reported regular hypoglycaemic symptoms such as shakiness had higher ISI (1.14 [0.63] vs 0.77 [0.46]; p=0.03).
Conclusion: These new findings identify a common subtype of PCOS in which lower visceral fat and increased insulin sensitivity are associated with normal blood glucose despite poor insulin secretion. Waist circumference less than 87 cm and 2 hour glucose less than baseline, sometimes accompanied by symptoms of RH, identifies women with PCOS who may have lower risks of future T2D and CVD. These findings have significant implications for patient education, screening and prevention.
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Metabolic flexibility and oxidative capacity independently predict insulin sensitivity in newly diagnosed patients with type 2 diabetes

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Background and aims: Inherited and acquired insulin resistance have been related to abnormal muscle mitochondrial function. Mitochondrial function should be reflected by maximal oxygen uptake (VO2max) and metabolic flexibility, defined as the difference between fasting and insulin-stimulated respiratory quotient (ΔRQ). However it is unclear, whether impaired ΔRQ results from impaired mitochondrial function or decreased glucose uptake due to insulin resistance. We hypothesized that VO2max and ΔRQ correlate with each other and with insulin sensitivity and that gender, genetic variations associated with higher insulin sensitivity or VO2max in peroxisome proliferator-activated receptors and co-factors (PPARγ, PPARδ, PGC-1α), NADH dehydrogenase 1 ß subcomplex 6 (NDUFB6) or ß-adrenergic receptor (ADRB) genes, glycemia or lipidemia would mediate such relationships.
Materials and methods: A total of 121 patients (85 males, 36 females), all participants of the GDS, within the first year of type 2 diabetes (T2D) diagnosis were included. The patients underwent comprehensive phenotyping including hyperinsulinemic-euglycemic clamps, indirect calorimetry and cycling spiroergometry as well as genotyping for PPARγ, NDUFB6, PGC-1α, PPARδ and ADRB2 genes.
Results: Males and females had comparable age (51±10 vs 52±11 years) but different body mass (BMI; 30.3±5.5 vs 33.8±7.6 kg.m-2, p=0.01) and VO2max (29±5 vs 27±4 ml.min-1.(kg fat free mass)-1, p=0.05). Insulin sensitivity (M: 9.7(7.3,12.2) vs 9.7(7.1, 11.9) mg.(kg fat free mass)-1.min-1) and ΔRQ (0.11±0.06 vs 0.11±0.05, p=0.99) were nearly identical between males and females. ΔRQ and VO2max did not correlate with each other, but both parameters associated with insulin sensitivity in both males and females (ΔRQ: males: β=1.91, p=0.002, females: β=2.46, p=0.014, VO2max: males: β=0.04, p<0.0001, β=0.04 females: p=0.02). When we examined also the effect of body mass index, age, fat mass and waist-to-hip ratio performing multiple regression analysis, ΔRQ and VO2max were remained independent predictors of insulin sensitivity in male (β=1.56, p=0.004 and β=0.02, p=0.006 respectively), but not in female participants. There was no association of the examined parameters with glycemic control, free fatty acids or tested gene variations.
Conclusion: The absence of a relationship between VO2max and ΔRQ despite their independent association with insulin sensitivity suggest that metabolic flexibility and oxidative capacity are different features of muscle mitochondrial function. Moreover, neither glycemia and lipidemia nor variants in genes related to oxidative metabolism affected the relationship between VO2max and ΔRQ in T2D patients.
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Excess in haemoglobin glycation and glucose variability in obese patients without known dysglycaemia

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Background and aims: We previously showed that, in obese patients without known dysglycemia, age was a determinant of hemoglobin glycation. A mathematical relation has been proposed between mean

glucose given by continuous glucose monitoring (CGM) and HbA1c levels. This study aimed to explore in overweight or obese patients the difference between the measured HbA1c level and HbA1c level predicted by this relation (ΔHbA1c), and the factors associated with this difference.
Materials and methods: We included 70 patients (46.5±14.3 years, BMI 35.2±6.8 kg/m2) without known dysglycemia. CGM was performed during 24 hours. Mean glucose and mean amplitude glycemic excursion (MAGE) were calculated. Predicted HbA1c level was calculated using the following formula (ADAG): mean glucose=1.649×HbA1c-2.645, and the difference between measured and predicted HbA1c was calculated (ΔHbA1c). An OGTT was performed.
Results: Measured HbA1c was 5.1% to 7.4%. According to OGTT, 24 patients had prediabetes (fasting hyperglycemia and/or glucose intolerance) and 13 had diabetes whereas 49 patients had an HbA1c ≥5.7%. The population was separated in quartiles of measured HbA1c (Q1 to Q4). For every quartile, measured HbA1c was higher than predicted value of HbA1c ($p<0.01$ to <0.0001), and ΔHbA1c increased significantly from Q1 to Q4 (0.60±0.23/0.77±0.34/0.85±0.25/1.13±0.31%; $p<0.008$). In the overall population, ΔHbA1c correlated with age ($r=0.536$, $p<0.0001$) and MAGE ($r=0.494$, $p<0.0001$). In multivariate analysis, ΔHbA1c remained associated independently with age, MAGE and HbA1c quartiles ($p=0.002$, 0.017, 0.006; adjusted r2 for the model=0.43).
Conclusion: In obese patients with dysglycemia detected on OGTT in half of them, measured HbA1c is higher than HbA1c predicted by mean glucose given by CGM. This possible excess in hemoglobin glycation is noticeably determined by 24-hour glucose variability in addition to age and higher measured HbA1c. This may partly explain why the sensitivity of HbA1c is greater than OGTT to detect dysglycemia in this population.

PS 048 Food intake and weight regulation

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Body composition, insulin resistance, and the effectiveness of orlistat versus metformin added to dietary treatment in obese premenopausal women

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Background and aims: Clinical studies have determined a strong correlation between insulin resistance and the amount of total and intraabdominal fat. We aimed to evaluate the effects of treatment with metformin and orlistat on body composition with a focus on glucose-insulin homeostasis in obese premenopausal women.
Materials and methods: 73 obese premenopausal women aged 31.9± 9.4 years were randomized to open label treatments: diet + metformin 1000 mg/d (n=37); or diet + orlistat 360 mg/d (n=36). Anthropometric measurements, dual-energy X-ray absorptiometry, oral glucose tolerance test, insulin resistance by the homeostasis model assessment (HOMA-IR), and insulin sensitivity by the Matsuda Index (ISI) were evaluated at the baseline and after 3 months.
Results: Treatment with orlistat was superior to metformin regarding weight loss (-9.4±2.3 vs. -4.9±1.3 kg), fat mass (-5.41±3.02 vs. -3.49 ±0.68 kg) , as well as an increase in the lean body mass (2.39±1.87 vs. 0.09±1.10 kg), improving the body composition. Similar reductions in the % of android and gynoid fat were achieved in both groups, with the greater decreases in android (-0.10±0.06 vs. -0.69±0.19 kg) and trunk fat (-3.83±0.73 vs. -0.72±0.68 kg), seen in the metformin group. Orlistat and metformin similarly improved the ISI (18.6±10.3 vs. 17.5±47.4) and postchallenge insulin (-19.0±50.5 vs. -18.8±40.2 nmol/L). High initial postload insulin and low ISI - though not HOMA-IR - correspond with reductions in total fat, trunk fat, waist in both groups as well as with the decrease in android fat in metformin group (table).
Conclusion: Orlistat treatment resulted in a greater weight loss and improvement in body composition, metformin - in a additional visceral fat reduction. Orlistat and metformin produced a comparable improvement in insulin/glucose homeostasis. High initial postload insulin and low ISI predict greater effects of treatment.

	Metformin group			Orlistat group		
3-month change	Log 120-insulin	Log ISI	Log HOMA-IR	Log 120-insulin	Log ISI	Log HOMA-IR
Waist cm	-0.40	0.38	-0.30	-0.51	0.40	NS
Fat %	-0.37	0.56	NS	-0.33	0.40	NS
Fat kg	-0.38	0.42	NS	-0.29	0.39	NS
Android fat kg	-0.38	0.31	-0.40	NS	0.30	NS
Android /gynoid fat	-0.40	0.35	-0.42	NS	NS	NS
Trunk fat kg	-0.40	0.47	-0.30	-0.30	0.31	-0.34

Table. Univariate regressions between 3-month changes in adiposity, and initial 120 OGTT insulin, ISI Matsuda, and HOMA-IR indices in both examined groups.

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The effect of laparoscopic greater curvature plication and Roux-en-Y gastric by-pass on metabolic control in obese subjects with type 2 diabetes mellitus: a 2-year follow-up

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Background and aims: Bariatric surgery is considered one of the most effective options in the treatment of obesity and its co-morbidities, including type 2 diabetes mellitus (T2DM). The aim of our study was to assess the effect of laparoscopic greater curvature plication (LGCP) and Roux-en-Y gastric by-pass (RYGB) on antropometric and biochemical parameters and metabolic control in obese subjects with T2DM.
Materials and methods: Forty-four subjects undergoing LGCP and 8 patients scheduled for RYGB were included into the study. Anthropometric, biochemical and hormonal examinations were performed before and 1, 6, 12 and 24 months after the procedure. In a selected subgroup of LGCP patients the postprandial incretin effect was assessed using a 2-hour liquid meal test (LMT - Fresubin Original 200 ml, Fresenius Kabi, Germany).
Results: Both LGCP and RYBP lead to a significant decrease in body weight that was sustained throughout the whole 24 months (BMI 42.4± 0.9 vs. 37.1±1.8 kg/m2, $p<0.05$ for LGCP and 50.3±4.2 vs. 37.1± 3.6 kg/m2, $p<0.05$ for RYGB). The same was true for glucose control (HbA1c 51.9±2.6 vs. 44.7±3.2 mmol/mol, $p<0.05$ for LGCP and 54.0± 7.7 vs. 41.7±4.7 mmol/mol, $p<0.05$ for RYGB). Both procedures also partially improved the lipid profile (HDL cholesterol 1.17±0.04 vs. 1.27 ±0.07 mmol/l, $p<0.05$ for LGCP and 1.15±0.09 vs. 1.45±0.16 mmol/l, $p<0.05$ for RYGB). Additionally, LGCP was accompanied by a reduction in systemic low-grade inflammation (hsCRP 3.2±0.6 vs. 1.3±0.3 mg/l, $p<0.05$). The incretin effect was temporarily restored 1 month after LGCP (GLP-1 at min 30 of LMT: 10.37±3.77 vs. 26.48±7.70 pM, $p<0.05$).
Conclusion: Laparoscopic greater curvature plication as well as Roux-en-Y gastric by-pass rapidly and sustainably reduce body weight and improve glucose control during the first 24 months after surgery. Partial restoration of the incretin effect might play a role in these processes.
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Insulin sensitivity indices derived from oral glucose tolerance tests: Are they valid after Roux-en-Y gastric bypass?

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Background and aims: Insulin sensitivity indices obtained from oral glucose tolerance tests (OGTTs) are frequently used in diabetes research and provide an easily obtained surrogate measure of insulin sensitivity. Roux-en-Y gastric bypass (RYGB) induces extensive weight loss and improvements in insulin sensitivity when evaluated by the hyperinsulinaemic clamp. Insulin sensitivity indices obtained from OGTTs have not been validated after RYGB and may be inaccurate due to surgical alterations of the gut resulting in accelerated glucose absorption and increased insulin secretion.
Materials and methods: We investigated insulin sensitivity in 20 obese subjects (10 with type 2 diabetes and 10 with normal glucose tolerance) before, 3 months (mo) and 1 year (y) after RYGB using a 4 h hyperinsulinaemic (40 mU/m2/min) euglycaemic (5.5 mmol/L) clamp combined with [6,6-2H2]-glucose tracer infusion as well as a 2 h 75 g OGTT. Fat free mass (ffm) was assessed by DEXA. Matsuda, Gutt and Oral Glucose Insulin Sensitivity (OGIS) indices were calculated from OGTTs and compared to the clamp-derived Rate of disappearance (Rd) of [6,6-2H2]-glucose (in mg/min/kg ffm). Pearson test was used to evaluate correlations.
Results: Before RYGB, the three OGTT-based insulin sensitivity indices correlated significantly with Rd (Matsuda vs Rd: $r=0.63$, $p<0.001$, Gutt vs Rd: $r=0.56$ $p=0.012$ and OGIS vs Rd: $r=0.62$, $p=0.005$). After RYGB, the relative improvements in Matsuda and Gutt were substantially larger than improvements in Rd at 3 months (Rd: +36±13%, Matsuda: +86±17% $p<0.05$, Gutt: +91±9.7% $p<0.01$) and 1 year (Rd: +57±14%, Matsuda: +164±28% $p<0.01$, Gutt: +145±18% $p<0.01$). Furthermore, the relative changes in Matsuda and Gutt did not correlate significantly to the relative changes in Rd (%Matsuda vs %Rd: 3 mo: $r=0.23$, $p=0.35$, 1y: $r=0.46$, $p=0.07$; %Gutt vs %Rd: 3 mo: $r=0.35$, $p=0.16$, 1 y: $r=0.46$, $p=0.06$). In contrast, the relative changes in OGIS were comparable to the relative changes in Rd both at 3 mo (Rd +36±13%, OGIS +31±5.9%, $p=0.34$) and 1 year (Rd: +57±14%, OGIS: +46±5.9%, $p=0.36$) and correlations between the relative change in OGIS and Rd were significant (%OGIS vs %Rd: 3 mo: $r=0.61$, $p=0.007$; 1 y: $r=0.50$, $p=0.04$).
Conclusion: Insulin sensitivity indices obtained from oral tests may be inaccurate after RYGB. Compared to the gold standard hyperinsulinaemic euglycaemic clamp, OGIS seems to perform better than Matsuda and Gutt indices in assessing changes in insulin sensitivity within the first year after RYGB.
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Metabolic improvements observed in subjects receiving EndoBarrier: a pooled analysis of clinical trials

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Background and aims: EndoBarrier therapy (EBT) involves the reversible endoscopic placement of an impermeable liner that anchors in the duodenum and bypasses the first two feet of the upper bowel, thus replicating features of bariatric surgery.
Materials and methods: EBT in obese subjects with≥1 comorbidity (diabetes, hypertension, hyperlipidemia) or morbidly obese with or without comorbidities from 6 clinical trials (n=211, age 46 yrs, BMI 38.9 kg/m2) was evaluated.
Results: Significant metabolic improvements were observed with EBT (Table). At 12 months, 57% of subjects achieved ≥10% weight loss while 55% of those with T2DM reached an A1C of ≤7%. The improvement in glycemic control occurred despite a shift to decreased concomitant anti-diabetic medication use. Moreover, 37% of T2DM subjects achieved both a ≥0.5% A1C reduction and ≥10% weight loss at 12 months.Reported AEs were mild (event rate 85.0%) or moderate (event rate 14.5%) in severity and mostly GI in nature. Hypoglycemia was observed in patients on insulin or sulfonylurea, but no severe hypoglycemia occurred. EBT did not induce hypoglycemia in non-diabetic subjects, suggesting no innate hypoglycemia potential. Early device removal was due to anchor migration, GI distress, liner obstruction, or GI bleed; all resolved w/o permanent sequelae. SAEs were reported infrequently at a rate of 3%.
Conclusion: EBT elicits metabolic benefits, with an acceptable safety profile, in obese subjects with comorbidities.

Table. Mean Metabolic Changes from Baseline			
	Baseline	Month 12[a]	Absolute Change
Total Weight, kg	108.1 ± 21.5	96.1 ± 19.9	−11.8 ± 8.6[b]
A1C in T2DM, %	8.4 ± 1.2	7.2 ± 1.2	−1.2 ± 1.3[b]
Lipid Panel			
Total cholesterol, mmol/L	4.8 ± 1.1	4.2 ± 0.9	−0.6 ± 0.8[b]
LDL, mmol/L	2.8 ± 0.9	2.4 ± 0.8	−0.4 ± 0.7[b]
HDL, mmol/L	1.2 ± 0.3	1.1 ± 0.3	−0.1 ± 0.2
Triglycerides, mmol/L	2.2 ± 1.8	1.7 ± 1.1	−0.4 ± 1.0[b]
Blood pressure			
Systolic, mmHg	138.1 ± 16.7	128.4 ± 15.7	−9.2 ± 17.5[b]
Diastolic, mmHg	83.5 ± 9.7	77.4 ± 11.0	−5.8 ± 11.9[b]

Data presented as mean ± standard deviation; [a] last observation carried forward (LOCF) if data not available at 12 months; [b] p-value ≤0.05, baseline vs. Month 12 or LOCF.

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Associations of glucagon-like peptide 1 with food processing in the brain are present in lean and obese humans

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Background and aims: Glucagon-like peptide 1 (GLP-1) is a peptide hormone that is released into the bloodstream after food intake. In addition to stimulating insulin release, it introduces satiety and contributes to the termination of food intake as indicated by research in animals and exogenous administration in humans. Here, we investigated whether endogenous GLP-1 associates with food-related brain activity and hunger.
Materials and methods: Twenty-four human volunteers underwent a 75 g oral glucose tolerance test that promotes GLP-1 secretion as part of another study. Food-cue induced brain activity was assessed by functional magnetic resonance imaging (fMRI) and GLP-1 concentrations were measured before and after glucose intake.
Results: The significant increase in GLP-1 levels negatively correlated with change in food-cue induced brain activity in the orbitofrontal cortex, a major reward area. This association was independent of concurrent alterations in insulin and glucose concentrations. Furthermore, GLP-1 was negatively correlated with hunger ratings. Both associations were present in lean and overweight participants. In contrast, postprandial insulin associated with orbitofrontal response only in lean individuals.
Conclusion: The postprandial release of GLP-1 alters reward processes in the orbitofrontal cortex and might thereby support the termination of food intake and reduce hunger. While obese persons showed brain insulin resistance, no GLP-1 resistance was observed. Our study provides novel insights in the central regulation of food intake by the incretin hormone GLP-1.
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Luminal trans,trans-2,4-decadienal reduces gastric emptying in rats

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Background and aims: Postprandial glycemia and lipidemia are strongly affected by the rate of gastric emptying, and slowing of gastric emptying contributes to reducing postprandial hyperglycemia and hyperlipidemia. Gut hormones including cholecystokin (CCK) are major endogenous factors for reducing postprandial gastric emptying. We recently demonstrated that unsaturated aldehydes stimulated CCK secretion from murine enteroendocrine cell line, STC-1. Among various aldehydes tested, *trans,trans*-2,4-decadienal (decadienal) had a potent CCK-releasing activity, however, its effect *in vivo* has not been studied. In the present study, we investigated whether oral administration of decadienal affects the rate of gastric emptying (one of CCK-related physiological effects) in rats, by using the acetaminophen absorption test and the phenol red recovery method.
Materials and methods: In fasted Male Sprague-Dawley rats, test liquids containing acetaminophen with or without decadienal (50-100 mg/kg) were orally administered. Tail vein blood was collected with time until 120 min, and plasma acetaminophen concentration was measured. In separated experiments, decadienal was orally administered together with phenol red. Luminal contents in the stomach, the proximal and the distal small intestine were respectively collected 15 min after the administration. Amounts of luminal phenol red were measured and the rate of gastric emptying was calculated. By using the methods above, we compared various aliphatic compounds having the same chain length (C10) with decadienal, and further investigated the effect of an intraperitoneal administration of decadienal, on the rate of gastric emptying.
Results: Oral administration of decadienal dose-dependently reduced the appearance of acetaminophen in the peripheral blood. Luminal phenol red recovered from the stomach was greater in decadienal-treated rats than control rats, which resulted in lower gastric emptying rate in decadienal-treated rats (13%) compared to control rats (57%) ($n=7$, $P<0.01$). This was reflected by smaller amount of phenol red collected from the small intestine in decadienal-treated rats compared to control rats. Oral administrations of aliphatic compounds (decanal, decanol and decanoic acid) having the same chain length with decadienal, and an intraperitoneal administration of decadienal did not reduce the rate of gastric emptying, respectively.
Conclusion: These results demonstrate that orally administered decadienal has inhibitory effect on gastric emptying through acting in the intestinal lumen, possibly on enteroendocrine cells.

PS 049 Animal models of diabesity

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Mice on high fat diet or normal diet have different metabolic responses to rosuvastatin treatment

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Background and aims: Rosuvastatin (Ros) is a member of the cholesterol lowering statin family prescribed to reduce the risk of cardiovascular disease. The beneficial effects of statin treatment are well documented. However, several reports have also indicated diabetogenic effects of statins. The underlying mechanisms are poorly understood. Here we aim to investigate the effects of rosuvastatin on glucose homeostasis and insulin secretion in mice on high fat diet (HFD) or normal diet (ND).
Materials and methods: C57BL/6 mice were put on ND or HFD for 12 weeks. 4 weeks into the experiment a subset of the mice were given rosuvastatin (Ros) in the drinking water (0.2 mg/mice/day; 15 mice per group). Every 4 weeks the mice were subjected to an oral glucose tolerance test (OGTT) and samples were taken for *in vivo* measurements of glucose and insulin. In agreement with earlier studies HFD mice had higher blood glucose levels than ND mice and comparison was performed ND *vs* ND+Ros and HFD *vs* HFD+Ros. Every second week it was verified that Ros treatment resulted in lowered blood cholesterol levels. At the termination of the experiment tissues were collected for *in vitro* experiments. Insulin secretion was measured with RIA. Exocytosis was recorded as changes in membrane capacitance using the patch clamp technique. Intracellular calcium was monitored with fura-2.
Results: After 4 weeks of Ros treatment the OGTT revealed a glucose lowering effect of Ros in ND mice (delta15 min(glucose) was reduced ~50%; $p \leq 0.01$ and total AUC(glucose) was reduced ~20%; $p \leq 0.05$; N= 14 ND; N=15 ND+Ros), but this was not accompanied by any changes in insulin response. In the HFD mice, that already had high blood glucose compared to the ND mice, total AUC or delta15 min for glucose was not altered after 4 weeks or 8 weeks of Ros treatment. However, acute insulin response during the OGTT was significantly decreased after Ros treatment for 8 weeks in the HFD group (delta15 min (insulin) was reduced ~65%; $p \leq 0.01$; n=15 HFD; n=13 HFD+Ros). On a cellular level, Ros treated mice, regardless of diet, had a ~20% ($p \leq 0.01$; n=24) lower insulin content. *In vitro* insulin secretion at 16.7 mM glucose was reduced by ~40% (n=4; $p \leq 0.05$) with Ros treatment in the ND mice. However, the insulin release relative to insulin content was not changed. No significant difference in insulin secretion by Ros from the HFD islets was detected. The exocytotic response from single beta-cells did not differ between the ND+Ros and ND (n=7). However, elevating glucose concentrations from 2.8 mM to 16.7 mM in isolated ND islets resulted in a delayed increase in intracellular calcium in the Ros treated group compared to ND alone. In addition, the dip in intracellular calcium preceding the increase in calcium was less prominent ($p<0.001$; N=3) in ND+Ros islets, indicating a reduced uptake of calcium into the ER.
Conclusion: The integrated physiological response to glucose is intact after 8 weeks of rosuvastatin treatment. Interestingly, the acute insulin response to glucose in HFD mice is more vulnerable to rosuvastatin treatment than it is in ND mice. Taken together, our data suggests that the metabolic state of the individual could influence the effects of rosuvastatin treatment on glucose homeostasis. On a cellular level rosuvastatin reduced insulin content and disturbed calcium handling in the islets, which may perturb insulin secretion in the long term.
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Adverse effect of ovariectomy on substrate utilisation in brown adipose tissue and on liver dicarbonyl stress in rats

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Background and aims: Ovariectomy leads to increase in body weight, visceral adiposity, insulin resistance, fatty liver and other disorders of lipid and glucose metabolism. However, the underlying mechanism of these disorders remains unclear. Increasing evidence indicates that brown adipose tissue (BAT) modulates energy balance. It also suggests that increased accumulation of dicarbonyls in the liver may increase oxidative stress, activate the inflammatory pathway and can play a key role in the development of other metabolic disorders. In this study, we examined the effect of ovariectomy on the utilization of energetic substrate in interscapular brown adipose tissue and liver dicarbonyl and oxidative stress.
Materials and methods: Female Wistar rats were ovariectomized (OVX) or sham-operated (controls) at 8 weeks of age and fed a standard diet for 4 month, in order to fully develop disorders associated with ovariectomy. BAT activity was determined *ex vivo* according to the utilization of ^{14}C-U-palmitic acid and ^{14}C-U-glucose for oxidation (incorporation into CO_2) and incorporation of ^{14}C-U-palmitic acid and ^{14}C-U-glucose into BAT lipids. Lipolysis in BAT was measured by the release of free fatty acids. The concentrations of methylglyoxal and glutathione in the liver were determined using the HPLC-method with fluorescence detection.
Results: Compared with sham-operated control rats, OVX rats exhibited markedly increased body weight (+10%, $p<0.05$) and visceral adipose tissue weight (+10%, $p<0.05$). Insulin sensitivity of white visceral adipose and muscle tissue, measured according to ^{14}C-U-glucose incorporation into lipids and glycogen, were significantly decreased (p 0.01) in OVX rats compared to controls. However, plasma glucose, insulin and triglycerides were not different between OVX and control rats. Severe hepatic triglycerides accumulation (12.76±1.10 vs 5.44±0.85 μmol/g, p <0.001) in OVX rats associated with significantly elevated level of methylglyoxal (12.78±2.7 vs 5.49±0.41 nmol/mg, p 0.01) may have contributed to the development of liver steatosis. Ovariectomy led to impaired balance in the reduced and oxidised forms of glutathione in the liver (GSH/GSSG: 15.4±1.3 vs 28.4±1.4, p 0.01), which may have increased oxidative and carbonyl stress. BAT of OVX rats exhibited a reduced fatty acid oxidation (-72%, $p<0.05$), lipogenesis (-26%, $p<0.05$) and decreased lipolysis (-25%, $p<0.05$), despite the fact that BAT weight was significantly increased ($p<0.05$) in OVX rats compared to controls.
Conclusion: Results indicate that reduced fatty acid and glucose utilization, together with lower lipolysis in brown adipose tissue in response to ovariectomy, may contribute to the development of obesity in the postmenopausal period. Severe liver steatosis induced by ovariectomy was associated with excessive formation of methylglyoxal and the reduced ratio of GSH/GSSG, which could participate in oxidative stress. Both low metabolic activity in BAT and liver dicarbonyls formation may be involved in the development of postmenopausal metabolic syndrome.
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Interspecies scaling of dynamic glucose and insulin using a mathematical model approach

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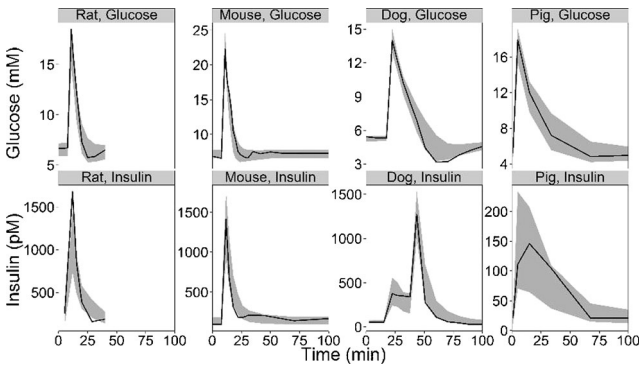
Background and aims: Mathematical models are useful tools in understanding complex relationships such as glucose homeostasis. The

integrated glucose insulin (IGI) model is a mathematical model that describes dynamic glucose and insulin concentrations in humans during glucose tolerance tests. The aim of this work was to investigate if the model can be scaled to describe intravenous glucose tolerance test (IVGTT) data from several preclinical species.

Materials and methods: Glucose and insulin concentrations from IVGTTs performed in healthy individuals of rats (n=16), mice (n=24), dogs (n=11), pigs (n=11) and humans (n=24) was available for analysis. In the first step allometric scaling based on individual body weights was investigated. The most suitable value for the allometric exponent was investigated for all parameters in the model, based on objective function value, parameter uncertainty and model complexity. In the second step species adaptations was investigated by scaling parameters with the weight of organs relevant to that parameter in each species. In the third step remaining species adaptations that could not be described by allometric scaling was investigated.

Results: Estimating one allometric exponent for clearances (0.85), one exponent for volumes (0.9) and fixing the exponent of rate constants to -0.25 described the data well and kept the scaling complexity low. Estimating the allometric exponent is of importance to be able to describe rodents well, since their body weights are far from humans. Of the investigated scaling relationships with species specific organ weights no relationship improved the fit for all animals. Dogs and pigs showed lower first phase secretion and stronger second phase secretion of insulin than the model predicted and these two parameters were estimated for dogs and pigs. Pigs also showed a slower first phase secretion and this parameter was also estimated for pigs. The model predicted slower glucose elimination in pigs than the data showed and insulin dependent glucose clearance was estimated separately for pigs. The result of the final model describing IVGTT in rats, mice, dogs and pigs is displayed in figure 1 (the solid line is the median based on the observed data. Shaded area is the 95% confidence interval around the median based on the simulated data).

Conclusion: The allometrically scaled IGI model developed in this project can accurately predict preclinical IVGTT data. In general glucose scales better than insulin, as insulin is a protein the kinetics are more complex and the amino acid sequence is not identical in the different species. When investigating animal models of diabetes it is of most importance that glucose scales well since insulin secretion often is diminished. The allometrically scaled IGI model can be used in drug development to facilitate better translations of preclinical research into clinic.



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Moderate exercise prevents pituitary dysfunction induced by a sucrose rich diet

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Background and aims: Increased consumption of sucrose, a major constituent of western diet, has been associated with the development of obesity and insulin resistance (IR). Altered hypothalamic-pituitary-adrenal axis function has been linked to to IR, in animal models and patients. Studies from our group have shown morphological and functional changes in the adrenal cortex of rats fed a sucrose-rich diet (SRD). The aim of this study was to evaluate the effects of SRD on basal activity of the pituitary gland, the underlying cellular processes, and the effect of exercise (E) on these parameters.

Materials and methods: Adult male Wistar rats were randomized into 4 groups (n=5), two of which received 30% w/v sucrose in the drinking water (SRD) for 15 weeks. A subset of each dietary group was subjected to E protocol (C-E and SRD-E) (treadmill walk, 15 min/day, 5 day/week, for 15 weeks, at 0.8 km/h speed). The sedentary (C-S and SRD-S) subset of each dietary group did not perform any physical activity. Insulin sensitivity was assessed at the 7th week of treatment by i.p. ITT. Statistical analyses were performed using Student's t test or ANOVA and significant differences were studied with Tukey's post hoc test.

Results: Our results showed that serum metabolites (fasting glucose, triglycerides and free fatty acids (FFA)) were increased in SRD-S animals by the end of the 15th week of treatment. Animals from the SRD-S group showed significantly lower plasma levels of ACTH (C-S: 96.9±2.6, SRD-S: 52.6±0.5, pg/ml), decreased pituitary POMC mRNA levels (C-S: 1.02±0.04, SRD-S: 0.64±0.03, fold induction), and lower serum corticosterone concentrations (C-S: 13.61±2.66, SRD-S: 2.66±0.33, ng/ml) than those from the C-S group. Analysis of pituitary levels of antioxidant enzymes and autophagy markers suggested the activation of these cellular pathways in the SRD-S group. E was able to prevent the induction of autophagy and antioxidant defense systems in the pituitary gland of SRD-E animals. Analysis of POMC mRNA (C-E: 1.46±0.06, SRD-E: 1.43±0.18, fold induction), plasma ACTH levels (C-E: 142.0±8.6, SRD-E: 130.6±20.2, pg/ml) and of serum corticosterone concentrations (C-E: 28.05±4.8, SRD-E: 50.40±3.33, ng/ml) showed that E prevented the effects of SRD on HPA axis basal activity. Regarding the analyzed serum biochemical parameters, E was able to prevent the increase in serum FFA levels. Given this, we analyzed the effects of palmitic acid (PA) on POMC-ACTH production in AtT-20 corticotroph cell-line. Cells were transiently transfected with a POMC-luciferase reporter plasmid, and then incubated with 4% BSA±PA. Our results showed that 24 h treatment with 1 mM PA significantly inhibited POMC promoter activity (4% BSA: 1.00±0.07, 4% BSA + 1 mM PA: 0.75±0.04, A.U.), and induced autophagy and oxidative stress. Similar results on POMC promoter activity were obtained incubating the cells with Rapamycin or H2O2, suggesting a role for these cellular processes on POMC regulation.

Conclusion: Administration of a SRD shows a significant effect on cellular mechanisms that influence POMC transcription and ACTH production in corticotroph cells. We also hypothesize that sustained elevated FFA serum levels could contribute to the observed pituitary dysfunction.

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A disrupted rhythm of appetite-regulating hormone is relevant to abnormal feeding behaviour in high fat diet induced obese mice

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Background and aims: A number of appetite-regulating molecules have been identified and discussed in obese subjects and animals. Ghrelin is a representative orexigenic gut hormone produced in the stomach. Pathophysiological roles of ghrelin in obesity is, however, largely unknown together with its diurnal dynamics. Neuromedin U (NMU) is distributed in the brain and gut, including supra-chiasmic nucleus (SCN) in the hypothalamus, a master biological clock. In contrast to ghrelin, NMU acts as an anorexigenic peptide. Because these two antagonistic peptides have never been discussed simultaneously, the aim of the present study is to explore the relevance of these peptides in the formation and progression of obesity, especially in relation to a diurnal rhythm of feeding and activity.
Materials and methods: Male C57BL/6J mice at 4 weeks old were housed in a control room under a 12 h light-dark cycle (light on 0700-1900) with ad libitum access to either control diet (CD: 10 kcal% fat) or high fat diet (HFD: 60 kcal% fat) for 12 weeks. At 16 weeks old, an individual mouse was transferred to a metabolic chamber (ARCO systems), and respiratory quotient (RQ), food consumption and locomotor activity (ACT) was measured on a minute by minute basis for 2 days. Then mice were sacrificed at 4 different time points (7:00, 13:00, 19:00, 25:00), and plasma for the determination of ghrelin by RIA and hypothalamus for NMU and stomach for clock genes such as BMAl1 and PER2 by RT-PCR were retrieved. To further elucidate a role of ghrelin for the regulation of feeding and ACT, 16 weeks old ghrelin knockout (GKO) mice fed CD were housed in ARCO systems for 2 days same as wild type (WT) mice.
Results: Body weight in HFD group was significantly higher than that in CD group at 16 weeks old. Food consumption and ACT in CD group were mainly observed during dark phase (D) with a very small ACT during light phase (L). RQ in CD group showed a clear diurnal rhythm with being low during L and high during D. In contrast, these distinct diurnal rhythms of food consumption, ACT and RQ were severely disrupted in HFD group with an increase of these parameters during L. Although plasma ghrelin concentration revealed a bimodal rhythm with a zenith at 7:00, 19:00 and a nadir at 13:00, 25:00 in CD group, a completely reverse rhythm was observed in HFD group. On the other hand, NMU expression in the hypothalamus showed a diurnal rhythm with being high during L and low during D in CD group, and a completely reverse rhythm was observed in HFD group. Furthermore, a diurnal phase of stomach BMAl1 expression shifted backward and PER2 shifted forward in HFD group compared to CD group. It is of interest that GKO mice fed CD showed indistinct rhythms of ACT and RQ, which are quite similar to those in WT mice fed HFD.
Conclusion: An indistinct rhythms of ACT and feeding observed in HFD group evokes abnormal binge eating in human obese subjects. Ghrelin may act as an initiator of feeding behavior from the results in GKO mice, and NMU may control circadian rhythms of clock genes taking its localization in SCN into account. It is suggested that abnormal diurnal rhythms of both appetite-regulating peptides in HFD group is quite relevant to the formation and progression obesity.
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Dose-dependent stimulatory effect of leptin on Leydig cell steroidogenesis in leptin-deficient obese mice

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Background and aims: Over the last decade, the evidence linking obesity to impaired male reproductive function has grown. Leptin is a known mediator and modulator of the hypothalamus-pituitary-testes axis, dysregulated in obesity; however, data have been inconsistent. The aim of the study was to elucidate the dose-dependent impact of leptin on male reproductive function and Leydig cell steroidogenesis in a mouse model of obesity.
Materials and methods: Leptin-deficient obese (ob/ob) male mice on a C57BL/6J LDLR-/- background were treated with 0.1, 0.5, or 3 mg/kg body weight/d murine recombinant leptin or saline for 12 weeks starting at 8 weeks of age. The effect of leptin on testicular weight, spermatogenesis, intratesticular testosterone, Leydig cell morphology, and macrophages was elucidated.
Results: Testis weight which is mostly indicative of overall spermatogenic activity was significantly and dose-dependently up-regulated between control (saline-treated; 1.69 mg/g bodyweight (BW)) and leptin-treated animals (2.56, 2.76, and 3.28 mg/g BW at 0.1, 0.5, or 3 mg/kg body weight/d, respectively) ($p<0.001$). Moreover, intratesticular testosterone content was restored dose-dependently after leptin treatment (314.4, 497.2, and 526.3 ng/g tissue at 0.1, 0.5, or 3 mg/kg body weight/d, respectively) compared to saline-treated mice (212.7 ng/g tissue) ($p< 0.001$). Restoration of intratesticular testosterone in leptin-treated animals was associated with a significant up-regulation of the steroidogenic enzymes Cyp11a1 and Cyp17. Testicular histology indicated that Leydig cells dose-dependently regain their usual morphology and clustering characteristic after leptin-treatment as compared to saline-treated animals. In contrast, macrophage markers were not different between leptin-treated and control mice.
Conclusion: The adipokine leptin might play an important role in the regulation of Leydig cell function and testicular physiology.
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BACE2 suppression protects from High Fat Diet (HFD)-induced metabolic effects

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Background and aims: BACE2 (β-site APP-cleaving enzyme 2) is a protease that has been found in the brain, where it is thought to play a role in the development of Alzheimer's disease (AD). It has also been localized in the pancreas, where it seems to play a physiological role, since BACE2-deficient mice elicit better glucose tolerance than control littermates. However, despite the potential link between AD and glucose homoeostasis deregulation in humans and rodents, the involvement of BACE2 in other metabolic disturbances, such as insulin resistance and obesity, has not been explored. Thus, the aim of the present study was to investigate the effect of BACE2 on whole-body glucose metabolism.
Materials and methods: BACE2-KO mice and their respective controls were used to analyze their phenotype after 16 weeks of high-fat diet (HFD) feeding. Insulin tolerance test (ITT) and glucose tolerance test

(GTT) were performed to evaluate metabolic phenotype, and the area under the curve (AUC) of the GTT was calculated as a measure of glucose homeostasis. The ability to secrete insulin in response to glucose (GSIS) was quantified with an ELISA kit. β-cell mass was analyzed by insulin immunostaining. mRNA expression of relevant genes from pancreatic islets, white adipose tissue and hypothalamus was analyzed by quantitative PCR.

Results: BACE2-KO mice fed with HFD showed a 32% reduction in body weight ($p<0.05$), with respect to their wild type counterparts, whereas no changes in food intake were observed. Moreover, BACE2-KO mice fed with HFD presented better glucose homeostasis (28% decrease in AUC of GTT) and did not exhibit insulin resistance when compared to wild type animals with the same feeding. Interestingly, white adipose tissue from these animals presented a weight reduction of 25% ($p<0.01$) and a lower expression of the inflammation marker ccl2. Furthermore, while wild type animals presented a remarkable decrease in the levels of hypothalamic anorexigenic markers pomc and cartpt when fed with HFD (65% reduction in the case of pomc, $p<0.01$), BACE2-KO animals did not show changes in any of the neuropeptides studied with respect to the animals fed with a regular diet.

Conclusion: Altogether, these results indicate that the inhibition of BACE2 seems to protect against HFD. Thus, targeting BACE2 may represent a good therapeutic strategy to ameliorate the pathological effects of obesity.

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PS 050 GLP-1 based therapies for obesity

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Significant effects of HM11260C on body weight over 20 weeks in obese subjects without diabetes: a randomised, double-blind, placebo controlled study

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Background and aims: HM11260C (HM) is a novel long acting GLP-1R agonist with a $T_{1/2}$ of~158 hrs resulting in a flat PK profile. This 20-week, randomized, double-blind, placebo (PBO) controlled, parallel group study was designed to evaluate the efficacy, safety, and tolerability of once a week (QW) or every other week (Q2W) doses of HM in obese subjects without diabetes.

Materials and methods: 297 subjects (mean age 43.4 yrs and BMI 35.5 kg/m^2) were randomized to one of four HM doses (4 mg QW, 6 mg QW, 6 mg Q2W, or 8 mg Q2W) or to PBO. HM was administered subcutaneously for 20 weeks. The diet and exercise regimens remained unchanged during the study.

Results: The body weight loss with HM 4 mg QW, 6 mg QW, 6 mg Q2W and 8 mg Q2W was 6.2 kg, 7.8 kg, 7.0 kg and 7.1 kg (LS Mean, $p<0.0001$ all HM treatment groups), whereas the body weight gain was observed with PBO (0.8 kg). More subjects in the HM treatment groups achieved significant body weight loss ≥5% or ≥10%, and the mean reductions in BMI from baseline were greater, compared with PBO. Changes from baseline in waist circumference were -6.00 cm, -9.45 cm, -7.11 cm, -8.00 cm and -0.21 cm (LS Mean) with HM 4 mg QW, 6 mg QW, 6 mg Q2W, 8 mg Q2W and PBO. The most frequent adverse events were gastrointestinal events which were observed relatively frequently and increased injection site reactions which were less common (Table 1).

Conclusion: All doses of HM meaningfully reduced body weight and were well tolerated. These results warrant further studies to assess titration schemes as well as the long-term efficacy and safety of HM in obesity.

Table 1. Summary of efficacy and safety of HM11260C over 20 weeks (Interim analysis)

	Placebo (n=27)	HM11260C 4 mg QW (n=27)	HM11260C 6 mg QW (n=27)	HM11260C 6 mg Q2W (n=27)	HM11260C 8 mg Q2W (n=27)
Baseline Characteristics (Safety Set)					
Body weight, kg	98.06 (11.081)	105.21 (23.573)	104.75 (17.600)	98.82 (17.705)	98.28 (15.578)
BMI, kg/m^2	35.096 (3.3666)	36.431 (5.4869)	37.762 (4.4860)	36.518 (5.5296)	35.811 (4.3200)
Efficacy (Full Analysis Set)					
Body weight change, kg [a]	0.8 (0.84)	-6.2 (0.82)* (p<0.0001)	-7.8 (0.89)* (p<0.0001)	-7.0 (0.84)* (p<0.0001)	-7.1 (0.82)* (p<0.0001)
Subjects with Body weight loss ≥5%, n (%) [b]	0 (0.0)	12 (44.4)* (p<0.0001)	13 (48.1)* (p<0.0001)	13 (48.1)* (p<0.0001)	16 (59.3)* (p<0.0001)
Subjects with Body weight loss ≥10%, n (%) [b]	0 (0.0)	5 (18.5) (p=0.0478)	7 (25.9)* (p=0.0019)	7 (25.9) (p=0.0033)	6 (22.2) (p=0.0207)
Waist circumference change, cm [a]	-0.21 (1.422)	-6.00 (1.359) (p=0.0042)	-9.45 (1.500)* (p<0.0001)	-7.11 (1.420)* (p=0.0009)	-8.00 (1.356)* (p=0.0001)
Waist/Hip circumferences change [a]	-0.01 (0.013)	-0.01 (0.012) (p=0.9874)	-0.04 (0.014) (p=0.1193)	-0.04 (0.013) (p=0.0994)	-0.03 (0.013) (p=0.2909)
Safety (Safety Set)					
Incidence of Nausea, n (%)	8 (29.6)	16 (59.3)	13 (48.1)	14 (51.9)	17 (63.0)
Incidence of Vomiting, n (%)	1 (3.7)	5 (18.5)	5 (18.5)	5 (18.5)	7 (25.9)
Injection site reactions, n (%)	6 (22.2)	5 (18.5)	5 (18.5)	3 (11.1)	4 (14.8)

[a] Data are LS Mean (SE). From a mixed-effects model for repeated measures (MMRM) over all post-baseline visits, with an unstructured covariance matrix, change from baseline in each evaluation variable as the outcome variable; treatment group, visit, and their interaction as factors and baseline each evaluation variable as a covariate.
[b] Fisher's exact test is to test whether the null hypothesis of equal proportions holds or not between the treatment group and placebo.
* p-value <0.0031 vs. placebo

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MEDI0382 a novel GLP1/glucagon co-agonist induces profound body weight loss and improves metabolism in rodents and non-human primates

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Background and aims: Oxyntomodulin (OXM), the endogenous GLP-1/Glucagon co-agonist peptide, is secreted from intestinal L-cells in response to meals. OXM reduces body weight in obese subjects and produces direct acute glucoregulatory effects in type 2 diabetic patients. Due to its short lifetime in circulation, the clinical utility of this hormone is limited. We synthesized stable, lipidated analogs of OXM that possessed the *in silico* selected ratios of GLP-1:glucagon activity ideal for clinical utility.
Materials and methods: Chinese hamster ovary cells stably expressing human GLP-1 and glucagon receptors were used to evaluate the ability of MEDI0382 to stimulate cAMP production. C57Bl/6 mice fed a 60% high-fat diet, diabetic *db/db* mice and cynomolgus monkeys were used to evaluate the metabolic effects of subcutaneously injected MEDI0382.
Results: The lead molecule, MEDI0382, is a highly potent agonist of GLP-1 and glucagon receptors. Once-daily subcutaneous dosing with MEDI0382 (10 and 30 nmol/kg) reduced body weight by 24.5% and 32.5% in male diet-induced obese (DIO) mice, as compared with vehicle-treated DIO mice. The effect was superior to liraglutide (40 nmol/kg). Liraglutide had a greater effect on cumulative food intake than both doses of MEDI0382, suggesting that the effect of MEDI0382 on body weight may be partly mediated by increased energy expenditure. A significant improvement in glucose tolerance was observed in drug-treated mice in an intraperitoneal glucose tolerance test. In diabetic *db/db* mice, a single dose of MEDI0382 reduced glucose excursion following an intraperitoneal glucose challenge. Repeated daily dosing of MEDI0382 for 2 months in cynomolgus monkeys resulted in dose-dependent reductions in body weight of 5% to 12% compared to starting bodyweights at the end of the treatment period.
Conclusion: In summary, our data indicate that MEDI0382 shows significant promise as a weight loss drug in overweight and obese subjects and the potential to lower glucose in people with type 2 diabetes.

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Early weight loss with liraglutide 3.0 mg is good predictor of clinically meaningful weight loss after 56 weeks

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Background and aims: Early identification of responders to weight loss (WL) medications is important in order to discontinue those unlikely to achieve WL targets; EU regulators are especially interested in identifying those unlikely to achieve ≥10% WL at 1 yr. This subgroup analysis of SCALE Obesity and Prediabetes and SCALE Diabetes trials reports key efficacy and safety outcomes in adults achieving ≥5% WL from baseline at Week (W) 16 on liraglutide (lira) 3.0 mg (early responders; ER), compared to those who did not (early non-responders; ENR). A WL target of ≥5% at W16 demonstrated the best balance between negative predictive value and sensitivity in identifying those unable to achieve ≥10% WL at W56.
Materials and methods: 2910 adults were randomized to lira as adjunct to diet & exercise (D&E): 2487 without T2D (BMI ≥30 or 27-29.9 kg/m2+≥1 comorbidity; 45 yr; 21% male; BMI 38 kg/m2; 61% with prediabetes), and 423 with T2D (BMI ≥27 kg/m2; 55 yr; 52% male; BMI 37 kg/m2); 2159 and 365 adults without and with T2D respectively completed 16 W of treatment. Lira was increased weekly by 0.6 mg to a 3.0 mg maintenance dose. Efficacy data are for 56 W completers. Mean and categorical WL was estimated by ANCOVA or logistic regression model respectively. Efficacy data are LS means or estimated proportions, safety data are observed proportions.
Results: 67.5% of W16 completers without T2D were ER to lira 3.0 mg, with a mean WL of 11.5% at W56. Proportions of ER with ≥5%, >10% and >15% WL at W56 with lira were 88.2%, 54.8% and 24.2%, respectively. ENR without T2D achieved mean WL of 3.8% at W56, and 36.9%, 8.3% and 1.8% had achieved ≥5%, >10% and >15% WL, respectively. 50.4% of W16 completers with T2D were ER to lira 3.0 mg, with mean WL of 9.3% W56. Proportions of ER with ≥5%, >10% and >15% WL at W56 with lira were 80.1%, 44.6% and 11.6%, respectively. ENR with T2D achieved mean WL of 3.6% at W56, at which time 33.3%, 5.8% and 1.3% had achieved ≥5%, >10% and >15% WL, respectively. Pooled across trials 93.4% of ENR failed to achieve ≥10% WL at W56. Across both trials, greater improvements in CV risk factors were seen in ER than ENR, consistent with greater WL. The overall safety profile was generally comparable between ER and ENR (Table). In those without T2D rates of hepatobiliary disorders appeared higher in ER than ENR. In patients with T2D rates of severe hypoglycaemia (ADA Criteria) were low in both ER and ENR (1.1% v 0.6%; all on SU) and the overall hypo rates (ADA criteria, documented symptomatic) were generally comparable between ER (28.3%) and ENR (21.0%).
Conclusion: Those failing to reach ≥5% WL at W16 are unlikely to reach ≥10% at W56. By contrast, ER to lira 3.0 mg as adjunct to D&E with and without T2D achieve a mean WL at W56 of 9.3% and 11.5%, respectively, with improvements in cardiometabolic markers with a similar safety profile to NR.

Table: Key efficacy and safety outcomes of early responders vs early non-responders on liraglutide 3.0 mg by trial

	SCALE Obesity and Prediabetes		SCALE Diabetes	
	Early responder N=1456	Early non-responder N=703	Early responder N=184	Early non-responder N=181
Change from baseline in cardiometabolic outcomes				
HbA_{1c} (% points)	-0.37	-0.25	-1.61	-1.22
SBP/DBP (mmHg)	-5.5/-3.6	-2.3/-1.4	-3.8/-0.6	-1.3/-1.5
HDL-C/LDL-C (%)	4.5/-3.4	-0.1/-2.5	9.0/3.3	0.5/-1.7
TC (%)	-3.0	-2.7	-0.8	-2.0
TG (%)	-15.9	-8.3	-20.7	-8.3
Safety outcomes				
Total AEs (%)	93.9	91.7	96.2	93.4
Serious AEs (%)	6.4	5.3	7.6	9.9
Hepatobiliary AEs (%)	3.5	2.1	0.5	1.7

Efficacy data are LS mean estimates of change from baseline (HbA_{1c}, SBP, DBP) and of relative change from baseline (lipids). Safety data are observed proportions.
AE, adverse event; bpm, beats per minute; GI, gastrointestinal; HbA_{1c}, glycated haemoglobin; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; SBP/DBP, systolic/diastolic blood pressure; TC, total cholesterol; TG, triglycerides

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Liraglutide 3.0 mg efficacy and safety by baseline BMI in the SCALE Diabetes trial: post-hoc analysis

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Background and aims: This analysis from the SCALE Diabetes trial compared efficacy and safety results for individuals with baseline BMI <or ≥35 kg/m^2 treated with liraglutide 3.0 mg for weight management or placebo.
Materials and methods: 846 adults were randomised (age 54.9 y, male 50%, BMI 37 kg/m^2, A1C 7.9%, T2D duration 7.3 years) 2:1:1 to liraglutide 3.0 mg, 1.8 mg, or placebo as adjunct to diet and exercise for 56 weeks. Data are LS means (efficacy) or observed means (LOCF).
Results: Overall withdrawals rates were 23.4% for liraglutide 3.0 mg vs 34.0% for placebo. At 56 weeks, greater mean and categorical weight loss, and greater improvements in glycaemic parameters, SBP, and IWQoL-Lite physical function score were seen with liraglutide 3.0 mg vs placebo in both subgroups. Treatment effects were independent of baseline BMI subgroup (p>0.05) (Table). BMI subgroups had broadly comparable adverse events (AEs). In both liraglutide 3.0 mg subgroups (BMI<or ≥35 kg/m^2) a greater proportion of subjects had nausea (34 vs 32%) and vomiting (11% vs 20%) than placebo (nausea: 12 vs 15%; vomiting: 8% vs 4%). Documented symptomatic hypoglycaemia (FPG ≤3.9 mmol/L) rates were similar with liraglutide 3.0 mg in both BMI subgroups (1.07 vs 0.70 events/PYE), as was severe hypoglycaemia, seen in three subjects with concomitant sulphonylureas.
Conclusion: In summary, effects of liraglutide 3.0 mg, as adjunct to diet and exercise, on weight loss, associated metabolic effects and safety profile were consistent across predefined baseline BMI subgroups.

Efficacy Endpoints	BMI <35 kg/m^2			BMI ≥35 kg/m^2			Interaction
	Liraglutide 3.0 mg/Placebo (n= 185/88) (45%/42%)			Liraglutide 3.0 mg/Placebo (n=227/123) (55%/58%)			P value
	Baseline	Change from baseline	ETD/OR†	Baseline	Change from baseline	ETD/OR†	
Body weight, kg	91.4/91.4	-5.7%/-1.8%	-4.0*	117.2/117.5	-6.1%/-2.1%	-4.0*	0.9498
% Weight loss ≥5% at 56 weeks	48.3/11.4		7.6†*	51.2/13.8		6.4†*	0.7180
% Weight loss >10% at 56 weeks	21.1/3.0		8.5†**	22.9/4.4		6.5†*	0.7263
BMI, kg/m^2	31.5/31.4	-1.8/-0.6	–1.2*	41.7/41.6	-2.6/-0.9	-1.7*	0.1728
% A1C	7.9/7.9	-1.2/-0.4	-0.9*	8.0/7.9	-1.4/-0.3	-1.0*	0.5295
FPG, mmol/L	8.8/8.5	-1.9/-0.1	-1.7*	8.8/8.7	-1.9/0.0	-1.8*	0.6594
SBP, mmHg	128.4/129.2	-1.9/-0.4	-1.9	129.3/129.2	-3.6/-0.4	-3.1***	0.5606
IWQoL-Lite Physical function score	75.2/77.9	12.1/9.4	1.6	55.7/60.1	18.0/8.5	7.1**	0.0528

*p<0.0001, **p<0.001, ***p<0.05, †OR
P-values are for the test of interaction between BMI subgroup and treatments liraglutide 3.0 mg and placebo.
ETD, estimated treatment difference; OR, odds ratio; FPG, fasting plasma glucose; SBP, systolic blood pressure; IWQoL-Lite, Impact of Weight on Quality of Life - lite

Clinical Trial Registration Number: NCT01272232
Supported by: Novo Nordisk

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The impact of gastrointestinal adverse events on weight loss with liraglutide 3.0 mg as adjunct to a diet and exercise programme

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Background and aims: To explore any associations between gastrointestinal adverse events (GI AEs) and weight loss with liraglutide 3.0 mg/day in addition to a diet and exercise programme in individuals without type 2 diabetes who had obesity (BMI ≥30 kg/m^2) or overweight (BMI 27-29.9 kg/m^2) with at least one comorbidity.
Materials and methods: The SCALE Obesity and Prediabetes trial was a randomised, double-blind, multi-centre trial in which individuals (mean age 45.1 years, 78.5% female, mean weight 106.2 kg, mean BMI 38.3 kg/m^2, 61% with prediabetes) were enrolled in a long-term weight management programme and randomised to liraglutide 3.0 mg (n=2487) or placebo (n=1244). These data are from an exploratory analysis based on groups of individuals defined by occurrence of GI AEs (0-16 weeks, 0-56 weeks). Weight loss at week 56 is presented as least squares means using LOCF, with p-values denoting whether or not GI AEs had a significant effect on treatment.
Results: Overall, liraglutide 3.0 mg was associated with a greater weight loss from baseline than placebo (8.0% vs. 2.6%, respectively, p<0.0001). As expected, more individuals on liraglutide 3.0 mg (68.3%) compared with placebo (40.3%) reported GI AEs; the most prevalent GI AEs were nausea (40.2 vs. 14.7%), diarrhoea (20.9 vs. 9.3%), constipation (20.0 vs. 8.7%) and vomiting (16.3 vs. 4.1%), occurring mostly within the first 16 weeks of treatment. There was no significant difference in weight loss between individuals who did or did not experience ≥1 episode of nausea/vomiting during 0-56 weeks, regardless of treatment (liraglutide 3.0 mg: nausea/vomiting, -7.8%, no nausea/vomiting, -8.1%; placebo: nausea/vomiting -2.5%, no nausea/vomiting -2.6%, p=0.81). Similar results were seen if all other types of GI AE combined were included. Moreover, no significant differences were observed at week 56 for weight loss in individuals who experienced 0, 1, 2-3, or ≥4 GI AEs in the first 16 weeks (7.7-8.2% with liraglutide 3.0 mg vs. 2.3-3.0% with placebo, p=0.24), or during the entire 56 weeks of treatment (7.7-8.4% with liraglutide 3.0 mg vs. 2.4-3.2% with placebo, p=0.55). Although those experiencing 0 GI AEs appeared to perform slightly better than the other groups, this may be explained by the higher withdrawal rate as the number of GI AE increase. These results were further supported by comparable mean weight loss profiles over time across the 0, 1, 2-3, or ≥4 GI AE groups.
Conclusion: The weight loss observed with liraglutide 3.0 mg is not explained by the occurrence of GI AEs, including nausea/vomiting.
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GLP-1 and GLP-2 responses to a fat-rich meal correlate to postprandial triglyceride-rich lipoproteins in obese men

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Background and aims: Nutrients, mainly glucose and lipids stimulate the gut incretin hormones glucagon-like peptide (GLP) 1, GLP-2 and glucose-dependent insulinotrophic peptide (GIP) that in animal models or if given in pharmacologic doses to humans regulate chylomicron secretion from the enterocytes. However, the effect of these gut incretins on human postprandial lipid metabolism is not fully clarified. We aimed to explore the responses of GLP-1, GLP-2 and GIP after glucose tolerance test (OGTT) or fat-rich meal, and the relationships between responses of incretins and postprandial triglyceride (TG)-rich lipoprotein after fat-rich meal.

Materials and methods: Glucose, insulin, GLP-1, GLP-2 and GIP were measured during OGTT (75 g glucose) and fat-rich meal (63 g carbohydrates, 56 g fat and 40 g protein) in 75 healthy obese (BMI 26-40 kg/m^2) male subjects with mean age of 49 years, LDL-cholesterol 3.3 mmol/L, HDL-cholesterol 1.2 mmol/L and TG 1.6 mmol/l. TG, apoB48 and apoB100 in TG-rich lipoproteins(chylomicrons, VLDL1 and VLDL2) were measured after fat-rich meal. We measured postprandial responses [area under curve (AUC)] for glucose, insulin, GLP-1, GLP-2, GIP in plasma, and TG, apoB48 and apoB100 in plasma and TG-rich lipoproteins separated by gradient density ultracentrifugation.
Results: The GLP-1, GLP-2 and GIP responses after fat-rich meal and OGTT correlated strongly ($r=0.73$, $p<0.0001$; $r=0.46$, $p<0.001$ and $r=0.69$, $p<0.001$, respectively). Glucose and insulin AUCs were lower, but the AUCs for GLP-1, GLP-2 and GIP were significantly higher after fat-rich meal than OGTT. The peak value for all hormones appeared at 120 minutes after fat-rich meal, compared to 30 minutes after OGTT. After fat-rich meal, the AUCs for GLP-1, GLP-2 and GIP correlated significantly with TG, apoB48 and apoB100 in chylomicrons.
Conclusion: In obese males, GLP-1, GLP-2 and GIP responses to a fat-rich meal are greater than following an OGTT. The balance between responses of GLP-1 and GLP-2 to a fat-rich meal may be involved in regulating postprandial responses of TG-rich lipoproteins.

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Effects of weight loss and long-term weight maintenance with diets varying in protein and glycaemic index on atrial natriuretic peptide in the Diet, Obesity, and Genes study

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Background and aims: Low atrial natriuretic peptide (ANP) concentrations are independently associated with risk of cardiovascular diseases and type 2 diabetes in obesity, while high natriuretic peptide levels appear to be protective. We tested the hypothesis that circulating ANP may be regulated by weight loss and dietary interventions.
Materials and methods: DiOGenes is a pan-European controlled dietary intervention study in overweight adults who first lost body weight on an 8-week low-calorie diet and were then randomized to 1 of 5 ad libitum diets for 26 weeks. The diets were either high (HPI) or low (LPI) protein or high (HGI) or low (LGI) glycemic index in 4 combinations or control. The concentrations of adiponectin and ANP (measured as mid-regional pro-atrial NP (MR-proANP)), were measured and analyzed together with anthropometrical and physiological parameters.
Results: Weight loss after 8-week low-calorie diet (mean±s.e.m; 11.2±3.5 kg; $p<0.001$) increased MR-proANP (from 49.7±20.3 pmol/l to 53.0±19.3 pmol/l; $p<0.001$) in the whole cohort and was pronounced in male subjects ($n=173$; 44.6±18.2 pmol/l vs. 52.5±21.0; $p<0.001$) than in women ($n=336$; 52.0±20.1 pmol/l vs. 52.8±18.2 $p=0.39$). During the 26-week weight maintenance period, MR-proANP decreased among the male subjects assigned to HGI diets (53.0±19.0 pmol/l vs. 47.0±19.0 $p=0.002$) as well as in groups with LGI diets (50.3±22.1 pmol/l vs. 45.2±19.4 $p=0.003$, $p=0.69$ for group comparison). A similar effect was observed in male subjects during HPI and LPI interventions (50.3±19.2 pmol/l vs. 45.6±16.6, $p=0.006$ for HPI; 53.5±22.3 pmol/l vs. 46.8±22.4, $p<0.001$ for LPI; $p=0.418$ for group comparison). No changes of MR-proANP were observed with all three types of diets in women (51.2±14.5 pmol/l vs. 49.7±15.8, $p=0.18$ for HGI; 54.3±21.4 pmol/l vs. 53.6±21.9, $p=0.63$ for LGI; $p=0.63$ for group comparison and 52.1±17.1 pmol/l vs. 51.0±19.9, $p=0.37$ for HPI; 53.7±20.1 pmol/l vs. 52.7±18.7, $p=0.45$ for LPI; $p=0.95$ for group comparison). Changes in circulating MR-proANP after weight loss correlated inversely with fasting insulin ($r=-0.12$, $p=0.01$), fasting glucose ($r=-0.18$, $p<0.0001$), waist circumferences ($r=-0.11$, $p=0.01$ and positively with adiponectin ($r=0.16$, $p=0.0004$) in the whole cohort.
Conclusion: Weight loss effectively increases circulating MR-proANP in male but not female subjects. The different types of diets were unable to prevent a decrease of ANP during the weight maintaining phase. Gender differences in the regulation of endogenous ANP may be causally associated with higher cardiovascular risk in males and could have implications for personalized medicine.
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Impact of obesity on bone mass throughout adult life: influence of gender and severity of obesity

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Background and aims: Cross-sectional studies have demonstrated that obesity improves areal bone mineral density (aBMD). However, it is not known if gender, aging or severity of obesity could modulate this effect and if bone sites are similarly impacted. The main aim of the study was to model the aBMD variation in obese patients from peak bone mass period until elderly age according to gender, bone localisation and severity of obesity. The secondary aim was to identify the relationship between aBMD and body composition during obesity.
Materials and methods: Obese patients were recruited in the Nutrition Clinic where they had been addressed for metabolic and physical assessment of their obesity. DXA (Hologic QDR-4500A, Hologic, Inc., Waltham, MA) measured the areal bone mineral density (BMD; g/cm2) of the whole body and at specific bone sites: the antero-posterior lumbar spine (L1-L4), the dominant arm radius and the hip. The soft tissue body composition , percentage of body fat mass and lean mass was derived from the whole body scan. We first estimated Z-scores of aBMD adjusted for age according to bone sites and gender using a linear mixed model. We then customized statistical tests to compare (1) the estimated value of Z-score (and change in Z-score with age) to 0 for each bone site for men and women, (2) the estimated value of Z-score (and change in Z-score with age) between men and women for each bone site, (3) the estimated value of Z-score (and change in Z-score with age) between each bone site for men and women. The effect of obesity grades was analyzed separately for men and women using the same type of linear mixed model and customized statistical tests. The relations between aBMD and age have also been studied for each bone site according to gender and grades of obesity using thin plate regression spline models. Statistical analyses were performed at the conventional two-tailed α level of 0.05 using SAS version 9.2
Results: Five hundred and four french obese patients (363 women and 141 men) with a mean BMI of 38.5±6.0 kg/m^2, aged from 18.1 to 81.9 years (mean age 49.6±14.6 years) were enrolled.Excepted at L1-L4 in men, Z-scores were significantly increased, above the age- and gender-related mean, both for women and men at WB (respectively 0.79 SD and 0.31 SD), hip (1.09 SD and 1.05 SD), radius (1.42 SD and 0.25 SD) and L1-L4 (0.85 SD for women only). The improvement of Z-scores appeared significantly more marked in women that in men in all bone sites, hip excepted. Further, differences with normal values were significantly accentuated by aging, without any depend-effect of gender. In women, whatever the BMI or the bone site, Z-scores were significantly higher than normal values, but difference appeared more marked at WB, L1-L4 and hip for those patients with a BMI above 40 kg/m^2. Lean mass, but not fat mass appeared an independent predictor of aBMD in men and women.
Conclusion: This study demonstrated for the first time that obesity induces an improvement of aBMD, which is however modulated by bone site localisation, severity of obesity, age and gender. The accentuation of peak bone combined to reduction of bone loss related to aging may explain that obese patients do present a lower prevalence of osteoporosis.

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Visceral fat obesity and metabolic syndrome increase serum DPP-4 levels in type 2 diabetes mellitus

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Background and aims: Dipeptidyl peptidase-4 (DPP-4) is a ubiquitously expressed transmembrane glycoprotein and separates N-terminal dipeptides from a variety of substrates including incretin such as glucagon-like peptide 1 (GLP-1) and gastric inhibitory polypeptide. Recently, it has been shown that DPP-4 is one of adipokines expressed and secreted from adipose tissue, and that DPP-4 expression in visceral fat is greater than in subcutaneous fat. Previous studies showed that serum DPP-4 levels were significantly elevated in obese subjects compared to non-obese. However, it remains unclear whether or not serum DPP-4 levels are associated specifically with visceral fat mass and metabolic syndrome (Mets) in type 2 diabetes mellitus (T2DM). Therefore, the aim of this study was to examine the association of serum DPP-4 with visceral fat accumulation and the presence of Mets in patients with T2DM.
Materials and methods: This is a cross-sectional study with 135 Japanese male patients with T2DM. None of them had hepatic or renal dysfunction and had taken DPP-4 inhibitors or GLP-1 receptor agonists so far. Visceral and subcutaneous fat areas were evaluated by performing computed tomography scan at the level of the umbilicus. Serum DPP-4 concentration was measured by using commercially available ELISA kits. We defined more than 100 cm^2 of visceral fat area as visceral fat obesity as well as dyslipidemia (triglyceride≥150 mg/dL, HDL <40 mg/dL, or usage of statins and fibrates) and/or hypertension (systolic blood pressure (BP)≥140 mmHg, diastolic BP≥90 mmHg, or usage of antihypertensive drugs) accompanied by visceral fat obesity as Mets. We analyzed the relationships between serum DPP-4 levels and various parameters by multiple regression analyses and logistic analysis.
Results: Means of age and duration of T2DM were 57.4 years old and 9.8 years, respectively. Mean serum DPP-4 level was 809.1 ng/mL. Of 135 patients, 78 and 74 were visceral fat obesity and Mets, respectively. In multiple regression analysis adjusted for age, duration of T2DM, body mass index, serum creatinine, and HbA1c, DPP-4 was significantly and positively associated with visceral fat area (β=0.15, p=0.01), but not subcutaneous fat area (β=-0.09, p=0.14). Un-paired Student *t*-test showed that serum DPP-4 was marginally higher in patients with visceral fat obesity than without it (832 ng/mL vs 778 ng/mL, p=0.09), and that serum DPP-4 was significantly higher in patients with Mets than without it (838 ng/mL vs 775 ng/mL, p=0.04). In logistic analyses adjusted for the confounding factors described above, serum DPP-4 was significantly and positively associated with visceral fat obesity and Mets [odds ratio (OR)=1.34, 95% confidence interval (CI)=1.04-1.72 per standard deviation (SD) increase, p=0.02; OR=1.87, 95%CI=1.17-2.98 per SD increase, p<0.01, respectively].
Conclusion: Although it is previously reported that serum DPP-4 levels were increased in obese patients, the present study showed for the first time that serum DPP-4 levels were associated positively and specifically with accumulation of visceral fat, which is evaluated by CT scan, in male patients with T2DM. In addition, serum DPP-4 levels were significantly increased in patients with Mets, suggesting that serum DPP-4 may be involved in not only glucose metabolism but also lipid metabolism and blood pressure.

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Analysis of the intestinal microbiota composition and its relationship with type 2 diabetes in patients with overweight and obesity in level II

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Background and aims: It is estimated that 11 million people are diabetic in Brazil, with a prevalence of T2DM. In recent years, studies indicate the gut microbiota as an important modulator of the disease. Environmental and genetic factors interact to control the host intestinal microbiota, triggering metabolic disorders such as obesity and insulin resistance. The objective of this study was to identify the fecal microbiota in adult diabetic patients and to evaluate changes in its composition after bariatric surgery. Two groups of eleven patients each were enrolled in this study.

Materials and methods: Group 1 were submitted to the Reducing gastroplasty in Roux-Y and fecal sample were collected before the surgery and after 6 and 12 months. Group 2 were followed through 6 and 12 months without surgery intervention and fecal samples were collected at inclusion in the trial and after 6 and 12 months. Fecal microbiota was analyzed using high throughput sequencing in MiSeq (Illumina) with v3-v4 16S rRNA primers.
Results: The fecal microbiota in a surgery group has an increase in bacterial abundancy and diversity when compared with non-surgery group. This data was observed with the genera belonging to the Phylum Bacteroidetes, with a remarkable increase of Bacteroides, and Actinobacteria, with an increase of Bifidobacterium in surgery group.
Conclusion: This data could contribute to a better understanding of the role of the microbiota in T2DM regulation.
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Gliadin intake alters intestinal microbiota, glucose and lipid metabolism, and adipose tissue and liver immune cells
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Background and aims: Dietary gluten induces type 1 diabetes and intestinal inflammation in celiac disease patients. Likewise, gluten has effects on intestinal inflammation and microbiota composition in non-celiac disease subjects. However, the effect of dietary gluten on metabolic performance in non-celiac disease conditions is still largely uncharacterised. Therefore, we aimed to elucidate the mechanisms involved in the development of gluten-induced metabolic dysregulation in obesity. We hypothesized that intake of the gluten component, gliadin, induces changes in the intestinal ecology compared to a gluten-free diet. This subsequently affects host physiology via changes in glucose and lipid metabolism, as well as leukocyte subsets in liver and adipose tissue, thus leading to a metabolic syndrome phenotype.
Materials and methods: Forty C57BL/6 mice were fed a high-fat diet with or without 4% gliadin for 22 weeks.
Results: Among the gliadin-fed mice, HbA1c levels and HOMA-IR were significantly higher, suggesting gliadin-dependent changes in glucose metabolism and development of insulin resistance. Analysis of RNA expression showed that the gluconeogenic enzyme Glucose-6-phosphatase (G6Pase) was upregulated in the liver, indicating that the increased blood glucose level was at least partly explained by increased hepatic glucose output. Principal coordinate analysis (PCoA) of intestinal content showed that gliadin-fed mice have an altered intestinal microbiota, which also was manifested as higher acetate and total levels of short-chain fatty acids in cecum. Since gliadin is known to be immune-regulating and obesity-induced insulin resistance rely strongly on immune cell signaling in metabolic organs, such as adipose tissue and the liver, we performed a deep-phenotyping of the immune cells in these tissues using flow cytometry. Monocyte subsets in blood showed no differences in systemic inflammation between treatments. However, significant alterations of the immune cell composition were evident in the epididymal adipose tissue and the liver. Total numbers of macrophages and T cells increased significantly in the adipose tissue of gliadin-fed mice. Furthermore, various T cell subsets in the adipose tissue exhibited a more pronounced inflammatory phenotype. Likewise, based on the major immune cells detected in the liver, gliadin-fed mice displayed alterations in their immune cell composition allowing for a gliadin-dependent segregation using a principal component analysis (PCA).
Conclusion: Gliadin intake caused significant changes in glucose regulation, intestinal microbiota, and in the leukocyte phenotypes of adipose tissue and liver. Collectively, this suggests that gliadin-intake affects several of the systems involved in regulating insulin sensitivity. The exacerbation of an insulin resistant phenotype in obese mice by gliadin indicates that excessive gluten intake might increase the severity of metabolic syndrome. Further analyses of the relationship between these gliadin phenotypes are needed to identify the mechanisms mediating a response to gluten in the host.
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Elevated plasma pigment epithelium-derived factor is reduced by metformin treatment
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Background and aims: Pigment epithelium-derived factor (PEDF) was recently found to be closely associated with metabolic syndrome and insulin resistance. However, there is a lack of evidence quantitating PEDF levels in different glucose tolerance state and whether metformin treatment could influence it remains unknown. We aimed to investigate serum PEDF levels in subjects with different glucose tolerance and the effect of metformin treatment on PEDF levels in type 2 diabetic patients.
Materials and methods: Circulating PEDF levels and metabolic profiles were assessed in 517 Chinese participants with normal glucose tolerance (NGT), impaired glucose regulation (IGR) and type 2 diabetes mellitus (T2DM), based on the guideline of International Diabetes Federation. Body mass index (BMI) classification was according to the World Health Organization, underweight (BMI <18.5 kg/m2), normal weight (18.5≤ BMI<25 kg/m2), overweight (25≤BMI <30 kg/m2), and obesity (BMI ≥30 kg/m2).63 diabetic patients were treated with metformin (Bristol-Myers Squibb) for a period of 24 weeks, initially administered at 500 mg once daily and gradually titrated every two weeks to meet glycemic targets (fasting plasma glucose ≤6.1 mmol/L) up to a maximum daily dose of 1500 mg. If the target was not achieved with maximum dose, then metformin was maintained as the maximum dose until the end of the 24 weeks. PEDF levels after 24-weeks metformin treatment in 63 patients with T2DM were also analyzed. Serum estradiol levels were determined in females.
Results: In all participants (42.6% men and 57.4% women, mean age was 49.7±14.5 years), 186 had NGT, 216 had IGR and 169 had newly diagnosed diabetes. Serum PEDF level was significantly higher in subjects with IGR [9.51 (7.6 - 11.4) ug/ml] and T2DM [9.7 (7.9 - 11.7) ug/ml], compared with the controls [8.5 (7.1 - 10.7) ug/ml, p=0.010] and metformin treatment significantly decreased PEDF levels in T2DM patients with weight loss (p=0.027).Spearman correlation analysis demonstrated that PEDF levels were positively and significantly correlated with risk factors regarding metabolic syndrome and visceral obesity such as body mass index, waist circumference, waist-to-hip ratio, fasting and 2 h glucose (p<0.001). Multiple stepwise regression analysis revealed that PEDF was associated with alanine aminotransferase (p<0.001), triglycerides (p=0.002) and diastolic blood pressure (p=0.002) after adjustment for age and gender. Subjects with a higher tertile of PEDF concentration exhibited a higher prevalence of overweight or obesity than those in the lower tertile (p<0.001). Furthermore, men exhibited remarkably higher serum PEDF levels [9.58 (7.92-11.92) ug/ml] than women [8.88 (7.35-10.77) ug/ml, p=0.003] and this difference still existed even after adjusting for BMI, waist circumference and waist-to-hip ratio (p= 0.009). We found that in post-menopause females, serum estradiol level

was negatively associated with PEDF (r=-0.216, p=0.013) and was an independent determinant of PEDF

Conclusion: PEDF concentration is significantly elevated in patients with IGR and could be reduced after metformin treatment in T2DM patients with weight loss. We for the first time identifying decreased estradiol level was an independent risk factor for the elevated PEDF and may contribute to the occurrence of T2DM in post-menopause females.

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PS 052 The future of obesity management?

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Effects of a novel potent and specific melanin-concentrating hormone receptor 1 antagonist, AZD1979, on body weight homeostasis in mice and dogs

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Background and aims: Melanin-concentrating hormone (MCH) exerts an orexigenic response and while rodents express one receptor for MCH (Mchr1), humans, non-human primates and dogs express two MCH receptors (MCHR1 and MCHR2). MCHR1 antagonists have been developed for the treatment of obesity and they generally lower body weight in rodents. However, the relative contribution of the effect on food intake and energy expenditure as well as if MCHR1 antagonists can lower body weight in species expressing both MCH receptors are not fully understood. We have discovered a novel potent small molecule MCHR1 antagonist, AZD1979, and the aims of this study were to investigate the mechanism for weight loss in diet-induced obese (DIO) mice and to study the effect of AZD1979 on body weight in dogs expressing both MCH receptors.

Materials and methods: The effect of AZD1979 (20-60 μmol/kg by oral gavage twice daily for up to 3 weeks) on food intake and body weight was compared to vehicle in DIO wildtype and mchr1 knock-out (KO) mice. The importance of food intake and energy expenditure for the effects was analyzed using continuous pair-feeding based on 30 min intervals in an automated system and by indirect calorimetry (CLAMS system). The effect of AZD1979 (22-216 μmol /kg by oral gavage once daily for 4 weeks) on body weight was also studied in dogs.

Results: AZD1979 dose-dependently reduced body weight (-17.1±1% vehicle adjusted, $p<0.001$ at 60 μmol/kg), body fat mass (60 μmol/kg, 19.2±1.5 g vs. vehicle, 27.7±1.7 g, $p<0.001$) and improved homeostasis model assessment (HOMA)-index of insulin sensitivity (mM glucose x nM insulin) (60 μmol/kg, 4.9±0.9 vs. vehicle, 9.8±1.8, $p<0.01$). Importantly, AZD1979 had no effect on food intake or body weight in Mchr1 KO mice. AZD1979 (60 μmol/kg) and pair-fed vehicle dosed DIO mice showed similar body weight reduction up to 11 days. Thereafter, AZD1979 dosed DIO mice had lower body weights compared to pair-fed vehicle dosed DIO mice (41.5±1 g vs. 44.2±1 g, $p<0.001$ dosing day 17), indicating a preserved energy expenditure. In line with this, AZD1979 (60 μmol/kg) had no effect on energy expenditure day 0-3 post initial dose but elevated energy expenditure during the light periods day 7-10 post initial dose ($p<0.05$). Finally, AZD1979 also dose-dependently reduced body weight in dogs (-5.2±1.6% vehicle adjusted, $p<0.01$ at 216 μmol /kg).

Conclusion: We have discovered a novel potent and specific MCHR1 antagonist, AZD1979 that affects both food intake and energy expenditure leading to body weight loss in species expressing one or two MCH receptors.

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FGF21 improves the adipocyte dysfunction related to seipin-deficiency

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Background and aims: Berardinelli and Seip Congenital Lipodystrophy (BSCL) is a rare genetic disease characterized by an almost complete lack

of adipose tissue. Bi-allelic mutations in BSCL2, encoding seipin, are responsible for the most severe form of BSCL. Seipin function remains largely unknown, however, previous studies have shown that seipin deficiency affects adipogenesis.

Materials and methods: We used seipin knock-out (SKO) mice and we developed a new inducible seipine knock-down cell line (SKD). We use the FGF21 analog from LY2405319.

Results: SKO mouse model displays severe lipodystrophy with residual dysfunctional adipose tissues. Interestingly, by comparing the profile of these residual fat pads in 4 and 12-week-old mice, we highlighted a decrease in adiponectin mRNA and plasma levels with aging (3,25±0, 41 vs 0,64±0,12 μg/mL in 4 vs 12-week-old mice), suggesting that beyond adipogenesis, seipin is involved in adipocyte maintenance. Aiming to prevent this impairment, we treated 6-week old SKO mice with an FGF21 analog (LY2405319, Eli-Lilly) known to target adipose tissue and to increase adiponectin secretion. FGF21 treatment during 28 days improved random fed glycemia (256±49 vs 147±28 mg/dL in control vs treated SKO), normalised insulin sensitivity, and increased adiponectin expression in adipose tissue and secretion (0,64±0,12 vs 1,53±0,47 μg/mL). To further decipher the molecular pathways altered by seipin deficiency in mature adipocytes, we developed a unique SKD cell-line from 3 T3-L1, with inducible expression of a shRNA targeting seipin mRNA. shRNA expression was induced after differentiation, and at day 9, the SKD cells and control cells exhibited similar Oil red O staining and mature adipocyte gene expression with a 60% decrease in seipin mRNA levels. At day 21, SKD cells displayed a strong decrease in Oil red O-positive cells and in mRNA levels of mature adipocytes markers. Importantly, FGF21 treatment significantly prevented this phenotype.

Conclusion: This study highlights an essential role of seipin in the maintenance of the mature adipocyte integrity, and identifies FGF21 as a potential therapeutic target for BSCL2. Our new cellular model is a useful tool to distinguish seipin function in the mature adipocyte as opposed to its established role in adipocyte differentiation.

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Dose dependent effect of all-trans retinoic acid on weight and glucose homeostasis

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Background and aims: All-trans retinoic acid (ATRA) is a potent derivative of Vitamin A. In recent years, the importance of vitamin A in obesity and type 2 diabetes has become apparent. We previously showed that treatment (tx) of db/db mice with ATRA promoted the remodeling of WAT and attenuated inflammation by reducing macrophage infiltration. The objective of this study was to determine the effects of different doses of ATRA on weight, glucose homeostasis and body composition in obese, diabetic db/db mice.

Materials and methods: Three month old female mice were divided into 4 groups: high dose group was treated with 0.6 mg/mouse (ATRA-H), median dose group-0.2 mg/mouse (ATRA-M), low dose group-0.06 mg/mouse (ATRA-L) and control grp was treated with corn oil. Tx was 5 days/week for 12 weeks by voluntary oral feeding. Body weight and random glucose levels were determined weekly, and body composition analysis was performed at baseline, at week 6 and 12.

Results: Baseline body weights were similar in the 3 groups (46.3±0.8 g (ATRA-H) vs 46.4±0.8 g (ATRA-M) vs 46.8±0.9 g (ATRA-L). High dose group lost 4.6±1.9 g, with a significant difference at wk 10 (41.6 ±1.4 g (ATRA-H) vs 43.6±2.2 g (ATRA-M), 47.7±2.8 g (ATRA-L), $p<.05$), whereas no weight loss was observed in low dose group. After 10 weeks of tx, random glucose levels significantly decreased in the high dose grp (239.5±32 mg/dL (ATRA-H) vs 407.8±32 mg/dL (ATRA-M) vs 418.5±28 mg/dL (ATRA-L), $p<.05$). High dose ATRA treatment significantly decreased fasting glucose levels at week 6 (277.3±32 mg/dL (ATRA-H) vs 445.6±17 mg/dL (ATRA-M) vs 420.7±46 mg/dL (ATRA-L), $p<.05$). Baseline % body fat was similar in all the groups, which significantly reduced after 6 wks of high dose ATRA treatment (56.6±0.9 (ATRA-H) vs 54.7±1. 7 (ATRA-M) vs 57.1±1.5 (ATRA-L). The db/db mice developed hepatic steatosis, which was markedly reduced with high dose of ATRA. This change was reflected in the liver triglyceride content, which decreased significantly with ATRA (48.9±6.1 ug Triglycerides/mg Liver control vs 23.4±3.2 1 ug Triglycerides/mg Liver ATRA, $p<0.05$). Obesity is accompanied by downregulation of adipose PPAR-δ expression and activity, leading to weight gain and insulin resistance. ATRA increased PPAR-δ gene expression in visceral white adipose which was statistically significant (1.1±0.1 control vs 6.7±3.4 ATRA, $p<0.001$).

Conclusion: We have shown that ATRA promoted weight loss, improved glucose homeostasis and reduced hepatic steatosis by upregulating PPAR-δ expression suggesting that it may be an effective treatment for obesity-associated diabetes.

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Role of cannabinoid receptor type 1 in glucocorticoid-induced lipolysis, insulin resistance and central obesity in human adipose tissue

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Background and aims: Glucocorticoids and the endocannabinoid system are both involved in the regulation of energy balance. Cannabinoid receptor type 1 (CNR1) is highly expressed in the central nervous system. However, it is also expressed in adipose tissue. CNR1 antagonistic drugs such as rimonabant have previously been developed and marketed in efforts to treat obesity and metabolic comorbidities. However, psychiatric side-effects, notably depression, lead to its withdrawal from the market. We recently showed that CNR1 is upregulated in human adipose tissue by the synthetic glucocorticoid dexamethasone. Here, we study the involvement of CNR1 in glucocorticoid-induced lipolysis and insulin resistance in human adipose tissue.

Materials and methods: Human subcutaneous (sc) and omental (om) adipose tissue, obtained from non-diabetic volunteers (13 M/30 F, 24-66 yr, BMI 20.7-55.5 kg/m2) undergoing kidney donation or bariatric surgery, was incubated with or without dexamethasone (0.003-3 μM, 24 h). In addition, sc adipose tissue acquired from non-diabetic volunteers (5 M/17 F, 23-72 yrs, BMI 21.3-32.8 kg/m2) by needle biopsies from the lower abdomen was incubated with or without dexamethasone (0.3 μM, 24 h) in the presence or absence of the CNR1 antagonist/inverse agonist AM281 (1 μM) during the last 4 h of incubation. In adipose tissue, CNR1 expression as well as sc adipocyte lipolysis and glucose uptake was assessed following the incubations above.

Results: Dexamethasone increased CNR1 expression dose-dependently in adipose tissue ($p<0.001$ sc: by 14-fold, om: by 29-fold). CNR1 gene expression was higher in sc than om adipose tissue in overweight-obese subjects, while levels were similar between depots in normal-weight subjects. Furthermore, CNR1 gene expression in both depots correlated positively with markers of insulin resistance and central obesity such as fasting plasma levels of insulin (sc: $r=0.52$, $p<0.001$; om: $r=0.40$, $p<0.05$), HOMA-IR ($r=0.56$, $p<0.001$; $r=0.38$, $p<0.05$) and waist circumference ($r=0.62$, $p<0.001$; $r=0.44$, $p<0.01$). In subcutaneous adipose tissue, pre-treatment with dexamethasone (0.3 μM) for 24 h increased the rate of isoprenaline-stimulated lipolysis by about 50%, compared with control ($p<0.01$), whereas AM281 prevented this effect ($p<0.01$).

Moreover, incubation of subcutaneous adipose tissue for 24 h with only the CNR1-specific agonist ACEA (1 μM), increased isoproterenol-stimulated lipolysis in adipocytes by about 20% ($p<0.01$). Dexamethasonealso reduced the rate of basal glucose uptake in sc by about 40% ($p<0.001$) and insulin-stimulated glucose uptake by about 24% ($p<0.01$). However, treatment with AM281 did not prevent these effects.

Conclusion: Our findings suggest that CNR1 is upregulated in states of insulin resistance and central obesity. Its expression may therefore be associated with the metabolic syndrome. Furthermore, CNR1 may be a mediator involved in glucocorticoid-regulated lipid metabolism in human adipose tissue. This study gives further support for a role of the peripheral endocannabinoid system in central obesity and insulin resistance. It supports the potential of peripherally restricted CNR1 antagonists for future treatment of type 2 diabetes.

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Peripheral oxytocin administration activates vagal afferent nerves to suppress feeding, ameliorating hyperphagia and obesity in diabetic db/db mice

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Background and aims: Hyperphagia and obesity cause type 2 diabetes and hamper dietetic treatment. Oxtytocin (Oxt) is produced in the paraventricular nucleus and supraoptic nucleus of hypothalamus and, as a classic neurohypophysial hormone, promotes labor and lactation. Oxt is also released as a neurotransmitter within various brain regions and plays a critical role in regulation of food intake and body weight, as well as social behavior and stress responses. Peripheral administration of Oxt, as well as central administration, suppresses feeding and ameliorates obesity and metabolic syndrome in high fat diet-induced obese mice. However, peripheral Oxt reportedly little enters the brain through blood-brain barrier. Hence, the route through which peripheral Oxt informs the brain is unclear. In this study, we aimed to clarify whether vagal afferents mediate the sensing and anorexigenic effect of peripherally injected Oxt in healthy and diabetic *db/db* mice.

Materials and methods: To evaluate the direct action of Oxt on vagal afferents, we measured the cytosolic Ca^{2+} concentration ($[Ca^{2+}]_i$) in single nodose ganglion neurons isolated from mice, by using fura-2 ratiometoric analysis. To examine the involvement of vagal afferents in Oxt-induced anorexigenic effect, we measured food intake after intraperitoneal injection of Oxt in the mice received systemic capsaicin treatment, subdiaphragmatic vagotomy or sham-operation.

Results: Oxt evoked membrane depolarization, increased action potential firings, and increased $[Ca^{2+}]_i$ in single nodose ganglion neurons. The Oxt-induced $[Ca^{2+}]_i$ increases were inhibited by Oxt receptor antagonist. Majority of Oxt-responsive nodose ganglion neurons also responded to cholecytokin-8, a postprandial gastrointestinal hormone known to reduce food intake via direct interaction with vagal afferents. Moreover, around 80% of Oxt-responsive nodose ganglion neurons contained cocaine- and amphetamine-regulated transcript (CART) as the neurotransmitter. Intraperitoneal injection of Oxt suppressed food intake and induced c-Fos expression in the nucleus tractus solitarius (NTS) of medulla, to which vagal afferents project. This feeding suppression and c-Fos expression in NTS were blunted by subdiaphragmatic vagotomy or capsaicin treatment. In obese diabetic *db/db* mice, leptin failed to but Oxt increased $[Ca^{2+}]_i$ in nodose ganglion neurons. Moreover, sub-chronic administration of Oxt decreased feeding and body weight gain.

Conclusion: Peripheral injection of Oxt suppresses food intake by direct activation of vagal afferents. Oxt exerts these effects in leptin-resistant obese diabetic *db/db* mice, and its sub-chronic administration suppresses feeding and body weight gain. This "vagal afferent-mediated peripheral Oxt signaling to brain" may provide a novel effective route to treat hyperphagia and obesity in diabetes.

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A monoclonal anti-aP2 antibody treats diabetes and fatty liver disease in obese mice

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Background and aims: The lipid chaperone aP2/FABP4 has been implicated in the pathology of many immunometabolic diseases, such as diabetes and atherosclerosis. While multiple lines of evidence also supports its involvement in human disease, targeting aP2 for therapeutic applications have not yet been accomplished. Recent studies in our laboratory have shown that aP2 is not simply an intracellular protein binding lipids but an active adipokine that contributes to hyperglycemia by promoting hepatic gluconeogenesis and interfering with peripheral insulin action. Multiple groups have now demonstrated that serum aP2 levels are markedly elevated in mouse models of obesity, and human serum aP2 levels strongly correlate with BMI, insulin resistance, dyslipidemia, and cardiovascular risk. Importantly, blocking aP2 in preclinical models showed strong anti-diabetic activity. These observations raised an exciting new strategy for targeting serum aP2 to treat metabolic disease with a monoclonal anti-aP2 antibody.Here, we report the identification of a highly effective, anti-aP2 mAb, CA33, and the characterization of its effects in vivo.

Materials and methods: In hyperinsulinemic-euglycemic clamp studies, we found that the anti-diabetic effect of CA33 was predominantly linked to the regulation of hepatic glucose output and peripheral glucose utilization. We also examined the properties of this antibody by structural and biochemical studies, identified its target epitopes, and demonstrated its target specificity.

Results: Treatment of mice with dietary or genetic obesity with CA33 lowered fasting blood glucose levels, improved glucose metabolism, increased systemic insulin sensitivity and reduced fat mass and liver steatosis.

Conclusion: We conclude that development of an anti-aP2 monoclonal antibody-mediated therapeutic is a feasible approach and would constitute a strong candidate for the treatment of diabetes and fatty liver disease.

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Prevention of progression from prediabetes to diabetes in 109 hypogonadal men treated with testosterone for up to 8 years

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Background and aims: While short-term studies using testosterone replacement therapy (TRT) in hypogonadal men with type 2 diabetes mellitus (T2DM) have yielded mixed results, long-term treatment has

shown beneficial effects of TRT. There is no information, however, whether TRT has benefits in hypogonadal men with prediabetes.

Materials and methods: Men presenting to urological offices with various complaints were screened for the presence of hypogonadism and, if found hypogonadal, offered TRT. Those who had received at least 1 year of treatment with TU were entered into two independent, prospective, observational, cumulative registry studies. 109 men with prediabetes, defined as a baseline HbA_{1c} from 5.7 to 6.4%, were analysed. TU was administered in 3-month intervals for up to 8 years. At each or each other visit, anthropometric and metabolic parameters were measured. Patients whose TRT was temporarily interrupted were excluded from the analysis.

Results: Mean age was 57.37±8.99 years. Mean weight decreased from 96.15±13.05 to 84.14±6.98 kg. Change from baseline was -14.58± 0.68 kg, percent change from baseline -14±0.65%. Waist circumference decreased from 103.8±6.88 to 94.32±4.53 cm. Change from baseline was -9.62±0.44 cm. BMI decreased from 30.55±4.35 to 27.04± 2.55 kg/m^2, change from baseline -4.66±0.23 kg/m^2. Waist-to-height ratio decreased from 0.58±0.04 to 0.53±0.03. All anthropometric measures were statistically significant vs. baseline ($p<0.0001$) and improved progressively with statistical significance compared to the previous year for 6 to 7 years. Fasting glucose decreased from 5.43±0.68 to 4.63± 0.6723 mmol/L ($p<0.0001$), change from baseline -0.94±0.11 mmol/L reaching a plateau after 1 year. HbA_{1c} decreased from 5.9±0.21 to 5.38± 0.26% ($p<0.0001$), change from baseline -0.59±0.04% with statistical significance compared to the previous year for the first 3 years. The triglyceride to HDL ratio, considered a surrogate parameter of insulin resistance, declined from 5.62±2.61 to 2.6±0.74 ($p<0.0001$). The product of fasting glucose and triglycerides (TyG Index), another surrogate for insulin resistance, improved from 4.04±0.17 to 3.81±0.14. No patient progressed from prediabetes to T2DM. All but 4 patients' last measured HbA_{1c} was <5.7%. Lipid patterns as well, blood pressure, liver transaminases and C-reactive protein all improved significantly. 3 patients dropped out, 2 due to relocation to a different city, 1 was lost to follow-up. There were no major adverse cardiovascular events during the full observation time.

Conclusion: Hypogonadal men with prediabetes defined by HbA_{1c} from 5.7 to 6.4% showed clinically meaningful and sustainable weight loss as well as improvements in glycaemic control when receiving long-term treatment with testosterone. No patient advanced from prediabetes to overt T2DM. TRT seems to be effective to improve anthropometric and metabolic parameters in hypogonadal men and in preventing progression from prediabetes to T2DM, thereby potentially reducing cardiometabolic risk.

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Effect of apelin on duodenal contraction: consequences on the gut-to brain axis in the control of glucose metabolism

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Background and aims: In the gut, the enteric nervous system (ENS) controls intestinal contractility allowing postprandial absorption of nutrients. In diabetic human and mice, ENS function is profoundly altered resulting in intestinal hyper-contractility. Our original concept is that "ENS/intestinal contraction" coupling, could also permit to modulate central nervous system activity (via afferent nervous messages), and in particular the hypothalamus, a key area able to control glucose utilization in peripheral tissues. In this study, we aim at demonstrating that apelin, a bioactive peptide present in the intestinal lumen, could target ENS neurons and so control activity of "ENS/intestinal contraction" coupling. These potential modifications could have consequences on hypothalamic Nitric Oxide release (NO, a neurotransmitter strongly involved in the control of glucose metabolism) leading to variations of glucose utilization in peripheral tissues.

Materials and methods: Animals were handled in accordance with the principles and guidelines established by the National Institute of Health and Medical Research. Male C57BL6/J mice are fed on normal or high-fat diet. ENS neurons expressing APJ, the apelin receptor, are identified by immunohistochemistry. Effects of apelin on duodenal contractility are measured ex vivo with an isotonic transducer (Krebs-Ringer is used as control), and in vivo by telemetry (water is used as control). Hypothalamic release of NO in response to apelin infusion in the digestive tract, is recorded in vivo via specific amperometric NO probe implanted in the hypothalamus (water is used as control). Peripheral glucose utilization is assessed following oral gavage of apelin coupled with tritiated glucose (water is used as control).

Results: Immunohistochemistry experiments reveal that APJ is expressed in ENS neurons. In normal mice, low-dose of apelin stimulates ex vivo duodenal mechanical activity (151±15,55% of basal contraction amplitude vs 90±11, 14 for control; $P<0,05$) and in vivo duodenal electrical activity (123±0,67 (fold increase) vs 114±0,56 for control; $P<0,001$). Average frequency of NO hypothalamic release is significantly reduced following infusion of low-doses of apelin in the digestive tract (0,11±0,003 Hz vs 0,12±0,002 for control; $P<0.001$) associated with a significant decrease in muscle glucose utilization (158±17,3 Log10[dpm/g tissue] vs 267±33,1 for control; $P<0.05$). In contrast, higher concentrations of apelin inhibit in vivo duodenal electrical activity, restore hypothalamic NO release and improve glucose utilization and tolerance in normal and obese/diabetic mice.

Conclusion: We demonstrate that apelin could target the ENS activity in order to modify hypothalamic NO release and then to control muscle glucose entry. Decrease the activity of the duodenum by acting on ENS neurons via bioactive peptides could be considered as a new therapeutic strategy for metabolic diseases.

PS 053 Adipokines and novel proteins in adipose tissue

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siRNA-mediated silencing of DPP4 improves insulin sensitivity of human primary adipocytes

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Background and aims: DPP4 is a ubiquitously expressed cell surface protease which is also released to the circulation as soluble DPP4 (sDPP4). DPP4 is able to clip numerous substrates e.g. the incretin hormones, and therefore represents an important drug target for the treatment of type 2 diabetes. Recently, we identified DPP4 as a novel adipokine oversecreted in obesity and thus potentially linking obesity to the metabolic syndrome. Furthermore, we could show that sDPP4 impairs insulin signaling in an autocrine and paracrine fashion in different cell types. However, it is still unknown which functional role DPP4 plays within adipose tissue. The aim of this study was to elucidate the influence of siRNA-mediated knockdown of DPP4 on differentiation and functionality of adipocytes.
Materials and methods: Primary human adipocytes were silenced with a specific DPP4 siRNA one day prior to start of differentiation. Markers of differentiation, inflammation and adipocyte functionality were measured by Western Blot, qRT-PCR and ELISA. Furthermore adipokine profiler arrays were performed with supernatants from day 14 of differentiation to monitor changes in the secretome. In these arrays the relative level of 58 human adipokines can be determined simultaneously. On day 14 of differentiation, cells were stimulated with 100 nM insulin and parameters of insulin signaling were monitored.
Results: Very high silencing efficiency was achieved at the DPP4 mRNA level (68±15%) which remained stable over the whole differentiation. DPP4 protein expression was diminished substantially to about 50% at day 14 of differentiation. DPP4 knockdown had no effect on protein or mRNA expression of the differentiation marker PPARγ. Functional adipocyte markers like HSL and Glut4 were slightly but not significantly reduced at the level of protein expression on day 14. Of the 58 adipokines spotted on the membrane of the profiler arrays, 31 proteins were detectable. Most of them were unaffected by DPP4 silencing in adipocytes. Slight changes were found in the secretion of Cathepsin L, MIF and IGFBP-6, which seemed to be decreased whereas IL-10 secretion seems to be upregulated. mRNA expression and release of adiponectin and MCP-1 were unchanged by silencing, whereas mRNA expression of IL-6 was significantly reduced in adipocytes with reduced DPP4 expression. Most interestingly, at the level of insulin signaling DPP4 knockdown affected the insulin-stimulated phosphorylation of the insulin receptor (Tyr1150/1151) and expression of the insulin receptor substrate 1(IRS1) which are increased by about 60% and 40%, respectively. In accordance to that, silencing induced a significant 1.7 -fold higher insulin-stimulated phosphorylation of Akt (Ser473) compared to control whereas basal levels remained similar. Further experiments to elucidate the underlying mechanisms and consequences of altered insulin signaling are ongoing at the moment.
Conclusion: In conclusion, knockdown of DPP4 improves the responsiveness towards insulin in primary human adipocytes without affecting adipocyte differentiation and secretory function. Taken together with our previous observation that sDPP4 induces insulin resistance in adipocytes and higher adipose DPP4 levels in obese insulin-resistant patients, DPP4 might be a potential marker of adipose tissue insulin sensitivity.

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A new role for GLP-2 in human adipose tissue: effects on differentiation and adipocyte metabolism

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Background and aims: Glucagon-like peptide-2 (GLP-2) is a proglucagon-derived peptide structurally related to GLP-1 that is released by enteroendocrine L cells, which plays an important role in the regulation of intestinal growth and function. Unlike GLP-1, GLP-2 has not been reported to modulate insulin secretion, however, meal-related GLP-2 secretion suggests a putative role for GLP-2 in metabolism regulation beyond its well-established intestinal function. The GLP-2 receptor (GLP-2R) shows significant homology with GLP-1R, but its expression has been described largely restricted to intestine, lungs and hypothalamus. Interestingly, our group has observed GLP-2R expression in human adipose tissue. This work aims to gain insight into the molecular function of GLP-2/GLP-2R axis on energetic metabolism, focusing on its potential modulatory function on adipose tissue.
Materials and methods: Clinical studies. Visceral (VAT) and subcutaneous (SAT) adipose tissue samples from lean and obese individuals with different degrees of insulin resistance were obtained during a scheduled surgical procedure (27 women and 21 men, aged 42,4±11,3 years and BMI range 23,3 to 42,4 kg/m2). Stroma-vascular (SVF) and adipocyte fractions were also obtained. Gene and protein expression studies were performed. in vitro studies. SGBS cell line was used as a model of human subcutaneous pre-adipocytes. Human adipocytes (hADSC) were isolated from adipose tissue from lean and obese patients. Effects of GLP-2 on proliferation, viability, gene expression and adipocyte metabolism were analysed.
Results: GLP-2R is expressed in SAT and VAT of lean and obese individuals mainly in the adipocyte fraction. Patient classification according to HOMA-IR shows a GLP-2R down-regulation in SAT on high-IR individuals, with independence on BMI, suggesting a putative relationship between insulin sensitivity and GLP-2R expression on AT. Accordingly, GLP-2R expression is down regulated at mRNA and protein level on adipocytes derived from hADCS isolated from obese patients. In vitro studies demonstrate no effects on adipocytes proliferation and viability during differentiation, but GLP-2 administration produce an increase on lipids accumulation with an up-regulation of lipogenic genes in differentiated adipocytes.
Conclusion: Our data suggest that adipose tissue may be a new target for GLP-2 activity, influencing the adipogenic and functional capacity of human adipocytes. Understanding the role of GLP-2 in the metabolic events that take place in AT my help to define more precisely new potential indications of clinical usefulness in metabolic imbalance.
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Adiponectin is required for adaptive thermogenesis by enhancing the browning of white adipose tissues in mice

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Background and aims: Adiponectin is a major adiocyte-secreted adipokine with multiple protective effects against a cluster of obesity-related cardiometabolic diseases. Plasma levels of adiponectin are inversely associated with obesity and insulin sensitivity. However, its role in adaptive thermogenesis remains unclear. This study aims to investigate whether adiponectin regulates thermogenic activity of adipose tissues in mice.

Materials and methods: Adiponectin-knockout mice and wild-type littermates were exposed to cold temperature to induce thermogenesis. The browning phenotypes of different adipose depots were analyzed by immunocytochemistry and real-time PCR. The thermogenic activity of adipose tissues was measured with the Seahorse analyzer.
Results: Chronic cold exposure caused a dramatic and selective elevation of adiponectin in stromal vascular fraction of subcutaneous white adipose tissue (scWAT),but not in classical brown adipose tissue. In scWAT, adiponectin co-localized with alternatively-activated M2 macrophages in response to cold challenge. Chronic cold exposure-induced accumulation of M2 macrophages, activation of biege cells and thermogenesis were obviously impaired in scWAT of adiponectin-knockout mice, whereas these impairments were reversed by replenishment with recombinant adiponectin. Further analysis found that adiponectin bound to the cell surface of M2 macrophages via its co-receptor T-cadherin and promoted the cell proliferation by activation of Akt, thereby leading to beige cell activation by secreting catecholamines.
Conclusion: Adiponectin is a key mediator for cold-induced browning and adaptive thermogenesis of white adipose tissue, by facilitating the crosstalk between adipocytes and immune cells.
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FNDC5 (irisin) expression is increased in subcutaneous adipose tissue in obese patients with type 2 diabetes one year after bariatric surgery
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Background and aims: Gastric bypass surgery improves glycaemic control, but the underlying mechanisms including potential changes in adipoinsular axis are incompletely understood. The aim of this study was to investigate potentially causal or associated alterations in gene expression of adipokines, myokines and hormones in the subcutaneous adipose tissue of diabetic and non-diabetic obese patients before and after gastric bypass.
Materials and methods: Biopsy specimens from the subcutaneous adipose tissue of 12 diabetic and 12 non-diabetic patients at and one year after gastric bypass were analyzed with quantitative real-time PCR for the expression levels of various adipokines and myokines.
Results: Non-diabetic subjects were found to express significantly higher levels of FNDC5 (irisin) in the subcutaneous adipose tissue than diabetic subjects before gastric bypass ($p=0.0167$). FNDC5 expression in the subcutaneous adipose tissue of diabetic patients was significantly increased one year after surgery ($p<0.001$). In non-diabetic subjects, there was no significant increase observable ($p=0.158$). The increase in FNDC5 in diabetic subjects correlated with reduction in BMI (Pearson's $r=0.718$, $p=0.009$). No correlation was observed between FNDC5 expression and early insulin response or insulin sensitivity index.
Conclusion: To the best of our knowledge, this is the first study to investigate the role of the novel myokine irisin in bariatric surgery mediated weight loss in diabetic patients. The results of this study demonstrate that exercise-independent weight loss significantly increases irisin expression levels in subcutaneous adipose tissue of diabetic subjects.
Supported by: EFSD/MSD

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Microdialysis and proteomics of the subcutaneous interstitial fluid reveals abundance of galectin-1 in type 2 diabetes patients
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Background and aims: It is well established that adipose tissue dysfunction conveys insulin resistance and type 2 diabetes (T2D). We have previously used microdialysis for in vivo studies of adipose tissue metabolism in human subjects. Our aim was to identify novel proteins in insulin resistant adipose tissue by applying a combination of subcutaneous microdialysis and proteomics in T2D patients and healthy controls.
Materials and methods: Eight newly diagnosed male T2D patients and 7 male healthy participants were recruited. Microdialysis was performed in the subcutaneous adipose tissue (SCAT) and the obtained dialysates were analysed with a tandem MS. For validation of our results, dialysates and serum samples were quantified for galectin-1 using ELISA. Moreover, isolated adipocytes were prepared from biopsies and cell size and galectin-1 gene expression were determined. In a larger group of T2D patients ($n=31$) and healthy controls ($n=20$) serum levels of galectin-1 were measured. Furthermore, another group of obese patients ($n=24$) was subject to a very low calorie diet (VLCD) for 16 weeks and galectin-1 gene expression was measured at week 0, 8, 16 and 18. In addition, SCAT biopsies were obtained from non-diabetic participants where glucose uptake, GLUT 1 and GLUT 4 expression were measured in the presence of galectin-1. Finally, insulin signalling was quantified in cultured endothelial cells through Western blot in the presence of galectin-1.
Results: Thirty-six different proteins differed significantly when comparing dialysates obtained from SCAT in T2D patients and healthy controls. One of these proteins showing a significant over-expression in T2D patients compared with the controls, was galectin-1. This was validated by ELISA analyses of the dialysate samples ($p<0.05$) and qPCR of isolated adipocytes ($p<0.05$). Moreover, circulating galectin-1 levels correlated with subcutaneous adipocyte size and waist/hip ratio ($p<0.05$, respectively). In the expanded groups there was also a significant difference in circulating galectin-1 levels when comparing T2D patients and healthy controls ($p<0.05$). In this larger sample, we also observed linear correlations between serum galectin-1, fasting glucose and fasting insulin at concentrations below 10 mmol/L and 10 mU/L respectively. The correlations were not observed in participants with higher fasting glucose or insulin levels. Furthermore, a VLCD diet reduced galectin-1 gene expression in adipose tissue biopsies ($p<0.05$). In addition, adipocytes showed a 20% reduction ($p<0.05$) in basal and insulin-stimulated glucose uptake after pre-incubation with galectin-1 for 24 h and GLUT4, but not GLUT1 mRNA expression was reduced. Finally, endothelial cells pre-incubated with galectin-1 for 24 hrs showed a significant decrease in AKT phosphorylation after 5 min of insulin stimulation ($p<0.05$).
Conclusion: Galectin-1 is an abundant protein in subcutaneous adipose tissue of T2D patients, with effects on both insulin signalling and glucose uptake. Circulating galectin-1 levels may be a biomarker for an insulin-resistant adipose tissue indicating an increased diabetes risk.
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Clock genes are implicated in the regulation of energy homeostasis in humans

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Background and aims: The circadian clock coordinates numerous metabolic processes to adapt physiological responses to light-dark and feeding regimens. The implication of the circadian clock in the control of energy balance, and consequently in the regulation of body weight, is widely studied in rodents, but not in humans. Here, we investigated (1) whether the expression of clock genes in human adipose tissue is changed by weight loss and weight regain and (2) whether these alterations are associated with metabolic parameters.
Materials and methods: Subcutaneous adipose tissue samples were collected before and after 8 weeks of weight loss on a 800 kcal/d hypocaloric diet (Modifast, Nutrition et Santé, France plus 200 g/d vegetables) and again after 6 month of weight maintenance at the same time of day. Only participants who achieved a weight loss of at least 8% after 8 weeks were selected for the study (n=50, age 40.8±0.9 years, BMI 34.2±0.6 kg/m2, mean +/- SEM). The expression of core clock genes, and key metabolic and inflammatory genes was determined by quantitative real-time PCR.
Results: The mean weight loss for the group was 10.8% of initial weight after the hypocaloric phase and 8.0% after the weight maintenance phase. The expression of core clock genes PER2 and NR1D1 was increased in the hypocaloric phase ($p=7\times10\text{-}6$ and $p=0.031$, respectively). Expression of BMAL1 and CLOCK did not change in the hypocaloric phase, but decreased during the weight maintenance period ($p=0.014$ and $p=0.012$, respectively). Clock gene expression levels and their weight loss-induced changes strongly correlated with each other at all investigation days ($p<0.001$). The expression levels of PER2, but not its weight loss-induced alterations, negatively correlated with BMI and body fat percentage ($p<0.05$). Moreover, BMAL1, CLOCK, and PER2 demonstrated a large number of correlations with genes involved in the inflammatory response (TLR4, NFKB, NFKBIA, NLRP3), energy metabolism (SIRT1) and fat metabolism (FASN, CPT1A, LPL, PPARG, PGC1A), as well as with adiponectin gene expression (ADIPOQ).
Conclusion: Clock gene expression in human subcutaneous adipose tissue is regulated by the body weight changes and associated with BMI, body fat percentage and expression of metabolic and inflammatory genes. This suggests that clock genes are implicated in the regulation of energy homeostasis in humans.
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Role of lipocalin 2 in human adipose tissue metabolism and glucocorticoid-induced insulin resistance

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Background and aims: Lipocalin 2 (Lcn2), a peptide produced by adipose tissue (AT), has been reported to be linked to obesity and related metabolic disorders. Agents promoting insulin resistance like glucocorticoids can induce Lcn2 expression and this can be reversed by thiazolidinediones. However, the role of Lcn2 in glucose and lipid metabolism is not fully explored in humans. We aimed to study effects of Lcn2 on glucose and lipid metabolism in human AT.
Materials and methods: Paired samples of human subcutaneous (SC) and omental (OM) AT obtained from non-diabetic subjects undergoing elective surgery (12 M/29 F, 24-66 yrs, BMI 20.7-55.5 kg/m2) were incubated with Dexamethasone (Dex), a synthetic glucocorticoid, (0.03-3 μM, 24 hr) to measure Lcn2 gene expression. Adipose tissue samples from non-diabetic subjects (3 M/19 F, 22-71 yrs, BMI 21.2-29.4) were treated with recombinant human (RH) Lcn2 (100 ng/ml, 24 hr) and used for gene (n=11) and protein expression (n=7). Isolated adipocytes were used for glucose uptake (n=8) and lipolysis (n=11) assays. Fasting blood samples were taken to measure the levels of plasma glucose, insulin and lipids, and anthropometric measurements were done.
Results: When considering both males and females, Dex significantly increased the Lcn2 gene expression in SC AT by 2.5 fold ($p<0.05$) in a dose-dependent manner. However, in OM AT, there was only a tendency to an increased Lcn2 expression by 1.4 fold (NS). Gender comparison shows that Dex increased Lcn2 expression in SC AT from females by 3.5 fold ($p<0.05$), but had no effect in males. No effect on Lcn2 expression in OM AT was seen in either gender. RH-Lcn2 treatment reduced the basal and insulin-stimulated (1000 μU/ml) glucose uptake in SC adipocytes by 18% and 25% respectively ($p<0.01$), similar in both genders. In addition, Lcn2 reduced the protein content of the glucose transporters GLUT1 and GLUT4 by ~40% ($P<0.01$) in SC AT. In paired samples, Lcn2 gene expression in OM depot was ~7.5 fold higher than in SC depot ($p<0.001$). Lcn2 expression in both SC and OM AT showed a tendency to be correlated with BMI (NS). The basal Lcn2 expression in SC AT was positively correlated with fasting glucose ($r=0.32$, $p<0.05$), SC adipocyte size ($r=0.509$, $p<0.002$) and OM adipocyte size ($r=0.397$, $p=0.017$). In OM AT, Lcn2 expression was positively correlated with fasting insulin ($r=0.359$, $p=0.034$) and HbA1C ($r=0.376$, $p=0.031$). In a stepwise multivariate analysis, glucose ($r=0.457$, $p<0.01$) and HbA1C ($r=0.377$, $p<0.05$) remained significant predictors of Lcn2 gene expression in SC and OM AT, respectively. In human SC AT, Lcn2 reduced PPARγ gene expression by 24% ($p<0.05$) and its target gene adiponectin by 34% ($p<0.01$). Finally, RH-Lcn2 did not affect basal or isoproterenol stimulated lipolysis in adipocytes.
Conclusion: Our results suggest that glucocorticoids increase Lcn2 expression in SC AT from females, and that this inhibits glucose uptake and reduces expression of GLUT1 and GLUT4 in adipocytes. Decreased PPARγ expression by Lcn2 suggests a possible role in the regulation of human adipogenesis. Lcn2 expression in AT is also positively correlated with blood glucose levels. In conclusion, the adipokine Lcn2 can contribute to development of insulin resistance in human AT, and it may be a mediator of metabolic adverse effects of glucocorticoid excess.
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Adipose tissue miR-15b, miR-20a, miR-20b, miR-27b, miR126, miR-130a, miR210, miR296 and Let-7f expression profile in relation to obesity and type 2 diabetes

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Background and aims: Adipose tissue expandability is an important factor determining the appearance of insulin resistance and Type 2 diabetes (T2D) associated to obesity. Several studies revealed the importance of miRNAs in the post-transcriptional regulation of adipose tissue functionality. MiR-15b, miR-20a, miR20b, miR-27b, miR126, miR130a, miR210, miR-296 and Let-7f have shown a differential expression profile between lean and obese subjects and between metabolically healthy and

type 2 diabete subjects in visceral adipose tissue (VAT). These microRNAs are principally involved in adipogenic, angiogenic and apoptotic regulation. Bioinformatic's analysis highlighted 50 target genes of these microRNAs; 16 among them:CDKN1A, E2F3, ESR1, GLUL, GPI, HIF1A, HIST2H3A, LRIG3, MYC, MEF2D, NUP210, STAT3, SERTAD2, UBA1, VMP1, VEGFA, showed significant expression differences in visceral adipose tissue in relation to obesity and type 2 diabetes. In summary, our data highlight the potential involvement of miR-15b, miR-20a, miR20b, miR-27b, miR126, miR130a, miR210, miR-296 and Let-7f in the regulation of apoptosis, adipogenesis and angiogenesis in adipose tissues and to have an association with obesity and type 2 diabetes.

Materials and methods: MicroRNAs and mRNA were extracted from visceral and subcutaneous adipose tissue biopsies from the following subjects: Lean (n=10), T2D lean subjects (n10) obese (n=10), T2D obese subjects (n=10). MicroRNAs miR-15b, miR20a, miR.20b, miR-27b, miR-126, miR-130a, miR-210, miR-296 and Let-7 were extracted and analyzed by qPCR from visceral and subcutaneous adipose tissues. In silico prediction of target genes was carried out using miRTarBase, Panther and David. The network and visualization using Cytoscape software.

Results: MiR-15b, miR-20a, miR20b, miR-27b, miR126, miR130a, miR210, miR-296 and Let-7f have shown a differential expression profile between lean and obese subjects and between metabolically healthy and T2D subjects in VAT. A prediction in silico by miRTarBase, Panther and David showed that 50 target genes share connections with at least two or three of the studied miRNA. 16 among them: CDKN1A, E2F3, ESR1, GLUL, GPI, HIF1A, HIST2H3A, LRIG3, MYC, MEF2D, NUP210, STAT3, SERTAD2, UBA1, VMP1, VEGFA, showed statistically significant expression differences in visceral adipose tissue in relation to obesity and type 2 diabetes

Conclusion: Our data highlight the potential involvement of MiR-15b, miR-20a, miR20b, miR-27b, miR126, miR130a, miR210, miR-296 and Let-7f in the regulation of apoptosis, adipogenesis and angiogenesis in adipose tissues and to have an association with obesity and type 2 diabetes.

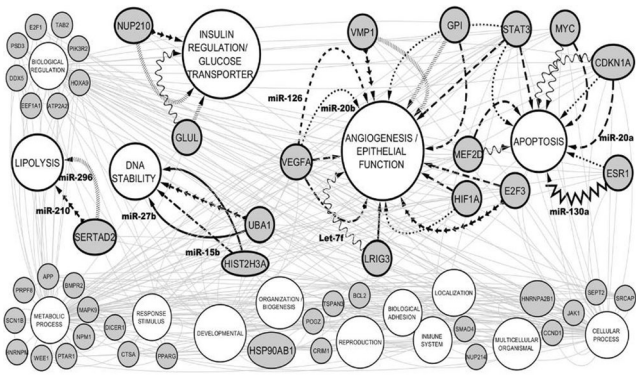

Fig.1 . Mapping of target genes that were share miRNA studied to biological process. Biological network were created using the Cytoscape software. This network is made of 50 gray nodes for target genes, 16 white nodes for biological processes and 317 edges that shown regulations between 9 miRNAs and 50 target gene. The 16 target genes: CDKN1A, E2F3, ESR1, GLUL, GPI, HIF1A, HIST2H3A, LRIG3, MYC, MEF2D, NUP210, STAT3, SERTAD2, UBA1, VMP1, VEGFA, that were differentially expressed in visceral adipose tissue in relation to obesity and type 2 diabetes are shown through miRNA involved in each process.

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PS 054 Fat on fire! Inflammation and obesity

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Hyperinsulinaemia increases proinflammatory factors genes expression in subcutaneous adipose tissue in young healthy subjects

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Background and aims: In obese and type 2 diabetic subjects adipose tissue is characterized by a chronic low-grade inflammation witch is correlated with an increased secretion of proinflammatory cytokines, such as macrophage inhibitory factor (MIF), monocyte chemotactic protein-1 (MCP-1) and IL-18, which might contribute to insulin resistance. Hyperinsulinemia, frequently present in type 2 diabetes and obesity, might be one of the drivers of the enhanced production of proinflammatory adipokines, however this issue remains controversial. It is also unclear if the alternations in expression of proinflammatory factors genes in adipose tissue are already present in young subjects with increased adiposity but without overt metabolic disturbances. The aim of the present study was to assess the regulation of proinflammatory factors genes expression in subcutaneous adipose tissue by hyperinsulinemia and their relationships with adiposity and insulin sensitivity in young subjects.

Materials and methods: We examined 150 young (age 23.39± 2.93 years) apparently healthy subject with normal glucose tolerance, 83 lean (BMI<25 kg/m2) and 67 with overweight or obesity (BMI between 25 and 40 kg/m2). In all subjects insulin sensitivity was evaluated by the 2 h euglycemic-hyperinsulinemic clamp. Additionally, in 20 subjects, clamp was prolonged to 6 hours. Biopsy of subcutaneous adipose tissue was performed before each clamp and after 6-hour clamp. Adipose tissue mRNA expression of MIF, MCP-1, IL-18 and subunits of nuclear factor κ B (NFκB) was analysed with Real-Time PCR.

Results: Obese subjects had higher adipose tissue MIF, MCP-1, IL-18, NFκB1 expression (all $p<0.05$). 6 h hyperinsulinemia increased the adipose tissue expression of MIF by 22% ($p=0.009$). Furthermore, 6 h hyperinsulinemia resulted in an increase in the expression of NFκB1 by 35%, ($p=0.004$) and NFκB2 by 54% ($p=0.008$) in subcutaneous adipose tissue. We found positive correlations between adipose tissue MIF, MCP-1, IL-18 expression and BMI ($r=0.17$, $p=0.04$; $r=0.29$, $p=0.001$ and $r=0.35$, $p<0.0001$, respectively), waist circumference ($r=0.21$, $p=0.01$; $r=0.22$, $p=0.009$ and $r=0.34$, $p<0.0001$, respectively) at baseline. Adipose tissue MIF and IL-18 expression was negatively related to insulin sensitivity ($r=-0.17$, $p=0.04$; $r=-0.18$, $p=0.03$, respectively) in the baseline state.

Conclusion: Our data show that 6 h hyperinsulinemia resulted in an increased expression of the proinflammatory factors in adipose tissue. This expression is already increased in young healthy people with excess body fat.

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Plasma chemerin is elevated in type 2 diabetes, is associated with impaired kidney function, and is predictive for cardiovascular events

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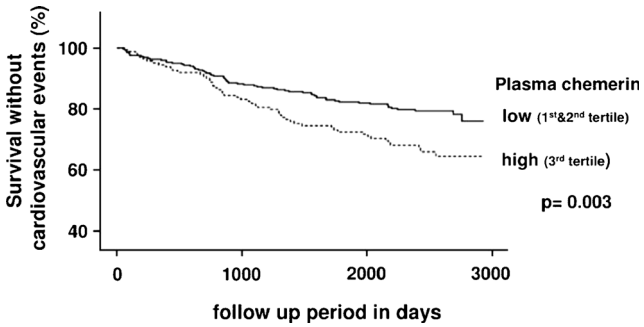
Background and aims: The association of the novel adipokine chemerin with cardiovascular event risk is unclear and is addressed in the present study.
Materials and methods: We measured plasma chemerin levels in 495 patients undergoing coronary angiography for the evaluation of established or suspected stable CAD.
Results: Chemerin was higher in patients with type 2 diabetes mellitus (T2DM, n=111) than in non-diabetic subjects (192±73 vs.170±65 ng/ml, p=0.001). Further, chemerin was significantly and independently associated with the glomerular filtration rate (GFR) in analysis of covariance using age, sex, and BMI as covariates (F=49.6, p<0.001). Prospectively, we recorded 186 cardiovascular events during a 8-year follow-up. Chemerin both univariately and after multivariate adjustment including baseline GFR significantly predicted cardiovascular events, with hazard ratios of 1.72 [95%CI 1.19-2.47], p=0.004 and 1.51 [1.03-2.23], p=0.037 for the top tertile of chemerin versus the first and second tertiles, respectively. A cardiometabo-chip-analysis revealed an association of two nearby located SNPs in TP53BP1 and CAPN3 rs2444030 nominal p-value=5.2 e-9, and rs3098423 nominal p-value=9.6 e-8) with chemerin concentration. Haplotype analysis for these two SNPs revealed a significantly impaired GFR associated with the fully mutated haplotype compared to all other haplotypes (OR=0.63, p=0.006).
Conclusion: We conclude that high chemerin is characteristic of T2DM, is associated with impaired kidney function, and is predictive for cardiovascular events.



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The role of the nuclear receptor FXR in obesity-induced adipose tissue inflammation

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Background and aims: Obesity plays a central role in the development of metabolic syndrome and related diseases. Understanding the mechanism behind the development is necessary to adopt the right clinical approach to prevent and treat metabolic disorders. Obesity is characterized by a state of low-grade inflammation consisting of a progressive infiltration of immune cells in adipose tissue. The molecular mechanisms and the contribution of the different cell types involved are still not clear. Recent observations suggested that adipocytes themselves may exert immunological functions. The nuclear receptor FXR plays major role in regulating bile acid metabolism, but it plays also a role in maintaining systemic glucose/lipid homeostasis. Although lower than in liver, FXR is expressed in adipose tissue and regulates adipocyte differentiation and function. FXR-KO mice, in the lean state, show impaired glucose tolerance and peripheral insulin resistance. By contrast, in mouse models of obesity (ob/ob and high fat diet (HFD), FXR-deficiency protects from obesity. Recently, has been shown that FXR plays a role in hepatocyte inflammation. The aim of this study was to elucidate the role of FXR in adipocytes on the development of obesity-induced inflammation.
Materials and methods: WT and FXR KO mice were fed a low fat diet (LFD) or a HFD. After 12 weeks, epididymal white adipose tissue (e-WAT) was collected and inflammatory phenotype was analyzed. Cytofluorimetric analysis was used to evaluate number and phenotype of immune cells infiltrating WAT. mRNA was isolated from WAT to analyze the expression of inflammatory mediators by quantitative PCR. In vitro differentiated primary adipocytes from WT and FXR KO mice were stimulated with cytokines (TNFα 20 ng/ml, IFNγ 20 ng/ml), LPS (100 ng/ml) or palmitic acid (PA, 0.5 mM). mRNA and proteins has been isolated to evaluate the expression of genes and proteins involved in adipocyte inflammation, respectively by quantitative PCR and western blot. Cytokines secretion in cell culture medium was evaluated using ELISA kits.
Results: HFD-induced accumulation of macrophages in e-WAT, measured by the number of macrophages per gram of WAT, was significantly lower in FXR-KO mice (p<0.05) compared to WT after 12 weeks of HFD. Moreover, gene expression of the macrophage marker EMR1 (F4/80) was lower in WAT of FXR-KO mice compared to WT on HFD (p<0.05). HFD drove M1 polarization of e-WAT macrophages in WT but not FXR-KO mice. Indeed, we found a significant decrease in CD11c+ M1 macrophages (p<0.001) and a significant increase in CD206+ M2 macrophages (p<0.005) in e-WAT of FXR KO mice. Gene expression analysis revealed a significantly decreased of inflammatory marker (TNFα, IL6, NLRP3, caspase1) (p<0.05) in WAT from HFD feeding FXR-KO mice. Our in vitro results showed a decreased inflammatory response of FXR-KO adipocytes. Gene expression analysis showed that FXR deficiency impairs the inflammatory response of adipocytes, in terms of gene expression of different cytokines (TNFα, IL-1β) (p<0.005) and of inflammasome proteins encoding genes (NLRP3 and caspase-1) (p<0.05). Moreover FXR-KO adipocytes did not show any increase of SOCS3 gene expression in response to cytokines. Furthermore, FXR-KO adipocytes stimulated with LPS and PA showed decreased IL1β secretion compared to WT (p<0.05).
Conclusion: Our results clearly suggest a role of FXR in the inflammatory response of adipocytes and in the crosstalk between adipocytes and immune cells in adipose tissue in the context of obesity-induced inflammation.

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Linagliptin-mediated DPP4 inhibition controls both macrophage migration and alternative activation and improves insulin resistance in diet-induced obese mice

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Background and aims: Obesity activates the innate immune system with subsequent recruitment of immune cells such as macrophages and T cells into metabolic tissues, leading to the development of insulin resistance. In particular, macrophage recruitment and polarization are pivotal in obesity-induced inflammation and insulin resistance. Dipeptidyl peptidase-4 (DPP4), also known as CD26, is widely expressed, including

in immune cells. However, the role of DPP4 in macrophage-mediated inflammation is unclear. In the present study, we showed that linagliptin, a DPP4 inhibitor, attenuates whole-body insulin resistance by regulating macrophage migration and alternative activation in both the adipose tissue and liver of high-fat diet (HFD)-induced obese (DIO) mice.

Materials and methods: Eight-week-old C57BL/6J mice were fed a HFD or a HFD containing linagliptin (HFD + Lina; 3 mg/kg body weight) for 8 weeks. The histological characteristics and DPP4 activity in the epididymal white adipose tissue (eWAT) and liver as well as insulin sensitivity were examined. To quantify DPP4+ macrophages in lean and obese mice, fluorescence-activated cell sorter (FACS) analysis was performed on stromal vascular (SV) cells from the eWAT and liver of DIO mice.

Results: DPP4 mRNA expression was significantly higher in the SV fraction than in the adipocyte fraction from the eWAT of DIO mice. Furthermore, an immunofluorescence study of eWAT in DIO mice revealed that F4/80+ macrophages expressed DPP4 in crown-like structures. FACS analysis showed that only a small percentage of adipose tissue macrophages (ATMs) and liver macrophages (LMs) co-expressed DPP4 in wild-type (WT) mice. However, the percentage of DPP4+ cells was significantly higher in both ATMs and LMs of DIO mice. Importantly, DPP4 activity in the eWAT and liver of DIO mice were 1.7-fold and 1.4-fold higher, respectively, than in WT mice ($p<0.01$), whereas linagliptin markedly reduced the DPP4 activity by 89% and 88% ($p<0.01$) in the eWAT and liver of DIO mice, respectively. Linagliptin administration improved HFD-induced glucose intolerance, hyperinsulinemia (HFD, 1.1±0.3 vs. HFD + Lina, 0.5±0.08 ng/mL; $p<0.05$, fasting state) and hepatic steatosis. Linagliptin also enhanced insulin signaling assessed by IRβ and Akt phosphorylation in both the eWAT and liver of DIO mice. F4/80+ macrophage migration into eWAT and liver as well as crown-like structure formation in eWAT were lower in HFD + Lina mice than in HFD mice, although adiposities were similar. These findings were associated with decreased mRNA expression of chemokine systems (MCP-1-CCR2 and RANTES-CCR5) and proinflammatory cytokines (IL-1β, IL-6, and TNFα) in eWAT and liver of HFD+Lina mice. Furthermore, FACS analysis revealed that HFD + Lina mice had 35% and 36% fewer CD11c+CD206-(M1) ATMs and LMs, respectively, and 25% and 164% more CD11c-CD206+(M2) ATMs and LMs, respectively, than HFD mice, resulting in predominance of the M2 over M1 ATM and LM population. However, the predominance of the Ly6C- over the Ly6Chi monocyte population was not observed in the peripheral blood or bone marrow of HFD+LY mice.

Conclusion: Linagliptin-mediated DPP4 inhibition reduces M1-polarized macrophage migration and induces a M2 dominant shift of macrophages within adipose tissue and liver, thereby attenuating obesity-induced inflammation and insulin resistance.

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The SUCNR1-pathway couples obesity-induced metabolic stress to the development of adipose tissue inflammation and diabetes

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Background and aims: Adipose tissue of obese animals and humans is characterized by adipocyte hypertrophy, oxidative stress, macrophage infiltration and enhanced production of pro-inflammatory cytokines. The enhanced inflammatory state of the adipose tissue significantly contributes to the development of insulin resistance and development of type 2 diabetes mellitus (T2DM). However, mechanisms by which local metabolic disturbances in adipose tissue in obesity lead to macrophage infiltration and the onset of chronic inflammation are not fully understood. The TCA cycle intermediate succinate has recently emerged as a metabolic signal induced by pro-inflammatory stimulations. In the present study, we aimed to investigate the role of succinate and its receptor SUCNR1 in obesity-induced inflammation and insulin resistance in type 2 diabetes mellitus (T2DM).

Materials and methods: To study the role of the SUCNR1-pathway, both animal and human studies were combined with various *in vitro* approaches. Human plasma samples of T2DM patients (n=58) and non-diabetic subjects (n=76) were used to determine circulating succinate levels. In addition, adipose tissue inflammation as well as glucose tolerance were determined in SUCNR1-/- and Wildtype (WT) mice that were fed a high fat diet (HFD) or low fat diet (LFD) for 16 weeks.

Results: Circulating levels of succinate were elevated in humans diagnosed with T2DM as compared to non-diabetic subjects (+60%; $P<0.01$). Interestingly, *ex vivo*, both hypoxia and hyperglycemia appeared to drive the release of succinate from adipose tissue. Therefore, we set out to evaluate the significance of the SUCNR1 in adipose tissue. Feeding mice a high fat diet (HFD) increased expression of SUCNR1 in the stromal vascular fraction (SVF) of the adipose tissue (4-fold; $P<0.01$), pointing towards a role of the SUCNR1 in immune cell function in enlarged adipose tissue. In response to HFD, WT and SUCNR1$^{-/-}$ mice had a similar increase in total body weight and adipose tissue mass. However, absence of the SUCNR1 in HFD-fed mice led to a reduction in total adipose tissue macrophage numbers (-39%; $P<0.05$) and Crown Like Structures (CLS; -53%: $P<0.01$), further confirmed by reduced F4/80 and Cd68 mRNA expression. *In vitro*, absence of the SUCNR1 in bone marrow derived macrophages strongly reduced the chemotaxis potential towards medium derived from apoptotic/hypoxic 3 T3 adipocytes (-59%; $P<0.05$). *In vivo,* the reduced macrophage infiltration in adipose tissue of obese SUCNR1-/- mice was accompanied by a significantly improved glucose tolerance as compared to WT mice (AUC -28%; $P<0.01$).

Conclusion: Overall, our results have identified succinate and its receptor as a driver of obesity-induced inflammation and an important contributor to the migration of macrophages into adipose tissue leading the systemic glucose intolerance in obesity-induced type 2 diabetes. As such, our data put the SUCNR1 forward as a promising therapeutic target to combat obesity-induced diabetes.

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MIP-1α ablation prevents insulin resistance induced by high-fat feeding or leptin deficiency

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Background and aims: Monocyte chemoattractant protein-1 (MCP-1) and its receptor CCR2 play a central role in the recruitment of adipose tissue macrophages (ATMs) and subsequent development of insulin resistance; however, a deficiency in either CCR2 or MCP-1 is insufficient to overcome obesity-induced ATM recruitment and insulin resistance. We have previously shown that other chemokine receptors, such as CCR5, also promote adipose tissue inflammation and insulin resistance in obese mice. The expression of macrophage inflammatory protein-1α (MIP-1α or CCL3), a ligand of CCR5, is elevated markedly in the white adipose tissue (WAT) of obese mice, although the mechanism by which MIP-1α affects insulin resistance and glucose homeostasis remains poorly understood. Here, we investigated the role of MIP-1α in obesity-induced adipose tissue inflammation and whole-body insulin resistance induced by high-fat (HF) feeding or leptin deficiency.

Materials and methods: Gene expression and the localization of chemokines and their receptors were examined in the WAT of genetically (ob/ob) and HF diet-induced obese (DIO) mice. To determine whether MIP-1α is required for obesity-induced ATM recruitment and insulin

resistance in vivo, we examined the metabolic phenotype of MIP-1α-/- mice. We also performed bone marrow transplantation (BMT) of MIP-1α-/- mice or wild-type (WT) C57Bl/6J mice donor cells into irradiated WT recipient mice to generate myeloid cell specific chimeric mice.
Results: MIP-1α expression was increased markedly compared to that of MCP-1 in the WAT of both DIO (MIP-1α, 5.9-fold; MCP-1, 3.2-fold; $p< 0.05$ vs. WT) and ob/ob mice (MIP-1α, 17.5-fold; MCP-1, 4.1-fold; $p< 0.05$ vs. WT) at 15 weeks of age. Immunofluorescence staining of WAT in DIO mice revealed strong MIP-1α expression in the crown-like structures (CLS) of F4/80+ macrophages. MIP-1α-/- mice fed normal chow had slightly better glucose tolerance. MIP-1α-/- mice fed a HF diet showed decreased macrophage infiltration and CLS formation in WAT compared with WT mice at 16 weeks, although the weight gain was similar in both groups. The HF diet-induced glucose intolerance and hyperinsulinemia (MIP-1α-/- vs. WT; 0.6±0.1 ng/mL vs. 2.3±0.3 ng/mL, $p<0.01$) were improved significantly in MIP-1α-/- mice. These findings were associated with decreased inflammatory cytokine expression (TNFα and iNOS), attenuated endoplasmic reticulum stress evaluated by XBP-1 splicing, and reduced hepatic steatosis. Next, we introduced a MIP-1α deficiency into ob/ob mice to generate double-knockout (DKO) mice. The DKO mice were strikingly resistant to the development of both insulin resistance and fatty liver disease. The DKO mice also exhibited decreased ATM recruitment and CLS formation, along with slightly reduced adipocyte size compared to ob/ob littermates. Importantly, mRNA expression of MIP-1α and other CCR5 ligands in WAT was higher in the stromal vascular fraction relative to the adipocyte fraction from DIO mice at 16 weeks. Furthermore, chimeric mice lacking MIP-1α in myeloid cells were protected from HF diet-induced hyperinsulinemia (MIP-1α-/-BMT 2.6±0.3 vs. WT-BMT 5.1±1.1 ng/mL, $p<0.05$), glucose intolerance, and hepatic steatosis.
Conclusion: MIP-1α deficiency prevents insulin resistance caused by either HF feeding or leptin deficiency, indicating a crucial role for MIP-1α in ATM recruitment and the subsequent development of insulin resistance independent of MCP-1/CCR2.
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Inflammation triggers specific microRNA profiles in human adipocytes and macrophages and in their supernatants
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Background and aims: The potential relevance of microRNAs (miRNAs) in adipose tissue is increasingly recognized, being intrinsically linked to different pathways, including obesity-related inflammation. In this study, we aimed to characterize the changes induced by inflammation on the miRNA pattern of human adipocytes and macrophages.
Materials and methods: An extensive profile of 759 common miRNAs was assessed in cells (human primary mature adipocytes, and the macrophage-like cell line THP-1) and in their supernatants (SN) using TaqMan low-density arrays. This profile was evaluated at the baseline and after administration of lipopolysaccharide (LPS, 10 ng/ml) and LPS-conditioned medium from M1 macrophages (MCM, 5%). Those miRNA that experienced the most dramatic changes were also studied in subcutaneous human adipose tissue before and ~2 years after bariatric surgery-induced weight loss.
Results: Differentiated adipocytes expressed 169 miRNAs, being 85 detectable in the SN. In M1 macrophages, 183 miRNAs were detected, being 106 present also in the SN. MCM and LPS led to increased number of detectable miRNAs in cells and SN in both adipocytes (+8.3% and +24.7%) and M1 macrophages (+1.4% and +5%, respectively). Under inflammatory conditions, adipocytes and M1 macrophages shared the expression of 147 (+9%) miRNAs, and 100 (+41%) common miRNAs were also shared in their SN. Twelve of these "inflammatory" miRNA were linked to inflammation in adipose tissue from obese subjects. Interestingly, miR-221 (2-fold, $p=0.002$), miR-222 (2.5-fold, $p=0.04$) and miR-155 (5-fold, $p=0.015$) were increased not only in inflamed adipocytes but also in their SN (15, 6 and 4-fold, respectively, all $p<0.001$). The expression of these specific miRNAs in human adipose tissue concordantly decreased after weight loss (-51%, $p=0.003$, -49%, $p=0.03$, and -54.4%, $p=0.005$, respectively).
Conclusion: Inflammation induces a specific miRNAs pattern in adipocytes and M1 macrophages with impact on the physiopathology of obesity-induced inflammation of adipose tissue. Variations regarding the cluster miR-221/222 and the miR-155 may participate in the crosstalk between obesity-related inflammation and AT/adipocytes impaired glucose uptake.
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The transcription factor Prep1 is a molecular link between insulin-resistance and inflammation in adipose tissue
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Background and aims: Mounting evidence have highlighted that the chronic low-grade systemic inflammation accompanying obesity impairs insulin signaling by secreting adipokines and may contribute to the development of insulin-resistance and type 2 diabetes mellitus. We have previously identified Prep1 as a homeodomain transcription factor, which plays an important role in glucose homeostasis. Prep1 hypomorphic heterozygous mice (Prep1i/+) expressing low levels of protein are protected from streptozotocin-induced diabetes and show improved insulin sensitivity both in muscle and in liver. Moreover, these mice feature reduced lipotoxicity and diet-induced steatohepatitis. In this study, we have focused our attention on the role of Prep1 on adipose tissue function.
Materials and methods: Adipose tissue of WT and Prep1i/+ mice have been characterized by histological analysis and Western blot and qRT-PCR experiments have been used to measure adipocyte protein and mRNA expression. Adipose tissue immune-phenotyping has been performed by cytofluorimetric analysis and metabolic profile of adipocytes has been evaluated by extracellular flux analyzer.
Results: Prep1 hypomorphic heterozygous mice feature a 23% reduction of total body lipid content compared to the WT littermates while the fresh weight of the epididymal fat pads was found 25% lower in Prep1i/+ mice ($p<0.01$). Histological analysis in adipose tissue of WT and Prep1i/+ mice revealed that Prep1 hypomorphic adipocytes feature a reduced cell volume compared to the adipocytes of WT mice. More detailed analysis of Prep1i/+ adipose tissue indicated increased number of small adipocytes, which are more insulin responsive, and a reduction of enlarged adipose cells that promote immune-inflammatory responses. Indeed, insulin receptor tyrosine and akt/PKB phosphorylation was markedly increased in Prep1 deficient mice and, in parallel, glucose uptake was 3-fold higher in Prep1i/+ animals ($p<0.05$). In addition, adipose tissue from Prep1 hypomorphic heterozygous mice showed reduced T cells infiltration and leptin, TNF-α and IL-8 expression, while adiponectin levels were increased.
Conclusion: All together, these data indicate that Prep1 deficiency reduces immune-inflammatory response improving glucose homeostasis in adipose tissue.

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The role of tight glycaemic control on inflammation factors and vasculoactive substances in newly diagnosed type 2 diabetes mellitus

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Background and aims: In conditions with insulin resistance, a low-grade inflammation in adipose tissue activates macrophages that together with adipocytes produce various inflammatory adipokines, procoagulant and vasculoactive substances. Considering the crosstalk between inflammatory pathways and insulin signalling cascade, these molecules may represent a link between inflammation and metabolic signals and mediate, at least in part, insulin resistance in peripheral tissues. However, little is known about the effect of intensive therapy targeting in tight glycemic control on these factors. In our study we aimed to investigate circulating levels of inflammatory markers including hsCRP, interleukin (IL)-6, IL-18 and von Willebrand factor (vWF) in patients with newly diagnosed type 2 diabetes, before and after 6-months intensive antidiabetic therapy resulting in tight glycemic control.
Materials and methods: 52 newly diagnosed T2DM (29 males, age: 54.7+2.1 years) were selected. None of the subjects had coexistent autoimmune or other inflammatory disease. Venous blood was obtained on admission (1st baseline sample without any medication therapy) and 6 months later (2nd sample under antidiabetic therapy with oral agents and/or insulin) for measurements of hsCRP, IL-6 IL-18 and vWF.
Results: After 6 months therapy (compared to baseline): 1) HbA1c decreased (6.01+0.1 vs 7.91±0.26, $p<0.05$) 2) BMI decreased (31+1 kg/m^2 vs 34.5+1 kg/m^2, $p=0.0001$) 3) hsCRP serum levels decreased (4.20+1.3 vs 9.29+1.51 mg/l, $p<0.05$) 4) IL-6 serum levels decreased (2.44+0.41 vs 3.45+0.26 pg/ml, $p<0.05$) 5) IL-18 serum levels decreased (77.57+6.64 vs 99.54±8.0 pg/ml, $p<0.05$) and vWF plasma levels decreased (97.14+8.58 vs 137.28+25.08%, $p<0.69$) 6) There were no statistically significant differences between different therapies.
Conclusion: Tight glycemic control with oral agents and/or insulin decreases the inflammatory factors, with additional beneficial effects on endothelial dysfunction wich may reduce procoagulant imbalance seen in untreated patients with type 2 diabetes.

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Impact of glucose-loading on the variation of CD4+ and CD8+ T cells

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Background and aims: Chronic inflammation is involved in the pathogenesis of obesity and type 2 diabetes. Obesity and diabetes were associated with the peripheral blood T cell composition as well as total peripheral white blood cell and leukocyte count. However, little is known about the effect of acute change of glucose metabolism by glucose-loading on the peripheral blood T cell compartment. The aim of this study is to examine the fluctuation of CD4+, CD8+ T cells and natural CD4+ CD25+FoxP3+ T-regulatory (Treg) cell variation following oral glucose tolerance test (OGTT) in patients with or without type 2 diabetes and its relation to fluctuation in glucose, insulin and free fatty acid (FFA) caused by OGTT.
Materials and methods: Twenty Japanese patients with type 2 diabetes (DM group) and 20 without diabetes (non DM group) were recruited into the present study. After obtaining the approval of the institutional review board and written informed consent from the subjects, a 75-g OGTT was performed. The plasma glucose and insulin levels were measured after an overnight 12-h fast and during a 75-g OGTT at 30, 60, 90 and 120 min. Cell numbers of leucocyte, lymphocytes and T cell compartment such as CD4+, CD8+, and Treg were calculated from their blood after an overnight 12-h fast and during a 75-g OGTT at 60 and 120 min. Insulin sensitivity was estimated using the Matsuda index and homeostasis model assessment of insulin resistance (HOMA-IR). Glucose-stimulated insulin secretion was evaluated based on the insulinogenic index and oral disposition index.
Results: Age was significantly higher, whereas insulinogenic index and oral disposition index were significantly lower in the DM group compared with those in the non DM group. There were no significant differences in sex, body mass index, Matsuda index, nor HOMA-IR between these two groups. Before glucose-loading, there were no differences in cell numbers of leucocyte (6466.7±2332.9/μL in the DM group vs 6290.0±1587.1/μL in the non DM group), lymphocytes (2001.2±636.7/μL vs 2336.6±1173.7/μL), CD4+ (896.5±290.9/μL vs 1036.2±429.0/μL), CD8+ (582.7±277.8/μL vs 622.6±290.5/μL), and Treg (78.6±38.1/μL vs 88.9±40.2/μL) between these two groups. The proportion of lymphocytes (DM group: 31.8±7.3% to 30.2±7.4/μL; $p<0.01$, and non DM group: 37.5±16.0% to 36.9±15.5%; $p<0.01$) and CD8+ (DM group: 28.4±7.4% to 26.3±6.4/μL; $p<0.01$, and non DM group: 27.1±4.9% to 25.3±5.6%; $p<0.01$) significantly decreased, whereas the proportion of CD4+ significantly increased (DM group: 45.2±6.1% to 47.6±5.9/μL; $p<0.01$, and non DM group: 46.3±9.0% to 47.7±9.8%; $p<0.01$) after 120 min of glucose-loading in both groups. The proportion of Treg was not affected. Furthermore, significant positive correlation was observed between the area under the curve for CD8+ and the change of FFA following OGTT ($r=0.40$, $p<0.05$), but not that of glucose nor insulin.
Conclusion: Although Treg population was not quantitatively different, CD4+ T cells were increased and CD8+ T cells decreased after glucose loading in both subjects with and without diabetes. These findings suggest that glucose loading dynamically affects the balance of circulating T lymphocyte subset regardless of glucose tolerance.

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Association of low-grade inflammation with early changes of glycaemic control, insulin sensitivity and secretion in recent-onset type 1 and type 2 diabetes

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Background and aims: Low-grade inflammation affects insulin sensitivity and beta-cell function already before the onset of diabetes and can contribute to the development of both type 1 (T1D) and type 2 diabetes (T2D). We hypothesized that biomarkers of low-grade inflammation also associate with the early progression of recent-onset T1D and T2D.

Materials and methods: In the prospective GDS study, new-onset T1D (n=42) and T2D (n=94) patients underwent detailed metabolic characterization within the first year after diabetes diagnosis and 2 years thereafter. Associations between changes of markers of low-grade inflammation with changes of HbA1c, insulin sensitivity and secretion as measured by intravenous glucose tolerance test were assessed using multivariable linear regression analysis adjusted for age, sex, body mass index (BMI), smoking status, as well as for 2-year changes of BMI, smoking status, and glucose-lowering treatment.
Results: Both T1D and T2D patients exhibited good glucometabolic control at baseline (mean HbA1c 7.1±1.6% and 6.4±1.0%) and after 2 years (mean HbA1c 7.0±1.2% and 6.6±1.1%). Two-year increases of high-sensitive C-reactive protein, soluble E-selectin and soluble intercellular adhesion molecule-1 in T2D and of interleukin-18 in T1D were associated with absolute increases of HbA1c by +0.5%, +0.9%, 1.7% and +1.2% (all $P<0.01$; changes per doubling of biomarker levels between baseline and 2-year follow-up). A comparable 2-year increase of sE-selectin associated with a relative decrease in pre-hepatic beta-cell function by -34.9% ($P<0.05$) in T2D.
Conclusion: During the initial course of disease, low-grade inflammation relates to worsening of glycemia in both T1D and T2D, whereas endothelial activation may contribute to decreasing beta-cell function in T2D.
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Metformin decreases TLR4 expression in type 2 diabetic patients monocytes and inhibits leptin secretion in peripheral blood mononuclear cells (PBMC)

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Background and aims: Several studies argue the importance of inflammation as key component of Type 2 Diabetes Mellitus (T2D). Among cytokines involved in T2D pathogenesis, serum levels of Leptin and High Mobility Group Box (HMGB)1 were found increased in T2D patients: Leptin modulates cytokines secretion in monocytes and CD4+ T-cells while HMGB1 actives immune system by promoting T-cell and B-cell reactivity. Moreover, HMGB1 receptors Toll like receptor 2 and 4 (TLR2 and 4) were found increased in monocytes of newly diagnosed T2D patients. Metformin represents the T2D first lane therapy, and it can decrease both HMGB1 and Leptin blood levels. We aimed to investigate the effects of Metformin therapy on TLR expression in T2D patients and to explain its anti-inflammatory mechanism.
Materials and methods: 50 Caucasian subject were recruited and divided in 3 groups: 10 healthy controls (HC), 20 T2D patients in therapy with Metformin (MET-T2D), and 20 newly diagnosed T2D patients without pharmacological treatment (Naïve-T2D). MET-T2D and Naïve-T2D showed BMI≤28, glucose value <126 mg/dl and HbAC1 values were <6%. Monocyte percentage was evaluated by flow cytometry and confirmed with complete blood count (CBC) analysis. TLR2 and TLR4 expression or mean fluorescence intensity (MFI) of each cohort were evaluated by flow cytometry. PBMCs of each group were isolated by Ficoll gradient, activated with IL-2 (200U/ml) for 72 h and treated with HMGB1 (2 ug/ml) for 48 h. Leptin levels were evaluated by ELISA assay. Results were analyzed with t-test and P values <0.05 were considered statistically significant.
Results: CBC analysis showed no variation in monocytes count among our groups. We, thus, analyzed TRL2 and TLR4 expression: we found Naïve-T2D showed an increase in amount of TLR2+/TLR4+ subpopulation compared to HC. Conversely, MET-T2D showed a dramatic decrease in TLR4 compared to Naïve-T2D and HC while TLR2 expression is not affected by Metformin. We confirmed these data by MFI analysis. To asses biological significance to TLR4 inhibition by Metformin, PBMCs from HC, Naïve-T2D and MET-T2D were isolated, activated with IL-2 and treated with HMGB1. We established that HMGB1 increases Leptin secretion in IL-2 activated HC PBMCs, while MET-T2D PBMCs showed no variations in Leptin secretion after HMGB1 treatment and IL-2 activation. Nevertheless, HMGB1/IL-2 tandem treatment increased Leptin secretion only in TLR4+ PBMC in Naïve-T2D patients, in fact we found a small percentage of Naïve-T2D in which TLR4 expression is highly deregulated. MET-T2D and TLR4- Naïve-T2D did not show any variation in Leptin secretion after HMGB1/IL-2 treatment.
Conclusion: Our data shows that Metformin therapy is associated with TLR4 deregulation in T2D patient's monocytes. The lack of TLR4 suggests a minor susceptibility to inflammatory stimuli in terms of Leptin secretion, providing a potential mechanism to explain Metformin anti inflammatory pleiotropic effect.

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Effects of empagliflozin in a murine model of diet-induced insulin resistance: A role for NLRP3 inflammasome inhibition?

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Background and aims: Empagliflozin is a potent and selective inhibitor of the sodium glucose co-transporter (SGLT)-2, approved as a new oral treatment for type 2 diabetes. However, to date, the potential ability of empagliflozin to affect the chronic inflammatory response associated with diabetes and recently recognized as a primary pathogenic factor in the development of insulin resistance, has not been investigated. Aim of this study was to evaluate the effects of a chronic treatment with empagliflozin in a murine model of diet-induced insulin resistance, focusing on inflammatory response in liver and kidney. In particular, as NLRP3 (nucleotide-binding and oligomerization domain [NOD]-like receptor [NLR] pyrin domain-containing 3) inflammasome has been recognized playing a key role in insulin-resistance and obesity-induced inflammation, the effect of empagliflozin on diet-induced NLRP3 activation was evaluated.
Materials and methods: Male C57BL/6 mice (n=20 per group) were provided with a standard (control group) or a high fat-high sugar (HFHS group) diet for 4 months. Over the last 2 months, subsets of animals were treated with empagliflozin (1-10 mg/kg) added to the diet. Body weight, water/food intake were recorded weekly. Insulin, interleukin (IL)-1β, IL-18, IL-6 and tumor necrosis factor (TNF) levels and albumin/creatinine ratio (ACR) were measured by ELISA. Phosphorylation of insulin signaling intermediates, NLRP3 expression and caspase-1 activation were evaluated by western blot on tissue specimens.
Results: In comparison to mice fed with standard diet, animals provided with HFHS diet exhibited a significantly bigger final body weight (25.2± 0.8 vs 34.6±3.0 g, $p<0.001$), higher fasting glycaemia (111.3±11,8 vs 171.3±21.4 mg/dl, $p<0.001$) and plasma total cholesterol (102.3±24.3 vs 138.9±10.9 mg/dl, $p<0.05$). HFHS mice shown hepatic steatosis and tubular vacuolar degeneration in the kidney. Moreover, a 4-fold higher liver triglyceride level ($p<0.01$), a 5-fold higher ACR ($p<0.001$) and a marked increase of NLRP3 expression ($p<0.01$) in liver and kidney were also measured in HFHS mice. A 10% reduction of body weight was induced by 10 mg/kg empagliflozin ($p<0.001$), and a dose-dependent

effect on fasting glycaemia, liver steatosis and renal tubular damage was measured in animals treated with the drug. Interestingly, the diet-related overexpression of NLRP3 was attenuated by empagliflozin in a dose dependent manner, and this effect was associated with a significant decrease in tissue caspase-1 activation and IL-1β production. Empagliflozin evoked a significant reduction of plasma TNF, liver IL-6 and ACR starting from 1 mg/kg.

Conclusion: The deleterious effects of a HFHS diet were reverted by empagliflozin in a dose-dependent manner. Our results clearly demonstrated the ability of the drug to affect NLRP3 inflammasome signaling, suggesting that this inhibition may contribute to the therapeutic effects exerted by empagliflozin.

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Hypoglycaemic episodes are associated with inflammatory status in patients with type 1 diabetes

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Background and aims: It is well documented that glycemic control is associated with inflammatory status in type 1 diabetes (T1DM) patients. The hyperglycemia-induced oxidative stress plays a key pathophysiological role in damaging endothelial function in diabetes. However, the individual contribution of different parameters and indices, for example glucose variability or hypoglycemic episodes, is not clear. The aim of the study was to examine the association of glycemic control parameters and selected blood markers of endothelial function and inflammation in T1DM patients.

Materials and methods: We included 101 patients with T1DM on insulin pumps (mean age - 28.7+10.8 years, mean T1DM duration - 8.5 +/- 2 years). All subjects completed a standardized questionnaire. Other sources of information included medical records, downloads from glucose meters and personal pumps. The list of glycemic control parameters included HbA1c, mean glucose level, standard deviation and four other indices based on self-monitoring blood glucose as well as the number of hypoglycaemias (glucose <55 mg/dL) from the period of last 7 days. Blood was collected for 4 inflammatory markers: interleukin 6 (IL-6), Vascular Cell Adhesion molecule 1 (VCAM), Intercellular Adhesion Molecule 1 (ICAM), E-selectin. Appropriate statistical analyses were performed for differences and associations. In forward stepwise multiple linear regression analysis, inflammatory factors were dependent variables, while as independent variables we used seven glycemic control indices and selected clinical as well as biochemical parameters.

Results: The examined cohort was characterised by good glycemic control as the mean HbA1c level was 7.1+0.8%, mean daily glucose - 141.5+27.1 mg/dL. Standard deviation reached 65.6+16.9, while number of hypos 5.6+4.0 over the observation period. In forward stepwise multiple linear regression analysis we observed that the number of hypoglycemias was consistently an independent predictor of the level of all investigated markers - sICAM ($p=0.0019$), VCAM ($p=0.021$), E-selectin ($p=0.048$) and IL-6 ($p=0.027$), respectively. None of the other glycemic parameter turned out to independently predict the inflammatory status of examined T1DM patients.

Conclusion: For the first time, we report the association between the number mild hypoglycemic episodes over the period of one week and the level of several inflammatory markers in T1DM patients with good glycemic control.

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Characterisation of the complement system among hospitalised type 2 diabetic patients with community acquired bacterial infections

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Background and aims: Infections are more frequent and severe in patients with type 2 diabetes mellitus (T2DM) than in nondiabetic population. The complement cascade is a complex protein system, which can be activated via different enzymatic pathways and plays an important role in response to pathogens. The goal of our study was to determine predictive association between increased risk of community-acquired bacterial infections and lectin complement pathway member ficolin-3 among patients with type 2 diabetes mellitus.

Materials and methods: In our prospective, matched pairs study 40 subjects with T2DM according to WHO criteria and 12 nondiabetic subjects were investigated. Patient inclusion criteria were clinical diagnosis of acute community acquired bacterial infections. Immunological, hematological, oncological illnesses were excluded. Activity of the ficolin- and MBL-lectin complement pathway member ficolin-3 and mannose binding lectin (MBL), activity of the alternative complement pathway, concentrations of classical complement pathway member C1q and complement activation end product C4d were measured from blood samples using standardized ELISA kits. Complement and laboratory parameters of 26 subjects from the T2DM and 9 subjects from the nondiabetic group were reassessed after a minimum of three-month infection-free period. Statistical analysis and correlations between C1q/WBC, C4d/WBC, C4d/CRP, F3/WBC and F3/CRP were performed using GraphPad Prism 5.

Results: We found higher HbA1c levels in the T2DM group (17/40 men; median [percentile 25-75] age: 73 [66.3-80.8] years, HbA1c: 7.6 [6.2-8.1]%, CRP: 146 [74.6-248.3] mg/l, white blood cell count (WBC): 10.2 [8.1-14] G/l; sepsis: 17/40; mortality: 8/40) than in the nondiabetic group (8/12 men; median [percentile 25-75] age: 74.5 [59.3-82] years, HbA1c: 5.5 [5.4-6] %, CRP: 118.7 [74.7-159.7] mg/l, white blood cell count (WBC): 10.95 [8.5-13.4] G/l; sepsis: 4/12; mortality: 1/12). Among T2DM subjects during acute infection no change was found in the MBL activity, however ficolin-3 activity, alternative complement pathway activity were higher ($p=0.0061$ and $p=0.0447$, respectively), C4d concentrations were lower ($p=0.0138$) than in an infection-free period. During infection, C4d concentrations were also lower in the T2DM group than in the nondiabetic group ($p=0.025$), however no difference was found in the MBL and ficolin-3 activity, alternative complement pathway activity, and C1q concentrations. In the absence of infection, lower C1q levels were found in T2DM than in nondiabetic subjects ($p=0.0212$), however during infection this difference could not be seen. Interestingly, between C1q and WBC during infection-free period a positive ($p=0.0087$, Spearman $r=0.51$), while having an infection a negative correlation ($p=0.0054$, Spearman $r=-0.53$) was found.

Conclusion: Our results show that T2DM may be associated with an alteration of not only the ficolin- and MBL-lectin, but also the classical and the alternative complement pathways. This could be a possible cause of the increased risk of infection among patients with T2DM.

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PS 056 Fatty acid metabolism: new insights

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Free fatty acid uptake in different abdominal adipose tissue compartments

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Background and aims: Abdominal adipose tissue (AT) contains subcutaneous (SC) deep and superficial layers and intraperitoneal and extraperitoneal visceral AT. There is limited information about differences and the effect of surgery in fatty acid uptake (FAU) in these compartments. We therefore aimed to study tissue-specific FAU in abdominal adipose tissue compartments before and after obesity surgery and compare the results to non-obese subjects.
Materials and methods: A total of 27 severely obese females (BMI 41.4 ±4.0 kg/m2, age 42±9 yrs, 11 T2D and 16 non-T2D) undergoing bariatric surgery and 15 age-matched non-obese healthy women (BMI 22.6± 2.8 kg/m2) were recruited into the study. FAU in superficial and deep abdominal SC AT and extra- and intraperitoneal visceral AT was assessed using PET with [18 F] fluoro-6-thia-heptadecanoic acid ([18 F] FTHA). Studies were repeated in obese subjects 6 months after surgery. The controls were studied once.
Results: During the follow-up, both BMI (41.4±4.0 vs 31.8±4.2 kg/m2, $p<0.001$) and body fat % (50.0±3.7 vs 42.8±4.3%, $p<0.001$) decreased, but remained abnormal. Before surgery obese had higher fasting glucose and FFA levels compared to controls, and FFA remained at the same level postoperatively. In obese, abdominal fat FAU (μmol*min-1*100 g-1) was higher in intraperitoneal than extraperitoneal AT (0.81±0.44 vs 0.46± 0.29, $p<0.001$) and in deep than superficial SC AT (0.38±0.18 vs 0.30 ±0.16, $p<0.001$). Similarly, deep vs superficial AT (0.30±0.16 vs 0.25± 0.14, $p<0.01$) and intra- vs extraperitoneal AT (0.56±0.27 vs 0.43±0.25, $p<0.01$) differed in controls, but no differences were found in tissue-specific FAU between obese and controls, when FAU was expressed per kilogram AT. The FAU per depots of AT was in obese 6-fold higher before surgery and 4-fold higher postoperatively as compared to controls. Surprisingly, the distribution of FAU between SC, and intra- and extra-abdominal depots was similar in obese and controls. Baseline SC FAU correlated with fasting glucose ($r=0.41$, $p=0.03$) and intraperitoneal FAU with high-sensitivity C-reactive protein ($r=0.47$, $p=0.02$).
Conclusion: Fatty acid uptake in abdominal AT depots is markedly enhanced in obesity and remains elevated after bariatric surgery. FAU per volume of AT differs between different abdominal AT depots, but remains similar in morbidly obese before and after bariatric surgery. Surgery did not alter FAU in the metabolically distinct abdominal fat compartments. The enhanced metabolic activity in intraperitoneal fat coupled with its association with a marker of low-grade inflammation may be the major contributing factor to the unfavorable glucose homeostasis observed in obese subjects before surgery.
Clinical Trial Registration Number: NCT01373892
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Respective roles of ATGL and HSL on adipose tissue lipolysis-mediated control of glucose metabolism and insulin sensitivity

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Background and aims: We and others showed that pharmacological and genetic inhibition of the neutral lipases, Adipose Triglyceride Lipase (ATGL) or Hormone-Sensitive Lipase (HSL), responsible of adipose tissue lipolysis, improves whole-body glucose metabolism and insulin sensitivity. The underlying mechanisms are not perfectly understood. Recently, our laboratory showed a potentially beneficial upregulation of adipose de novo lipogenesis (DNL) following HSL down regulation in vitro in human adipocytes (see abstract from Morigny et al, EASD 2015). Here, we aimed at deciphering in vivo the roles of ATGL and HSL haploinsufficiency on insulin sensitivity.
Materials and methods: ATGL+/-HSL+/-, ATGL+/-, HSL+/- and wildtype (WT) male C57BL/6J mice were fed 3 months either a chow or a 60% high fat diet and phenotyped at various time points.
Results: Whole-body insulin sensitivity, measured by QUICKI, indicated a higher sensitivity in ATGL+/-HSL+/- fed a chow diet in comparison to WT mice (0.47±0.01 vs 0.41±0.01, respectively, $p=0.02$). When challenged 3 months with high fat diet, ATGL+/- (0.36±0.01, $p=0.02$), HSL+/- (0.36±0.01, $p<0.01$) and double haploinsufficient mice (0.38± 0.01, $p<0.001$) showed a significantly higher insulin sensitivity in comparison to WT mice (0.33±0.01). Compared to WT mice fed high fat diet, lipase haploinsufficiency resulted in an improvement of insulin signalling pathway (AktSer473 phosphorylation) and increased expression of master regulator genes of glucose metabolism and DNL in white adipose tissue (WAT). The improvement was more pronounced in ATGL+/-HSL+/- mice. There was no difference in fat mass and WAT inflammation between genotypes. In the liver, glycogen content was increased and hepatic steatosis was decreased. Measurements of respiratory quotient suggested a shift from fat to glucose metabolism in the haploinsufficient mice.
Conclusion: Altogether these results shed new light on the role of adipose neutral lipase in the control of glucose metabolism and insulin sensitivity. Whether the mechanisms mediating these improvements are similar or different following ATGL and HSL inhibition is currently under investigation.
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Partial inhibition of hormone-sensitive lipase improves insulin sensitivity by induction of de novo lipogenesis and elongase ELOVL6 in human adipocytes

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Background and aims: Partial inhibition of Hormone-Sensitive Lipase (HSL) improves in vivo and in vitro insulin sensitivity. In human adipocytes, HSL inhibition improves glucose metabolism and increases de novo lipogenesis (DNL). Recent studies report a close relationship between adipose DNL, under the control of the transcription factor Carbohydrate Responsive Element Binding Protein (ChREBP), and insulin sensitivity. Consequently, we hypothesized that the improvement of insulin sensitivity observed during HSL inhibition is driven by an increase of DNL.
Materials and methods: In human hMADS adipocytes (human multipotent adipose-derived stem cells), downregulation of HSL (siHSL),

ChREBP (siChREBP), Elongation of Very-Long-Chain Fatty Acids 6 (siELOVL6) or, as control, Green Fluorescent Protein (siGFP) expression has been achieved by siRNA transfection. Glucose metabolism has been measured by radiolabelled glucose. Insulin signaling has been assessed by Western Blot. Fatty acid (FA) composition of phospholipids (PL) has been determined by gas chromatography.

Results: siHSL adipocytes show improved glucose transport, oxidation and DNL in parallel to an increase of ChREBP gene expression (+32%, $p<0.001$). These cells also present an improved insulin signaling. All these beneficial effects are lost with dual inhibition of HSL and ChREBP. HSL downregulation promotes an increase in PL oleate (+18%, $p<0.001$) combined with a slight decrease of PL palmitoleate whereas the opposite profile is observed in siChREBP adipocytes. Among ChREBP-induced genes, the elongase ELOVL6 gene expression shows the highest induction in siHSL cells (+93%, $p<0.001$). Inhibition of ELOVL6 decreases PL oleate in favor of an important increase of PL palmitoleate and results in a significant reduction of the beneficial effects of HSL inhibition on insulin sensitivity.

Conclusion: We have demonstrated that partial inhibition of HSL improves insulin sensitivity and increases DNL pathway through ChREBP induction. ChREBP by a positive control of ELOVL6 is responsible of an increase in oleate synthesis. Increased PL oleate by ELOVL6 could modify plasma membrane fluidity and thereby improve insulin signaling.

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Mitochondrial oxidative pathways are downregulated in adipocytes in obesity - a twin study

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Background and aims: Low mitochondrial activity in adipose tissue is suggested to be an underlying factor in obesity and its metabolic complications. However it is still unknown whether this is due to malfunction of adipocytes or of other cells in adipose tissue.

Materials and methods: We studied young adult monozygotic (MZ) Finnish twin pairs discordant (n=14, ΔBMI>3 kg/m2) and concordant (n=5, ΔBMI<3 kg/m2) for obesity, aged 22-36-years by assessing abdominal body fat distribution (MRI), liver fat content (MR spectroscopy), insulin sensitivity (OGTT) and global gene expression (Affymetrix U133 plus 2.0 chips) of isolated adipocytes, collagenase digested and washed from subcutaneous abdominal adipose tissue biopsies.

Results: The obese (mean BMI 30.0±1.0 kg/m^2) and lean (BMI 23.9±0.8 kg/m^2) co-twins of the discordant pairs had a mean 17.6±1.9 kg, $p<0.001$ difference in body weight. The obese co-twins had more subcutaneous (6068.9±437.4 vs. 3526.1±293.6 cm^3, $p<0.001$), intra-abdominal (1193.1±169.4 vs. 540.5±81.9 cm^3, $p<0.001$) and liver fat (2.93±0.79 vs. 0.62±0.07%, $p<0.001$), and were more insulin resistant (HOMA-index 1.6±0.2 vs. 1.1±0.2, $p<0.01$; Matsuda-index 6.4±0.7 vs. 8.3±0.9, $p=0.02$). The concordant pairs were very similar in their measures. The significantly differentially expressed genes in adipocytes between the discordant co-twins (2538 transcripts, adjusted p-value, FDR<0.05) were related to mitochondrial oxidative phosphorylation, branched chain amino acid catabolism, glucocorticoid receptor signaling and IL-8 signaling. More than one-tenth of these transcripts (n=278) were mitochondria-related and almost all of them downregulated (244/278, 88%) in the obese co-twins. Pathway analysis of the mitochondria-related transcripts revealed reduced oxidative phosphorylation, TCA-cycle, fatty acid beta oxidation, branched chain amino acid degradation and ketone body production and breakdown (all $p<0.001$) in the obese co-twins. These downregulated pathways correlated negatively with intra-abdominal and liver fat, HOMA-index, hs-CRP and positively with adiponectin ($p<0.05$ all) in individual twins.

Conclusion: We report global downregulation of mitochondrial oxidative pathways in obese adipocytes, associating with metabolic disturbances, in young adult MZ twins.

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The adipokine adipocyte fatty acid-binding protein in high altitude

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Background and aims: Different adipose tissue-secreted adipokines are dysregulated in hypoxia *in vitro*. However, the *in vivo*-effect of prolonged hypobaric hypoxia on serum levels of the insulin resistance-inducing and proatherogenic adipokine adipocyte fatty acid binding-protein (AFABP) has not been comprehensively studied in humans, so far.

Materials and methods: AFABP serum levels were quantified in all participants of the High Altitude Medical Research Expedition Himlung 2013 (N=39) byenzyme-linked immunosorbent assay. This expedition is an observational cohort study of human adaption to hypobaric hypoxia and has measured clinical and anthropometric data of the subjects at six time points at different altitudes, i.e. pretest: 550 m; base camp 1: 4800 m; camp 2: 6025 m, base camp 2: 4800 m; camp 3: 7050 m; post: 550 m (Figure 1). Associations were studied using multivariate regression models with relative changes (ratios=parameter at maximum altitude / parameter at start altitude) of the respective parameters.

Results: Median [interquartile range] AFABP serum concentrations increased during the ascent from 11.2 [6.6] μg/l at pretest to a maximum of 34.2 [16.1] μg/l at the peak level, i.e. 7050 m ($p<0.05$). Barometric pressure was the strongest and negative predictor of AFABP after adjustment for age, gender, and body mass index ($p=0.003$). In accordance with these findings, altitude was the strongest positive predictor of AFABP when included in this multivariate model instead of barometric pressure ($p=0.004$). Interestingly, serum levels of the control and obesity-associated adipokine leptin decreased during the ascent (2.8 [4.8] μg/l at pretest as compared to 1.1 [1.7] μg/l at 7050 m) ($p=0.003$) but no independent associations with relative changes of leptin could be demonstrated.

Conclusion: Circulating AFABP is increased during a high altitude ascent and is independently associated with barometric pressure, as well as with altitude level. These data are in accordance with the hypothesis that hypoxia could contribute to an adverse metabolic profile in humans. Further studies need to elucidate the pathophysiological significance of AFABP upregulation in high altitude/hypoxia.

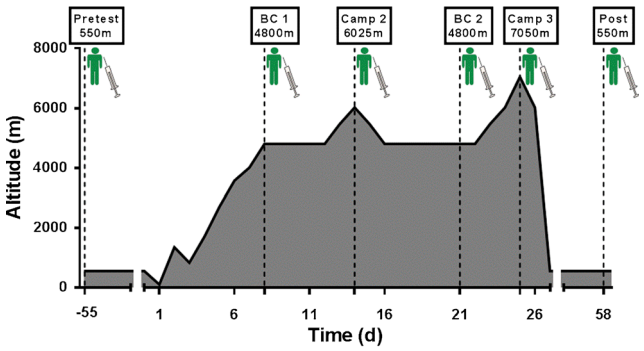

Figure 1

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Human brown adipose tissue oxygen consumption after meal is similar to cold activated consumption

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Background and aims: Diet-induced thermogenesis in human brown adipose tissue (BAT) has not been widely examined. Therefore, we aimed to compare BAT oxygen consumption during cold activation and after standardised meal.

Materials and methods: Healthy lean and obese study subjects (n=14, age: 36.2±12.0 years, BMI range: 23.3 - 34.2 kg/m^2) of both genders (10 F/4 M) were studied at two different scanning sessions, 1) after standardised meal (542 kcal, 58% carbohydrates, 25% fats, 17% proteins) at room temperature (RT) and 2) after overnight fasting during acute cold exposure, using PET-CT. Radioactive oxygen [^{15}O]O_2 and [^{15}O]H_2O were given to measure oxygen consumption and perfusion in BAT, respectively. Indirect calorimetry was performed to assess the differences in whole body resting energy expenditure (REE) and whole body substrate oxidation between post-prandial state and cold exposure.

Results: Supraclavicular BAT oxygen consumption during cold was 1.7 ±0.6 ml/100 g/min while after standardised meal it was 2.0±1.0 ml/100 g/min (p=0.81). There was no relation between BAT oxygen consumption measured during cold and post prandial state (r=-0.15; p=0.66). As expected, post-prandial plasma insulin level was higher than plasma insulin levels during fasting and cold exposure (p<0.001). Whole body REE during cold and post-prandial state was at the same level (7.7±1.0 MJ/day and 7.4±0.9 MJ/day, p=0.50, respectively). During post-prandial state whole body glucose oxidation was higher than in cold (p<0.001), while in cold whole body fat oxidation was higher compared to post-prandial state (p<0.001). Post-prandial BAT oxygen consumption correlated negatively with waist circumference (r=-0.77; p=0.006).

Conclusion: Similar oxygen consumption in post-prandial state and in cold suggests that human BAT has diet-induced thermogenic capacity. However, mechanism of action and substrate utilisation responsible for enhanced BAT oxygen consumption in these two states appear to be different. Since BAT is insulin sensitive we speculate that BAT oxygen consumption in post-prandial state is potentially due to insulin mediated substrate utilisation.

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Clinical and laboratory characteristics of 14 patients with Berardinelli-Seip syndrome

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Background and aims: Congenital generalized lipodystrophy (CGL) or Berardinelli-Seip syndrome (BS) is a rare disease that affects 1: 10.000.000 live births. This condition is inherited in an autosomal recessive pattern. These individuals have total or near total absence of subcutaneous tissue since birth. This condition is associated to severe insulin resistance and, in general, these patients develop early metabolic disorders such as diabetes and hypertriglyceridemia. Others clinical manifestations are hepatic steatosis and hepatic cirrhosis, muscle hypertrophy and acanthosis nigricans. The aim of this study was to describe the clinical and laboratory characteristics of patients with BS residents in the state of Ceara-Brazil.

Materials and methods: We studied 14 patients with BS. They performed a complete clinical evaluation, image exams and collected laboratory tests for metabolic evaluation. Data were analyzed in STATA 11.2 program. Results were expressed as mean±standard deviation (SD).

Results: 71.43% of patients were women; the mean age was 10.78± 8.29 years old. BS family history was present in 21.43% and inbreeding in the family 64.29%. All patients had muscle hypertrophy, prominence of veins, acanthosis nigricans and no clinical evidence of peripheral adipose tissue. The mean body fat percentage of these individuals, rated by bioelectrical impedance analysis, was 8.56±3.53%. Diabetes was present in 50% of subjects. The age mean in diabetes diagnosis was 7.73± 6.52 years old. After adjusted for sex, age and height, 88.89% had high blood pressure; 69.23% hypertriglyceridemia; 84.62% low HDL-c and 25% hypercholesterolemia. Imaging studies showed hepatomegaly in 92.86%, hepatic steatosis in 28.57%, splenomegaly in 35.71%, nephromegaly in 61.54% and left ventricular hypertrophy in 40% of the subjects. Laboratory findings were: A1C: 7.06±2.30%, fasting glucose: 109.14±78.60 mg / dL, insulin: 28.04±31.18 μUI / mL, HOMA-IR: 3.83 ±3.21, total cholesterol: 192.46±179.83 mg / dL, HDL-c: 29.84± 8.73 mg / dL, LDL-c: 80.82±28.04 mg / dL, triglycerides: 188.08± 186.09 mg / dL, leptin: 1.19±0,26 ng / mL and albumin / creatinine ratio: 85.80±113,20 mg / g.

Conclusion: The patients with BS have important metabolic disturbances since childhood, with multiple cardiovascular risk factors. Thus, it is necessary an early therapeutic intervention in this population to minimize the complications inherent to these condition.

PS 057 The cancer connection

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Type 2 diabetes and a reduced risk of prostate cancer: a case for a spurious association

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Background and aims: Several studies from western populations indicate that men with Type 2 Diabetes (T2D) are paradoxically at reduced risk of developing incident prostate cancer (PC), but recent data from Asian-Pacific populations indicate that, here, there is a positive association between T2D and PC. In addressing aetiological inference, we hypothesise that such contrasting relationships reflect methodological rather than biological mechanisms.
Materials and methods: We used the Salford Integrated record database linked with the Northwest Cancer Intelligence service (UK) (1995-2010). We identified incident male cohorts with (N=5,069) and without T2D (N=41,812), who were matched (1: 3) by year of birth and smoking status. We explored two broad methodological models to estimate risk: (A) a fixed ever/never method based on prevalent T2D in 1999 (commonly used in the literature); and a (B) time-varying approach allowing for date of T2D diagnosis to be a varying exposure, until the diagnosis of PC. Risk estimates were expressed as hazard ratios (HRs) and their 95% confidence intervals (CIs) using a split-interval approach and age as the timescale.
Results: Using model A, there were 1,098 new diagnoses of PC, the risk of incident PC in men with T2D was reduced (HR: 0.54, 95% CIs: 0.31, 0.94), similar to estimates reported in the literature. Using model B, mean follow-up time was 6.80 years we observed that within 6 months following T2D diagnosis, there was an increased 'spike' co-diagnosis of PC. Analysing beyond 2 years, the association between T2D was null (HR: 0.96, 95% CIs: 71, 1.29).
Conclusion: These findings support the hypothesis that the apparent protective association between T2D and risk of PC might be spurious, and reflect methodological rather than true biological mechanisms. These analyses need to be repeated in a larger independent dataset.
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Deterioration of glycaemic control as a clue to finding occult cancer

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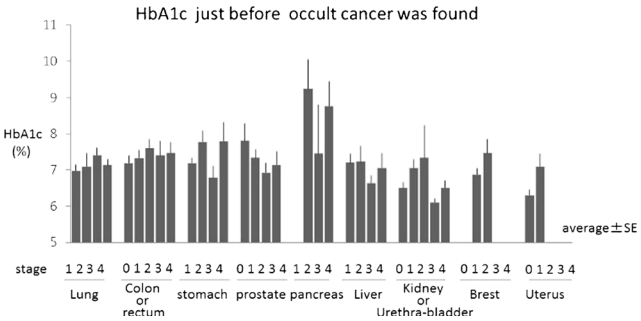
Background and aims: Hyperglycemia, insulin resistance and hyperinsulinemia have been implicated in promoting carcinogenesis. On the other hand, cancer associated factors worsen insulin resistance, and glycemic control of cancer bearing diabetic patients often deteriorates. Either way, deteriorated glycemic control is believed to be an important clue to finding occult cancer in patients with diabetes.
Materials and methods: We tried to confirm whether deteriorated glycemic control was a clue to finding occult cancer in patients with diabetes and investigated whether it applies to every type of cancer. We investigated the medical records for diabetic patients with cancer going back over a period of 8 years. Their trends in glycemic control in relation to types and stages of cancer were analyzed.
Results: During 8 years from 2006 to 2013, 755 diabetic patients were newly diagnosed with cancer. Lung cancer was most common and found in 167 patients, followed by 114 colorectal, 110 gastric, 72 prostate, 40 pancreas, 36 hepatocellular, 26 kidney or urethra-bladder, 24 breast cancers and 20 uterine cancers. Cancers with diagnosed patient numbers under 20 were excluded for analysis. HbA1c significantly elevated in gastric cancer as the stage advanced. However, gastrointestinal bleeding causes apparent decrease in HbA1c regardless of glycemic control, and should be given attention. HbA1c of patients with pancreatic cancer rose significantly in stage 2. Although statistically not significant, HbA1c of patients with hepatocellular carcinoma tended to increase as the stage advanced. HbA1c of patients with kidney or urethra-bladder elevated in stage 0 or 1 more significantly than stage 4. In patients with uterus cancer, HbA1c rose significantly as the stage 0 to 1 advanced. On the other hand, HbA1c of lung or prostate cancer-bearing patients was not elevated even in advanced stages.
Conclusion: Deterioration of glycemic control can be a promising clue to detect occult gastric, pancreatic, kidney or urethra-bladder, and uterus cancers. However, lung or prostate cancers had little effect on glycemic control. Keeping good glycemic control is not only essential for prevention of diabetic complication, but also helps early detection of pancreatic cancer, improvement of personal prognosis and the saving of medical economics.



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p38 MAPK inhibitor suppresses diabetic pancreatic tumour growth and epithelial-mesenchymal transition-mediated metastasis via reducing inflammation

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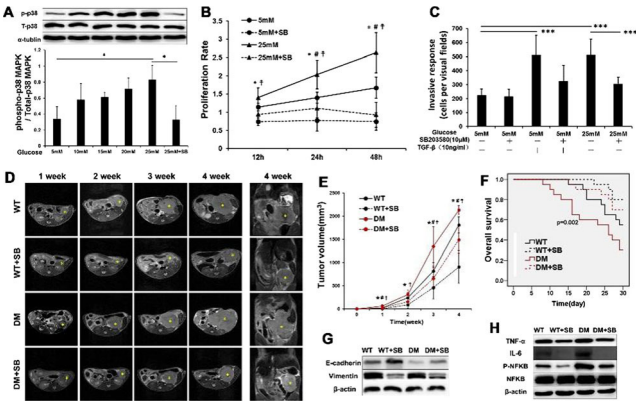
Background and aims: Diabetes contributes to chronic inflammation and promotes tumor progress. p38 mitogen-activated protein kinase (MAPK), as an essential kinase of inflammation, plays an important role in tumor growth. However, the effects of p38 MAPK on epithelial-mesenchymal transition (EMT)-mediated diabetic pancreatic cancer metastasis remains unclear. The aim of this study was to investigate the effects of p38 MAPK inhibitor on pancreatic cancer growth and metastasis in diabetic animals.
Materials and methods: C57BL/6 mice were intra-peritoneally administered with streptozotocin for 5 days and then fed with high fat diet in following 3 weeks to establish animal models of type 2 diabetes. p38 MAPK inhibitor, SB203580, was intravenously injected into pancreatic cancer mice for 7 consecutive days after surgical orthotopic implantation.
Results: Glucose does-dependently increased the phosphorylation of p38 MAPK ($P<0.05$, Fig. A) and time-dependently increased the proliferation ($P<0.05$, Fig. B) of pancreatic cancer cells which were both significantly reduced by SB203580. Transwell assay showed that SB203580 significantly inhibited both high glucose and TGF-β induced cancer cell invasion ($P<0.001$, Fig. C). The orthotopically implanted tumors in wild-type controls and diabetic mice treated with SB203580 or saline were monitored by MRI in 1, 2, 3 and 4 weeks (n=20/group, Fig. D). Tumor volume measurement showed that the diabetic cancers grew much more rapidly than non-diabetic controls ($P<0.05$, Fig. E), while SB203580 treatment significantly suppressed the tumor growth both in non-

diabetic and diabetic mice (P<0.05, Fig. E). Survival curves showed that SB203580 could significantly increase the survival of diabetic and non-diabetic pancreatic cancer mice (P=0.02, n=20/group, Fig. F). In addition, Western blot analysis of tumors showed that diabetes decreased the expression of E-cadherin while increased the expression of vimentin; however, SB203580 treatment could reverse the above EMT process (P <0.05, n=6/group, Fig. G). Furthermore, the pro-infammatory factors (IL-6,TNF-α, NFκB) in diabetic pancreatic tumors were significantly reduced in SB203580-treated groups (P<0.05, n=6/group, Fig. H).

Conclusion: Diabetes induced the phosphorylation of p38 MAPK and inflammation, which promoted the proliferation and EMT-mediated metastasis of pancreatic cancers; while SB203580 suppressed the diabetes-induced tumor growth and EMT. Our results indicate that p38 MAPK inhibitor may provide a novel intervention strategie for diabetic pancreatic cancer patients.



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Differential expression of the E2F family in the carcinogenesis accelerated by liver steatosis

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Background and aims: Obesity and type 2 diabetes are major risk factors for non-alcoholic steatohepatitis (NASH) and hepatocellular carcinoma (HCC). Although various factors including insulin resistance and oxidative stress have been implicated in the development of HCC, the precise mechanism of the carcinogenesis remains to be determined. The E2F family is a group of transcription factors that plays pivotal roles in the regulation of cellular proliferation and apoptosis. The family is currently divided into pro-proliferative (E2F1-E2F3) and anti-proliferative (E2F4-E2F8) members. We tested a hypothesis that the differential regulation of the E2F family is involved in the development of HCC associated with steatosis.

Materials and methods: We used wild-type C57BL/6 mice, adipocyte-specific nSREBP-1c transgenic mice that exhibit liver steatosis and insulin resistance, and liver-specific cyclin D1 (Ccnd1) transgenic mice that develop HCC at around the age of 60 weeks or later. A Ccnd1/nSREBP-1c double transgenic mouse line was generated to assess the effect of liver steatosis on the development of HCC. Gene expressions were measured by DNA microarray analysis and quantitative RT-PCR. Protein expressions were determined by the Western blot analysis.

Results: Wild-type mice fed a high-fat/high-sucrose diet from the age of 8 weeks became obese and developed diabetes before the age of 20 weeks. The expression of E2f2 was higher in the liver compared with mice fed normal chow, while that of E2f8 was lower. Similarly, the expressions of E2f2 and E2f8 were increased and decreased, respectively, in the liver from nSREBP-1c transgenic mice. However, in Ccnd1 transgenic mice that had no liver steatosis, the expressions of E2f2 and E2f8 were comparable to those of wild-type mice. At the age of 52 weeks, small hepatic tumors were detected in 10 of 48 Ccnd1 transgenic mice. In contrast, Ccnd1/nSREBP-1c double transgenic mice that had liver steatosis developed multiple hepatic tumors with histological features corresponding with those of human HCC (34 of 34 mice, p<0.00001). The expressions of E2f2, Ccna2 and Ccnb1 were significantly increased in the background liver tissue, whereas the expression of E2f8 was markedly reduced. Immunohistochemical analysis showed that the proliferating cell nuclear antigen was positive in the nuclei of the non-tumor tissue.

Conclusion: We demonstrated that steatosis accelerated carcinogenesis in the liver of the cancer-susceptible mice. Both Ccna2 and Ccnb1 are target genes of E2F2 and E2F8; E2F2 enhances the expression of the cyclin genes whereas E2F8 represses it. Although the expression levels of E2F members in human HCC and their association with prognosis are still controversial, these observations suggest that the up-regulation of E2F2 and the down-regulation of E2F8 may be involved in the development of HCC in patients with obesity and type 2 diabetes, probably through the modulation of cyclin gene expression.

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Glucose-induced CCL5 release by human adipocytes promotes breast cancer invasiveness and distant metastasis

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Background and aims: Type 2 diabetes (T2D) is associated with an excess risk of several forms of cancer. In particular, women with diabetes have a statistically significant 20% increased risk of breast cancer. Additionally, patients with breast cancer and preexisting diabetes have an increased of metastasis and of all-cause mortality compared with their nondiabetic counterparts. Breast cancer grows and metastasizes to a predominantly adipocyte-dominated host environment. T2D induces adipocyte alterations leading to an imbalanced adipokine production that in turn may promote cancer phenotypes. We have studied the mechanisms by which adipocytes integrate inputs from T2D-induced alterations and promote breast cancer cell motility and invasiveness.

Materials and methods: Human Stromal Vascular cells were isolated by adipose tissue biopsies, differentiated in adipocytes and incubated in the presence of different concentrations of glucose. Adipocyte conditioned media and co- cultures were established to investigate MDA-MB-231 breast cancer cell motility and invasiveness. Adipocyte- released factors were determined by ELISA multiplex. CCL5 and IGF1 expression in peritumoral adipose tissue was evaluated by immunochemistry.

Results: We have obtained evidence that human adipocytes exposed to high glucose concentration (25 mM) induce breast cancer cell proliferation, migration and invasion. Screening for several cytokines revealed that adipocytes cultured in high glucose medium release 1.5 and 2-fold higher amount of IGF1 and CCL5, respectively, compared to those cultured in low glucose medium (5.5 mM glucose). Moreover, adipocyte-released factors control IGF1, but not CCL5 expression in cancer cells. Inhibition of IGF-1 pathway almost completely prevented the effect of adipocytes on breast cancer cell growth but only slightly interfered with cancer cell motility. At variance, inhibition of CCL5 action by specific peptides and antibodies reduces adipocyte promoting effect on breast cancer cell migration and invasion. Interestingly, CCL5/RANTES expression in peritumoral adipose tissue of women with Triple Negative

Breast Cancer correlated with lymph node (p-value=0.04) and distant metastases (p-value=0.001). A positive correlation was also observed between CCL5 expression and T2D. Finally, Kaplan-Meier curves showed a significant negative correlation between CCL5 staining in the peritumoral adipose tissue and overall survival of patients (p-value= 0.039).

Conclusion: Hyperglycemia induces adipocyte release of CCL5 that, in turn, contributes to cancer cell motility, cancer lymph node and distant metastases, which may contribute to reduce the overall survival of patients.

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Chronic hyperglycaemia enhances the malignant molecular communication between the human pancreatic stellate and cancer cells

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Background and aims: The association between diabetes mellitus (DM) and pancreatic cancer (PaC) was established by epidemiologic studies. It was concluded that DM is not only an early manifestation, but also an etiologic factor of PaC. We hypothesized a role for pancreatic stellate cells (PSC) in the hyperglycemia induced deterioration of pancreatic cancer. Therefore studied two human cell lines (RLT-PSC, T3M4) in hyperglycemic environment.

Materials and methods: In case of 'chronic hyperglycemia' (CHG) exposure RLT-PSC cells were cultured in a medium containing 15.3 mmol/L glucose for 21 days. Subsequently, cells were starved for 24 hours in FBS-free medium and thereafter for 48 hours in FBS-free medium either with or without TGFβ1. The effect of CHG on PSCs was sutided using mRNA expression array (GeneChip PrimeView, Affymetrix) with real-time PCR (ABI Gene Expression TaqMan®) validation and bioinformatic pathway analysis (MetaCore, Thomson Reuters). Main results were also confirmed using protein studies (ELISA, WB, IC). The effect of PSC-conditioned media and hyperglycemia on T3M4 pancreatic cells was assessed in proliferation and migration assays.

Results: The stress fiber formation (IC αSMA) indicated that PSCs tend to trans-differentiate to a myofibroblast-like state after CHG exposure. Glucose transporters (types-1, -2, and -3) were expressed on RLT-PSC and T3M4 cells. The phosphorylation of p38 and ERK1/2 was increased with a consecutive upregulation of CDC25, SP1, cFOS and p21, and with downregulation of PPARg after PSCs were exposed to chronic hyperglycemia.

CXCL12 levels increased significantly in PSC supernatant after cells were exposed to CHG regardless of TGF-β1 treatment (3.09-fold with and 2.73-fold without TGF-β1, $p<0.05$). Treatment of PSCs - kept in normal glucose concentration with TGF-β1 treatment in a 2.69-fold increase of CXCL12 levels. When the TGF-β1 treatment was applied subsequently after that PSCs were exposed to CHG it resulted in a significant (3.78 fold, $p<0.05$) IGFBP2 protein level elevation in the supernatant. In cancer cells, hyperglycemia induced an increased expression of CXCR4, a CXCL12 receptor that was also induced by PSC's conditioned medium. The receptor-ligand interaction increased the phosphorylation of ERK1/2 and p38. Conditioned medium of PSC increased pancreatic cancer cell proliferation and this effect could be partially inhibited by a CXCR4 inhibitor (AMD3100). The PSC conditioned medium (even under normal glucose concentration) could increase the ERK1/2 and p38 phosphorylation.

Conclusion: Hyperglycemia induces increased CXCL12 production by the human PSCs, and its receptor, CXCR4 on cancer cells. The ligand-receptor interaction activates MAP kinase signaling that further increases the cancer cell proliferation and migration. The upregulation of the SP1 transcription factor in PSCs after CHG exposure may result in the increased CXCL12 and IGFBP2 production.

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Insulin and IGF-1 promote pancreatic stellate cells activation and galectin-3 expression: potential role in promotion of pancreatic cancer

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Background and aims: Epidemiological studies indicate that the risk of pancreatic cancer (PC) is increased in type 2 diabetic patients. Insulin and insulin-like growth factor (IGF-1) play an important role in carcinogenesis. Insulin resistance and associated hyperglycemia, hyperinsulinemia, and abnormalities in insulin/IGF receptor pathways have been suggested to be the underlying mechanisms contributing to development of diabetes-associated pancreatic cancer. Activated pancreatic stellate cells (PSCs) are key stroma cells responsible for pancreatic fibrogenesis and pancreatic cancer progression. The aim of this study was to elucidate PSCs functional responses to insulin and IGF-1.

Materials and methods: In primary mouse PSCs and a novel immortalized mouse pancreatic stellate cell line (imPSCs), we examined the effects of insulin and IGF-1 on PSCs activity. Cells were stimulated with 100 nmol/L insulin or 10 nmol/L IGF-1. We determined expression of α-smooth muscle actin (α-SMA) and galectin-3, measured matrix synthesis of collagen I (Col-I) and fibronectin (FN), and analyzed the expression of insulin/IGF-1 signaling pathway components and their operation as profibrotic and/or proliferative pathways and assessed the potential contributions of PSCs to a procarcinogenic microenvironment.

Results: PSCs express receptors for both insulin (IR) and insulin-like growth factor 1 (IGF-1R), and respond to insulin/IGF-1 stimulation with increased tyrosine phosphorylation at specific autophosphorylation sites. Akt/mTOR/p70S6K signaling were activated.α-SMA expression were induced when PSCs were incubated with either insulin (1.75±0.06 of control, $P<0.01$) or IGF-1 (8.16±0.04 of control, $P<0.01$). Galectin-3 production of PSC was enhanced after incubation with insulin (1.83± 0.07 of control, $P<0.01$) or IGF-1 (9.80±0.06 of control, $P<0.01$). Col-I and FN production were also increased ($P<0.05$). IGF-1 was four to five times more potent than insulin. Results in imPSCs were in accordance with primary mouse PSCs.

Conclusion: Our study suggests that insulin and IGF-1 play an important role in the activation of PSCs and progression of fibrosis. Increased expression of galectin-3 and abnormalities in insulin/IGF receptor signaling network may be involved in development and progression of diabetes-associated pancreatic cancer.

PS 058 You are what you eat!

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The postprandial glucose response is not a good reflection of the actual rate of glucose release from a starchy food: an exploratory study using dual stable isotope technique

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Background and aims: We have shown that guar gum added to a flat bread flour lowers postprandial blood glucose and insulin dose-dependently, probably due to the viscosity-inducing effects of the fibre. However, the glucose influx rate from a starchy food source is not established by measuring only total blood glucose, as this is the net result of glucose absorption, glucose disposal into tissues and hepatic glucose production rates. The aim of this study was therefore to measure the actual rate of glucose release from the food and related glucose fluxes, and to obtain more insight into the mechanism of glucose lowering, by using the dual isotope technique and measuring incretin hormones.
Materials and methods: In a double-blind, randomized, placebo-controlled, cross-over design, 12 healthy males consumed each of three flatbread test products labelled with ^{13}C-wheat flour: a control (C), or C with the incorporation of 15% chickpea with either 2% (GG2) or 4% (GG4) guar gum. By a 6 h primed-continuous infusion of D-[6,6-$^2H_2$] glucose the appearance of exogenous glucose (RaE), endogenous glucose production (EGP) and glucose clearance (GCR) rates could be calculated. Plasma glucose, insulin, GIP and GLP-1 were measured for 4 h postprandially. All data were calculated as 2 h and (except glucose) 4 h area-under-the-curve (AUC). As the study was explorative, statistical significance was assessed by comparing the confidence intervals of the changes after GG2 and GG4 compared to C.
Results: Compared to C, GG2 reduced glucose by 14% (NS) and insulin by 16% ($P<0.05$), while 4 h AUC values for RaE, GCR and EGP were reduced slightly by 3, 5 and 3%, respectively (all NS). Changes after GG4 were more distinct and all significant (all $P<0.05$): glucose was reduced by 26% and insulin by 23%, while RaE, GCR and EGP were reduced by 11, 12 and 64%, respectively. The 2 h and 4 h effect sizes were similar for RaE and GCR, but the 2 h values for EGP were bigger, namely 36 and 80% greater suppression after GG2 and GG4 respectively (both $P<0.05$) compared to C. Postprandial changes in GIP and GLP-1 did not differ significantly between products.
Conclusion: Using the dual isotope technique, we found that the lower glucose response to GG2 and GG4 was not clearly dominated by a reduced absorption rate, as might be expected for a viscous fibre, but was perhaps even more due to post-absorptive effects (GCR and EGP). The data suggest that small initial changes in RaE could potentially result in larger post-absorptive effects. The lower GCR was in accordance with the lower insulin response after the test meals, but EGP was not. EGP was paradoxically more suppressed after the test meals than after the control, especially during the first 2 h, which could also not be explained by the other incretin hormones. These data reinforce the view that the rate of glucose release from carbohydrate-rich foods cannot be assumed from the postprandial glucose response, but must be measured using labelled tracers.
Clinical Trial Registration Number: NCT01734590
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Metabolic and molecular effects of a high-protein diet in subjects with type 2 diabetes

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Background and aims: Previous studies reported both favourable and adverse impacts of high-protein diet in type 2 diabetes. In our clinical study we compared effects of two isocaloric high-protein diets of animal (AP) and plant (PP) origin on metabolic markers, liver fat content and signalling pathways in white blood cells and adipose tissue.
Materials and methods: Individuals with type 2 diabetes (age 65± 6 years, BMI 30.5±3.6 kg/m^2, HbA1c 7.0±0.6%, n=30) were randomized to either high-animal (meat and dairy foods) or high-plant (dietary pulses) protein diet (30% protein, 40% carbohydrates, 30% fat) for 6 weeks. Before and after the diet intervention, magnetic resonance imaging, hyperinsulinemic euglycemic clamps, and meal tolerance tests were performed, and blood and subcutaneous adipose tissue samples were collected. Key proteins of the Akt/mTOR signalling pathway were analysed in isolated white blood cells and adipose tissue using PathScan Antibody Array (Cell Signaling).
Results: Levels of liver parameters (AST, ALT, GGT) in blood improved after diet intervention in both groups. Liver fat content and HbA1c levels were reduced in all subjects (AP: -43.6%, $p<0.001$; PP: -37.1%, $p<0.001$, and AP: - 0.58%, $p<0.05$; PP: - 0.41%, $p<0.001$, respectively). The clamp-derived insulin sensitivity improved significantly only in the AP group (ΔM-value=0.88 mg/kg BW/min, $p<0.05$). In the PP group there was a significant reduction of plasma creatinine (-7.79 µmol/l, $p<0.01$) and improvement of the glomerular filtration rate (from 75.95 to 88.15 ml/min/1.73 m2, $p<0.001$) which was not found in the AP group. We did not observe any alteration of the Akt/mTOR pathway in white blood cells after the dietary intervention. However, in adipose tissue the phosphorylation of AMPK, Erk1/2 and 4E-BP1 was approximately 2-fold higher in the AP group, whereas in the PP group the phosphorylation of Bad and PDK1 was significantly increased.
Conclusion: In diabetic subjects, the 6-week high-protein diet leads to an improvement of glucose metabolism and decrease of liver fat content independently from the protein origin. The high-protein diet has no adverse effects on kidney parameters, moreover the kidney function improved in the plant protein group. On molecular level, we observed the dietary-induced increase of the phosphorylation of some proteins of Akt/mTOR pathway in subcutaneous adipose tissue, but not in blood cells, indicating a differential modulation of intracellular signalling pathways.
Clinical Trial Registration Number: NCT02402985
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Effects of medium-chain saturated dairy fat on the body composition of abdominal obese subjects (DairyHealth): a 12-week, randomised, double-blinded, intervention study

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Background and aims: Obesity, reduced insulin sensitivity and hypertension are important risk factors for cardiovascular disease (CVD) and type 2 diabetes (T2D). These risk factors are influenced by the dietary behavior. Thus the consumption of dairy products e.g. whey protein and medium-chain saturated fatty acids (MC-SFA) may have a beneficial impact.

Materials and methods: We conducted a 12-week randomized, double-blinded, parallel controlled, diet intervention study in 63 abdominal obese subjects. Subjects were randomized into one of four diets in a 2×2 factorial square design: 60 g/day of whey or casein combined with 63 g/day of milk fat with either high (8.5 g/day) or low content of MC-SFA (6.9 g/day). Body composition was assessed by dual-energy x-ray absorption scan at baseline and after intervention. We estimated insulin sensitivity using the homeostatic model assessment (HOMA-IR) and the Matsuda Index. In addition, diurnal blood pressure (24-h BP) was measured. Two-way ANOVA analysis was used to examine the difference between the whey and casein supplementation and between the high and low MC-SFA supplementation, as well as to elucidate potential interactions between the protein and the MC-SFA.
Results: Fifty-two subjects completed the intervention. We found that lean body mass increased by 981 g (95%CI: 248, 1713; p=0.010) by the diet supplemented with milk fat high rather than low in MC-SFA, whereas total body fat percentage was lowered by 0.70% (95%CI: 0.10, 1.31; p=0.024). These changes were independent of the protein type. We found no difference in HOMA-IR, the Matsuda Index, glycated hemoglobin, or 24-h BP between protein type or fat composition.
Conclusion: In conclusion, supplementation with milk fat enriched in MC-SFA via a feasible cattle feeding regimen resulted in increased lean body mass and lower total body fat percentage in abdominal obese subjects underlining the importance of the fat quality in dairy products.
Clinical Trial Registration Number: NCT01472666
Supported by: DSF, Arla Foods, Danish Dairy Research Foundation

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Differential effects of carbohydrate vs fat overfeeding on liver fat content and lipid metabolism in healthy overweight males

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Background and aims: Ectopic fat accumulation is thought to be an important contributor to insulin resistance and diabetes. Excess energy consumption has been shown to increase liver fat content and reduce insulin sensitivity, but it is unclear to what extent this is influenced by dietary composition. We investigated the degree to which liver fat, liver and lipid markers are affected by 2 weeks of overfeeding at 25% excess energy given as either carbohydrate or fat.
Materials and methods: After 1 week of ingesting an isocaloric diet, 21 overweight but healthy males (40.3±2.5 yrs; BMI 31±1.0 kg m-2) underwent 3 T H1Magnetic Resonance Spectroscopy (H1 MRS, Philips Achieva) of their liver and magnetic resonance imaging (MRI) of their abdomen to measure liver fat content and visceral fat respectively. Fasting serum insulin, glucose, triacylglyceride (TAG), free fatty acids (FFAs), lipoapoproteins, total cholesterol and liver function were also obtained. Subjects were then randomised into 2 groups of ingesting either a hyperenergetic (25% excess) high fat (HF) n=11, (42.2±2.4 yrs, BMI 30.5±0.9 kg m-2) or high carbohydrate (HC) n=10, (38.3±2.4 yrs, BMI 31.5±1.0 kg m-2) diet for two weeks. The diets comprised either high carbohydrate (65% CHO, 20% Fat, 15% Prot) or high fat (38% CHO,47% Fat, 15% Prot). After two weeks, H1MRS liver, MRI abdomen and fasting bloods were repeated.
Results: After 2 weeks of excess energy consumption, there was an overall main effect of time on liver fat content (increased by 2.2±0.8%; p<0.05) which approached significance in the high carbohydrate group (increased by 2.8±1.8%; p=0.06) but not in the high fat group (increased by 1.6±1.1%; p=0.3). Fasting TAG in the whole group increased by 0.33 ±0.13 mmol/l (p<0.05), and this was notably higher in the high carbohydrate group where TAG increased by 0.45±0.14 mmol/l (p<0.05). In the whole group both Apolipoprotein A and B increased by 0.08±0.01 g/L, p<0.01 and 0.07±0.01 g/L p<0.01 respectively. The increase in Apolipoprotein A and B was greater in the high fat group (0.13± 0.03 g/dL and 0.10±0.03 g/L; p<0.01 respectively. There were no changes in body mass, visceral fat, HOMA-IR, or fasting insulin, glucose, FFAs, total cholesterol or liver function after 2 weeks of overfeeding in either the whole group or between dietary groups.
Conclusion: Two weeks excess energy consumption increased liver fat content, TAG, Apolipoprotein A and B. This deleterious effect of overfeeding on liver fat and markers of liver function and lipid appears to be more pronounced in the high carbohydrate-fed group.

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Angiotensin converting enzyme increases in response to a high fat diet

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Background and aims: Angiotensin-converting enzyme (ACE) catalyzes the conversion of angiotensin I to angiotensin II which leads to vasoconstriction. Pharmaceutical inhibition of ACE serves as effective treatment in hypertension and cardiovascular diseases, but also improves insulin sensitivity. On the contrary, carriage of the D-allele of the ACE insertion/deletion (I/D) polymorphism is associated with increased ACE serum levels and impaired glucose tolerance. ACE is not only expressed in vascular endothelial cells but also in adipose tissue and its levels are altered in conditions of obesity and weight loss. We aimed to investigate effects of an isocaloric high-fat diet (HF) on ACE considering possible nutrigenetic influences.
Materials and methods: In the NUGAT (NUtriGenomic Analysis in Twins) study 46 healthy twin pairs went from a 6-week carbohydrate-rich low-fat diet (LF) to a 6-week HF diet under isocaloric conditions. Clinical investigation days took place after 6 weeks LF and after 1 and 6 weeks of HF (HF1, HF6). At each investigation day subcutaneous adipose tissue biopsies were taken for gene expression analysis on Agilent 8×60K microarrays. Serum parameters were analyzed in blood samples using ELISA. To assess insulin sensitivity intravenous glucose tolerance tests (ivGTT) were performed and incremental areas under the curve (AUC) calculated. Genomic DNA extracted from whole blood was genotyped using Illumina HumanOmniExpressExome BeadChips. For heritability estimates the '*ACE*' structural equation model was applied.
Results: Heritability estimates >80% prove a high genetic component of circulating ACE levels (p<0.001). After six weeks HF circulating ACE levels increased by 15% (HF6 161±49 ng/ml vs. LF 139±41 ng/ml; p< 0.001) paralleled by an increase in adipose tissue gene expression (1.41-fold, p<0.001). 34% of the increase in circulating ACE levels as regulatory response to the HF diet is explained by additive genetic factors (p<0.01). Interestingly, in homozygous carriers (GG) of the rs4343 polymorphism, which serves as a surrogate marker for ACE I/D polymorphism, the increase in serum levels was nearly double that of non-carriers (AA) or heterozygous carriers (AG) (p<0.001). Whereas no change in glucose tolerance was observed for AA/AG-carriers, glucose tolerance significantly declined in GG-carriers after six weeks of HF ($\Delta AUC_{glucose}$, recessive model: p=0.009).
Conclusion: Despite the known strong heritable component for ACE levels, we could show a considerable increase in ACE serum levels and gene expression in adipose tissue in response to a high fat diet. These results point out that ACE potentially constitutes a molecular link between dietary fat intake and cardiovascular diseases as well as impaired glucose metabolism. The extent of this relationship seems to be nutrigenetically modulated.
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High fat diet induced association of anti-inflammatory phospholipid and CRP levels in human adipose tissue in the NUGAT study

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Background and aims: Lipid metabolism and signaling might be either controlled genetically or by environmental factors. The aim of the study was to correlate lipidomic and genomic data of human subjects to identify specific gene-modules responsible for the regulation or connected with the function of specific lipid metabolites.
Materials and methods: In the NUGAT-Study 46 healthy mono- and dizygotic twin-pairs first were standardized for their nutritional behavior by a carbohydrate-rich low-fat diet for 6 weeks (LF), immediately followed by a high-fat diet for 1 week (HF1) and additional 5 weeks (HF6). At each CID periumbilical fat biopsies were taken for determination of gene expression on Agilent 8×60K gene micro Arrays. Plasma was measured for lipid metabolites and cytokines (ELISA). Weighted gene Co-Expression Network Analysis (WGCNA) and regularized Canonical Covariance Analysis (rCCA) were used for identification of co-expressed gene-networks and their correlation with lipidome data.
Results: Analysis of the 5000 strongest regulated genes revealed a gene-module highly correlated with CRP ($r=0.31$; $p=6\times10\text{-}4$) and highly associated ($r=-0.32$, $p<0.0004$) with an anti-inflammatory/anti-bacterial lysophosphatidylethanolamine specimen (LPE18:1). Application of rCCA showed an increasing association of a set of genes involved in lysophosphatidic acid (LPA) receptor signaling with the decreasing concentrations of LPE specimen. When stratifying the cohort for individuals with a CRP<1 or CRP≥1 at HF6, LPE18:1 levels decrease more pronounced in individuals with high CRP ($p=0.042$). Remarkebly, in individuals with low CRP the detected inflammatory gene-module is not associated with LPE18:1 ($r=-0.04$; $p=0.7$) while highly associated in people with high CRP ($r=-0.6$; $p=0.008$).
Conclusion: Our data show for the first time in humans that high-fat diet induces a gene set with inflammatory character which might be involved in lysophosphatidic acid (LPA) receptor signaling. Specifically we identify down regulation of the anti-inflammatory phospholipid LPE and increasing synchronization of LPE concentration with gene expression patterns as a mediator of inflammatory processes in the adipose tissue in a CRP associated manner.
Clinical Trial Registration Number: ClinicalTrials.gov, NCT01631123
Supported by: BMBF

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Dysregulation of sirtuins and key metabolic genes in skeletal muscle of pigs with intrauterine growth restriction is associated to alterations of circulating IGF-1

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Background and aims: Prenatal and early postnatal lives are important determinants of future health, and intrauterine growth restriction (IUGR) - associated low birth weight predisposes to the development of metabolic and cardiovascular disease in adult life, but the mechanisms are largely unknown. We hypothesize here that IUGR might confer gene expression alterations, predisposing to metabolic disease.
Materials and methods: Using a porcine model of spontaneous IUGR, we determined in utero (71, 112 days post-conception) and early-postnatal (2 days post-birth) IGF-1, insulin and leptin levels, and in parallel we investigated skeletal muscle (Longissimus dorsi) gene expression of sirtuins and key metabolic genes (IRS1, GLUT4, HK2 and GAPDH).
Results: In IUGR, we observed impaired IGF-1, insulin and leptin serum levels. Gene expression of sirtuin 1, 5, 6, 7, GLUT4 and HK2 exhibited significant correlations with gestational age or fetus/newborn body weight. SIRT1 and HK2 expression displayed an age- and weight-dependent downregulation in controls, which was lost in IUGR pigs. Conversely, SIRT2 and GLUT4 were upregulated in IUGR pigs. Within the set of genes studied, we found a significant correlation between IGF-1 levels and gene expression, indicating that IGF-1 is limiting in IUGR. IUGR-dependent gene alterations were partly linked to epigenetic changes on histone H3 acetylation and methylation.
Conclusion: Our observations indicate that several sirtuins and metabolic genes display specific gene expression trajectories during fetal and early postnatal life, which are altered in IUGR, and are related to hormonal dysregulations observed in IUGR. Given the importance of these genes in metabolic control, their perinatal alterations might contribute to the predisposition to metabolic disease and diabetes in adulthood.
Supported by: INRA multicentric study start grant

PS 059 You are how you eat!

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Association between eating rate and obesity: a systematic review and meta-analysis

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Background and aims: Considering the epidemic and deleterious impact of obesity and its complication such as type 2 diabetes, a better understanding of the association between diet therapy and obesity would be beneficial from both clinical and public health care perspectives. Epidemiological evidence has shown that diet therapy based on not only the types and amounts of foods, but also eating behaviours play an important role in the management of obesity. Recently, several epidemiologic studies have reported the significant association between eating rate and obesity. However, the influence of eating rate on the obesity remains inconclusive. Therefore, we undertook a systematic review with a meta-analysis of published epidemiological studies to provide a reliable estimate of the association between eating rate and obesity.
Materials and methods: A systemic search of MEDLINE, EMBASE and CINAHL was conducted to identify studies that reported quantitative estimates for indices of obesity based on the category of eating rate. Interventional studies or those conducted for children were excluded. The reference lists of identified articles were manually scanned to identify other relevant studies. Two independent researchers extracted the data. A summary estimate was calculated using a random-effects model. Subgroup analyses were conducted to identify the sources of heterogeneity.
Results: Twenty-three published studies were eligible for inclusion, of which 20 were cross-sectional studies, 2 were longitudinal studies and 1 provided results from both study designs. Twenty one cross-sectional studies were included in the meta-analysis. The mean difference in BMIs between individuals who ate quickly and those who ate slowly was 1.78 kg/m^2 (95% CI, 1.53-2.04 kg/m^2). The pooled odds ratio of eating quickly on the presence of obesity was 2.15 (95% CI, 1.84-2.51). There was evidence of significant quantitative heterogeneity in the magnitudes of the association across studies (I^2=78.4%, P value for heterogeneity< 0.001 for BMI, I^2=71.9%, P value for heterogeneity<0.001 for obesity), which may be partially explained by differences in the type of study population (a weaker association was observed for BMI in individuals with diabetes than in those without; mean difference 1.32 kg/m^2 [95% CI, 0.90-1.74 kg/m^2] versus mean difference 1.89 kg/m^2 [95% CI, 1.64-2.15 kg/m^2], respectively, P value for heterogeneity=0.038). A funnel plot showed a symmetric pattern, and formal statistical testing did not identify the presence of publication bias. Three longitudinal studies also showed that a faster eating rate was associated with increased BMI and a higher risk of incident obesity over time.
Conclusion: Eating quickly is positively associated with excess body weight. Although further studies are needed to conclude a causal relationship between eating rate and obesity, more emphasis may be placed in clinical practice on slowing the speed of eating.
Supported by: Health and Labour Sciences Research Grants: Research on Food Safety

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The effect of meal frequency in a reduced-energy regimen on the gastrointestinal and appetite hormones in patients with type 2 diabetes: a randomised crossover study

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Background and aims: Appetite and gastrointestinal (GI) hormones participate in energy homeostasis, feeding behavior and regulation of body weight. We demonstrated previously the superior effect of two meals a day, breakfast and lunch (B2) on body weight, hepatic fat content, insulin sensitivity and feelings of hunger compared to the same diet divided into six smaller meals a day (A6). The aim of this secondary analysis was to investigate the effect of frequency of meals on the appetite and GI hormones.
Materials and methods: In a randomized, crossover study, we assigned 54 patients with T2D to follow two regimens of a hypocaloric diet, each for 12 weeks: either 6 meals (A6) or two meals (B2). The diet in both regimens had the same macronutrient and energy content. After each regimen participants underwent a standard meal test. The concentrations of glucagon-like peptide-1 (GLP-1), gastric inhibitory peptide (GIP), pancreatic polypeptide (PP), peptide YY (PYY), amylin, leptin and ghrelin were determined using multiplex immunoanalyses. For statistical analysis 2×2 crossover ANOVA was used.
Results: Body weight decreased in both regimens ($p<0.001$), more in B2 ($p<0.001$). Fasting leptin and GIP decreased (both $p<0.05$) in response to both regimens with no difference between the treatments. Fasting ghrelin decreased in A6 ($p<0.001$), with a trend toward an increase ($p=0.08$) in B2 ($p<0.05$). Fasting PP increased in B2 ($p<0.05$) with a trend toward an increase in A6 ($p=0.09$) with no significant difference between both regimens. Neither GLP-1 nor PYY did change in either regimen. The decrease in body weight correlated negatively with changes in fasting ghrelin ($r=-0.4$, $p<0.05$) and the postprandial suppress of ghrelin correlated positively with its fasting level ($r=0.9$, $p<0.001$).
Conclusion: We demonstrated that a hypocaloric diet with lower meal frequency leads to greater decrease in body weight in patients with T2D and the changes in body weight correlated negatively with changes in fasting ghrelin. The effect of meal frequency on other measured GI peptides was not proven.
Clinical Trial Registration Number: NCT01277471
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Effect of different diurnal patterns of meal composition on metabolic parameters in healthy men

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Background and aims: A recent animal study has shown a potential benefit of a fat-rich diet at the beginning of the active phase and a carbohydrate-rich diet at the end (versus the opposite order) on glucose tolerance, beta-oxidation and body weight. We therefore aim to determine the effects of two different diurnal patterns of meal composition on metabolic parameters and circadian gene expression in subcutaneous adipose tissue (SAT) in humans.

Materials and methods: In a cross-over study, 20 men (age 46.4± 14 years, BMI 27.5±4 kg/m^2) consumed isocaloric carbohydrate-rich meals in the morning and fat-rich meals in the evening (diet A) or isocaloric fat-rich meals in the morning and carbohydrate-rich meals in the evening (diet B) for four weeks each. At the end of each intervention period, two 850 kcal meal tolerance tests, a carbohydrate-rich (MTT HC) or a fat-rich (MTT HF), were performed at 09.00 am and 03.40 pm, respectively, according to the participant´s previous dietary regimen. Insulin, C-peptide, glucose, and other routine lipid, liver and kidney markers were determined in plasma. Gene expression in SAT was measured at three time points (08.45 am, 12.15 pm and 07.00 pm) during each investigation day using quantitative real-time PCR.
Results: Mean body weights were not affected by the diets. Both diets decreased fasting blood glucose, insulin, and total cholesterol levels. As expected, MTT HC induced a larger increase in glucose and insulin levels than MTT HF. Postprandial glucose, insulin and C-peptide levels were higher in the afternoon compared to the morning for both MTT HC and MTT HF. In the afternoon, postprandial peak of insulin secretion was delayed and insulin excursion pattern showed a second peak independent of the meal composition. Postprandial NEFA levels were significantly higher in the afternoon compared to the morning only for MTT HC. Analysis of core clock gene expression in SAT (BMAL1, CLOCK, PER1-3, CRY1-2, NR1D1 and RORA) revealed profound reorganization of circadian oscillations induced by the change of the diurnal pattern of meal composition.
Conclusion: Our results show that glucose tolerance decreases in the afternoon independent of meal composition. Still, different diurnal patterns of meal composition induce large differences in circadian oscillations in SAT underlining the important role of food composition for the entrainment of peripheral circadian clocks in humans.
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Extension of overnight fast until noon, triggers increased postprandial hyperglycaemia and reduced and delayed insulin response after lunch and dinner in type 2 diabetes

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Background and aims: Reduction of postprandial hyperglycemia is a major target in the treatment of type 2 diabetic patients. Skipping breakfast has been consistently associated with higher HbA1c and overall PPHG in T2D. We aimed to explore the effect of eating vs. skipping breakfast on PPHG after subsequent isocaloric lunch and dinner.
Materials and methods: In a crossover design, 22 type 2 diabetic patients, since 7.79±2.1 yrs, aged 58.7±4.9 yrs, BMI 28.4±2.8 kg/m2 and HbA1c 7.7±0.394% were randomly assigned to 2 meal test days: one day with breakfast, lunch and dinner (YesB) and another day the breakfast was omitted and only lunch and dinner were consumed (NoB). Postprandial plasma glucose, insulin, free fatty acids (FFA), glucagon and intact glucagon-like peptide-1 (iGLP-1) after isocaloric (700 kcals; % CH:protein:fat 50:30:20) lunch and dinner were assessed and calculated as AUC180min.
Results: Compared with YesB, AUC180 after lunch in the NoB group was higher by 37% for glucose (41804±310 vs 30566±185 mg/dl*min, p<0.0001), lower by 17% for insulin (5156±86 vs 6213±116 μU/ml*min, p<0.0001) and lower by 19% for iGLP-1 (p<0.0001). Plasma FFA and glucagon were suppressed by 41.3% and 14.8%, respectively (p <0.0001). Insulin peaked 30 min after lunch in the YesB group vs 60 min after lunch in the NoB group. After dinner, in the NoB vs YesB, AUC180 increased by 26.6% for glycemic response (47310±88 vs 37368± 247 mg/dl*min, p<0.0001), reduced by 7.9% for insulin (5150±99 vs 5593±147 μU/ml*min, p<0.0001) and reduced by 16.5% for iGLP-1 (p <0.0001). Plasma FFA and glucagon were less suppressed by 29.6% and 11.5%, respectively (p<0.0001) in the NoB compared with the YesB group. Insulin peaked 60 min after dinner in the YesB group vs 90 min after dinner in the NoB group. Glycemic response AUC after lunch and dinner positively correlated with AUC180 for FFA (R2=0.78, p<0.0001) and with AUC180 for glucagon (R2=0.73, p<0.0001) and was inversely correlated with AUC180 iGLP-1 (R2=0.79, p<0.0001).
Conclusion: Skipping breakfast increased postprandial hyperglycemia after lunch and dinner. This was accompanied in NoB, by less iGLP-1, lower and delayed prandial insulin release, and less suppressed plasma FFA and glucagon. Breakfast consumption should be a strategy toward reduction of postprandial hyperglycemia in type 2 diabetic individuals.
Clinical Trial Registration Number: NCT02287103

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Dietary habits and physical activity of Greek children in relation to the presence of diabetes in a household member

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Background and aims: To explore the relationship between dietary habits and amount of physical activity of Greek children aged 6-9 years old and the presence of diabetes in a family member living in the same household.
Materials and methods: Analysis was based on cross-sectional data obtained from the Greek arm of the second round of the Childhood Obesity Surveillance Initiative (COSI) study, established by the World Health Organization. The study population was nationally representative and consisted of primary school children aged 6-9 years old. A family record form was used to gather information on a voluntary basis on simple indicators of the children's dietary intake and physical activity/ inactivity patterns, the family's socioeconomic characteristics and the presence of diabetes on a household member. Univariate and multivariate relationships between variables were studied using Chi-square and logistic regression tests.
Results: A total of 1821 children (884 boys and 937 girls) were included in the analysis. There were 104 children (5.7%) reporting a family member with diabetes in their household. Mean BMI was similar between children of families with and without diabetes in the household (18.8±3.4 Kg/m2 vs. 18.7±3.3 Kg/m2 respectively). The presence of a household member with diabetes was associated with less frequent breakfast consumption (p=0.04), lower consumption of fresh fruits (p=0.051) and fish (p=0.053), higher consumption of diet soft drinks (p<0.001), flavored milk (p=0.016), foods like potato chips, corn chips, popcorn or peanuts (p=0,003) and foods like pizza, fried potatoes, hamburger, sausage or meat pies (p=0.001). In addition, it was associated with lower participation in sport or dancing clubs (p=0.002 and p=0.007 respectively), fewer hours spent playing outside (p=0,008) and more hours spent on computer games (p<0.001). The above associations remained significant after adjustment for BMI and socioeconomic status.
Conclusion: The presence of diabetes in a household member is associated with several unhealthy nutritional and physical activity habits in Greek children aged 6-9 years old, despite no difference in BMI and even after adjustment for family socioeconomic status. These findings may be attributed to the transition of unhealthy lifestyle choices of parents with diabetes to their children.

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Nutritional regulation of incretin secretion in controls, impaired glucose tolerance and type 2 diabetes

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Background and aims: While studies suggest that excessive sugar intake is associated with the development of type 2 diabetes (T2DM) and non-alcoholic fatty liver, it is unclear whether this is primarily related to sugar metabolites such as glucose and fructose or to the postprandial hormonal responses, particularly incretins, glucose-dependent insulinotropic peptide (GIP) and glucagon like pepetide-1 (GLP-1). We investigated the effects of sucrose and its isomer Palatinose ingestion on endogenous GIP and GLP-1 release and their relation to hepatic insulin clearance (HIC) in normal (NGT), impaired glucose tolerant (IGT) and T2DM participants.
Materials and methods: In a randomized, within-subject crossover study 15 NGT (control), 10 IGT and 10 T2DM subjects were studied for 180 min consuming 50 g either Palatinose or sucrose solutions which are dimers of glucose and fructose but resorbed either distally or proximally due to different 1,6 vs 1,2 linkage to evaluate glucose and insulin responses. Postprandial GIP and GLP-1 levels were assessed and related to HIC.
Results: Following oral sucrose, blood glucose levels peaked significantly ~2 fold after 30 min which were 38% ($p<0.001$) and 26% ($p<0.05$) higher than Palatinose intake in NGT and IGT subjects, respectively. In T2DM, maximal postprandial glucose excursions after sucrose and Palatinose intake were at 60 min and were ~20% significantly lower with Palatinose ingestion ($p<0.01$). Palatinose intake resulted in similar insuliniAUC levels in NGT, IGT and T2M (10.44±2.2, 10.9±2.3 and 11.5±3.3 nmol/l x180 min, respectively). InsuliniAUC levels after sucrose ingestion were 88%, 32% and 55% higher than Palatinose intake in NGT, IGT and T2DM ($p<0.01$, $p<0.05$ and $p=0.1$, respectively). Following sucrose intake, plasma concentrations of GIP increased significantly with 4.1, 3.1 and 2.8 fold in NGT, IGT and T2DM subjects, respectively. It reached its peak after 15 min sucrose load, while by Palatinose intake GIP levels peaked at 60 min with 2 fold increase in NGT and IGT and 1.5 fold increase in T2DM subjects compared to baseline. Palatinose intake compared with sucrose increased secretion of active GLP-1 (GLP-1iAUC) ~77% ($p<0.01$), 85% ($p<0.01$) and 84% ($p<0.05$) in NGT, IGT and T2DM groups, respectively. Sucrose caused only minor increases. Compared with sucrose, Palatinose intake improved HIC rate ~32% ($p<0.001$), 30% ($p<0.05$) and 37% ($p<0.05$) in NGT, IGT and T2DM, respectively. Across all groups, there was an inverse association between GIPiAUC and HIC ($r=-0.44$, $p<0.001$). In contrast, GLP-1iAUC was positively associated with the increased HIC ($r=0.4$, $p=0.001$).
Conclusion: Palatinose possesses a favorable profile for diabetes nutrition by lowering postprandial endogenous GIP levels, increasing GLP-1 concentrations and saving insulin secretion which ultimately results in improved management of blood glucose. Palatinose improves HIC in IGT and T2DM subjects. An inverse relationship between HIC and postprandial GIP concentrations and positive association between GLP-1 nad HIC suggest that the pattern of incretin secretions determines the rate of HIC. In particular, modulation of GIP secretion can be considered as a potential therapeutic target for improving hepatic glucose metabolism and the treatment of fatty liver.
Clinical Trial Registration Number: NCT02219295

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Altered mitochondrial metabolism is involved with increased amplifying pathways modulation of insulin secretion in protein malnourished obese mice

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Background and aims: Glucose-induced insulin secretion (GIIS) stimulates dependent and independent mechanisms of KATP channels. Low protein and high-fat diets, during important stages of development favor obesity progress inducing several changes in pancreatic islets. The changes on the independent mechanisms of KATP channels, also called amplifying pathways, induced by the combination of these two treatments are unknown. Our aim was investigating the role of these pathways on GIIS modulation of malnourished obese mice.
Materials and methods: After weaning, 21 days old male C57BL-6 mice were randomly assigned into the control group (C) which received a normo-protein diet (14% protein) during 105 days; the control-high fat diet (HFD) (CH) received normo-protein diet for 45 days and after that was treated with a HFD (60% fat) for 60 days. The protein restricted R and RH groups were fed with a low protein diet (LPD) (6% protein), receiving the same HFD treatment. Insulin tolerance was assessed by euglycemic-hyperinsulinemic clamp. In order to investigate amplifying pathways control of insulin secretion, we incubated islets with glucose (G2.8; 11.1; 22.2 mM) in the presence of K+ (30 mM) and diazoxide (250 μm). Insulin content was measured by radioimmunoassay. Protein kinases A and C and mitochondrial metabolism were also assessed.
Results: HFD decreased glucose uptake (C=25.6±2.1/CH=15.9±2.36; R=50.0±5.1/RH=18.18±3.9 mg/kg/min). GIIS was higher in animals fed with HFD (G22.2 C=1.58±0.18/CH=3.0±0.3; R=1.08±0.12/RH=1.8±0.3 ng/mL/hour/islet). The influence of amplifying pathways in insulin secretion was lower in R animals (G22.2 C=0.757±0.1; R=0.33±0.09 ng/mL/hour/islet). There were no differences among C and CH in all glucose concentrations. Additionally, the role of amplifying pathways was higher in RH (G22.2 R=0.33±0.09; RH=1.67±0.22). Whereas PKA and PKC content were not altered, ATP production displayed a trend to be reduced in RH. Basal mitochondrial membrane potential and glutamate dehydrogenase (GDH) protein content were nearly 40 and 50% higher, respectively.
Conclusion: Higher amplifying pathway modulation of insulin secretion of obese protein malnourished mice is associated with impaired mitochondrial function and GDH content. This could be one of the mechanisms reprogrammed by early protein malnutrition, since this effect does not appear in animals fed with a normal protein plus HFD.
Supported by: FAPESP, CNPq, CAPES

PS 060 Treating with tablets 2: type 2 diabetes oral antidiabetic drugs update

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Metformin associated lactic acidosis-incidence in the years of 2004-2012

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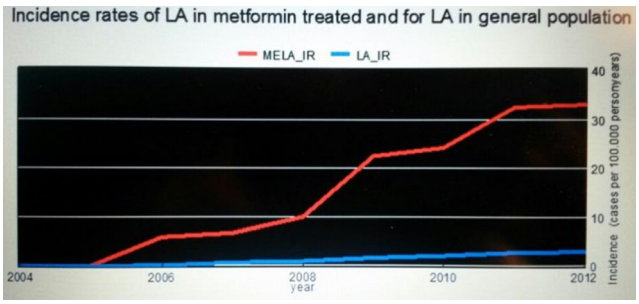
Background and aims: Over the last decade Metformin has become frontline treatment in the overwhelming majority of patients with type 2 diabetes, including in elderly patients with possible increased risk of adverse effects. We examined if the annual incidence of lactic acidosis (LA) overall and associated with metformin use has increased in Denmark over a 9-year period.
Materials and methods: We conducted a nationwide cross-sectional study in Denmark in the period of 2004-2012, using population-based hospital and prescription registries. We identified all people in Denmark exposed to metformin in this period, and assessed first-time hospital contacts with LA (ICD-10 diagnosis code E87.2A) from the Danish National Patient Register. For each calendar year, we assessed the annual incidence rate (IR) of first-time LA events per 100.000 person-years among metformin-exposed people and among Denmark's entire background population.
Results: As shown in the Figure below the annual IRs for LA in Denmark increased substantially over time, both for LA in the entire background population (LA_IR, red graph) and for LA among metformin exposed individuals (MELA_IR, blue graph). In the general population, the incidence of LA increased from 0.01-0.1 per 100,000 person years in the early study years 2004-2006 to 3.0 per 100,000 person years in 2012. Among metformin users, the incidence of LA increased from 0.0-5.9 per 100,000 person years in the early study years 2004-2006 to 33,0 per 100,000 person years in 2012, corresponding to a 11-fold higher incidence of LA in metformin users versus the general population.
Conclusion: During the last decade, there has been a substantial rise in the incidence of LA among metformin users in Denmark, as well as in the background population. These finding raise concern that caregivers may not always comply with contraindications for metformin use.



Supported by: 13/36915

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Metformin inhibits pancreatic stellate cells proliferation and migration

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Background and aims: Metformin treatment is associated with a decreased risk and better prognosis of pancreatic cancer (PC) in patients with type 2 diabetes. The effects of metformin are mediated by activation of AMPK and a reduction in mTOR signaling in cancer cells. Activated pancreatic stellate cells (PSCs) are key stroma cells responsible for pancreaticfibrogenesis and PC progression. However, the impact of metformin on PSCs has not been studied. Assuming a protective role of metformin, we investigated its effects on PSCs.
Materials and methods: In primary mouse PSCs and a novel immortalized mouse pancreatic stellate cell line (imPSCs), cells were cultured with different concentrations of metformin (2.5, 5, 10 mmol/L). Cell proliferation was determined by MTT assay. PSCs migration was assessed by a wound healing assay. AMPK and mTOR, the important regulatory molecules responsible for metformin action, were investigated for their possible involvements in metformin-induced proliferation and migration.
Results: Metformin dose- and time-dependently decreased PSCs proliferation (78±3% of control, $P<0.01$). Metformin inhibited PSCs migration (73±4% of control, $P<0.01$). Metformin addition induced AMPK phosphorylation, reaching a maximum within 24 hours (4.52±0.03 of control, $P<0.01$). Metformin application showed inhibition of phosphorylated Akt (0.38±0.06 of control, $P<0.01$), mTOR (0.12±0.05 of control, $P<0.01$) and P70S6K (0.18±0.02 of control, $P<0.01$), maximal inhibitory effects were achieved at 48 hours.
Conclusion: Our findings indicated that metformin inhibited the proliferation and migration of PSCs. Metformin activated AMPK and reduced AKT/mTOR/P70S6K phosphorylation. Further studies designed to characterize the possible molecular mechanisms by which metformin affects PSCs are awaited.

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Once-daily delayed release metformin in the morning is superior in efficacy to evening and twice-daily administration but at lower systemic exposures

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Background and aims: Metformin delayed-release (MetDR) is a gut-restricted formulation designed to release drug only when it reaches the ileum, a region of the gut where GLP-1 secreting L-cells are abundant and absorption is poor, leading to low bioavailability. MetDR was previously shown to have glucose-lowering activity comparable to immediate-release and extended-release metformin (both of which are absorbed in the upper bowel with ~50% bioavailability) but with ~40% lower doses and significantly lower (>75%) systemic exposure, consistent with the gut being metformin's primary site of action. As the intestine is a major site of Met accumulation (300-1000X plasma), timing and frequency of daily metformin delivery to the lower bowel may not be important with repeated dosing (ie, at steady-state, the intestine effectively becomes a metformin reservoir that provides sustained activity). We tested this in the present study by comparing the pharmacokinetics (PK) and glucose-lowering efficacy of a total daily dose of 1000 mg MetDR administered twice-daily (BID) or once-daily (QD) in the morning (QAM) or evening (QPM).
Materials and methods: 1000 mg MetDR QAM or QPM were compared to 500 mg MetDR BID in a 7-day randomized crossover design ($n=26$, mean FPG 168 mg/dL) in subjects with T2D not on Met ≥2 wks prior to dosing. All treatments were administered with meals and were separated by a 6- to 12-day washout.

Results: PK results indicate a diurnal effect in both the rate and extent of metformin absorption with QAM having the fastest gut transit time and the lowest plasma exposure compared to QPM. The time to reach the ileum was 4.5-5.8 h for morning dosing vs 7.1-8.5 h for evening dosing. QAM resulted in a 29% decrease in 24-h Met plasma exposure (AUC) compared to QPM and BID ($p<0.005$ for both). Despite these differences in exposure, QAM dosing resulted in the greatest reduction (9%) in plasma glucose AUC0-24 from baseline ($p=0.003$) compared to a 5% reduction for both QPM ($p=0.002$) and BID ($p=0.099$). QAM dosing also resulted in a 10% reduction in 24-h maximum plasma glucose (Cmax) from baseline ($p=0.0006$) compared to a 9% reduction for QPM ($p=0.0007$) and a 6% reduction for BID ($p=0.039$). All treatments were well tolerated consistent with previous studies of MetDR.
Conclusion: Met DR delivered once-daily in the morning results in lower Met plasma exposure but the greatest reduction in plasma glucose compared to evening or BID administration. The observation that MetDR provides 24-h glucose lowering with once daily administration supports the previous findings that Met works predominantly in the lower bowel and that the contribution of systemic exposure to glucose lowering is small at best. As MetDR is being developed to limit metformin exposure in patients with renal impairment, once-daily morning administration of MetDR is the preferred dosing regimen.
Clinical Trial Registration Number: NCT01804842

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Optimisation of metformin discontinuation in diabetic patients explored with 18 F-FDG PET/CT

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Background and aims: It is known that metformin increases 18 F-FDG PET/CT intestinal uptake and that it is reduced after its discontinuation for 3 days. The aim of this study was to determine if in patients with type 2 diabetes mellitus (T2D), 24 or 48 h metformin discontinuation could be useful to reduce 18 F-FDG PET/CT intestinal uptake keeping patient's glucose levels (GL) in normal range and improving PET/CT quality.
Materials and methods: 101 T2D patients referred to a 18 F-FDG PET/CT study were included. Drug regimen included metformin in 81 patients (group A) and other oral antidiabetic drugs (OAD) in 20 patients (group B). 30 patients without T2D served as the control group (group C). Group A was divided in three subgroups: 31 patients (group A1) were taking metformin, 21 patients (group A2) were asked to discontinue metformin for 24 h before 18 F-FDG PET/CT and the remaining 29 patients (group A3) were asked to discontinue metformin for 48 h. 18 F-FDG PET/CT intestinal uptake was assessed both, qualitatively (normal, mild, moderate or intense) and semi-quantitatively, using SUVmax values and tissue-to-background ratio (TBR). Results were compared among the groups. GL were assessed before PET/CT performance in all patients.
Results: Intense intestinal uptake in group A3 (17%) was significantly lower ($p<0.001$) than in group A2 (32%) and A1 (68%) and comparable to group B (15%) and C (7%). Intestinal 18F-FDG uptake in group A was reduced after metformin discontinuation for 24 hours (TBR A1=2,32 vs TBR A2=3,24; p=ns) and significantly after 48 hours (TBR A3=1,82 vs A1 y A2; $p<0.001$). There were no significant differences among A3 (TBR=1, 82), B (TBR=1, 69) and C (TBR=1,40). There were also no significant differences in age and body mass index (BMI) among the different groups. GL were higher in group A (122±19) than in groups B (119±19) and C (95±11) ($p<0.01$).
Conclusion: Metformin discontinuation for 48 hours is feasible and better than 24 hours to reduce 18 F-FDG intestinal uptake in T2D and it significantly improved PET/CT studies quality. Although GL increased with discontinuation, they were in normal range according to guidelines.

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Development of oedema is associated with an improved glycaemic response in patients initiating thiazolidinediones: a MASTERMIND study

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Background and aims: Oedema is a common and serious side effect of thiazolidinedione therapy. A stratified medicines approach would aim to give thiazolidinediones to patients likely to have a good glycaemic response but to not develop oedema. We investigated whether oedema was associated with glycaemic response to thiazolidinedione therapy.
Materials and methods: We retrospectively studied 11,459 patients initiating a thiazolidinedione from UK primary care data (Clinical Practice Research Datalink), and identified medical records of new oedema in the subsequent twelve months. Response was defined as change in HbA1c at twelve months and was adjusted for baseline HbA1c, baseline BMI, gender and compliance (medication possession ratio). In secondary analyses we restricted oedema classification to patients with concomitant weight gain. As a comparison the same analysis was performed in 13,089 patients initiating a sulfonylurea.
Results: The 5% of patients with recorded oedema on thiazolidinediones had a mean (CI) 2.2 (1.1-3.2)mmol/mol greater fall in HbA1c ($p<0.001$) compared to thiazolidinedione patients without oedema. This improved response increased when oedema was associated with weight gain, with a 2.5 (1.1-4)mmol/mol greater HbA1c fall when weight gain >3 kg ($p<0.001$) and a 3.6 (1.8-5.4)mmol/mol greater fall when weight gain >5 kg ($p<0.001$). Oedema was recorded in 3.7% of sulfonylurea patients and was not associated with response (HbA1c fall difference 1 (-0.5-2.5)mmol/mol, $p=0.2$), even when associated with weight gain >3 kg ($p=0.19$).
Conclusion: Patients with Type 2 diabetes who develop oedema on initiating thiazolidinediones have an improved glycaemic response, and more severe oedema may be associated with greater reductions in HbA1c. An association between oedema and glycaemic response was not observed in patients initiating sulfonylureas. This supports glycaemic lowering and fluid retention being mediated by a common pathway of thiazolidinedione drug action.
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Combined intervention with pioglitazone and n-3 fatty acids in metformin-treated diabetic patients

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Background and aims: Marine n-3 fatty acids eicosapentaenoic (EPA) and docosahexaenoic (DHA) acids help in primary prevention of cardiovascular disease, but their impact on glucose homeostasis in type 2

diabetic (T2D) patients remains unclear. We aimed to evaluate the effect of a combination intervention using EPA+DHA and a suboptimal doses of insulin-sensitizing drug pioglitazone on insulin resistance, body weight, serum lipid concentrations and compensation of diabetes in obese patients with T2D already treated with metformin.

Materials and methods: In a double-blind, placebo-controlled trial, 69 patients, 38-70 years of age (66% men) were randomly assigned to 24-week-intervention using: (i) corn oil (5 g/day; Placebo), (ii) pioglitazone (15 mg/day; Pio), (iii) EPA+DHA (2.75 g/day; Omega3), or (iv) pioglitazone and EPA+DHA (Pio+Omega3). The primary endpoints were the changes from baseline in insulin sensitivity and in triacylglycerol clearance assessed using hyperinsulinemic-isoglycemic clamp and a meal test. Secondary endpoints included changes in fasting glycemia and HbA1c, glucose and NEFA clearance and inflammatory markers and adipokines.

Results: Omega3 and Pio+Omega3 increased EPA+DHA content in plasma phospholipids. Pio and Pio+Omega3 increased body weight and adiponectin levels. Both fasting glycemia and HbA1c were increased by Omega3, but unchanged by Pio + Omega3. Insulin sensitivity was improved by Pio + Omega-3. Triacylglycerol and NEFA clearance were increased by Pio+Omega3. See table.

Conclusion: Besides preventing a modest negative effects of Omega-3 on glycemic control, the combination of pioglitazone and EPA+DHA can be used to increase insulin sensitivity and postprandial lipid clearance in T2D patients on stable metformin therapy.

Table: Differences (Δ) between week 24 and baseline values for BMI, HbA1C, metabolic clearance of glucose (M) and adiponectin. Differences (Δ) between week 24 and baseline values for triacylglycerol and NEFA clearance calculated as area under the curve (AUC)

	Placebo	Pio	Omega3	Pio+Omega3
ΔBMI (kg/m^2)	-0.4 (0.8)	0.4 (0.9)^{a,c}	-0.5 (1.3)	0.7 (0.9)^{a,c}
ΔHbA1C (%IFCC)	0.00 (0.60)	0.00 (0.60)	0.65(1.20)^{a,b}	0.00 (0.45)^{c}
ΔM (mg/kg.min)	-0.60 (1.84)	0.29 (1.64)	-0.58 (1.12)^{b}	0.53 (1.13)^{c}
ΔADIPONECTIN (ug/ml)	0,4 (1,0)	3,5 (2,3)^{a,c}	0,9 (0,7)	3,7 (2,2)^{a,c}
ΔNEFA AUC	12 (7)	-4 (4)	5 (3)	-9 (5)^{a}
ΔTG AUC	-8 (17)	-44 (45)	-27 (25)	-95 (26)^{a,c}

Significant differences (1-way ANOVA) compared with Placebo(a), Pio(b), and Omega3(c)

Clinical Trial Registration Number: EudraCT number 2009-011106-42
Supported by: Ministry of Health (CR) NT13763-4, NT14250-3 and IN 00023001

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Single-dose colesevelam has no effect on postprandial GLP-1 levels, but increases CCK levels and decreases gallbladder refilling and gastric emptying in type 2 diabetes

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Background and aims: The well-established antidiabetic effect of the bile acid sequestrant, colesevelam (COL) has been proposed to be glucagon-like peptide-1 (GLP-1)-dependent since long-term COL treatment seems to increase postprandial GLP-1 levels in rodents and patients with type 2 diabetes. We evaluated the acute effect of oral COL on postprandial plasma GLP-1, insulin and glucose levels as well as plasma cholecystokinin (CCK), gallbladder volume, gastric emptying, appetite and food intake.

Materials and methods: In this placebo-controlled, double-blinded study we included twelve patients with type 2 diabetes (age: 60.8±8.8 years [mean ± SD]; BMI: 29.8±3.0 kg/m2; HbA1c: 6.5±0.5% (48±6 mmol/mol)). On 2 separate days, after 1-week washout of antidiabetic drugs, the patients received COL (3.75 g) or placebo suspended in a 302 kcal-liquid meal with 1.5 g paracetamol (for evaluation of gastric emptying). At baseline and during 240 min blood was sampled, gallbladder volume was evaluated by ultrasound and appetite was evaluated by visual analogue scale. At the end of each day ad libitum food intake was evaluated.

Results: Acute administration of COL did not affect postprandial plasma responses (incremental AUCs) of GLP-1 (995±678 vs 1156±845 min×pmol/l, p=0.78), insulin (3112±4252 vs 3115±2396 min×pmol/l, p=0.99) or glucose (321±197 vs 350±255 min×mmol/l, p=0.91), but significantly increased the postprandial CCK response compared to placebo (760±262 vs 391±248 min×pmol/l, p=0.005). Meal-induced gallbladder emptying (AUC0-60 min for gallbladder volume) was not affected by COL whereas gallbladder refilling (AUC60-240 min) was significantly reduced. COL significantly reduced gastric emptying vs placebo whereas food intake and appetite was unaffected.

Conclusion: Acute administration of COL does not seem to affect postprandial plasma levels of glucose, GLP-1 or insulin, appetite or food intake in patients with type 2 diabetes, but appears to have a pronounced stimulatory effect on postprandial CCK levels, which may explain the reduced gallbladder refilling observed, and an inhibitory effect on gastric emptying. Whether these changes contribute to the long-term antidiabetic effect of COL remains to be elucidated.

Clinical Trial Registration Number: NCT02050074
Supported by: University Hospital, Gentofte, Copenhagen

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Effects of pentoxifylline on proteinuria and glucose control in patients with type 2 diabetes

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Background and aims: Pentoxifylline is a methylxanthine derivative with significant anti-inflammatory, anti-fibrotic, and anti-proliferative properties. Some studies demonstrated that pentoxifylline might have renoprotective effects in chronic kidney disease. However, these studies had limitations due to the small sample sizes and the heterogeneity of patients' characteristics. Therefore, we investigated whether pentoxifylline could reduce proteinuria further in patients with diabetic nephropathy and residual proteinuria despite receiving adequate therapy with an angiotensin-converting enzyme inhibitor (ACEI) or an angiotensin II receptor blocker (ARB). We also studied the effects of pentoxifylline on glycemic control and insulin resistance.

Materials and methods: This was a prospective, randomized, double blinded, active control, multicenter study. The ethics committee of the Ministry of Food and Drug Safety approved the study protocol. A total of 174 patients with type 2 diabetes and albuminuria >30 mg/g of creatinine

during treatment with ACEI or ARB were assigned randomly to receive pentoxifylline (1200 mg, daily (n=87) or placebo (n=87). The study endpoint was the effect of pentoxifylline on proteinuria and glucose control, renal function, and inflammatory parameters.
Results: Six months of treatment with pentoxifylline did not change the amount of proteinuria. Although the estimated glomerular filtration rate (eGFR) decreased in the control group, there was no significant difference from the pentoxifylline group. However, pentoxifylline reduced fasting plasma glucose, HbA1c, and homeostatic metabolic assessment (HOMA-IR) significantly.
Conclusion: Although we did not find any beneficial effects regarding reducing proteinuria or preserving renal functions when pentoxifylline was added to ACEIs or ARBs, pentoxifylline therapy improved glucose control and insulin resistance in patients with type 2 diabetic nephropathy.

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Implementing an optimised glucose-lowering strategy in daily medical practice with first- and second-line gliclazide MR 60 mg

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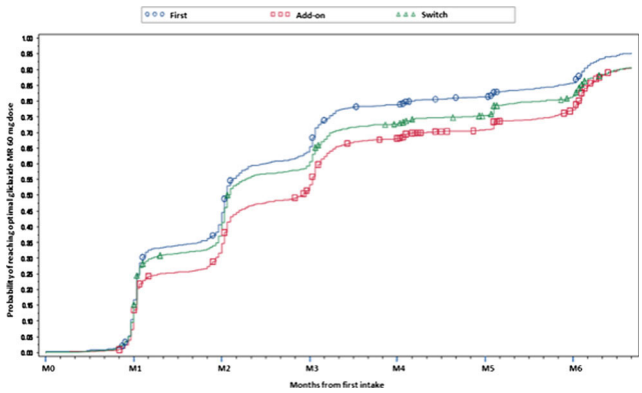
Background and aims: Intensive glucose control with gliclazide modified release (MR) has been reported to significantly reduce major macrovascular or microvascular events in people with type 2 diabetes (T2D). A new and convenient scored formulation (MR 60 mg) has been developed to facilitate medication adherence. This study explored the temporal titration profile of gliclazide MR 60 mg when used as either a first- or second-line prescription.
Materials and methods: This was a 6-month long, international, multicentre, non-comparative, open-label observational study. Between 7/2011 and 2/2014, 7170 individuals aged ≥35 years with T2D, HbA1c ≥7.5% and not on insulin were enrolled. At Visit 1, they were prescribed 30-120 mg once daily (QD) of the gliclazide MR 60 mg formulation as a first line (FIRST - newly diagnosed and/or pharmacotherapy naïve), add-on (ADD) or switch from a previous oral antihyperglycaemic treatment strategy (SWITCH). At months 1, 2 and 3, gliclazide MR 60 mg dose was uptitrated (≥120 mg) based on fasting plasma glucose (FPG) and at the discretion of the investigator. The primary evaluation criterion was the time between first gliclazide MR intake and first occurrence of optimal glycaemic control (usually HbA1c <7%). Values are mean(SD); significance set at P<0.05.
Results: Women comprised 58.5% of the cohort. Baseline (BL) age was 58.9(10.6) years, BMI 30.1 (5.0) kg/m2, and T2D duration 5.1(4.4) years. FIRST (n=1911), ADD (n=2831) and SWITCH (n=1683) groups had BL HbA1c 8.83(1.39)%, 8.77(1.25)% and 8.75(1.30)% respectively. Probability of the entire cohort reaching optimal gliclazide MR dosing at months 1, 2, 3 and 6 was 0.15, 0.39, 0.59 and 0.92 respectively. Similar trends were observed in the three groups (Fig). Mean HbA1c changes from BL to month 6 were FIRST: -1.98(1.36)%, ADD: -1.74(1.28)% and SWITCH: -1.61(1.33)% (all P<0.01); these corresponded with 71.8%, 65.0% and 57.7% of the respective groups documenting an HbA1c ≤7%. Overall, 65.3% achieved an HbA1c ≤7.0% with an average time to efficient dose of 80.1 days. Weight loss occurred across the board (kg differences between BL and month 6 — FIRST -1.45(4.31), ADD -1.27(5.01) and SWITCH -1.30(4.22)). Severe hypoglycaemic incidents were rare, with 4 (0.06%) participants experiencing an episode. The majority (95.5%) indicated a greater likelihood of adherence with the gliclazide MR 60 mg QD regime vs their previous therapy.
Conclusion: Within 6 months, progressive gliclazide MR 60 mg QD titration produced a significant mean 1.78% improvement in HbA1c with ~90% optimal dose achievement. There was concomitant weight loss and few severe hypoglycaemic incidents. In this large, real world study, progressive uptitration with gliclazide MR 60 mg QD appears to be efficacious and safe in people with uncontrolled T2D independent of their prior antihyperglycaemic treatment.



Clinical Trial Registration Number: ISRCTN00943368
Supported by: Institut de Recherches Internationales Servier (France)

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Efficacy and safety of repaglinide added to sitagliptin in Japanese patients with type 2 diabetes: a randomised 24 week open-label trial

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Background and aims: Although sitagliptin (SITA) improves glycemic control without increasing risk of hypoglycemia and is weight-neutral, SITA can't ameliorate postprandial glucose sufficiently in many patients with type 2 diabetes. Repaglinide (REPA), a potent insulin secretagogue has been reported to increase early insulin secretion and improve postprandial hyperglycemia, and might be effective in combination with SITA. However, there has been no report that shows the efficacy of REPA in combination with SITA, previously. The aim of this study is to investigate the efficacy and safety of REPA as add-on to SITA, compared with switching to REPA in Japanese type 2 diabetes patients poorly controlled with SITA.
Materials and methods: Thirty patients inadequately controlled with SITA 50 mg once daily [range of glycated hemoglobin (HbA1c) was 7.0-8.5%] were randomized 1:1 to REPA as add-on therapy to SITA (0.5 mg of REPA thrice daily and 50 mg of SITA once daily, n=15: AD group) or switch from SITA to REPA (0.5 mg of REPA thrice daily, n=15: SW group). A meal tolerance test was carried out at weeks 0 and 24. All data are expressed as mean±standard deviation (SD).
Results: Treatment groups were balanced at baseline (HbA1c, 7.6±0.4% in AD group and 7.6±0.4% in SW group; fasting plasma glucose, 8.5±1.4 mmol/L in AD group and 8.5±1.7 mmol/L in SW group; body weight, 66.4±8.0 kg in AD group and 71.4±15.1 kg in SW group). The mean HbA1c changes from baseline to week 24 were -0.8±0.7 and -0.2±0.4% in AD and SW groups, respectively, and the difference between two groups was significant (P=0.008). The mean changes in area under the curve (AUC) of glucose from 0 to 180 min from baseline to week 24 in AD group was -271.1±200.2 mmol*min/L in AD group compared with those in SW group (-31.2±342.6 mmol*min/L) (P=0.032). Mean insulin secretion relative to glucose elevation (ISG) (ISG: AUC0-180 insulin / AUC0-180 glucose) in AD group significantly increased in comparison with that in baseline (P=0.020) and tended to be higher than that in SW group, but the differences are not statistically significant (mean changes from baseline to week 24 were 1.23±1.24 μIU/mmol in AD group and those in SW group were 0.35±1.19 μIU/mmol; P=0.069). The mean change in AUC0-180 of glucagon decreased in AD group and increased in SW group, but the difference was not significant (-5485±9633 pg•min/mL in AD group and 2915±12050 pg•min/mL in SW group; P=0.058). There was no significant difference in changes in bodyweight between the two groups (AD group 0.4±1.6 kg, SW group 0.5±1.3 kg; P=0.807). The incidence of hypoglycemic events was low (three in AD group), and none of the patients developed severe hypoglycemia or severe.
Conclusion: The combination therapy of SITA and REPA is effective and well tolerated in Japanese type 2 diabetes without significant weight gain and severe hypoglycemia.
Clinical Trial Registration Number: UMIN (no. R000013214)

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Within-sulfonylurea-class evaluation of the time to intensification with insulin or triple oral therapy (ZODIAC-43)

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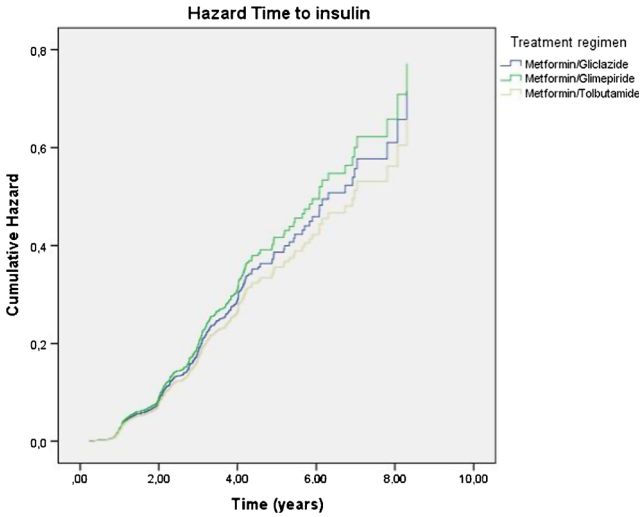
Background and aims: The Dutch type 2 diabetes mellitus (T2DM) guideline advices sulfonylureas (SUs), specifically gliclazide, as the first intensification step after metformin and the addition of insulin as a second intensification step. There is evidence for important within-class SU differences, but data are lacking concerning within-class SU differences and the time to a second intensification with either insulin or a third oral agent in daily practice. This study aims to investigate the relationships between three different SUs, either gliclazide, glimepiride or tolbutamide, used as dual therapy next to metformin, and the time needed for treatment intensification with either insulin or oral triple therapy in patients with T2DM.
Materials and methods: From a large prospective cohort study (the ZODIAC cohort, n=82.167), patients were selected who used monotherapy with metformin for at least one year and subsequently intensified with one out of three SUs (gliclazide, glimepiride or tolbutamide), the so called new users. Patients with an eGFR below 30 mL/min per 1.73 m2 were excluded. Time-dependent Cox proportional hazard analyses, adjusted for age, gender, diabetes duration, updated mean eGFR and BMI were used to evaluate the primary outcome, first receipt of insulin, and the secondary outcome, first receipt of insulin or triple oral therapy.
Results: From the 3507 selected patients 47.0% were female, the mean age (SD) 61.0(11.4), median diabetes duration (interquartile range (IQR)) 6.8 years (IQR 4.5-9.4), median HbA1c 6.8 (IQR 6.4-7.4), median BMI 29.7 (IQR 26.8-33.3) and the mean eGFR 83.2 mL/min per 1.73 m2 (20.0). 2.5 and 5 years after intensification 13% and 32% of patients were using insulin. The cumulative hazard ratio (HRs) for the primary endpoint is shown in figure 1. Metformin/gliclazide was taken as a reference category. HR for time to insulin or triple oral therapy (the secondary endpoint) for metformin/glimepiride was 1.042 (95% CI 0.778-1.394) and for metformin/tolbutamide 0.845 (95% CI 0.632-1.129). There were no significant between group differences in both primary and secondary outcome.
Conclusion: In this study, intensification with either gliclazide, glimepiride or tolbutamide on top of metformin, was not associated with a difference in risk for need for a second intensification with insulin or triple oral therapy. 87% and 68% of patients were without insulin 2.5 and 5 years, respectively, after starting a SU. In this large Dutch primary care population, there were no within-SU-class differences concerning the time for the need of further treatment intensification with either insulin or a third oral agent.



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Low baseline beta cell function is associated with higher rates of hypoglycaemia with glimepiride in elderly patients with type 2 diabetes inadequately controlled with metformin

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Background and aims: Elderly patients with type 2 diabetes (T2D) pose a challenge for management of glycemia owing to excess fragility and risk of adverse outcomes during hypoglycemia episodes. We therefore sought to identify risk factors for the development of hypoglycemia (any reported symptomatic event and events of plasma glucose concentration <54 mg/dL regardless of symptoms) in elderly patients when saxagliptin (SAXA) or glimepiride (GLIM) is added to metformin.

Materials and methods: A post hoc analysis of data from the GENERATION trial that enrolled 720 patients aged ≥65 years was conducted. β-cell function was assessed using HOMA-2%β.

Results: The proportion of patients experiencing any hypoglycemia event was lower with SAXA vs GLIM (5.8% vs 34.8%). Regardless of treatment, patients with baseline HOMA-2%β≤median value of 39.1% had a higher event rate of hypoglycemia of 1.27 events/patient year compared with patients with baseline HOMA-2%β>median, 0.82 events/patient year (RR=1.300 [95% CI, 1.084, 1.560]). In the GLIM-treated patients, the hypoglycemia event rate in patients with baseline HOMA-2%β≤median was higher (2.29 events/patient year) compared with patients with baseline HOMA-2%β>median (1.60 events/patient year; RR=1.278 [95% CI, 1.057, 1.545]); corresponding SAXA hypoglycemia event rates were too low to draw meaningful conclusions at 0.16 vs 0.09 events/patient year (RR=1.446 [0.687, 3.043]), respectively. The association between lower β-cell function at baseline and increased incidence of hypoglycemia was particularly strong for patients ≥75 years (RR=2.572 [95% CI: 1.787-3.701], $P<0.001$).

Conclusion: In patients with lower β-cell function, the addition of a sulfonylurea is associated with increased risk of hypoglycemia compared with patients with higher β-cell function. These findings are especially relevant to elderly patients in whom hypoglycemia associated morbidity is higher.

Clinical Trial Registration Number: NCT01006603

Supported by: AstraZeneca

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Comparative efficacy of anti-diabetic agents on nonalcoholic fatty liver disease in patients with type 2 diabetes mellitus: a systematic review and meta-analysis

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Background and aims: Nonalcoholic fatty liver disease (NAFLD) has a high prevalence in patients with type 2 diabetes mellitus (T2DM). It deteriorates abnormal glucose and lipid metabolism, and accelerates the development of diabetic complications. In this study, we sought to provide a comprehensive assessment regarding the effects of anti-diabetic agents on NAFLD in patients with T2DM.

Materials and methods: MEDLINE, EMBASE and the Cochrane Central Register of Controlled Trials were searched for both randomized and non-randomized clinical trials focused on T2DM patients with NAFLD. Thiazolidinediones (TZDs), glucagon-like peptide-1 receptor (GLP-1R) agonists, dipeptidyl peptidase-4 inhibitors, sodium-glucose transport proteins type 2 inhibitors, sulfonylureas, insulin and metformin were explored, regardless of comparing with placebo, each other or non-treated controls. Observational trials were also recruited to expand exploration. Relative changes of hepatic fat content (HFC) and liver histology were evaluated as primary outcomes. We also assessed body composition, glucose and lipid metabolism, and inflammatory factors. Data was collected and analyzed by STATA.

Results: 1,196 participants from 19 randomized controlled trials (RCTs) and 14 non-randomized studies were included. Evidence from RCTs and observational studies suggested TZDs for 12-72 weeks were associated with greater HFC reduction (WMD -22.27%, 95%CI -26.98 to -17.56, p=0.000, as compared with placebo; WMD -27.88%, 95%CI -47.41 to -8.35, p=0.005, as compared with metformin; -35.7% vs. -7.69%, p<0.05, as compared with glibenclamide; a range of -30% to -52.1%, all p<0.05, in observational studies) and improved liver histology (including steatosis, ballooning necrosis, inflammation and NAFLD score as compared with placebo and metformin, all p<0.05). GLP-1R agonists had beneficial effects on HFC after 26-50 weeks intervention (-61% vs. -41% for exenatide/pioglitazone combination vs. pioglitzone monotherapy, p<0.05; 0.1 vs. 0 for liraglutide vs. glimepiride, p=0.045, changes from baseline of CT value), and insulin/metformin combination with 3-7 months improved HFC (a range of -17.6% to -48%, all p<0.05, compared with baseline). Nevertheless, initiating metformin alone showed no benefit on HFC or liver histology, at least in 16-48 weeks. Neither did dapagliflozin for 24 weeks. Additionally, nateglinide for 18 months was reported in a small sample-size RCT to ameliorate HFC (-27%, p value was unavailable) and liver histology (steatosis and portal inflammation, both p<0.05). Sitagliptin therapy of one year also provided benefit on nonalcoholic steatohepatitis score (p=0.04) in an observational study. What's more, we observed the reduction of TG, improvement of HbA_{1c} and alleviation of inflammation all paralleled to the amelioration of NAFLD. In spite that weight loss has been proved beneficial on NAFLD, it is definitely not the mere factor that contributed, since TZDs could still improve HFC and liver histology significantly with greater weight gain than placebo and metformin.

Conclusion: For T2DM with NAFLD, administrating TZDs and GLP-1R agonists seems providing more identified advances in attenuating HFC. Further RCTs with high quality, long-term duration and large-size samples are warranted to assess the efficacy of various hypoglycemic agents on clinical outcomes associated with NAFLD in T2DM.

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A network meta-analysis to assess options for treatment intensification for patients with type 2 diabetes inadequately controlled on dual therapy

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Background and aims: Augmenting existing therapies with medicines that have alternative modes of action in terms of glucose lowering and benefits in terms of other micro- and macrovascular risk factors beyond HbA1c reduction (e.g. weight loss and blood pressure reduction) may improve outcomes. In the absence of direct evidence comparing antihyperglycaemic agents (AHAs) in triple therapy, indirect comparisons can be used to inform clinical and policy decisions. A network meta-analysis (NMA) was conducted to assess the relative efficacy of canagliflozin (CANA), an agent that inhibits sodium glucose co-transporter 2 (SGLT2), compared to other non-insulin AHAs as add-on to metformin plus sulphonylurea (MET+SU) and as add-on to MET plus pioglitazone (MET+PIO).
Materials and methods: Two separate networks of evidence informed by a systematic literature review were constructed to estimate the relative efficacy of CANA in combination with MET+SU or MET+PIO in lowering HbA1c at 26±4 weeks via Bayesian NMA. After screening the trials for appropriateness of pooling, comparisons of CANA were feasible versus dapagliflozin (DAPA), empagliflozin (EMPA), liraglutide (LIRA), exenatide (EXEN), sitagliptin (SITA), linagliptin (LINA), saxagliptin (SAXA), PIO and insulin in the MET+SU network and EMPA, EXEN, and LINA in the MET+PIO network. Relative efficacy was evaluated based on absolute differences (Δ) and Bayesian pairwise probabilities (P); P ≤30% and P ≥70% were chosen to indicate a smaller and larger effect, respectively.
Results: As add-on to MET+SU, HbA1c reductions (%) were higher for CANA 300 mg compared to EMPA 10 and 25 mg (Δ=-0.36 to -0.41, P=100%), DAPA 10 mg (Δ=-0.32, P=99%), EXEN 5 μg (Δ=-0.23, P=96%), and SITA, SAXA, and LINA (Δ=-0.20 to -0.39, P=100%) and comparable to LIRA 1.8 mg (Δ=0.08, P=31%) and EXEN 10 μg (Δ=-0.01, P=53%). HbA1c reductions with CANA 100 mg were greater compared to EMPA 10 and 25 mg (Δ=-0.11 to -0.16, P ≥81%), SAXA, and LINA (Δ=-0.10 to -0.14, P ≥75%), similar compared to DAPA 10 mg (Δ=-0.07, P=68%), EXEN 5 μg, and SITA (Δ=0.02 to 0.05, P ≥33%), and lower compared to LIRA 1.8 mg (Δ=0.33, P=3%). As add-on to MET+PIO, CANA 300 mg was associated with greater HbA1c reductions versus all comparators: EMPA 25 and 10 mg (Δ=-0.17 to -0.27, P ≥88%), LINA (Δ=-0.20, P=84%), and EXEN 10 μg (Δ=-0.24, P=96%). CANA 100 mg conferred HbA1c reductions greater than EMPA 10 mg (Δ=-0.13, P=81%) and EXEN 10 μg (Δ=-0.10, P=76%), and were deemed similar compared to EMPA 25 mg (Δ=-0.03, P=58%) and LINA (Δ=-0.06, P=62%).
Conclusion: An NMA of add-on therapies to MET+SU indicates that glycaemic reductions at 26 weeks were greater for CANA 300 mg and at least as large for CANA 100 mg compared to EXEN, other SGLT2 inhibitors, and dipeptidyl peptidase-4 (DPP-4) inhibitors. CANA 300 mg was found to be comparable to LIRA 1.8 mg in terms of HbA1c reduction. An NMA of add-on therapies to MET+PIO indicates that CANA 300 mg was associated with consistently greater HbA1c reductions versus EMPA, EXEN, and LINA, while CANA 100 mg was deemed at least similar. These results suggest that CANA may be a good therapeutic option as an add-on to common dual therapies for T2DM.
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The impact of delaying treatment intensification in type 2 diabetes: evidence from UK general practice

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Background and aims: A key objective of treating type 2 diabetes mellitus (TD2M) is obtaining and maintaining good glycaemic control, generally based on measurement of glycated haemoglobin (HbA1c). Keeping HbA1c below target levels, which vary across international and local guidelines, reduces the risk of vascular complications. Evidence from UK, Europe and USA, however, indicates that health care professionals are commonly slow to intensify treatment in patients with poor glycaemic control (often termed "clinical inertia"). Our study aimed to evaluate longitudinal clinical changes and resource burden in patients intensified at varying times after loss of glycaemic control in the UK.
Materials and methods: Adults with incident T2DM were identified in the UK Clinical Practice Research Datalink during the period 1 January 2000 to 31 March 2014. Patients who were newly initiated on metformin monotherapy and who had lost good glycaemic control on medication (HbA1c>7%) were included. Patients were grouped according to time to intensification of second line therapy: Group A, rapid intensification within 365 days of treatment failure; Group B, delayed intensification from day 366 to 1824 post treatment failure; Group C, never intensfied post treatment failure. Patients were followed up from day 365 (index date) post first treatment failure for five years, until end of study, switch to insulin, migration or death. The study evaluated a variety of longitudinal clinical changes including HbA1c, weight and diabetes prescribing.
Results: 6,710 patients with T2DM met the study inclusion criteria. Key baseline characteristics of the evaluated patients are described below. There was a delay in treatment intensification to second-line therapy or no treatment intensification in 60.5% of patients in the 5-year follow-up period. Group A achieved a significant decline in HbA1c from baseline 1 year post index date compared to Groups B & C (-1.13% Group A; +0.26% Group B, +0.16% Group C). A significantly higher proportion of patients achieved HbA1c target<7% in Group A (Group A (45.8%); Group B (19.1%), p<0.0001). Using an adjusted hazard model, Group A was found to achieve the HbA1c target from the index date significantly faster than Group B (hazard ratio 3.25 (95% CI 2.87, 3.69)). The most commonly used second-line intensification agent was sulphonylureas in both Groups A and B throughout the observation period but was associated with signficant weight gain (+1.3 kg per patient) in the adjusted models. Similar results were seen with thiazolidinediones users (+1.0 kg). Only patients taking DPP-4 inhibitors had consistent weight loss over 5 years of -0.6 kg.
Conclusion: There was a substantial level of clinical inertia. Patients that were rapidly intensified post treatment failure achieved a significant reduction in HbA1c and got to target faster. A significantly higher proportion achieved the HbA1c target <7% in Group A compared with Group B, despite a higher baseline HbA1c.

Variable	Group A (n=2,647)	Group B (n=2,452)	Group C (n=1,611)	Test* p value
Age (mean SD)	57.5 (11.08)	58.4 (11.29)	63.4 (12.55)	<0.001
Gender (% male)	64.4	60	56.2	<0.001
HbA1c % (mean SD)	8.5 (1.3)	7.9 (1.0)	7.6 (0.8)	<0.001
Weight kg (mean SD)	96.2 (21.1)	94.2 (20.1)	91.2 (20.3)	<0.001
BMI (mean SD)	33.2 (6.6)	32.8 (6.2)	32.1 (6.1)	<0.001
Proportion obese	59.8	56.7	51.2	<0.001
HDL-c (mean SD)	1.1 (0.3)	1.2 (0.3)	1.2 (0.4)	<0.001
Triglycerides (mean SD)	2.4 (1.4)	2.2 (1.3)	1.2 (1.8)	<0.001
Cardiovascular disease %	25.7	24.7	31.8	<0.001
Hypertension %	54.8	53.3	60.1	<0.001
* three way anova				

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Patient preferences for attributes of type 2 diabetes mellitus treatments in Germany

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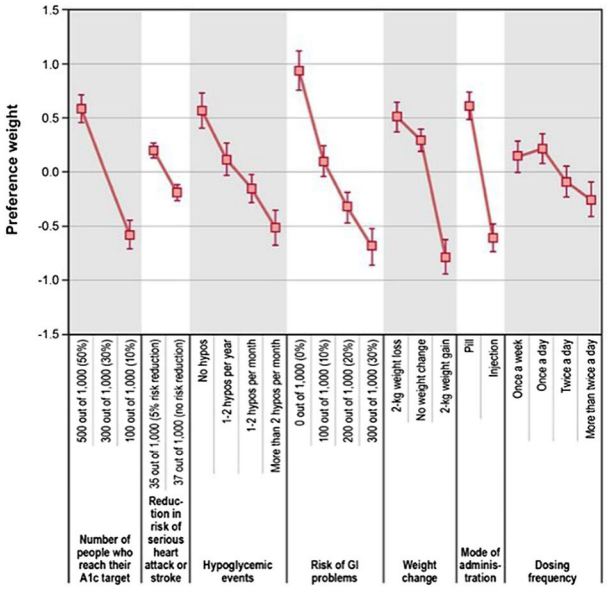
Background and aims: Understanding T2DM patients' preferences for attributes of T2DM treatments may help to explain how each of these attributes differentially affect patients' perception and behavior. Our aim was to quantify the relative preferences of a set of attributes describing T2DM treatments for T2DM patients in Germany.

Materials and methods: A stated-preference discrete-choice experiment (DCE) survey was designed to elicit preferences for T2DM treatment attributes among patients in Germany taking a prescription T2DM medication for>2 years. The survey collected background characteristics and information on respondent demographics, health history, current health status (EQ-5D), and experience with T2DM and T2DM treatments. The DCE included a series of 8 choices between pairs of hypothetical T2DM treatments defined by these seven attributes: chance of reaching target HbA1c (A1c), reduction in risk of serious heart attack or stroke, frequency of hypoglycemia, risk of gastrointestinal side effects (GI SEs), weight change, mode of administration (MoAdmin; pill or injection), and dosing frequency. Random-parameters logit was used to analyze the DCE data. Interactions between patient characteristics and preference estimates were explored and results for prespecified subgroups analyzed. A preference for an attribute was dominant if a respondent always selected the medicine with the better level of that attribute. Minimum additional benefit (MAB) was defined as the minimum increase in probability of reaching target HbA1c for which respondents would accept a worse level of another attribute.

Results: Of 1,198 individuals that responded to the survey, 531 (44.3%) were eligible to participate, and 474 consented and completed the survey and were included in this analysis. Mean age was 61 years, 58% male, and 54% with T2DM diagnosed>7 yrs ago. Based on the DCE analysis, Figure 1 below, GI SEs were most important to patients, followed by weight changes, MoAdmin and A1c. Eighteen percent of respondents always selected the treatment administered by pills, 7% dominated on weight change, and 4% on A1c. Based on the DCE, the MAB analysis found that patients would require that the probability of reaching their target HbA1c is 56 additional percentage points higher to change from a 0% to 30% risk of having GI SEs. Other high MAB levels were reported by moving from 2-kg weight loss to 2-kg weight gain (MAB of 44), moving from 0% to 20% risk of GI SEs (43) and moving from pill to injection (42).

Conclusion: Our findings suggest that this sample of German T2DM patients was willing to trade-off efficacy for improved GI SEs and a better mode of administration (pills). Patients focused on reduction in the risk ofT2DM-related GI-SEs, weight changes and mode of administration as key attributes that they value highly. Given the variety of T2DM medications available, the results suggest that careful discussion about patient preferences could help improve patient satisfaction with T2DM drugs.

Figure 1: Relative importance of the attributes over the ranges presented to respondents



GI = gastrointestinal; HbA1c = glycated hemoglobin; hypo = hypoglycemic event.

Note: All levels are different from each other within attributes at the 5% level except "once a week" and "once a day" ($P = 0.48$); "once a week" and "twice a day" ($P = 0.06$); and "twice a day" and "more than twice a day" ($P = 0.16$), in the dosing frequency attribute.

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PS 062 SGLT-2 inhibitors: early stage efficacy

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Dapagliflozin decreases post-prandial glucose without an increase in C-peptide or insulin

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Background and aims: Control of postprandial glucose (PPG) is important for type 2 diabetes mellitus (T2DM) management and risk reduction. The combination of PPG, insulin and C-peptide are measures of β-cell function, insulin resistance and success of glycaemic control.
Materials and methods: In a small subgroup of patients from a 4-year, phase 3 trial of dapagliflozin (DAPA, ≤ 10 mg/d) vs glipizide (GLIP, ≤ 20 mg/d) as add-on to metformin (MET) in patients with T2DM, PPG was assessed by oral glucose tolerance test at 52 and 104 weeks.
Results: At 52 weeks, mean area under the curve (AUC0-180 mins) for PPG was reduced from baseline in both groups, but was more pronounced with DAPA compared with GLIP (-636.44 [76.4] mmol/L/min vs -398.31 [82.67] mmol/L/min, respectively). Reductions were sustained at 104 weeks (DAPA, -517.12 [104.93] mmol/L/min; GLIP, -404.6 [107.0] mmol/L/min). At both time points, DAPA-mediated reductions in PPG were not associated with an increase in AUC for circulating insulin; this finding was the opposite for GLIP. In addition, increases in C peptide AUC were greater with GLIP than with DAPA at both time points (Table).
Conclusion: Compared with GLIP, DAPA is associated with a sustained reduction in PPG and improvement in glycaemic control without an increase in C-peptide or insulin over 2 years, suggestive of a potential protective effect of β-cell function and alleviation of β-cell stress and insulin resistance.

	AUC Change From Baseline			
	52 weeks		104 weeks	
	DAPA (N = 400)	GLIP (N = 401)	DAPA (N = 400)	GLIP (N = 401)
Glucose (mmol/L/min)				
n	34	29	24	23
Mean	–636.44	–398.31	–517.12	–404.60
95% CI	(–789.18, –483.68)	(–563.63, –232.99)	(–728.46, –305.78)	(–620.11, –189.08)
Diff vs GLIP	–238.12		–112.53	
95% CI	(–463.55, –12.69)		(–415.05, 189.99)	
Insulin (μU/L/min)				
n	35	29	24	23
Mean	–333.7	1860.3	–346.1	1828.5
95% CI	(–1055.0, 387.7)	(1068.2, 2652.4)	(–1438.5, 746.2)	(713.9, 2943.1)
Diff vs GLIP	–2194.0		–2174.7	
95% CI	(–3266.9, –1121.0)		(–3739.1, –610.3)	
C-peptide (nmol/L/min)				
n	35	29	24	23
Mean	11.65	97.48	28.14	115.06
95% CI	(–23.20, 46.47)	(59.22, 135.74)	(–17.61, 73.85)	(68.38, 161.73)
Diff vs GLIP	–85.83		–86.95	
95% CI	(–137.63, –34.06)		(–152.29, –21.58)	
Data are adjusted mean changes from baseline derived from longitudinal repeated measures analyses				

Clinical Trial Registration Number: NCT00660907
Supported by: AZ

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Combined treatment with saxagliptin + dapagliflozin improves beta cell function and reduces insulin levels by increased insulin clearance

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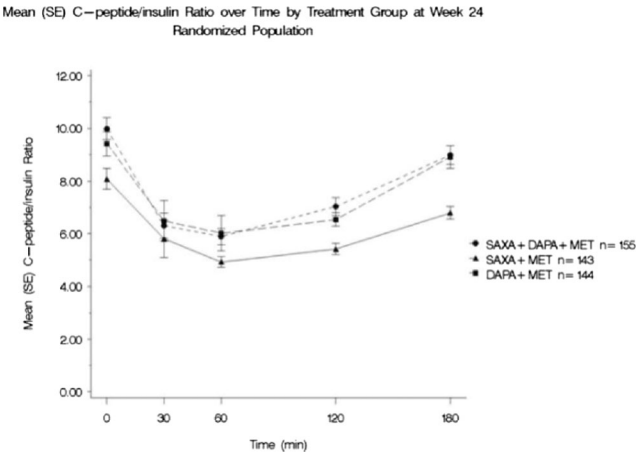
Background and aims: Dapagliflozin (DAPA) alone and in combination with saxagliptin (SAXA+DAPA) reduced, while SAXA increased, insulin levels during a meal tolerance test (MTT). We asked if this was driven by increased insulin clearance.
Materials and methods: C-peptide/insulin ratio was used as a marker for insulin clearance during an MTT and β-cell function was assessed by HOMA-2 in a study testing SAXA 5 mg+DAPA 10 mg vs SAXA and DAPA alone in patients with type 2 diabetes poorly controlled with metformin.
Results: At week 24, SAXA+DAPA and SAXA increased mean (95% CI) C-peptide AUC from baseline, whereas no change was observed for DAPA (SAXA+DAPA: 40.2 [9.2, 71.3 ng/mL]; SAXA: 95.4 [63.4, 127.4] ng/mL; DAPA: 14.5 [-17.6, 46.8] ng/mL). In contrast, change from baseline in insulin AUC was reduced with SAXA+DAPA (-1120.4 [-1633.9, -606.9] μU/mL), and DAPA (-1018.6 (-1550.5, -486.8), but increased with SAXA (661.2 [131.1, 1191.3] μU/mL). Curves for C-peptide/insulin at baseline were similar between the 3 treatment groups. At week 24, C-peptide/insulin was unaltered by SAXA but increased similarly after SAXA+DAPA and DAPA (Figure), mainly due to decreased insulin AUC with DAPA. All treatments improved β-cell function compared with baseline (SAXA+DAPA: 20.6% [16.5, 24.8]; DAPA: 17.0% [12.7, 21.4]; SAXA: 11.0% [6.6, 15.5]).
Conclusion: The data suggest that at 24 weeks, SAXA+DAPA and DAPA improved β-cell function and increased insulin clearance in patients with type 2 diabetes poorly controlled with metformin.



Clinical Trial Registration Number: NCT01606007
Supported by: AstraZeneca

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Baseline fasting plasma glucose may predict response to dapagliflozin when added to metformin

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Background and aims: Dapagliflozin (DAPA) is a highly selective, orally active sodium-glucose co-transporter 2 (SGLT2) inhibitor that increases urinary glucose excretion and reduces hyperglycaemia independently of insulin.
Materials and methods: In a 52-week, double-blind, active-controlled, non-inferiority trial, patients with type 2 diabetes mellitus (T2DM) inadequately controlled on metformin were randomised to DAPA (≤10 mg/d; n=406) or glipizide (GLIP; ≤20 mg/d; n=408). As previously reported, the response rate (reduction in HbA1c and body weight from baseline to 52 weeks) was significantly higher in the DAPA group (66.9 vs 21.3% [difference, 45.7%; 95% CI, 39.3-51.6%]). We report the results of a post hoc analysis of baseline factors, which may predict response to DAPA (C-peptide; fasting plasma glucose [FPG]; C-peptide:FPG ratio; estimated glomerular filtration rate).

Results: In both arms, response rate increased as baseline FPG increased (Table). Differences in response rate between baseline FPG categories were significant for DAPA categories <7.8 vs ≥9.6 mmol/L (<141 vs ≥173 mg/dL [difference, 20.0%; $P<0.001$]) and for ≥7.8-<9.6 vs ≥9.6 mmol/L (≥141-<173 vs ≥173 mg/dL [difference, 11.8%; $P=0.044$]) but not for any GLIP categories. In both groups (full analysis set), greater HbA1c decreases occurred with higher baseline FPG. Body weight change showed no obvious pattern with baseline FPG.
Conclusion: These data suggest that baseline FPG may predict the likelihood of combined HbA1c and body weight reduction in DAPA-treated T2DM patients.

Baseline FPG, mmol/L (mg/dL)	DAPA (N=400)		GLIP (N=401)	
	Outcome	n	Outcome	n
	Response rate (%)*		Response rate (%)*	
<7.8 (<141)	57.5	127	17.3	133
≥7.8 to <9.6 (≥141 to <173)	65.7	143	21.3	122
≥9.6 (≥173)	77.5	129	24.8	145
	Change from baseline in HbA1c, mean ± SD (%)			
<7.8 (<141)	−0.26 ± 0.58	128	−0.20 ± 0.92	135
≥7.8 to <9.6 (≥141 to <173)	−0.44 ± 0.70	142	−0.52 ± 0.94	122
≥9.6 (≥173)	−0.83 ± 0.82	129	−0.84 ± 1.08	143
	Change from baseline in weight, mean ± SD (kg)			
<7.8 (<141)	−2.94 ± 3.87	128	1.57 ± 3.20	135
≥7.8 to <9.6 (≥141 to <173)	−2.89 ± 3.33	142	1.63 ± 3.11	122
≥9.6 (≥173)	−3.88 ± 3.76	129	1.18 ± 3.69	143
SD, standard deviation; *proportion of patients with reduction in HbA1c and body weight from baseline to 52 weeks.				

Clinical Trial Registration Number: NCT00660907
Supported by: AZ

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Initial combinations of empagliflozin and metformin in patients with type 2 diabetes

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Background and aims: Metformin (MET) is the recommended first-line therapy for patients with type 2 diabetes (T2DM), but initial combination therapy may provide more robust glucose-lowering efficacy.
Materials and methods: In this Phase III study, 1364 drug-naïve patients with T2DM were randomized to empagliflozin (EMPA) 12.5 mg bid+MET 1000 mg bid (n=170), EMPA 12.5 mg bid+MET 500 mg bid (n=170), EMPA 5 mg bid+MET 1000 mg bid (n=172), EMPA 5 mg bid+MET 500 mg bid (n=170), EMPA 25 mg qd (n=168), EMPA 10 mg qd (n=172), MET 1000 mg bid (n=171) or MET 500 mg bid (n=171). The primary endpoint was change from baseline in HbA1c at week 24. Key secondary endpoints were changes from baseline in fasting plasma glucose (FPG) and weight.
Results: EMPA+MET bid led to statistically superior and clinically meaningful reductions in HbA1c vs EMPA qd and MET bid doses (Table). EMPA+MET bid led to significant reductions in FPG vs EMPA qd and MET bid and in weight vs MET bid (Table). Adverse events (AEs) were reported in 56.7-66.3% of patients across groups. The percentage of patients with confirmed hypoglycaemic AEs (plasma glucose ≤70 mg/dL and/or requiring assistance) was low in all groups (0-1.8%); none required assistance.
Conclusion: EMPA+MET bid significantly reduced HbA1c vs EMPA qd and MET bid and was well tolerated.

		EMPA 12.5 mg bid+ MET 1000 mg bid (n=169)	EMPA 12.5 mg bid+ MET 500 mg bid (n=165)	EMPA 5 mg bid+ MET 1000 mg bid (n=167)	EMPA 5 mg bid+ MET 500 mg bid (n=161)	EMPA 25 mg qd (n=164)	EMPA 10 mg qd (n=169)	MET 1000 mg bid (n=164)	MET 500 mg bid (n=168)
HbA1c (%)	Baseline	8.66 (0.09)	8.84 (0.10)	8.65 (0.10)	8.68 (0.10)	8.86 (0.10)	8.62 (0.10)	8.55 (0.09)	8.69 (0.08)
	Change from baseline at week 24	-2.08 (0.08)*§	-1.93 (0.08)*†	-2.07 (0.08)*‡	-1.98 (0.08)*†	-1.36 (0.08)§	-1.35 (0.08)	-1.75 (0.09)	-1.18 (0.08)
	Patients with HbA1c ≥7% at baseline who reached HbA1c <7% at week 24, n/N (%)	111/163 (68.1%)*§	91/159 (57.2%)*†	112/161 (69.6%)*‡	98/153 (62.7%)*†	51/158 (32.3%)	69/159 (43.4%)	92/159 (57.9%)	63/166 (38.0%)
FPG (mg/dL)	Baseline	167.9 (3.2)	171.2 (3.4)	163.7 (3.3)	165.9 (3.1)	176.9 (3.8)	170.0 (3.0)	169.0 (3.8)	172.6 (3.0)
	Change from baseline at week 24	-51.0 (2.4)*†	-44.0 (2.4)*†	-47.8 (2.4)*†	-45.5 (2.4)*†	-28.0 (2.5)	-32.9 (2.4)	-32.1 (2.4)	-17.2 (2.5)
Weight (kg)	Baseline	83.7 (1.5)	82.9 (1.5)	83.0 (1.5)	82.3 (1.5)	83.4 (1.6)	83.9 (1.5)	83.8 (1.6)	82.9 (1.7)
	Change from baseline at week 24	-3.8 (0.3)†	-3.0 (0.3)†	-3.5 (0.3)†	-2.8 (0.3)†	-2.4 (0.3)	-2.4 (0.3)	-1.3 (0.3)	-0.5 (0.3)
Adverse events (AEs)	Patients with AEs consistent with urinary tract infection (%)	12.4	11.2	7.6	5.9	8.4	7.6	10.0	8.2
	Patients with AEs consistent with genital infection (%)	2.9	5.3	2.9	1.8	4.8	6.4	2.9	2.3

Baseline values are mean (SE). Changes are adjusted mean (SE) from mixed model repeated measures analysis based on observed cases in patients treated with ≥1 dose of trial medication who had a baseline HbA1c and ≥1 on-treatment HbA1c measurement. *p<0.001 vs EMPA qd dose; †p<0.001 vs MET bid dose; ‡p<0.01 vs MET bid dose; §p<0.05 vs MET bid dose (superiority tests for change from baseline in HbA1c; non-confirmatory tests for HbA1c <7% control rates and for changes from baseline in FPG and weight); ¶p=ns for non-inferiority vs MET 1000 mg bid.

Clinical Trial Registration Number: NCT01719003
Supported by: Boehringer Ingelheim and Eli Lilly and Company

734

Efficacy and safety of canagliflozin monotherapy: results from 2 studies in patients with type 2 diabetes mellitus

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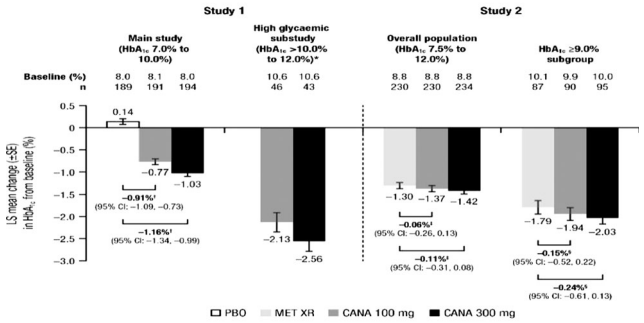
Background and aims: Canagliflozin (CANA) is an SGLT2 inhibitor approved for the treatment of type 2 diabetes mellitus (T2DM). The efficacy and safety of CANA monotherapy was assessed in patients with T2DM inadequately controlled with diet and exercise enrolled in 2 randomised, double-blind, Phase 3 studies.
Materials and methods: In Study 1, the efficacy and safety of canagliflozin 100 and 300 mg versus PBO were assessed at Week 26 (N=584; mean age, 55.4 y; HbA1c, 8.0%; BMI, 31.6 kg/m2); change in HbA1c was also assessed in a separate substudy of patients with high baseline HbA1c (HbA1c >10.0% to ≤12.0%; N=91) that was not PBO controlled. In Study 2, the efficacy and safety of canagliflozin 100 and 300 mg versus metformin extended release (MET XR) were assessed at Week 26 (N=712; mean age, 55.0 y; HbA1c, 8.8%; BMI, 32.6 kg/m2); change in HbA1c was also assessed in a subset of patients with baseline HbA1c ≥9.0% (n=280). The proportion of patients achieving HbA1c <7.0% was evaluated in both studies at Week 26. Safety was assessed based on adverse event (AE) reports.
Results: In Study 1, CANA 100 and 300 mg provided significant reductions in HbA1c from baseline at Week 26 versus PBO (least squares [LS] mean changes of -0.77% and -1.03% vs 0.14%); HbA1c reductions were -2.13% and -2.56% with CANA 100 and 300 mg in patients with baseline HbA1c >10.0% to ≤12.0% in the substudy of Study 1 (Figure). In Study 2, CANA 100 and 300 mg showed noninferiority in lowering HbA1c versus MET XR at Week 26 (LS mean changes of -1.37%, -1.42%, and -1.30%, respectively); HbA1c reductions were -1.94%, -2.03%, and -1.79%, respectively, in patients with baseline HbA1c ≥9.0% (Figure). In Study 1, a greater proportion of patients achieved HbA1c <7.0% with CANA 100 and 300 mg versus PBO at Week 26 (44.5% and 62.4% vs 20.6%). In Study 2, the proportion of patients achieving HbA1c <7.0% at Week 26 with CANA 100 and 300 mg and MET XR was 38.8%, 42.8%, and 43.0%, respectively. In Study 1, the overall AE incidence was 61.0%, 59.9%, and 52.6% with CANA 100 and 300 mg and PBO, respectively. In Study 2, the overall AE incidence was 37.1%, 39.9%, and 37.6% with

CANA 100 and 300 mg and MET XR, respectively. In both studies, the incidence of serious AEs and AEs related to discontinuation was low across groups. Rates of genital mycotic infections were higher with CANA in both studies; rates of osmotic diuresis-related AEs were low overall but higher with CANA.

Conclusion: CANA provided reductions in HbA1c and was generally well tolerated over 26 weeks as monotherapy in patients with T2DM inadequately controlled with diet and exercise, including those with high baseline HbA1c.

Figure. Change from baseline in HbA_{1c} at Week 26.



CI, confidence interval. aPatients were randomised to receive CANA 100 and 300 mg (1:1). bP <0.001 vs PBO. cNoninferiority P = 0.001 vs MET XR. dStatistical comparison vs MET XR not performed.

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Canagliflozin is superior to sitagliptin in reducing both HbA_{1c} and body weight in patients with type 2 diabetes mellitus

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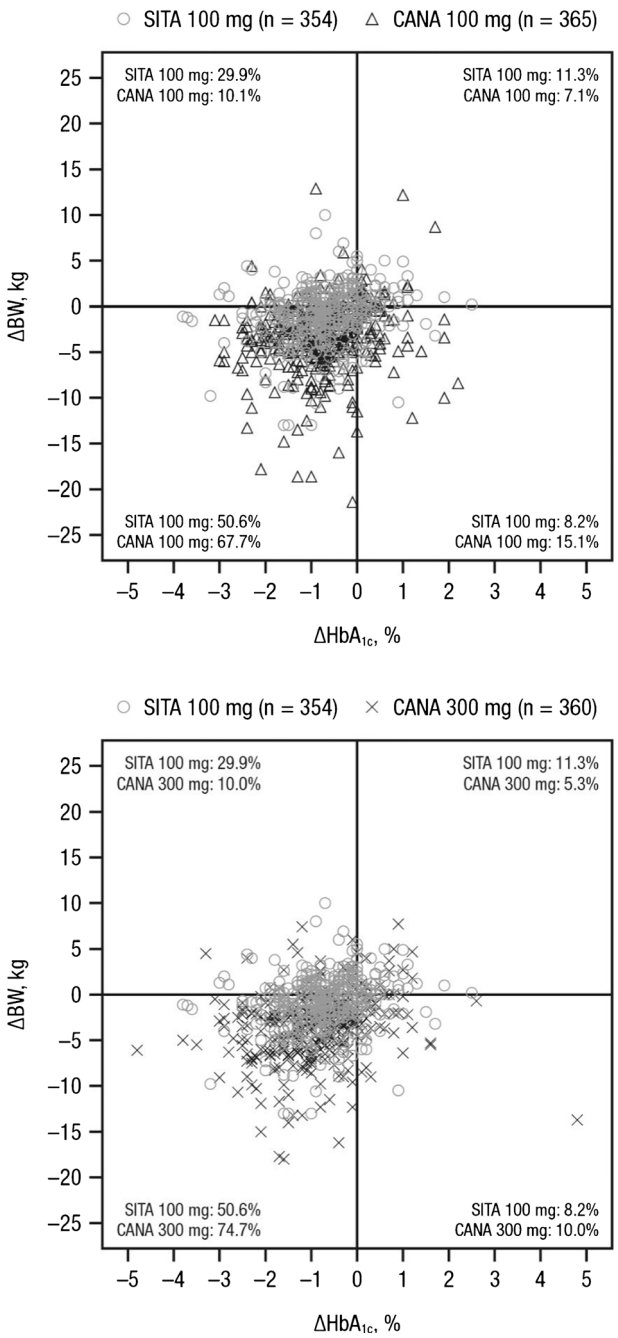
Background and aims: Many patients with type 2 diabetes mellitus (T2DM) are obese or overweight, so treatments that improve glycaemic control and reduce body weight (BW) may be beneficial. Canagliflozin (CANA), a sodium glucose co-transporter 2 (SGLT2) inhibitor approved for the treatment of T2DM, inhibits renal glucose absorption by lowering the renal threshold for glucose and increasing urinary glucose excretion, resulting in lowered plasma glucose levels and a net caloric loss. CANA has been shown to provide reductions in HbA1c and BW compared with sitagliptin (SITA) in 2 studies as add-on to metformin (MET) or MET plus sulphonylurea (SU). This analysis evaluated the proportion of patients who responded to treatment in terms of both HbA1c and BW in these studies at Week 52.

Materials and methods: This analysis used data from two, 52-week, randomised, double-blind, Phase 3 studies of CANA 100 and 300 mg versus SITA 100 mg as add-on to MET (Study 1: N=1,284; mean baseline HbA1c, 7.9%; BW, 87.2 kg) and CANA 300 mg versus SITA 100 mg as add-on to MET plus SU (Study 2: N=755; mean baseline HbA1c, 8.1%; BW, 88.3 kg).

Results: In Study 1, least squares (LS) mean changes from baseline in HbA1c at Week 52 were -0.73%, -0.88%, and -0.73% with CANA 100 and 300 mg and SITA 100 mg, respectively; LS mean percent changes in BW were -3.8%, -4.2%, and -1.3%, respectively. In Study 2, LS mean changes from baseline in HbA1c were -1.03% and -0.66% at Week 52, and LS mean percent changes in BW were -2.5% and 0.3% with CANA 300 mg and SITA 100 mg, respectively. A greater proportion of patients in Study 1 had reductions in both HbA1c and BW with CANA 100 and 300 mg versus SITA 100 mg (67.7%, 74.7%, and 50.6%, respectively; differences [95% confidence interval (CI)] of 17.1% [9.7, 24.5] and 24.2% [17.1, 31.3], respectively; Figure). Similar to Study 1, a greater proportion of patients in Study 2 had reductions in both HbA1c and BW with CANA 300 mg (70.6%) versus SITA 100 mg (37.8%; difference [95% CI] of 32.8% [25.7, 39.8]). The overall incidence of adverse events (AEs) was similar across treatment groups in both studies; CANA was associated with increased rates of genital mycotic infections and osmotic diuresis-related AEs compared with SITA. In Study 1, the incidence of documented hypoglycaemia (≤3.9 mmol/L) was 7%, 7%, and 4% with CANA 100 and 300 mg and SITA 100 mg, respectively; in Study 2, the incidence of documented hypoglycaemia was 43% and 41% with CANA 300 mg and SITA 100 mg, respectively.

Conclusion: CANA provided greater attainment of reduction in both HbA1c and BW compared with SITA at 52 weeks and was generally well tolerated in patients with T2DM as add-on to MET or MET plus SU.

Figure. Change from baseline in both HbA_{1c} and BW with CANA versus SITA at Week 52 (Study 1).



Clinical Trial Registration Number: NCT01106677, NCT01137812
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736

Canagliflozin provides reductions in HbA_{1c}, body weight, and blood pressure in patients with type 2 diabetes on metformin with or without other antihyperglycaemic agents

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Background and aims: Canagliflozin (CANA) is a sodium glucose cotransporter 2 (SGLT2) inhibitor developed for the treatment of adults with type 2 diabetes mellitus (T2DM). This post hoc analysis evaluated the efficacy and safety of CANA in patients with T2DM inadequately controlled with metformin +/- other antihyperglycaemic agents (AHAs; ie, sulphonylurea or pioglitazone) based on pooled data from Phase 3 studies.

Materials and methods: Data were pooled from 3 randomised, double-blind, placebo (PBO)-controlled studies (N=1,729; mean age, 56.2 years; HbA1c, 8.0%; body mass index, 32.2 kg/m2; estimated glomerular filtration rate, 88.5 mL/min/1.73 m2). Patients received CANA 100 or 300 mg or PBO once daily for 26 weeks. Efficacy endpoints were evaluated at 26 weeks; safety was assessed based on adverse event (AE) reports.

Results: Relative to PBO, CANA 100 and 300 mg provided least squares (LS) mean reductions in HbA1c (-0.65% and -0.82%), body weight (-2.2% and -2.8%), and systolic blood pressure (BP; -4.0 and -4.4 mmHg) at Week 26 (Table). Overall AE rates were 59.9%, 59.0%, and 62.3% with CANA 100 and 300 mg and PBO, respectively; serious AE rates were 3.1%, 3.1%, and 4.0%, respectively. Over 26 weeks, higher rates of genital mycotic infections in males and females and osmotic diuresis-related AEs were seen with CANA 100 and 300 mg versus PBO. Rates of urinary tract infections were higher with CANA 100 mg versus CANA 300 mg and PBO. Rates of volume depletion-related AEs were low and similar across groups.

Conclusion: CANA improved glycaemic control and reduced body weight and BP compared with PBO, and was generally well tolerated in patients with T2DM as add-on to metformin +/- other AHAs.

Table. Summary of Efficacy Endpoints at Week 26 (mITT, LOCF)

Parameter*	PBO (n = 454)	CANA 100 mg (n = 638)	CANA 300 mg (n = 637)
Baseline HbA_{1c}, %	8.0 (0.9)	8.0 (0.9)	8.0 (0.9)
Change, %	–0.22 (0.04)	–0.87 (0.03)	–1.04 (0.03)
Difference vs PBO		–0.65 (–0.75, –0.56)	–0.82 (–0.92, –0.72)
Baseline body weight, kg	90.0 (22.6)	90.9 (22.4)	89.0 (22.4)
% change	–0.6 (0.2)	–2.8 (0.1)	–3.4 (0.1)
Difference vs PBO		–2.2 (–2.7, –1.8)	–2.8 (–3.2, –2.4)
Baseline systolic BP, mmHg	128.8 (12.9)	128.3 (12.9)	128.9 (12.9)
Change, mmHg	–0.5 (0.5)	–4.5 (0.5)	–5.0 (0.5)
Difference vs PBO		–4.0 (–5.3, –2.6)	–4.4 (–5.8, –3.1)

mITT, modified intent to treat; LOCF, last observation carried forward; SD, standard deviation; SE, standard error; ANCOVA, analysis of covariance; CI, confidence interval. *Mean (SD) baseline, LS mean (SE) change from baseline using ANCOVA and PBO-subtracted LS mean (95% CI).

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Supported by: Janssen Research & Development, LLC

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Changes in HbA_{1c} and weight in type 2 diabetes patients initiating dapagliflozin treatment in routine UK primary care

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Background and aims: Dapagliflozin is a sodium-glucose co-transporter-2 (SGLT2) inhibitor that lowers blood glucose in type 2 diabetes (T2DM) by reducing renal glucose reabsorption. This study

examines the characteristics of patients initiating dapagliflozin treatment in routine clinical care and assesses subsequent changes in HbA1c and body weight.

Materials and methods: This retrospective observational study was conducted using the Clinical Practice Research Datalink which contains records from 684 primary care practices in the UK. The study cohort consisted of patients with T2DM given a first prescription for dapagliflozin between Nov 2012 and Sept 2014 who were registered ≥6 months prior to that prescription and remained registered for ≥3 months after dapagliflozin initiation. Changes in HbA1c and weight were reported for patients with a measure pre-initiation and at least one measure during dapagliflozin treatment (up to 12 months follow-up).

Results: Of 2401 patients with ≥1 prescription for dapagliflozin, 1732 fulfilled the inclusion criteria. Mean age was 57.5 (SD:10.5) years, weight was 103.1(SD:23.0) kg, HbA1c was 80.1(SD:17.9) mmol/mol and time since T2DM diagnosis was 9.5 (SD:6.0) years: 58% of patients were male. A history of retinopathy was present in 36.9%, nephropathy in 9.8% and neuropathy in 20.4%. The most common usages of dapagliflozin treatment were dual therapy with metformin (435, 25%), triple therapy (480, 28%) and add-on to insulin (332, 19%). 1091 patients had HbA1c values and 970 had weight recorded before initiation of dapagliflozin and on treatment. Reductions in HbA1c and weight were observed with each of the more common treatment combinations and overall (see table). A greater reduction was observed in patients with higher baseline HbA1c (upper tertile, 17.8 (15.4-20.1) mmol/mol) compared to those with a lower baseline (lower tertile, 2.6 (1.3-3.2) mmol/mol) as measured 14-90 days post-initiation.

Conclusion: Reductions in HbA1c and weight observed in a routinely treated UK T2DM population were consistent with results from the dapagliflozin clinical trial program. Reductions were observed with dapagliflozin in combination with metformin, two glucose-lowering agents and insulin.

Reduction in HbA1c and weight from baseline

	Overall		Dual (MET)		Triple		Add-on to Insulin	
	N	Mean(95%CI)	N	Mean(95%CI)	N	Mean(95%CI)	N	Mean(95%CI)
14-90 days post index								
HbA1c (mmol/mol)	562	**9.7** (8.5-10.9)	141	**9.6** (7.3-11.8)	172	**10.4** (8.3-12.9)	101	**10.5** (8.0-12.9)
HbA1c (%)	562	**0.89** (0.78-0.99)	141	**0.88** (0.67-1.08)	172	**0.95** (0.76-1.15)	101	**0.96** (0.73-1.18)
Weight (kg)	513	**2.6** (2.3-2.9)	137	**3.2** (2.7-3.7)	147	**2.8** (2.3-3.3)	93	**1.5** (0.7-2.2)
91-180 days post index								
HbA1c (mmol/mol)	552	**10.2** (8.9-11.5)	162	**9.1** (6.7-11.5)	151	**10.5** (8.0-13.0)	100	**7.4** (4.6-10.2)
HbA1c (%)	552	**0.93** (0.81-1.05)	162	**0.83** (0.61-1.05)	151	**0.96** (0.73-1.19)	100	**0.68** (0.43-0.93)
Weight (kg)	499	**4.3** (3.8-4.7)	155	**5.4** (4.7-6.1)	131	**4.3** (3.6-4.9)	102	**2.7** (1.8-3.7)
180+ days post index								
HbA1c (mmol/mol)	342	**12.6** (11.0-14.3)	101	**11.8** (8.8-14.9)	104	**12.9** (9.7-16.1)	56	**13.4** (9.9-16.8)
HbA1c (%)	342	**1.16** (1.01-1.31)	101	**1.08** (0.80-1.36)	104	**1.19** (0.89-1.47)	56	**1.22** (0.91-1.53)
Weight (kg)	342	**4.6** (4.0-5.3)	95	**6.3** (5.0-7.6)	101	**4.4** (3.3-5.5)	52	**3.2** (1.7-4.7)

PS 063 SGLT-2 inhibitors: long-term and advanced combination therapy

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Simultaneous reduction in both HbA_{1c} and body weight with canagliflozin compared with glimepiride in metformin-treated patients with type 2 diabetes mellitus over 104 weeks

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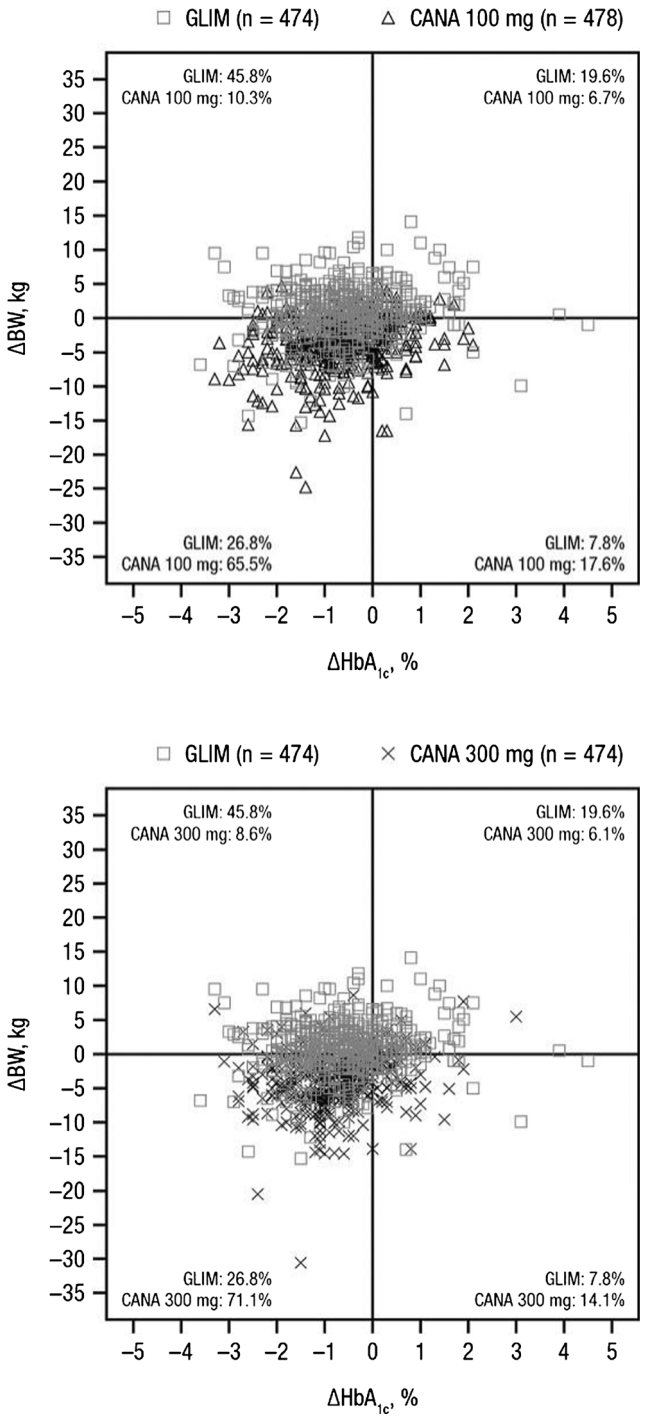
Background and aims: Canagliflozin (CANA), a sodium glucose co-transporter 2 (SGLT2) inhibitor developed to treat type 2 diabetes mellitus (T2DM), lowers the renal threshold for glucose and increases urinary glucose excretion, leading to decreased plasma glucose levels and a net caloric loss. Glimepiride (GLIM) is a sulphonylurea that stimulates insulin secretion. In patients with T2DM on background metformin (MET), CANA showed durable glycaemic improvements and reductions in body weight (BW) versus GLIM over 104 weeks; this post hoc analysis evaluated the proportion of patients who had decreases in both HbA1c and BW with CANA versus GLIM.

Materials and methods: In this randomised, double-blind study, patients with T2DM (N=1,450; mean baseline HbA1c, 7.8%; BW, 86.6 kg) received CANA 100 or 300 mg or GLIM as add-on to MET over a 52-week core period, followed by a 52-week extension (n=1,050). Using individual patient data, the proportion of patients achieving both change from baseline in HbA1c <0% and BW <0 kg was assessed at Weeks 52 and 104.

Results: At Week 52, least squares (LS) mean changes from baseline in HbA1c were -0.82%, -0.93%, and -0.81% with CANA 100 and 300 mg and GLIM, respectively; LS mean percent changes in BW were -4.2%, -4.7%, and 1.0%. At Week 104, LS mean changes from baseline in HbA1c were -0.65%, -0.74%, and -0.55% with CANA 100 and 300 mg and GLIM, respectively; LS mean percent changes in BW were -4.1%, -4.2%, and 0.9%. The proportion of patients with HbA1c reductions at Week 52 was 83.9%, 86.9%, and 81.9% with CANA 100 and 300 mg and GLIM, respectively; 84.1%, 87.3%, and 32.3% had reductions in BW. A greater proportion of patients had reductions in both HbA1c and BW at Week 52 with CANA 100 and 300 mg versus GLIM (72.4%, 78.5%, and 26.8%, respectively; differences [95% confidence interval (CI)] of 45.6% [39.7, 51.5] and 51.7% [46.0, 57.3]). The proportion of patients with HbA1c reductions at Week 104 was 75.7%, 79.7%, and 72.6% with CANA 100 and 300 mg and GLIM, respectively; 83.1%, 85.2%, and 34.6% had reductions in BW (Figure). A greater proportion of patients at Week 104 had reductions in both HbA1c and BW with CANA 100 and 300 mg versus GLIM (65.5%, 71.1%, and 26.8%, respectively; differences [95% CI] of 38.7% [32.6, 44.7] and 44.3% [38.4, 50.2]). At Week 104, the overall incidence of adverse events was 73%, 78%, and 78% with CANA 100 and 300 mg and GLIM, respectively. The incidence of documented hypoglycaemia (≤3.9 mmol/L) at Week 104 was lower with CANA 100 and 300 mg versus GLIM (7% and 8% vs 41%).

Conclusion: CANA provided greater attainment of reduction in both HbA1c and BW compared with GLIM at 52 and 104 weeks and was generally well tolerated in patients with T2DM as add-on to MET.

Figure. Change from baseline in both HbA_{1c} and BW with CANA 100 and 300 mg versus GLIM at Week 104.



Clinical Trial Registration Number: NCT00968812
Supported by: Janssen Research & Development, LLC

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Distribution of weight loss with canagliflozin in patients with type 2 diabetes mellitus over 104 weeks

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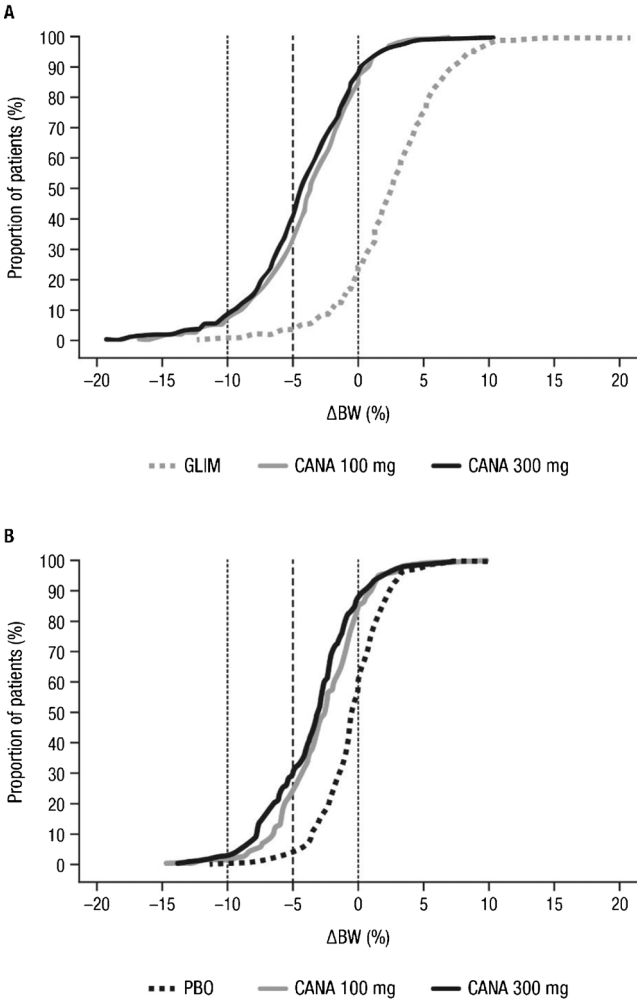
Background and aims: Canagliflozin (CANA), a sodium glucose co-transporter 2 inhibitor, lowers plasma glucose levels in patients with type 2 diabetes mellitus (T2DM) by increasing urinary glucose excretion. This also results in a mild osmotic diuresis and net caloric loss, which leads to reductions in body weight. This analysis assessed the distribution of body weight changes in two randomised, double-blind, Phase 3 studies of CANA.
Materials and methods: Study One assessed CANA 100 and 300 mg versus glimepiride (GLIM) as add-on to metformin in a 52-week core period, followed by a 52-week extension period (N=1,450; mean age, 56.2 y; HbA1c, 7.8%; body weight, 86.6 kg). Study Two assessed CANA 100 and 300 mg versus placebo (PBO) in older patients (55-80 years) on various background antihyperglycaemic therapies in a 26-week core period, followed by a 78-week extension period (N=714; mean age, 63.6 y; HbA1c, 7.7%; body weight, 89.5 kg). Percent change from baseline in body weight was a key endpoint in both studies. The cumulative distribution of body weight changes for each study was determined, as well as the proportion of patients with ≥5% and ≥10% weight loss.
Results: In Study One, body weight reductions were seen with CANA 100 and 300 mg versus an increase with GLIM at Week 52 (-4.2%, -4.7%, and 1.0%); these changes were sustained at Week 104 (-4.1%, -4.2%, and 0.9%). In Study Two, CANA 100 and 300 mg and PBO provided body weight reductions at Week 26 (-2.4%, -3.1%, and -0.1%) and at Week 104 (-3.0%, -3.8%, and -0.6%). In both studies, a greater proportion of patients in the CANA 100 and 300 mg groups experienced weight loss compared with those in the comparator groups (Figure). In Study One, approximately 85% to 90% of patients treated with CANA and approximately 30% to 35% of patients treated with GLIM had a reduction in body weight at Weeks 52 and 104. The proportion of patients with ≥5% body weight reduction with CANA 100 and 300 mg and GLIM was 33%, 43%, and 6% at Week 52, and 35%, 40%, and 7%, at Week 104. In Study Two, approximately 80% to 85% of patients treated with CANA and approximately 60% of patients treated with PBO experienced weight loss at Weeks 26 and 104. The proportion of patients with ≥5% body weight reduction with CANA 100 and 300 mg and PBO was 24%, 28%, and 4% at Week 26, and 28%, 33%, and 11% at Week 104. The proportion of patients with weight loss ≥10% was low across groups in both studies, but higher with CANA versus GLIM or PBO.
Conclusion: CANA 100 and 300 mg provided body weight reductions in a greater proportion of patients with T2DM compared with GLIM and PBO over 104 weeks; most patients experienced body weight reductions with CANA, and a greater proportion achieved ≥5% and ≥10% weight loss.

Figure. Cumulative distribution plots of body weight changes in (A) Study One at Week 52 and (B) Study Two at Week 26.



BW, body weight.

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Dapagliflozin added to metformin is effective in achieving combined improvements in HbA_{1c} and weight without hypoglycaemia over 4 years

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Background and aims: The goal of type 2 diabetes (T2D) management is to achieve optimal glycaemic control whilst avoiding side effects, including hypoglycaemia and weight gain. Dapagliflozin (DAPA) increases glucosuria in a glucose-dependent and insulin-independent manner, resulting in reductions in hyperglycaemia and weight, with a low risk of hypoglycaemia. Key 4-year results (1×52 week double-blind treatment period; 2× double-blind extension periods of 52 and 104 weeks) from a study comparing DAPA with the sulfonylurea, glipizide (GLIP), as add-on to metformin (MET) in patients with inadequately controlled T2D (HbA_{1c} >6.5 - ≤10%) have been reported previously. The aim of this post-hoc analysis was to investigate the proportion of patients achieving composite outcomes including reductions in HbA_{1c} and weight and no hypoglycaemic episodes with DAPA compared with GLIP over 4 years.
Materials and methods: HbA_{1c} reduction was considered to be a decrease ≥0.5%. Weight reduction was considered to be any decrease in weight ≥3% or ≥5%. HbA_{1c} and weight reductions were defined as change from baseline and percent change from baseline, respectively, to Week 208. Major (symptomatic episodes requiring third-party assistance and prompt recovery after glucose or glucagon administration, and capillary or plasma glucose <3.0 mmol/L [<54 mg/dL]) and minor (symptomatic or asymptomatic episode, with capillary or plasma glucose <3.5 mmol/L [<63 mg/dL] regardless of need for external assistance, that does not qualify as a major episode) hypoglycaemic events were considered when defining "no hypoglycaemic events". Binomial exact P-values and 95% CI for the difference in proportions between treatment groups were calculated using StatXact (r) PROCs (v9.0), Cytel Inc., USA.
Results: The proportion of patients achieving the composite outcomes of interest over 4 years (Table) was consistently higher in those receiving DAPA + MET (24.5-56.0%) compared with those receiving GLIP + MET (2.1-12.9%). This difference was significant for all endpoints explored, but was largest when the proportion of patients experiencing no hypoglycaemia was included in the analysis.
Conclusion: This post-hoc analysis demonstrates that over 4 years DAPA is more effective than GLIP, when added to MET, at achieving combined improvements of HbA_{1c} and weight without hypoglycaemia. 27.1% of DAPA-treated patients still benefited from both HbA_{1c} and weight reduction (≥0.5% and ≥3%, respectively) with no hypoglycaemic events after 208 weeks compared with 2.9% of GLIP-treated patients. This suggests that DAPA can positively impact long-term disease management in patients with T2D.

Proportion (%) and risk difference (%) of patients achieving composite outcomes including reduction in HbA_{1c} and weight and no hypoglycaemic episodes at 208 weeks with dapagliflozin compared with glipizide, each in addition to metformin.

Composite outcome	DAPA + MET (N=400) n/N# (%)	GLIP + MET (N=401) n/N# (%)	Risk difference vs GLIP + MET % (95% CI)
HbA_{1c} reduction ≥0.5% AND Weight reduction ≥3% AND No major/minor hypoglycaemia	42/155 (27.1)	4/140 (2.9)	24.2 (16.9, 32.2)*
HbA_{1c} reduction ≥0.5% AND Weight reduction ≥3%	48/155 (31.0)	18/140 (12.9)	18.1 (8.7, 27.4)†
Weight reduction ≥3% AND No major/minor hypoglycaemia	89/159 (56.0)	6/140 (4.3)	51.7 (42.9, 59.9)*
HbA_{1c} reduction ≥0.5% AND Weight reduction ≥5% AND No major/minor hypoglycaemia	38/155 (24.5)	3/140 (2.1)	22.4 (15.4, 30.1)*
HbA_{1c} reduction ≥0.5% AND Weight reduction ≥5%	42/155 (27.1)	9/140 (6.4)	20.7 (12.5, 29.0)*
Weight reduction ≥5% AND No major/minor hypoglycaemia	67/159 (42.1)	5/140 (3.6)	38.6 (30.2, 47.0)*

N# is the number of patients in the full analysis set with non-missing baseline and Week 208 values. n is the number of responders. *P<0.0001; †P=0.0002. DAPA, dapagliflozin; GLIP, glipizide; MET, metformin.

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Triple therapy with saxagliptin and dapagliflozin add-on to metformin: safety of dual add-on versus sequential add-on

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Background and aims: Triple oral therapy with dual add-on of saxagliptin (SAXA) plus dapagliflozin (DAPA) to metformin (MET) was previously shown to reduce HbA1c to a greater extent than dual therapy with either SAXA or DAPA add-on to MET and was an effective treatment option in patients with high baseline HbA1c poorly controlled with MET. We assessed the safety and tolerability of triple therapy with concomitant dual add-on of SAXA and DAPA to MET versus the sequential single add-on of SAXA to dual therapy of DAPA plus MET and the single add-on of DAPA to dual therapy of SAXA plus MET in 3 randomized trials of adults with type 2 diabetes (T2D).
Materials and methods: In the dual add-on trial, patients on stable MET (baseline HbA1c 8.0%-12.0%) received SAXA 5 mg/d plus DAPA 10 mg/d for 24 weeks. In the sequential add-on trials, patients (baseline HbA1c 7%-10.5%) on stable MET+SAXA 5 mg/d or stable MET+DAPA 10 mg/d received add-on DAPA 10 mg/d or SAXA 5 mg/d, respectively for 24 weeks. Safety results from the sequential add-on trials were pooled and compared with the dual add-on trial.
Results: Events of hypoglycemia were uncommon and similar in the 2 groups. Overall adverse events (AEs) and serious AEs (SAEs) were similar between treatment regimens (Table). The most common AE was nasopharyngitis (3.9% vs 3.5% for dual vs sequential add-on, respectively). Urinary tract infections were more frequent in patients receiving sequential add-on therapy; genital infections were reported only with the sequential single add-on of DAPA to dual therapy of SAXA plus MET. There were no events of hypotension/dehydration/hypovolemia. There was 1 event of hepatic cancer that was subsequently identified as pancreatic cancer that had metastasized to the liver and was considered unrelated to drug treatment.
Conclusion: In a cross-study comparison, dual add-on of SAXA and DAPA to MET had a similar safety and tolerability profile compared with single add-on of either SAXA or DAPA to existing dual therapy in patients poorly controlled on MET.

	Patients, n (%)	
Adverse event	**SAXA+DAPA Dual Add-on to MET (N=179)**	**SAXA+DAPA Sequential Add-on to MET (N=313)**
Any AE	87 (48.6)	163 (52.1)
AE related to study medication	12 (6.7)	20 (6.4)
AE leading to discontinuation	1 (0.6)	9 (2.9)
Any SAE	2 (1.1)	10 (3.2)
SAE related to study medication	0	1 (0.3)
SAE leading to discontinuation	0	3 (1.0)
Genital infections	0	8 (2.6)
Discontinuation due to genital infection	0	1 (0.3)
Urinary tract infections	1 (0.6)	16 (5.1)
Discontinuation due to urinary tract infection	0	1 (0.3)
Renal impairment/ failure	3 (1.7)	4 (1.3)
Heart failure	0	1 (0.3)
Hypoglycemia	2 (1.1)	4 (1.3)
Confirmed*	0	1 (0.3)
Major†	0	0

*Glucose value ≤50 mg/dL.
†Symptomatic episode requiring third party assistance due to severe impairment in consciousness or behavior with a glucose value <54 mg/dL and prompt recovery after glucose or glucagon administration.

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A randomised, double-blind phase 3 trial of dapagliflozin add-on to saxagliptin + metformin in type 2 diabetes

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Background and aims: SGLT2 and DPP-4 inhibitors have complementary mechanisms of action. We compared the efficacy and safety of dapagliflozin (DAPA) versus placebo (PBO) as add-on to saxagliptin (SAXA)+metformin IR (MET) in adults with type 2 diabetes (T2D).
Materials and methods: Patients on stable MET (stratum A; baseline A1C 8.0%-11.5%) or stable MET+DPP-4 inhibitor (stratum B; A1C 7.5%-10.5%) for ≥8 weeks received open-label SAXA 5 mg/d+MET for 16 (stratum A) or 8 weeks (stratum B, any DPP-4 inhibitor was replaced with SAXA). At the end of the open-label period, patients with inadequate glycemic control (A1C 7%-10.5%) were randomized to PBO or DAPA 10 mg/d plus open-label SAXA+MET. Primary end point was change in A1C from baseline to week 24. Secondary end points included fasting plasma glucose (FPG), 2-hour postprandial glucose (PPG), body weight, and the proportion of patients achieving A1C<7%.
Results: DAPA+SAXA+MET resulted in a greater A1C reduction than PBO+SAXA+MET (-0.82% vs -0.10%) (Table). More patients achieved A1C <7% and greater reductions in FPG, PPG, and weight were observed with DAPA+SAXA+MET vs PBO+SAXA+MET. AEs were similar across treatment groups with a low overall risk of hypoglycemia (~1%). More patients developed genital infections with DAPA (5%) vs PBO (0.6%).
Conclusion: Addition of DAPA to SAXA+MET improves glycemic control and is well tolerated in patients with T2D inadequately controlled with SAXA+MET.

End point at 24 weeks	**DAPA+SAXA+MET n=160**	**PBO+SAXA+MET n=160**
A1C,%		
n	158	158
Baseline mean (SD)	8.24 (0.97)	8.16 (0.99)
Adjusted mean change from baseline at 24 weeks (95% CI)	–0.82 (–0.96, –0.69)	–0.10 (–0.24, 0.04)
Mean difference (95% CI) vs PBO	–0.72 (–0.91, –0.53), *P*<0.0001	
FPG, mg/dL		
n	158	157
Baseline mean (SD)	179 (48.7)	177 (46.8)
Adjusted mean change from baseline at 24 weeks (95% CI)	–33 (–38.3, –27.2)	–5 (–11.1, 0.6)
Mean difference (95% CI) vs PBO	–28 (–35.4, –19.6), *P*<0.0001	
2-h PPG, mg/dL		
n	134	132
Baseline mean (SD)	240 (60.9)	241 (57.1)
Adjusted mean change from baseline at 24 weeks (95% CI)	–74 (–81.5, –65.5)	–38 (–46.1, –29.9)
Mean difference (95% CI) vs PBO	–36 (–46.3, –24.7), *P*<0.0001	
Body weight, kg		
n	158	158
Baseline mean (SD)	85.8 (18.4)	88.2 (18.1)
Adjusted mean change from baseline at 24 weeks (95% CI)	–1.9 (–2.34, –1.48)	–0.4 (–0.86, 0.04)
Mean difference (95% CI) vs PBO	–1.5 (–2.12, –0.89), *P*<0.0001	
Patients with A1C<7%		
	58/158	21/158
Adjusted % (95% CI)	38 (30.9, 45.1)	12 (7.0, 17.9)
Mean difference % (95% CI) vs PBO	26 (16.7, 34.4), *P*<0.0001	

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Impact of canagliflozin added-on to insulin and metformin in type 2 diabetes: a substudy of the CANVAS trial

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Background and aims: Canagliflozin (CANA) is a sodium glucose co-transporter 2 (SGLT2) inhibitor developed for the treatment of adults with type 2 diabetes mellitus (T2DM). The CANagliflozin cardioVascular Assessment Study (CANVAS) is a cardiovascular (CV) outcomes trial in patients with T2DM and a history or high risk of CV disease. The efficacy and safety of CANA vs. placebo (PBO) were evaluated in a subset of patients from CANVAS who were on ≥30 IU/d of insulin and ≥2,000 mg/d of metformin.
Materials and methods: Patients on ≥30 IU/d of insulin and ≥2,000 mg/d of metformin (N=432; mean age, 61 yrs; HbA1c, 8.2%; fasting plasma glucose [FPG], 9.2 mmol/L; body mass index [BMI], 34.9 kg/m2; insulin dose, 93 IU/d) were randomised to receive CANA 100 or 300 mg or PBO once daily for 18 weeks. Efficacy endpoints were evaluated at 18 weeks; safety was assessed by adverse event (AE) reports.
Results: Relative to PBO, CANA 100 and 300 mg provided significant least squares (LS) mean reductions in HbA1c (-0.7% and -0.8%; P <0.001), FPG (-0.9 and -1.4 mmol/L; P ≤0.001), and body weight (-1.7% and -2.7%; P <0.001) at Week 18 (Table). PBO-subtracted LS mean reductions in systolic blood pressure were -3.5 and -6.1 mmHg (P <0.001) with CANA 100 and 300 mg. The overall incidence of AEs was 66%, 67%, and 61% with CANA 100 and 300 mg and PBO, respectively, with low rates of AE-related discontinuations across groups. The incidence of hypoglycaemia (≤3.9 mmol/L) was similar with CANA 100 and 300 mg and PBO (42%, 47%, and 46%), with low rates of severe episodes (1%, 2%, and 3%). Genitourinary AEs were consistent with the reported frequency in the SGLT2 inhibitor class.
Conclusion: Short-term CANA treatment improved glycaemic control, reduced body weight, and was generally well tolerated as add-on to insulin and metformin in patients with T2DM.

Table. Summary of Efficacy (mITT, LOCF) Endpoints at Week 18 (Subset Receiving ≥30 IU/day + Metformin)*

	PBO (n = 145)	CANA 100 mg (n = 139)	CANA 300 mg (n = 148)
HbA_{1c} baseline, %	8.2 ± 0.8	8.2 ± 0.9	8.2 ± 0.8
Change	0.03 ± 0.05	−0.64 ± 0.06	−0.79 ± 0.05
Difference vs PBO		−0.66 (−0.82, −0.51)†	−0.82 (−0.96, −0.67)†
FPG baseline, mmol/L	9.1 ± 2.5	9.3 ± 2.5	9.3 ± 2.7
Change	0.03 ± 0.2	−0.9 ± 0.2	−1.3 ± 0.2
Difference vs PBO		−0.9 (−1.5, −0.4)‡	−1.4 (−1.9, −0.8)†
Body weight baseline, kg	102.3 ± 22.8	99.7 ± 20.9	101.1 ± 18.0
% change	0.0 ± 0.2	−1.7 ± 0.2	−2.7 ± 0.2
Difference vs PBO		−1.7 (−2.3, −1.1)†	−2.7 (−3.3, −2.1)†
Systolic BP baseline, mmHg	138.3 ± 17.7	136.2 ± 16.6	141.7 ± 16.5
Change	−1.7 ± 1.1	−5.2 ± 1.1	−7.7 ± 1.0
Difference vs PBO		−3.5 (−6.4, −0.5)	−6.1 (−9.0, −3.1)†

mITT, modified intent to treat; LOCF, last observation carried forward; BP, blood pressure; SD, standard deviation; SE, standard error; ANCOVA, analysis of covariance; CI, confidence interval. *Mean ± SD baseline value, LS mean ± SE change from baseline using ANCOVA, and PBO-subtracted LS mean (95% CI) value. †P <0.001 vs PBO. ‡P = 0.001 vs PBO.

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Efficacy and safety of dapagliflozin in patients with type 2 diabetes on insulin ± metformin regimens

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Background and aims: This analysis evaluated the efficacy and safety of dapagliflozin (DAPA) in patients with type 2 diabetes (T2D) inadequately controlled with high doses of insulin (INS)±up to 2 oral antidiabetes agents.
Materials and methods: In this post hoc analysis of a 24-week, placebo (PBO)-controlled trial of DAPA in patients with T2D inadequately controlled with INS±up to 2 oral antidiabetes agents, 587 patients were stratified by INS regimen (basal, sliding scale, or basal/sliding scale) and metformin (MET) use.
Results: Mean T2D duration was >10 years and baseline HbA1c was 8.4%-8.8% across groups. With each of the 3 INS regimens, DAPA 5 and 10 mg/d significantly reduced HbA1c (all P<0.001 vs PBO) and body weight (BW, all P<0.01, except DAPA 5 mg/d + basal INS) from baseline at week 24. DAPA reduced HbA1c and BW with MET (all P<0.0001 vs PBO) or without MET (HbA1c, all P<0.0001; BW, P= 0.0439 and <0.0001 for DAPA 5 and 10 mg/d, respectively), but reductions were numerically larger with MET and significantly more patients receiving MET had HbA1c <7% at 24 weeks (P<0.01 vs PBO). Genital and urinary tract infections were more frequent with DAPA than PBO. Hypoglycemia was more common with DAPA (45%, 48%) than PBO (42%); 1 major hypoglycemia event occurred in each DAPA dose group and 2 in the PBO group.**Conclusion:** In patients withT2D, including those with progressive disease (inadequately controlled on INS, T2D duration >10 years), DAPA was effective and well tolerated with each INS regimen, with no increase in major hypoglycemia. The effects of DAPA appeared greater in patients receiving MET in addition to INS.

End points at Week 24									
Stratified by INS Regimen	**Basal INS**			**Sliding Scale INS**			**Basal/Sliding Scale INS**		
		DAPA +INS		PBO +	DAPA +INS		PBO+	DAPA +INS	
HbA1c, %	PBO + INS (n=44)	5 mg/d (n=31)	10 mg/d (n=32)	INS (n=60)	5 mg/d (n=76)	10 mg/d (n=65)	INS (n=89)	5 mg/d (n=104)	10 mg/d (n=97)
Baseline mean (SD)	8.45 (0.83)	8.69 (0.76)	8.75 (0.89)	8.54 (0.70)	8.65 (0.85)	8.56 (0.79)	8.41 (0.77)	8.56 (0.95)	8.53 (0.82)
Adj mean change from baseline (95% CI) [P value vs PBO]	−0.23 (−0.45, −0.01)	−0.83 (−1.09, −0.57) [0.0008]	−0.99 (−1.24, −0.73) [<0.0001]	−0.36 (−0.54, −0.19)	−0.78 (−0.93, −0.62) [0.0008]	−0.81 (−0.98, −0.63) [0.0005]	−0.33 (−0.48, −0.17)	−0.89 (−1.03, −0.75) [<0.0001]	−0.94 (−1.09, −0.80) [<0.0001]
Patients with A1C<7%									
Adj % (95% CI) or % [P value vs PBO]	4.5	6.5 [NS]	18.8 [NS]	14.4 (6.8, 22.0)	19.0 (10.5, 27.5) [NS]	18.6 (9.2, 28.0) [NS]	8.0 (2.2, 13.9)	24.2 (16.5, 32.0) [0.0010]	23.4 (15.2, 31.5) [0.0026]
Body weight, kg									
Baseline mean (SD)	92.8 (19.3)	95.7 (16.3)	92.6 (18.0)	93.3 (17.2)	91.4 (17.5)	95.2 (17.2)	95.8 (21.1)	94.2 (17.6)	95.0 (16.3)
Adj mean change from baseline (95% CI) [P value vs PBO]	−0.4 (−1.1, 0.3)	−1.2 (−2.0, −0.4) [NS]	−2.3 (−3.1, −1.5) [0.0007]	0.1 (−0.6, 0.8)	−1.1 (−1.7, −0.5) [0.0096]	−1.6 (−2.2, −0.9) [0.0007]	0.2 (−0.3, 0.7)	−0.9 (−1.4, −0.5) [0.0013]	−1.5 (−1.9, −1.0) [<0.0001]

Stratified by Metformin use	**+MET**			**−MET**		
		DAPA + INS			DAPA + INS	
HbA1c, %	PBO + INS (n=93)	5 mg/d (n=94)	10 mg/d (n=92)	PBO + INS (n=100)	5 mg/d (n=117)	10 mg/d (n=102)
Baseline mean (SD)	8.50 (0.74)	8.67 (0.83)	8.50 (0.78)	8.42 (0.79)	8.57 (0.94)	8.64 (0.85)
Adj mean change from baseline (95% CI) [P value vs PBO]	−0.27 (−0.41, −0.12)	−0.89 (−1.03, −0.75) [<0.0001]	−0.95 (−1.09, −0.80) [<0.0001]	−0.37 (−0.52, -0.22)	−0.80 (−0.94, −0.67) [<0.0001]	−0.86 (−1.01, −0.72) [<0.0001]
Patients with A1C<7%						
Adj % (95% CI) [P value vs PBO]	7.7 (2.3, 13.0)	23.0 (15.3, 30.7) [0.0012]	26.0 (17.6, 34.4) [0.0003]	9.6 (3.7, 15.5)	18.1 (11.4, 24.8) [NS]	17.0 (10.0, 24.0) [NS]
Body weight, kg						
Baseline mean (SD)	99.8 (18.7)	98.5 (17.7)	96.4 (16.9)	88.9 (18.8)	89.3 (16.0)	93.0 (16.7)
Adj mean change from baseline (95% CI) [P value vs PBO]	−0.1 (−0.6, 0.5)	−1.6 (−2.1, −1.1) [<0.0001]	−1.9 (−2.4, −1.4) [<0.0001]	0.1 (−0.4, 0.6)	−0.6 (−1.0, −0.2) [0.0439]	−1.5 (−1.9, −1.0) [<0.0001]

Adj=adjusted; NS=nonsignificant.

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Efficacy and safety of canagliflozin added to background insulin in patients with type 2 diabetes mellitus in subgroups by baseline insulin dose

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Background and aims: Across Phase 3 studies, canagliflozin (CANA), an SGLT2 inhibitor, was associated with improvements in glycaemic control and reductions in body weight and blood pressure (BP) in patients

with type 2 diabetes mellitus (T2DM) on a range of background antihyperglycaemic agents, including insulin. This analysis evaluated the efficacy and safety of CANA in patients with T2DM who were on background insulin with or without other antihyperglycaemic agents in subgroups by baseline insulin dose.

Materials and methods: This post hoc analysis assessed efficacy and safety in the subset of patients enrolled in the CANagliflozin cardioVascular Assessment Study (CANVAS; patients with T2DM who had a history or high risk of cardiovascular disease) who were on insulin ≥30 IU/d by tertiles based on baseline insulin dose (<54, 54-<90, and ≥90 IU/d). Patients (N=1,718; mean age, 63 y; HbA1c, 8.3%; body weight, 97.0 kg; eGFR, 74.9 mL/min/1.73 m2; T2DM duration, 16.6 y; insulin dose, 83 IU/d) received CANA 100 or 300 mg or placebo (PBO) once daily over 18 weeks. Patient characteristics across tertiles were generally similar, except for baseline body weight, which increased as insulin dose increased.

Results: During the study, 6%, 10%, and 9% of patients across treatment groups in the <54, 54-<90, and ≥90 IU/d tertiles decreased their insulin dose; 2%, 1%, and 2% increased their insulin dose. In general, more patients decreased their insulin dose with CANA than PBO; the proportion with increases in insulin was similar across groups. Relative to PBO, CANA 100 and 300 mg provided reductions in HbA1c, body weight, and fasting plasma glucose (FPG) over 18 weeks that were similar across tertiles (Table). PBO-subtracted reductions in systolic BP (SBP) were also observed with CANA 100 and 300 mg across tertiles. The incidence of overall adverse events (AEs) with CANA 100 and 300 mg and PBO was 62.8%, 54.6%, and 53.5% in the <54 IU/d tertile; 57.9%, 64.7%, and 58.0% in the 54-<90 IU/d tertile; and 70.3%, 76.8%, and 65.0% in the ≥90 IU/d tertile; rates of serious AEs and AEs leading to discontinuation were low across groups. Increased incidence of male and female genital mycotic infections and osmotic diuresis-related AEs was seen with CANA versus PBO across tertiles. The incidence of documented hypoglycaemia episodes was higher with CANA 100 and 300 mg compared with PBO across tertiles. The incidence of severe hypoglycaemia episodes was low across groups in all tertiles.

Conclusion: Consistent with its insulin-independent mechanism of action, CANA provided improvements in glycaemic control, as well as body weight and SBP reductions, and was generally well tolerated compared with PBO across tertiles by baseline insulin dose in patients with T2DM over 18 weeks.

Table. Summary of Efficacy Outcomes and Hypoglycaemia Incidence by Baseline Insulin Tertile at Week 18 (mITT, LOCF)*

	Baseline insulin dose <54 IU/day			Baseline insulin dose 54-<90 IU/day			Baseline insulin dose ≥90 IU/day		
	PBO (n = 187)	CANA 100 mg (n = 172)	CANA 300 mg (n = 207)	PBO (n = 181)	CANA 100 mg (n = 209)	CANA 300 mg (n = 190)	PBO (n = 197)	CANA 100 mg (n = 185)	CANA 300 mg (n = 190)
HbA1c baseline, %	8.3 (0.9)	8.4 (1.0)	8.2 (0.9)	8.2 (0.9)	8.3 (0.9)	8.3 (0.9)	8.2 (0.8)	8.3 (0.9)	8.3 (0.9)
ΔLSM (95% CI)†		-0.64 (−0.80, −0.48)	-0.71 (−0.87, −0.56)		-0.70 (−0.84, −0.56)	-0.77 (−0.91, −0.62)		-0.59 (−0.74, −0.45)	-0.72 (−0.87, −0.57)
Body weight baseline, kg	88.6 (20.8)	88.1 (19.6)	88.0 (19.8)	95.6 (18.3)	94.5 (18.8)	95.4 (18.9)	108.2 (22.7)	107.8 (20.5)	107.6 (18.2)
ΔLSM (95% CI)		-2.2 (−2.8, −1.7)	-2.6 (−3.2, −2.1)		-1.9 (−2.5, −1.3)	-2.3 (−2.9, −1.7)		-1.6 (−2.1, −1.1)	-2.3 (−2.8, −1.8)
FPG baseline, mmol/L	9.0 (2.7)	9.2 (2.6)	9.1 (2.6)	9.8 (2.8)	9.6 (2.5)	9.3 (2.9)	9.3 (2.7)	9.4 (2.7)	9.7 (3.1)
ΔLSM (95% CI)		-1.4 (−1.9, −0.9)	-1.6 (−2.1, −1.1)		-1.4 (−1.9, −0.9)	-1.5 (−2.0, −1.0)		-1.0 (−1.5, −0.4)	-1.7 (−2.2, −1.1)
SBP baseline, mmHg	137.0 (15.8)	136.1 (16.5)	137.5 (16.0)	138.9 (16.6)	136.3 (17.2)	137.6 (17.3)	138.6 (16.0)	138.6 (16.7)	139.5 (17.1)
ΔLSM (95% CI)		-3.7 (−6.4, −1.1)	-2.5 (−5.0, 0.05)		-2.9 (−5.3, −0.5)	-5.2 (−7.6, −2.7)		-1.3 (−4.0, 1.3)	-5.8 (−8.5, −3.2)
Documented hypoglycaemia, n (%)‡	50 (26.7)	78 (45.3)	78 (37.7)	64 (35.4)	107 (51.2)	96 (50.5)	94 (47.7)	94 (50.8)	111 (58.4)
Severe hypoglycaemia, n (%)	3 (1.6)	2 (1.2)	2 (1.0)	5 (2.8)	6 (2.9)	8 (4.2)	6 (3.0)	2 (1.1)	6 (3.2)

mITT, modified intent-to-treat; LOCF, last observation carried forward; CI, confidence interval; SD, standard deviation; LSM, least squares mean; SE, standard error.
*Baseline data are mean (SD). †Interaction *P* value for treatment by insulin dose tertile = 0.87. ‡Blood glucose ≤3.9 mmol/L (70 mg/dL) or severe episodes.

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Supported by: Janssen Research & Development, LLC

PS 064 SGLT-2 inhibitors: cardio-renal implications

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Cystatin C and creatinine-based estimates of glomerular filtration rates in dapagliflozin phase 3 clinical trials

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Background and aims: Sodium-glucose cotransporter 2 (SGLT2) inhibitor efficacy depends on the kidneys' ability to filter glucose and declines with lower glomerular filtration rate (GFR). Estimates of GFR based on creatinine ($eGFR_{cr}$) are routinely used but are affected by factors, such as muscle mass and diet, and may be inaccurate under certain conditions. Estimates based on cystatin C ($eGFR_{cys}$) appear to correlate better with morbidity and mortality than $eGFR_{cr}$. KDIGO guidelines suggest measuring $eGFR_{cys}$ when $eGFR_{cr}$ is 45-59 ml/min/1.73 m^2 with no evidence of kidney damage and/or when $eGFR_{cr}$ may be unreliable. This analysis compared the proportion of patients with type 2 diabetes (T2D) with eGFR <60 and ≥60 mL/min/1.73 m^2 in 9 dapagliflozin (DAPA) clinical trials when GFR was estimated using creatinine versus cystatin C.

Materials and methods: Using pooled data from 9 DAPA phase 3 trials, the proportion of patients with T2D with eGFR 30-<60 and ≥60 mL/min/1.73 m^2 was determined using both $eGFR_{cr}$ and $eGFR_{cys}$.

Results: The correlation between $eGFR_{cr}$ and $eGFR_{cys}$ was poor; r^2=0.25. At least 60% of patients who had moderate renal impairment (eGFR 30-<60) at baseline based on $eGFR_{cr}$ had an $eGFR_{cys}$ ≥60. Among patients with $eGFR_{cr}$ ≥60, ≥95% remained ≥60 with $eGFR_{cys}$. Decreases in HbA1c, body weight, and systolic blood pressure with DAPA were similar in patient subgroups defined by either eGFR estimate, and statistically significant and clinically meaningful with DAPA 10 mg in most subgroups.

Conclusion: The data suggest that in patients with T2D, renal function as assessed by $eGFR_{cr}$ may be underestimated, rendering such patients ineligible to receive medications limited by renal function (eg, metformin, SGLT2 inhibitors).

	$eGFR_{cr}$, mL/min/1.73 m^2			
	30–<60		≥60	
	Placebo (n=256)	DAPA (n=333)	Placebo (n=1821)	DAPA (n=2499)
Patients with $eGFR_{cys}$, n	254	324	1779	2441
Distribution of $eGFR_{cys}$, mL/min/1.73 m^2, n (%)				
30–<60	99 (38.7)	107 (32.1)	54 (3.0)	63 (2.5)
60–<90	130 (50.8)	162 (48.6)	704 (38.7)	830 (33.2)
>90	25 (9.8)	53 (15.9)	1021 (56.1)	1548 (61.9)

Clinical Trial Registration Number: NCT00528372, NCT00528879, NCT00680745, NCT00673231, NCT00984867, NCT00855166, NCT01031680, NCT01042977, NCT00859898
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747

Effect of longer-term canagliflozin treatment on eGFR in patients with type 2 diabetes mellitus and various degrees of baseline renal function

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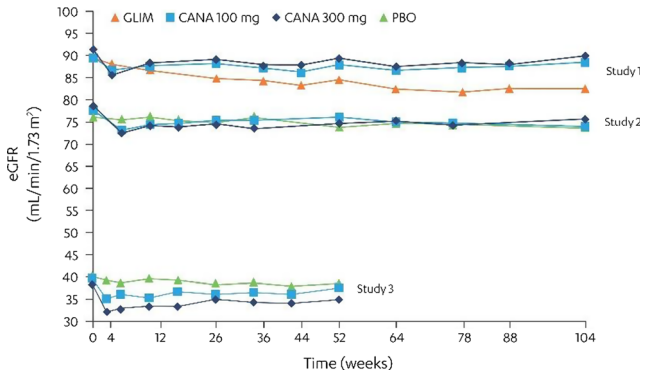
Background and aims: Canagliflozin (CANA), a sodium glucose cotransporter 2 inhibitor, is associated with a small, transient reduction in estimated glomerular filtration rate (eGFR) after treatment initiation,

which stabilizes or attenuates towards baseline levels with continued treatment in short-term studies (i.e. 26 weeks). This analysis evaluated the effect of CANA on renal function in patients with type 2 diabetes mellitus (T2DM) for up to 2 years.

Materials and methods: eGFR over time was compared between CANA and glimepiride (GLIM) or placebo (PBO) using data from 3 clinical studies: Study 1, patients aged 18-80 years (mean eGFR 90.2 mL/min/1.73 m2) received CANA 100 mg or 300 mg, or GLIM for 2 years; Study 2, patients aged 55-80 years (eGFR 77.5 mL/min/1.73 m2) received CANA 100 mg or 300 mg, or PBO for 2 years; and Study 3, patients aged≥ 25 years with Stage 3 chronic kidney disease (eGFR 39.4 mL/min/1.73 m2) received CANA 100 mg or 300 mg, or PBO for 1 year.

Results: Following an initial decrease with CANA, eGFR stabilized or attenuated toward baseline within the first 3–6 weeks and remained unchanged for up to 2 years; eGFR progressively declined with GLIM but not with PBO (Figure). Consistent absolute effects of CANA on eGFR were observed in all patients across study groups, regardless of baseline renal function. Reductions in the urine albumin to creatinine ratio were also seen with CANA 100 mg and 300 mg, versus increases with GLIM and PBO.

Conclusion: In summary, changes in eGFR with CANA were transient and attenuated over time.



Clinical Trial Registration Number: NCT00968812; NCT01106651; NCT01064414

Supported by: Janssen Scientific Affairs, LLC

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The effect of renal impairment on the pharmacokinetics and pharmacodynamics of ertugliflozin in subjects with type 2 diabetes mellitus

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Background and aims: Ertugliflozin is a highly selective and potent inhibitor of Sodium Glucose co-Transporter 2 (SGLT2) that is under development for the treatment of T2DM. Ertugliflozin inhibits renal glucose reabsorption resulting in urinary glucose excretion (UGE), thereby reducing elevated plasma glucose and HbA1c. The glycemic efficacy of SGLT2 inhibitors depends on the amount of glucose filtered through the kidney. As renal impairment (RI) is a co-morbidity of T2DM, this study evaluated the effect of RI on the pharmacokinetics, pharmacodynamics (as measured by 0 to 24 hour urinary glucose excretion [UGE24]), safety, and tolerability of ertugliflozin in subjects with T2DM.

Materials and methods: In this Phase 1, multi-centre, open-label study, a single 15 mg oral dose of ertugliflozin was administered in the fasted state to healthy and T2DM subjects with normal renal function (estimated glomerular filtration rate not normalized for body surface area [eGFR] ≥90 mL/min) and in T2DM subjects with mild RI (eGFR 60-89 mL/min), moderate RI (eGFR 30-59 mL/min) and severe RI (eGFR <30 mL/min). Serial blood samples and urine samples at specified intervals were collected predose and for 96 hours post-dose for pharmacokinetic evaluation and for measurement of UGE24.

Results: Geometric mean (CV%) values for ertugliflozin exposure parameters (area under the concentration-time curve [$AUC_{0\text{-inf}}$] and peak concentration [C_{max}]) are presented in the table below. Ertugliflozin exposures were similar in healthy subjects and T2DM subjects with normal renal function. Based on an analysis of variance, the geometric mean ratio (GMR) [90% confidence interval (CI)] for $AUC_{0\text{-inf}}$ in T2DM subjects with mild, moderate, and severe RI compared to healthy and T2DM subjects with normal renal function (pooled) was 1.56 [1.28, 1.91], 1.70 [1.39, 2.08], and 1.55 [1.24, 1.94], respectively. Regression analysis of the change from baseline in UGE24 versus eGFR showed a decrease in urinary glucose excretion with declining renal function.

Conclusion: Systemic exposure of ertugliflozin was increased by less than 2-fold in subjects with varying degrees of renal impairment compared to subjects with normal renal function. The observed increase in exposure in renal impairment is not anticipated to be clinically meaningful. As expected for this mechanism, UGE24 decreased with declining renal function due to a decrease in the filtered glucose load. A single 15 mg dose of ertugliflozin was well tolerated in subjects with normal renal function and in T2DM subjects with renal impairment.

Renal Function	C_{max} (ng/mL)	$AUC_{0\text{-inf}}$ (ng·hour/mL)
Healthy Normal (n=8)	219 (26)	1236 (27)
T2DM Normal (n=6)	216 (35)	1199 (42)
T2DM Mild RI (n=8)	313 (30)	1908 (28)
T2DM Moderate RI (n=8)	306 (23)	2075 (19)
T2DM Severe RI (n=6)	196 (28)	1895 (23)

Note: Values presented are geometric mean (%CV)

Clinical Trial Registration Number: NCT01948986

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Empagliflozin moderately increases LDL-cholesterol levels through reduced LDL catabolism while it increases slightly the reverse cholesterol in hamsters

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Background and aims: The slight increase of LDL-cholesterol (LDL-C) level described with SGLT2 inhibitors could result of the haemoconcentration associated with glucosuria and osmotic diuresis. Because chronic treatment with SGLT2 inhibitors shifts substrate utilization from carbohydrate to lipid with a moderate increase of ketone bodies, we investigated in hamster, a preclinical model with a human-like cholesterol metabolism, whether other mechanisms could contribute to this increase in LDL-C levels.

Materials and methods: Diet-induced dyslipidemic hamsters were treated orally (2 weeks) with vehicle or empagliflozin 30 mg/kg/day.
Results: Empagliflozin increased urinary glucose excretion (1200-fold, $p<0.001$), reduced fasting glycemia and insulin ($p<0.05$), and moderately but significantly increased ketone bodies and LDL-C levels In the liver, empagliflozin increased HMG-CoA reductase activity and cholesterol levels and reduced LDL-receptor protein levels by 20% ($p<0.05$). In line with lower LDL-receptors in response to higher hepatic cholesterol level, empagliflozin reduced LDL-3H-C catabolism by 20% ($p<0.05$), indicating that the raise in LDL-C may be due to lower LDL-C catabolism. Interestingly, empagliflozin increased LDL-derived 3H-C fecal excretion by 26% ($p<0.05$). This effect was related to a 40% reduction in intestinal cholesterol absorption determined after oral gavage of 14C-cholesterol-oil. We next evaluated whether the reverse cholesterol transport was affected by injecting hamsters (i.p.) with 3H-cholesterol labeled macrophages. Empagliflozin increased macrophage-derived 3H-tracer fecal excretion by 29% ($p<0.05$).
Conclusion: In conclusion, empagliflozin promotes LDL-derived cholesterol fecal excretion and reverse cholesterol transport despite a moderate increases in LDL-C levels possibly through lower LDL-C catabolism in response to a moderate increase in ketone bodies and a switch in substrate utilization.

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Potential relevance of changes in haematocrit to changes in lipid parameters with empagliflozin in patients with type 2 diabetes

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Background and aims: Empagliflozin (EMPA), when given as monotherapy or as add-on therapy, has consistently reduced HbA1c in patients with type 2 diabetes (T2DM). Changes in lipid parameters have also been observed following treatment with EMPA. To test our hypothesis that these changes in lipids could be partly due to haemoconcentration as a result of increased urinary volume and subsequent volume contraction during EMPA treatment, we used pooled data from four 24-week, Phase III, randomised trials to analyse the contributions of haematocrit (HCT) changes, a marker of changes in plasma volume, to changes in LDL-cholesterol (LDL-C), HDL-cholesterol (HDL-C), triglycerides (TG) and apolipoprotein B (Apo B).
Materials and methods: Patients with T2DM received placebo (PBO), EMPA 10 mg or EMPA 25 mg for 24 weeks as monotherapy or add-on therapy (N=2477; mean [SD] age 55.6 [10.2] years, HbA1c 7.99 [0.85] %, BMI 28.7 [5.5] kg/m^2). Total changes in LDL-C, HDL-C, TG and Apo B, and changes associated with (i.e., potentially contributed to by) and independent of changes in HCT were assessed using baseline-adjusted ANCOVA models.
Results: Increases in LDL-C, HDL-C and Apo B, and decreases in TG were observed with EMPA 10 mg and 25 mg; although changes from baseline in Apo B and TG at week 24 did not reach statistical significance with both doses of EMPA and EMPA 25 mg, respectively (table). At baseline, mean (SE) HCT was 41.9 (0.1) % in the EMPA groups and 41.5 (0.2) % in the PBO group. At week 24, the PBO-corrected adjusted mean (SE) percentage changes from baseline in HCT were 5.2 (0.3) % and 5.5 (0.3) % with EMPA 10 mg and EMPA 25 mg, respectively (both $p<0.001$). Changes in HCT were significantly associated with changes in LDL-C, HDL-C, TG and Apo B (table). The contribution of changes independent of changes in HCT varied among the lipid parameters; these were only significant for changes in HDL-C and TG.
Conclusion: Changes in HCT were associated with changes in LDL-C, HDL-C, TG and Apo B. As the observed increase in HCT may reflect haemoconcentration, the changes seen in these lipid parameters following 24 weeks' treatment with EMPA in patients with T2DM may be partly due to haemoconcentration. Changes in HDL-C and TG that were independent of changes in HCT were more pronounced than for the other lipid parameters; it may be hypothesised that improvements in metabolic control following treatment with EMPA in patients in T2DM may drive HCT-independent changes in HDL-C and TG.

EASD 2015: Contributions of haematocrit changes to lipid changes with empagliflozin

	LDL-cholesterol		HDL-cholesterol		Triglycerides		Apolipoprotein B	
	EMPA 10 mg (n=793)	EMPA 25 mg (n=798)	EMPA 10 mg (n=816)	EMPA 25 mg (n=816)	EMPA 10 mg (n=816)	EMPA 25 mg (n=816)	EMPA 10 mg (n=822)	EMPA 25 mg (n=811)
Baseline*	2.57 (0.03)	2.56 (0.03)	1.26 (0.01)	1.27 (0.01)	1.95 (0.05)	1.96 (0.07)	0.90 (0.01)	0.91 (0.01)
PBO-corrected percentage change from baseline								
Total change†	3.30‡ (0.32, 6.29)	4.38‡ (1.40, 7.36)	5.58‡ (4.09, 7.07)	5.25‡ (3.76, 6.74)	−7.12‡ (−11.51, −2.73)	−3.75 (−8.15, 0.64)	1.33 (−0.78, 3.43)	1.91 (−0.20, 4.02)
Change independent of percentage change in HCT†	1.33 (−1.84, 4.50)	2.29 (−0.89, 5.47)	4.09‡ (2.51, 5.68)	3.66‡ (2.07, 5.26)	−12.08‡ (−16.75, −7.41)	−9.02‡ (−13.72, −4.32)	−1.40 (−3.64, 0.84)	−0.97 (−3.23, 1.29)
Change associated with percentage change in HCT†	1.97‡ (0.94, 3.00)	2.09‡ (1.00, 3.19)	1.49‡ (0.97, 2.01)	1.58‡ (1.03, 2.14)	4.96‡ (3.42, 6.49)	5.27‡ (3.64, 6.90)	2.73‡ (1.98, 3.47)	2.88‡ (2.09, 3.67)

Baseline values are mean (SE). *mmol/l for LDL- and HDL-cholesterol and triglycerides, g/l for Apolipoprotein B.
†Contribution of percentage changes are adjusted mean (95% CI) based on ANCOVA with last observation carried forward imputation in randomised patients who received ≥1 dose of study medication and including values after rescue medication.
‡p<0.05.

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Contrasting influences of renal function on blood pressure, body weight and HbA_{1c} reductions with empagliflozin: pooled analysis of phase III trials

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Background and aims: The SGLT2 inhibitor empagliflozin (EMPA) reduces HbA1c, weight and blood pressure (BP) in patients with type 2 diabetes (T2DM). While glucose lowering with EMPA is dependent on renal function, it is less well understood how chronic kidney disease (CKD) influences BP modulation and weight loss with EMPA.
Materials and methods: In five randomised Phase III trials, 2286 patients with T2DM received EMPA 25 mg or placebo (PBO) for 24 weeks as monotherapy or add-on therapy. Using pooled data from these trials, we assessed changes from baseline in systolic BP (SBP), HbA1c and body weight with EMPA 25 mg vs PBO in subgroups by baseline eGFR (MDRD equation), adjusting for differences in baseline SBP (SBP analyses only), baseline body weight (body weight analyses only), baseline HbA1c, region, treatment, study, baseline eGFR and treatment by baseline eGFR interaction between groups.
Results: In patients with normal renal function, or stage 2 or 3 CKD, EMPA significantly reduced HbA1c, SBP and body weight vs PBO (Table). As expected, PBO-corrected HbA1c reductions with EMPA decreased with decreasing baseline eGFR and PBO-corrected reductions in body weight tended to decrease, with decreasing baseline eGFR. In contrast, PBO-corrected reductions in SBP with EMPA appeared to be maintained with decreasing baseline eGFR (Table).
Conclusion: Unlike HbA1c and possibly body weight, reductions in SBP with EMPA in patients with T2DM appeared to be maintained in patients with lower baseline eGFR, indicating that SBP modulation with EMPA may involve pathways other than urinary glucose excretion such as diuretic effects, weight loss, reduced arterial stiffness or direct vascular effects.

	HbA1c (%)		SBP (mmHg)		Body weight (kg)	
	PBO	EMPA 25 mg	PBO	EMPA 25 mg	PBO	EMPA 25 mg
eGFR ≥90 mL/min/1.73m² (normal renal function), n	343	348	343	348	343	348
Baseline	8.08 (0.05)	8.02 (0.05)	127.2 (0.8)	126.4 (0.8)	74.6 (0.9)	76.6 (1.0)
Change from baseline at week 24	-0.04 (0.04)	-0.88 (0.04)	-1.8 (0.7)	-5.0 (0.6)	-0.2 (0.1)	-2.1 (0.1)
Difference vs PBO (95% CI)		-0.84 (-0.95, -0.72)***		-3.2 (-4.9, -1.5)***		-1.9 (-2.3, -1.5)***
eGFR ≥60 to <90 mL/min/1.73m² (CKD stage 2), n	516	518	516	518	516	518
Baseline	8.03 (0.04)	7.94 (0.04)	130.5 (0.7)	131.5 (0.7)	80.7 (0.9)	81.8 (0.8)
Change from baseline at week 24	-0.07 (0.03)	-0.67 (0.03)	-0.2 (0.5)	-4.2 (0.5)	-0.3 (0.1)	-2.3 (0.1)
Difference vs PBO (95% CI)		-0.60 (-0.70, -0.51)***		-4.0 (-5.4, -2.6)***		-2.0 (-2.3, -1.7)***
eGFR ≥30 to <60 mL/min/1.73m² (CKD stage 3), n	239†	234†	239†	234†	239†	234†
Baseline	7.98 (0.05)	7.99 (0.05)	134.7 (1.1)	135.5 (1.2)	83.8 (1.2)	84.0 (1.3)
Change from baseline at week 24	-0.03 (0.06)	-0.40 (0.06)	1.7 (0.8)	-3.8 (0.8)	-0.2 (0.2)	-1.5 (0.2)
Difference vs PBO (95% CI)		-0.38 (-0.52, -0.24)***		-5.5 (-7.6, -3.4)***		-1.4 (-1.8, -0.9)***
eGFR <30 mL/min/1.73m² (CKD stage 4), n	46	42	46	42	46	42
Baseline	8.14 (0.14)	7.94 (0.15)	144.2 (3.4)	140.9 (3.3)	82.8 (3.0)	77.1 (2.5)
Change from baseline at week 24	-0.12 (0.12)	-0.16 (0.12)	5.5 (1.8)	-1.1 (1.8)	-0.1 (0.4)	-1.5 (0.4)
Difference vs PBO (95% CI)		0.04 (-0.37, 0.29)		-6.6 (-11.4, -1.8)**		1.5 (-2.5, -0.4)**

Baseline data are mean (SE), changes are adjusted mean (SE) from ANCOVA with LOCF imputation in randomised patients who received ≥1 dose of study medication and had a baseline HbA1c value. Data after initiation of anti-diabetes rescue therapy were set to missing.
HbA1c: p<0.001 for interaction between treatment and baseline eGFR; SBP: p=0.262 for interaction between treatment and baseline eGFR; body weight: p=0.092 for interaction between treatment and baseline eGFR.
**p<0.01 vs PBO; ***p<0.001 vs PBO.
†n=102 with eGFR ≥30 to <45 mL/min/1.73m²; ‡n=97 with eGFR ≥30 to <45 mL/min/1.73m².

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Blood-pressure lowering effects of canagliflozin across a range of background antihyperglycaemic treatment regimens used in patients with type 2 diabetes mellitus

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Background and aims: Hypertension is a common comorbidity in patients with type 2 diabetes mellitus (T2DM) and is an important risk factor for cardiovascular disease. Therefore, lowering blood pressure in patients with T2DM without meaningfully increasing heart rate is of benefit in reducing the risk of cardiovascular complications. In this analysis, the effect of the sodium glucose co-transporter 2 inhibitor canagliflozin (CANA) on blood pressure and heart rate was studied in patients with T2DM across a broad range of antihyperglycemic treatment regimens.
Materials and methods: In four Phase 3 clinical trials (26 weeks) and two substudies (18 weeks), CANA 100 mg and 300 mg were compared with: placebo (PBO) as monotherapy; dual therapy (add-on to metformin [MET] or a sulfonylurea [SU]); triple therapy (add-on to MET + SU or MET + pioglitazone [PIO]); and add-on to insulin (± other antihyperglycemic agents). In three active-controlled, Phase 3 clinical trials (52 weeks), CANA was compared with sitagliptin (SITA) 100 mg as dual therapy (add-on to MET; CANA 100 mg and 300 mg) or triple therapy (add-on to MET + SU; CANA 300 mg only); and to glimepiride (GLIM) as dual therapy (add-on to MET; CANA 100 mg and 300 mg). Efficacy and safety endpoints reported here include change from baseline in systolic blood pressure (SBP), diastolic blood pressure (DBP), and heart rate. The last observation carried forward imputation method was employed.
Results: Treatment with CANA 100 mg and 300 mg was associated with reductions in SBP and DBP compared with PBO, with generally no increase in mean heart rate across the range of treatment regimens evaluated. PBO-subtracted changes (least square [LS] mean change from baseline and 95% CIs) are reported for each endpoint (Table). When added to MET, CANA 100 mg and 300 mg provided greater reductions in SBP and DBP compared with SITA (differences in LS mean change from baseline [95% CI] with CANA 100 mg and 300 mg, respectively, vs. SITA: SBP −2.9 mmHg [−4.5, −1.3] and −4.0 mmHg [−5.6, −2.4]; and DBP −1.4 mmHg [−2.4, −0.5] and −1.5 mmHg [−2.5, −0.5]). When added to MET, CANA 100 mg and 300 mg provided greater reductions in SBP and DBP compared with GLIM (differences in LS mean change from baseline [95% CI] with CANA 100 mg and 300 mg, respectively, vs. GLIM: SBP −3.5 mmHg [−4.9, −2.1] and −4.8 mmHg [−6.2, −3.4]; and DBP −1.7 mmHg [−2.6, −0.8] and −2.4 mmHg [−3.3, −1.5]). As add-on to MET + SU, CANA 300 mg lowered SBP (−5.9 mmHg [−7.6, −4.2]) and DBP (−2.7 mmHg [−3.8, −1.7]) compared with SITA. No clinically meaningful differences in heart rate were observed with CANA across the studies.
Conclusion: Compared with placebo and commonly used antihyperglycemic agents, CANA reduced SBP and DBP without changing heart rate in patients with T2DM whether used alone, in combination with oral antihyperglycemic agents, or with insulin.

	SBP, mmHg		DBP, mmHg		Heart rate, bpm	
	Overall (PBO + CANA) baseline mean (SD)	PBO-subtracted LS mean change from baseline (95% CI)	Overall (PBO + CANA) baseline mean (SD)	PBO-subtracted LS mean change from baseline (95% CI)	Overall (PBO + CANA) baseline mean (SD)	PBO-subtracted LS mean change from baseline (95% CI)
Monotherapy (n = 584)						
CANA 100 mg (n = 192)	127.7 (13.0)	−3.71 (−5.86, −1.57)	78.0 (7.9)	−1.57 (−2.94, −0.19)	72.1 (9.9)	−1.33 (−2.84, 0.18)
CANA 300 mg (n = 195)		−5.42 (−7.56, −3.28)		−2.03 (−3.41, −0.66)		−0.70 (−2.20, 0.79)
Add-on to MET monotherapy (n = 1,284)						
CANA 100 mg (n = 365)	128.2 (13.0)	−5.36 (−7.28, −3.44)	77.7 (8.2)	−2.47 (−3.72, −1.22)	74.6 (9.4)	−0.95 (−2.36, 0.46)
CANA 300 mg (n = 360)		−6.58 (−8.50, −4.65)		−2.37 (−3.62, −1.11)		−0.24 (−1.65, 1.17)
Add-on to MET + SU (n = 469)						
CANA 100 mg (n = 156)	130.5 (13.5)	−2.24 (−4.72, 0.24)	78.7 (8.3)	−1.13 (−2.68, 0.42)	74.9 (9.8)	−0.16 (−1.90, 1.58)
CANA 300 mg (n = 154)		−1.62 (−4.11, 0.87)		−0.53 (−2.08, 1.02)		−0.53 (−2.28, 1.22)
Add-on to MET + PIO (n = 342)						
CANA 100 mg (n = 113)	127.2 (12.2)	−4.07 (−6.88, −1.25)	76.5 (8.2)	−2.36 (−4.20, −0.53)	73.0 (9.7)	1.02 (−0.97, 3.02)
CANA 300 mg (n = 112)		−3.46 (−6.28, −0.64)		−2.56 (−4.40, −0.73)		−0.08 (−2.07, 1.91)
Add-on to SU monotherapy* (n = 127)						
CANA 100 mg (n = 40)	136.2 (12.9)	−0.10 (−6.45, 6.25)	79.3 (9.5)	−2.07 (−5.61, 1.47)	71.4 (8.8)	−4.03 (−7.68, −0.37)
CANA 300 mg (n = 39)		−1.77 (−8.21, 4.67)		−2.34 (−5.97, 1.29)		−3.75 (−7.41, −0.10)
Add-on to insulin* (n = 1,718)						
CANA 100 mg (n = 559)	137.8 (16.7)	−2.58 (−4.06, −1.09)	76.4 (10.1)	−1.02 (−1.89, −0.15)	72.8 (11.3)	−1.11 (−2.10, −0.12)
CANA 300 mg (n = 577)		−4.38 (−5.85, −2.90)		−1.83 (−2.70, −0.97)		−0.22 (−1.20, 0.76)

Patients were randomly assigned to treatment in a 1:1:1 ratio in all studies except for Add-on to MET monotherapy, where the randomization ratio was 2:2:1.
*Study duration 18 weeks.
SD, standard deviation.

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The effects of empagliflozin on blood pressure and markers of arterial stiffness and vascular resistance by subgroups of age, sex and degree of hypertension in type 2 diabetes

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Background and aims: Empagliflozin improves glycemia and reduces weight, blood pressure as well as central and peripheral hemodynamic parameters. Differential effects of empagliflozin on this by age, sex and degree of hypertension are unknown. We assessed the hypothesis that empagliflozin would reduce blood pressure (BP), pulse pressure (PP), a validated surrogate marker of arterial stiffness being determined by the cardiac output and the stiffness of elastic central arteries like the aorta and wave reflection (PP=systolic BP - diastolic BP), and mean arterial pressure (MAP), a measure reflecting the cardiac cycle and is determined by the cardiac output, systemic vascular resistance, and central venous pressure (MAP = ([2 × diastolic BP] + systolic BP)/3) across these subgroups. It was also postulated that greater reductions would be seen in those with highest baseline systolic BP and advanced age.
Materials and methods: Overall 2477 patients were analyzed from four 24-week phase III randomized trials (on no, one or two background glucose lowering drugs) of empagliflozin 10 mg or 25 mg (n=1652) versus placebo (n=825). At screening, patients in these trials had type 2 diabetes, HbA1c ≥7% and ≤10%, a body mass index (BMI) ≤45 kg/m^2 and were on a diet and exercise regimen.

Results: Mean ± SD age was 55.6±10.2 years, HbA1c 7.99±0.85%, systolic BP/diastolic BP 129.1±15.0/78.3±8.8 mmHg, heart rate 74.2±9.6 bpm and BMI 28.7±5.5 m/kg^2 for the overall population and demographics and baseline characteristics were generally balanced between treatment groups. HbA1c was significantly reduced with empagliflozin (pooled) compared to placebo at week 24 (adjusted mean [SE]):-0.65% (0.03), p<0.001). The overall adjusted mean difference vs placebo in change from baseline in systolic BP at week 24 was -3.6 mmHg (95% CI -4.5, -2.7; p<0.001) and in DBP was -1.3 mmHg (95% CI -1.9, -0.8; p <0.001) and change from baseline in heart rate vs placebo was -0.8 bpm(95% CI -1.4, -0.2; p=0.012). Systolic BP, diastolic BP, PP and MAP were reduced in all subgroups. For systolic BP and MAP, greater reductions were observed in those with highest systolic BP whereas PP was reduced most in those with advanced age (Table).

Conclusion: Reductions in BP and arterial stiffness are two of the effects of SGLT2 inhibitors that might ameliorate cardiovascular risk in patients with type 2 diabetes. EMPA-REG OUTCOME™, reporting 2015, will evaluate if these benefits will translate into CV risk reduction.

Table. Adjusted mean changes from baseline in systolic and diastolic BP, pulse pressure and mean arterial pressure for empagliflozin relative to placebo after 24 weeks of treatment.

	Adjusted Mean† (SE) Difference Empagliflozin (EMPA 10/25 mg) vs. Placebo (PBO) at Week 24 in 4 pooled 24-week phase III trials (N=2477)			
Subgroup	**Systolic BP (mmHg)**	**Diastolic BP (mmHg)**	**Pulse pressure (mmHg)**	**MAP (mmHg)**
Age (yrs), (N)				
<50 PBO=222/EMPA=464	-3.3 (0.9)***	-1.1 (0.6)	**-2.2 (0.7)****	-1.8 (0.6)**
50 to 64 PBO=459/EMPA=871	-3.4 (0.6)***	-1.8 (0.4)***	**-1.6 (0.5)****	-2.3 (0.4)***
65 to 74 PBO=119/EMPA=276	-4.0 (1.2)**	-0.3 (0.8)	**-3.6 (0.9)*****	-1.6 (0.8)
≥75 PBO=25/EMPA=41	-8.3 (2.8)**	-0.1 (1.7)	**-8.2 (2.2)*****	-2.8 (1.9)
Interaction p-value across subgroups	*p=0.3648*	*p=0.2837*	***p=0.0107***	*p=0.7884*
Sex, (N)				
Male PBO=424/EMPA=927	-3.8 (0.6)***	-1.5 (0.4)***	-2.3 (0.5)***	-2.3 (0.4)***
Female PBO=401/EMPA=725	-3.4 (0.7)***	-1.2 (0.4)**	-2.2 (0.5)***	-1.9 (0.5)***
Interaction p-value across subgroups	*p=0.5982*	*p=0.5461*	*p=0.8506*	*p=0.5255*
Systolic BP (mmHg)				
SBP < 130 PBO=462/EMPA=891	**-2.6 (0.6)*****	-0.8 (0.4)*	-1.7 (0.5)***	**-1.4 (0.4)*****
SBP 130-140 PBO=201/EMPA=412	**-4.0 (1.0)*****	-1.7 (0.6)**	-2.4 (0.7)**	**-2.5 (0.6)*****
SBP >140 PBO=162/EMPA=349	**-6.3 (1.1)*****	-2.3 (0.7)***	-3.6 (0.8)***	**-3.6 (0.7)*****
Interaction p-value across subgroups	***p=0.0130***	*p=0.1233*	*p=0.1242*	***p=0.0266***

Adjusted mean (SE) from ANCOVA with LOCF imputation in randomized patients who received ≥1 dose of study medication and had a baseline HbA1c value. Data after initiation of anti-diabetes rescue therapy were set to missing. *p<0.05, **p<0.01, ***p<0.001 vs PBO.

Supported by: Boehringer-Ingelheim and Eli Lilly

PS 065 SGLT-2 inhibitors: safety and tolerability

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No increased risk of cardiovascular events with dapagliflozin in elderly patients with type 2 diabetes mellitus, cardiovascular disease and hypertension

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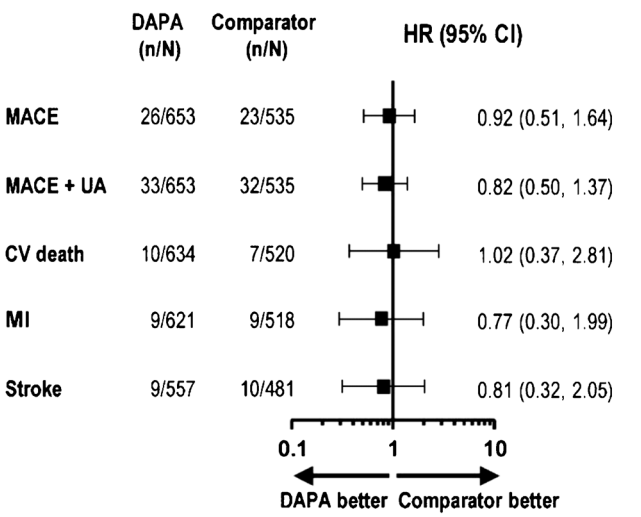
Background and aims: Dapagliflozin (DAPA) reduces plasma glucose in patients with type 2 diabetes mellitus (T2DM) by inhibiting renal glucose reabsorption, leading to increased glucosuria. DAPA is also associated with blood pressure and weight reduction. In a previous pooled analysis of 21 phase 2b/3 trials in T2DM (≤4 years), the hazard ratio (HR) for the pre-specified primary endpoint of major adverse cardiac events (MACE: cardiovascular [CV] death, myocardial infarction, and stroke) plus unstable angina for DAPA (all doses [2.5, 5, 10, 20 and 50 mg]; N=5936) versus comparator (N=3403) was 0.79 (95% CI: 0.58, 1.07). The HR for MACE was 0.77 (95% CI: 0.54, 1.10).

Materials and methods: This *post hoc* pooled analysis of the data assessed CV safety in a high-risk subgroup of elderly (≥65 years) patients with a history of CV disease and hypertension (n=707 for DAPA; n=556 for comparator).

Results: Baseline characteristics were similar between treatment groups. For MACE, the HR was 0.92 (95% CI: 0.51, 1.64). For MACE plus unstable angina, the HR was 0.82 (0.50, 1.37). Additional HRs are shown in the figure. The overall adverse events profile was similar between treatment groups.

Conclusion: These analyses suggest that DAPA is not associated with increased CV risk in elderly patients with T2DM, comorbid CV disease and hypertension. The impact of DAPA on CV events is being prospectively tested in the ongoing DECLARE-TIMI58 study.



n=patients with event: N=number of patients in studies where an event was observed; CI=confidence interval; CV=cardiovascular; DAPA=dapagliflozin (2.5, 5, 10, 20 and 50 mg doses); HR=hazard ratio; MACE=major adverse cardiac event; MI=myocardial infarction; UA=unstable angina; Cox analysis is stratified by study

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Safety and tolerability of combinations of empagliflozin/linagliptin for 52 weeks in subjects with type 2 diabetes

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Background and aims: Two 52-week Phase III studies evaluated the efficacy and safety of once daily combinations of empagliflozin/linagliptin (EMPA/LINA) as monotherapy or add-on to metformin in subjects with type 2 diabetes (T2DM). Using pooled data from these trials, we assessed the safety and tolerability of EMPA/LINA.
Materials and methods: A total of 1363 subjects were treated with EMPA 25 mg/LINA 5 mg (n=273), EMPA 10 mg/LINA 5 mg (n=272), EMPA 25 mg (n=276), EMPA 10 mg (n=275), or LINA 5 mg (n=267). Adverse events (AEs) were assessed descriptively in subjects who took ≥1 dose of study drug.
Results: Total exposure was 251, 255, 256, 249, and 243 patient-years in the EMPA 25 mg/LINA 5 mg, EMPA 10 mg/LINA 5 mg, EMPA 25 mg, EMPA 10 mg, and LINA 5 mg groups, respectively. The percentage of subjects with any AE(s) was similar across groups (Table). The percentage of subjects with confirmed hypoglycaemic AEs (plasma glucose ≤70 mg/dL and/or requiring assistance) was low in all groups (1.1%-2.2%); none required assistance. Events consistent with urinary tract infection were reported in similar percentages of subjects in all groups. Events consistent with genital infection were reported in higher percentages of subjects on EMPA/LINA or EMPA than LINA 5 mg, and in a greater proportion of female than male subjects.
Conclusion: EMPA/LINA as monotherapy or add-on to metformin for 52 weeks was well tolerated in subjects with T2DM, with safety profiles similar to individual components, including a low risk of hypoglycaemia.

	EMPA 25 mg/ LINA 5 mg (n=273)	EMPA 10 mg/ LINA 5 mg (n=272)	EMPA 25 mg (n=276)	EMPA 10 mg (n=275)	LINA 5 mg (n=267)
Subjects with AE(s), n (%)	201 (73.6)	193 (71.0)	196 (71.0)	206 (74.9)	188 (70.4)
Subjects with serious AE(s), n (%)	12 (4.4)	16 (5.9)	19 (6.9)	16 (5.8)	10 (3.7)
Deaths, n (%)	0	2 (0.7)	2 (0.7)	2 (0.7)	0
Subjects with investigator reported drug-related AEs, n (%)	41 (15.0)	37 (13.6)	48 (17.4)	42 (15.3)	32 (12.0)
Subjects with AE(s) leading to treatment discontinuation, n (%)	12 (4.4)	10 (3.7)	9 (3.3)	16 (5.8)	6 (2.2)
Subjects with confirmed hypoglycaemic AE(s)*, n (%)	5 (1.8)	3 (1.1)	6 (2.2)	6 (2.2)	4 (1.5)
Subjects with AE(s) consistent with urinary tract infection†, n (%)	31 (11.4)	34 (12.5)	33 (12.0)	38 (13.8)	34 (12.7)
Female, n/N (%)	25/128 (19.5)	27/114 (23.7)	28/132 (21.2)	29/129 (22.5)	29/125 (23.2)
Male, n/N (%)	6/145 (4.1)	7/158 (4.4)	5/144 (3.5)	9/146 (6.2)	5/142 (3.5)
Subjects with AE(s) consistent with genital infection‡, n (%)	11 (4.0)	12 (4.4)	18 (6.5)	18 (6.5)	7 (2.6)
Female, n/N (%)	4/128 (3.1)	9/114 (7.9)	14/132 (10.6)	11/129 (8.5)	4/125 (3.2)
Male, n/N (%)	7/145 (4.8)	3/158 (1.9)	4/144 (2.8)	7/146 (4.8)	3/142 (2.1)
Subjects with events consistent with volume depletion§, n (%)	2 (0.7)	5 (1.8)	2 (0.7)	1 (0.4)	4 (1.5)
Subjects with hypersensitivity reactions¶, n (%)	3 (1.1)	2 (0.7)	2 (0.7)	2 (0.7)	1 (0.4)
Subjects with pancreatitis¶, n (%)	1 (0.4)	0	0	0	1 (0.4)

*Plasma glucose ≤70 mg/dL and/or requiring assistance. †Based on 70 preferred terms. ‡Based on 89 preferred terms. §Based on 8 preferred terms. ¶Based on 3 Standardised MedDRA Queries (SMQs). ¶Based on 1 SMQ and 1 preferred term.

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Efficacy and safety of canagliflozin in patients with type 2 diabetes mellitus living in hot climates

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Background and aims: In hot climates, patients with type 2 diabetes mellitus (T2DM) treated with canagliflozin (CANA), an SGLT2 inhibitor, may be at increased risk for adverse events (AEs; eg, dehydration). This post hoc analysis assessed the efficacy and safety of CANA in a pooled population of patients living in hot climates (ie, countries between the Tropics of Cancer and Capricorn: Colombia, Costa Rica, Guatemala, Hong Kong, India, Malaysia, Mexico, Peru, Philippines, Singapore, and Thailand).
Materials and methods: This analysis pooled data from 4 randomised, placebo (PBO)-controlled, Phase 3 studies. Patients (N=611; mean age, 53.3 y; HbA1c, 8.0%; BMI, 28.9 kg/m2) received CANA 100 or 300 mg or PBO once daily for 26 weeks.
Results: Relative to PBO, CANA 100 and 300 mg lowered HbA1c (-0.88% and -0.98%), body weight (-2.2% and -3.0%), and systolic BP (-3.5 and -5.8 mmHg) over 26 weeks. Overall AE incidence was 58.6%, 61.1%, and 57.1% with CANA 100 and 300 mg and PBO, with a similar safety profile as the overall population (Table). The incidence of genital mycotic infections in men and women and osmotic diuresis-related AEs was higher with both CANA doses versus PBO. While 3.6% of patients in the hot climate dataset were at high risk for volume-related AEs (eGFR <60 mL/min/1.73 m2, on loop diuretic, and/or aged ≥75 y), rates of these AEs were low across groups. Consistent with the overall population, documented hypoglycaemia rates were low but higher with CANA 100 and 300 mg versus PBO in patients not on sulphonylurea (SU; n=535; 6.5%, 4.0%, 1.5%), with a higher incidence across groups in those on SU (n=76; 39.1%, 18.5%, 19.2%).
Conclusion: *In summary, CANA reduced HbA1c, body weight, and BP, and was generally well tolerated in patients with T2DM living in hot climates over 26 weeks.*

Table. Summary of Overall Safety and Selected AEs at Week 26 (Regardless of Rescue Medication)

	Hot Climate Population			Overall Pooled Population		
Parameter, n (%)	PBO (n = 163)	CANA 100 mg (n = 222)	CANA 300 mg (n = 226)	PBO (n = 646)	CANA 100 mg (n = 833)	CANA 300 mg (n = 834)
Any AE	93 (57.1)	130 (58.6)	138 (61.1)	384 (59.4)	501 (60.1)	494 (59.2)
AE leading to discontinuation	2 (1.2)	2 (0.9)	5 (2.2)	20 (3.1)	36 (4.3)	30 (3.6)
AE related to study drug*	18 (11.0)	38 (17.1)	39 (17.3)	85 (13.2)	171 (20.5)	191 (22.9)
Serious AE	1 (0.6)	5 (2.3)	7 (3.1)	22 (3.4)	28 (3.4)	22 (2.6)
Deaths	0	0	0	2 (0.3)	1 (0.1)	1 (0.1)
Urinary tract infection	12 (7.4)	21 (9.5)	16 (7.1)	26 (4.0)	49 (5.9)	36 (4.3)
Male genital mycotic infection†	0	3 (3.7)	5 (6.4)	2 (0.6)	17 (4.2)	15 (3.7)
Female genital mycotic infection‡	1 (1.1)	9 (6.4)	10 (6.8)	10 (3.2)	44 (10.4)	49 (11.4)
Osmotic diuresis-related AEs	1 (0.6)	11 (5.0)	5 (2.2)	5 (0.8)	56 (6.7)	47 (5.6)
Volume-related AEs	0	2 (0.9)	2 (0.9)	7 (1.1)	10 (1.2)	11 (1.3)

*Possibly, probably, or very likely related to study drug, as assessed by investigators.
†PBO, n = 71; CANA 100 mg, n = 81; CANA 300 mg, n = 78 in the hot weather population; PBO, n = 92; CANA 100 mg, n = 141; CANA 300 mg, n = 148 in the overall population.
‡PBO, n = 334; CANA 100 mg, n = 408; CANA 300 mg, n = 404; in the hot weather population; PBO, n = 312; CANA 100 mg, n = 425; CANA 300 mg, n = 430 in the overall population.

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Assessment of dehydration parameters with dapagliflozin in patients with type 2 diabetes mellitus during Ramadan fasting month

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Background and aims: The population-based Epidemiology of Diabetes and Ramadan (EPIDIAR) study showed that 79% of the 12,243 people in their study fasts during Ramadan.4,8 There will be changes in glycaemic, metabolic profile and biochemistry parameters among patients who fasts during Ramadan. Dapagliflozin is a novel class of glucose-lowering medications which acts via blocking the sodium-glucose cotransporter (SGLT 2) in the proximal renal tubule and leads to urinary glucose excretion. We assessed the dehydration parameters in Type 2 diabetic patienst on either Dapagliflozin or sulphonylurea during Ramadan fasting month.
Materials and methods: In this 12-weeks, randomized, open-label, two-arm parallel group study, 119 patients with T2DM on sulphonylurea(SU) and metformin therapy randomised to either switch to Dapagliflozin 10 mg od(n=58) or remain on pre-study therapy sulphonylurea(n=52). The primary endpoint was to assess the proportion of patient with dehydration, defined as loss of 1.8% of body weight within 13 hours period of fasting daily; which is weight difference between after sunirise('sahur') and before sunset ('iftar'). Other assessment of dehydration included 4 parameters; medical history questions (feeling thirsty, recent fever, recent vomiting and diarrhoea, vigorous exercise and frequency of passing urine), physical examination parameters (dry mucus membrane, dry tongue, reduce skin turgor and altered mental status), blood biochemistry (blood urea, serum creatinine, serum sodium, plasma osmolarity, uric acid and blood ketone) and urine biochemistry analysis (urine osmolarity, urine sodium and urine specific gravity). Data analysed via IBM SPSS Statistics Version22.
Results: There is no difference in the proportion of patient with dehydration 73.1%(n=38) vs 81.6%(n=38); p=0.258, defined as 1.8% weight low within 13 hours of fasting in both Dapagliflozin and sulphonylurea group. More patients in the Dapagliflozin group, 43.1% (n=25) vs 23.1% (n=12); p=0.026 complained of thirst sensation. Other parameters such as BMI, postural hypotension, changes in standing heart rate, physical examinations were similar in both groups. There is higher mean blood ketone (0.2 (0.20) versus 0.1(0.10)mmol/L; p=0.002) and mean haematocrit level (41.6(3.60)% versus 40.0(4.70)%;p=0.009) in Dapagliflozin group versus sulphonylurea group respectively. There is a significantly higher mean urine osmolarity (805(116.23) vs 652(220.19) mOsm/L; p=0.001) and a significantly lower mean urine sodium (85(43.0) vs 120(50) mmol/24 hr; p<0.005) in Dapagliflozin group compared to sulphonylurea group, at Week 6 and Week 12. Adjustment of 1.8% of weight loss to age, gender, HbA1c, urine osmolarity and blood ketone did not show any significant correlation via binary logistic regression analysis.
Conclusion: Clinical dehydration assessment of 1.8% loss of body weight within 13 hours of fasting, physical examination and blood biochemistry (except blood ketone and haematocrit) parameters were similar between both groups, postulating that Dapagliflozin does not pose a higher risk of dehydration in Ramadan fasting month. In conclusion, Dapagliflozin is well-tolerated in diabetic patients fasting during Ramadan month without an increase risk of dehydration.
Supported by: AstraZeneca

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Incidence of genital mycotic infections decrease over time in patients with type 2 diabetes mellitus treated with canagliflozin over 2 years

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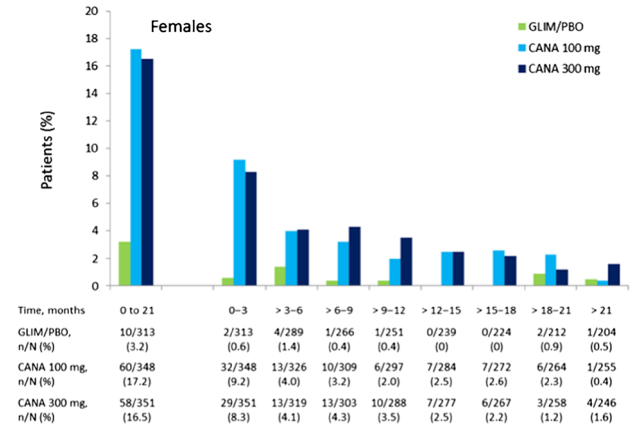
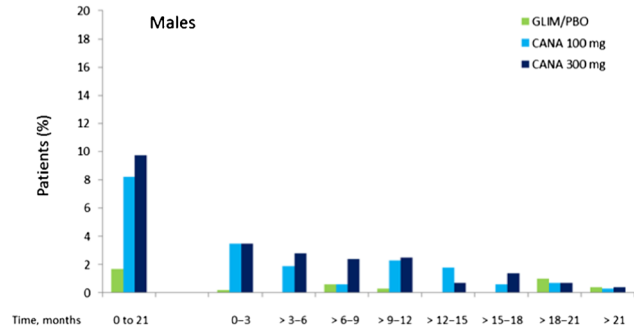
Background and aims: Patients with type 2 diabetes mellitus (T2DM) are at increased risk of genital mycotic infections (GMIs), and those with poorly controlled diabetes have higher risks. Sodium glucose co-transporter 2 inhibitors, such as canagliflozin (CANA), improve glycemic control by inhibiting renal glucose reabsorption and increasing urinary glucose excretion, a mechanism of action associated with an increased incidence of GMIs.
Materials and methods: The incidence of GMIs was evaluated over 2 years in a pooled analysis of 2 randomized clinical studies including 2,164 patients with T2DM; patients received CANA 100 mg or 300 mg, or glimepiride (GLIM) in Study 1 or placebo (PBO) in Study 2. The incidence of GMIs was monitored and evaluated overall, and at 3-month intervals.
Results: The cumulative incidence of GMIs was higher with CANA 100 mg and 300 mg versus GLIM/PBO in both females (17.2% and 16.5% vs 3.2%) and males (8.2% and 9.7% vs 1.7%). The highest incidence of GMIs occurred in the first 3 months of treatment in both females and males, and then declined with time (Figure). GMIs were characterized by the investigators as generally mild to moderate in intensity and responded to standard treatment. In both CANA-treated groups, around 1% of patients (a total of 4 men and 6 women) discontinued treatment due to GMIs.
Conclusion: In summary, in patients with T2DM, GMIs associated with CANA occurred early in treatment, were mild to moderate in intensity, and decreased over time.

Fig. Incidence of GMIs in female and male patients with T2DM treated with CANA or GLIM/PBO



Time, months	0 to 21	0–3	> 3–6	> 6–9	> 9–12	> 12–15	> 15–18	> 18–21	> 21
GLIM/PBO, n/N (%)	10/313 (3.2)	2/313 (0.6)	4/289 (1.4)	1/266 (0.4)	1/251 (0.4)	0/239 (0)	0/224 (0)	2/212 (0.9)	1/204 (0.5)
CANA 100 mg, n/N (%)	60/348 (17.2)	32/348 (9.2)	13/326 (4.0)	10/309 (3.2)	6/297 (2.0)	7/284 (2.5)	7/272 (2.6)	6/264 (2.3)	1/255 (0.4)
CANA 300 mg, n/N (%)	58/351 (16.5)	29/351 (8.3)	13/319 (4.1)	13/303 (4.3)	10/288 (3.5)	7/277 (2.5)	6/267 (2.2)	3/258 (1.2)	4/246 (1.6)



Time, months	0 to 21	0–3	> 3–6	> 6–9	> 9–12	> 12–15	> 15–18	> 18–21	> 21
GLIM/PBO, n/N (%)	7/406 (1.7)	1/406 (0.2)	0/383 (0)	2/355 (0.6)	1/341 (0.3)	0/320 (0)	0/310 (0)	3/297 (1.0)	1/282 (0.4)
CANA 100 mg, n/N (%)	31/376 (8.2)	13/376 (3.5)	7/362 (1.9)	2/351 (0.6)	8/341 (2.3)	6/331 (1.8)	2/318 (0.6)	2/306 (0.7)	1/294 (0.3)
CANA 300 mg, n/N (%)	36/370 (9.7)	13/370 (3.5)	10/352 (2.8)	8/330 (2.4)	8/319 (2.5)	2/302 (0.7)	4/292 (1.4)	2/279 (0.7)	1/274 (0.4)

Clinical Trial Registration Number: NCT01106651; NCT00968812
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Genital infections and relation to body mass index in patients with type 2 diabetes mellitus treated with dapagliflozin

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Background and aims: Treatment with dapagliflozin (DAPA), a highly selective sodium glucose co-transporter 2 (SGLT2) inhibitor, increases glucosuria and reduces hyperglycaemia in patients with type 2 diabetes mellitus (T2DM). Genital infections are a common side effect of treatment with SGLT2 inhibitors, likely resulting from this increase in glucosuria. Clinical trials have reported a greater frequency of vulvovaginitis, balanitis and related genital infections with DAPA versus placebo. In addition, patients with T2DM are generally at increased risk of genital infections. Because obesity is a potential risk factor for some infections, it could be speculated that patients with a higher body weight are at increased risk of genital infections. DAPA has been shown to promote modest weight loss and may therefore be a useful option when it is desirable to avoid weight gain or induce weight loss, particularly in overweight or obese patients. This pooled analysis investigated whether there was a relationship between baseline body mass index (BMI) and the frequency of genital infections in patients with T2DM treated with DAPA, in order to determine whether overweight or obese patients have a clinically important increased susceptibility to this adverse event (AE).
Materials and methods: Data were pooled from 13 studies of up to 24 weeks' duration comprising 2,360 patients treated with DAPA 10 mg and 2,295 patients treated with placebo. The frequency of genital infections was compared between three BMI subgroups (<30, ≥30 to <35 and ≥35 kg/m^2).
Results: Genital infection AEs were reported in 130 (5.5%) patients receiving DAPA and 14 (0.6%) patients receiving placebo; none of which were serious. Baseline HbA1c was similar in DAPA-treated patients with and without a genital infection (8.12 and 8.18%, respectively). AEs of genital infections were more common in females than males treated with DAPA (64.6 and 35.4%, respectively). The frequency of genital infection AEs in DAPA-treated patients was similar between the BMI subgroups (4.3% [38/882], 5.9% [47/796], 6.6% [45/682] in patients with BMI <30, ≥30 to <35 and ≥35 kg/m^2, respectively). Discontinuations due to genital infections were low in DAPA-treated patients (N=5) and were similar in all BMI subgroups (0.2% [2/882], 0.1% [1/796], 0.3% [2/682] in patients with BMI <30, ≥30 to <35 and ≥35 kg/m^2, respectively). There were several limitations to this *post hoc* pooled analysis, including the low numbers of cases of genital infection, the short duration of the analysis and the exclusion of patients with a BMI of >45 kg/m^2.
Conclusion: The frequency of genital infection was low in patients with T2DM treated with DAPA 10 mg. Although these findings were hypothesis-generating only, there was a trend towards a greater frequency of genital infections in patients with a higher baseline BMI. This observed trend is unlikely to be clinically relevant and there is currently no need to adjust advice about the risk of genital infections in overweight or obese patients versus lean patients.
Clinical Trial Registration Number: NCT00263276, NCT00972244, NCT00357370, NCT00528372, NCT00528879, NCT00855166, NCT00859898, NCT00680745, NCT00683878, NCT00984867, NCT00673231, NCT01031680, NCT01042977
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Low carbohydrate diet and SGLT-2 inhibitors dissimilarly influenced on body weight and adiposity in normal mice

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Background and aims: Carbohydrate is one of major energy sources, but its excess intake can induce metabolic disorders including obesity and diabetes. Low-carbohydrate diet (LCHD) has been favourably indicated to prevent obesity, dyslipidaemia and hyperglycaemia mostly in the short-term studies. While, a new class of glucose lowering agents, sodium-glucose cotransporter-2 inhibitor (SGLT-2i), exerts its therapeutic activity independently on insulin secretion by facilitating glucose excretion through the kidneys. SGLT-2i improves glycaemic control and potentially promotes body weight reduction. However, it is still obscure whether dietary carbohydrate-restriction via LCHD functions similarly to urinary glucose disposal by SGLT-2 inhibitors. Moreover, it is not clear whether LCHD is still beneficial to long-term metabolism or not. In current study, we investigated how differently LCHD and SGLT-2i can influence on metabolic changes including body weight, adiposity and glycaemia using normal mice.
Materials and methods: We conducted an eight-week study using non-diabetic C57Bl/6 mice. Mice were fed either LCHD (carbohydrate : protein : fat (C:P:F)=16:40:44%cal) or normal diet (ND, C:P:F=68:20:12%cal) ad libitum and treated with either SGLT-2i (ipragliflozin 3 mg/kg) or saline by daily oral gavage. Mice were randomized into four groups (each n=5~10): LC group fed with LCHD, Ipra group treated with ipragliflozin, LC + Ipra group combined with LCHD and ipragliflozin and C group as controls. To evaluate metabolic changes, we monitored body weight and glycaemia. We also compared glucose excursions, glucose-stimulated insulin secretion and insulin sensitivity among the groups by 2 g/kg OGTT or intraperitoneal insulin tolerance

test (0.6 U/kg). Then, we conducted the calorie-adjusted pair-feeding (PF) study, where mice were similarly divided to four groups: LCPF, IpraPF, LC + IpraPF, controlPF, respectively.

Results: There was no significant difference in non-fasted glucose levels among the groups fed ad libitum. However, not LCHD but SGLT-2i treatment reduced glucose excursion after OGTT compared to controls (AUC glucose, LC: 1698±77.04, Ipra*: 1190±62 C: 1730±102 mmol/l x min, * $p<0.0001$ vs C). Both LCHD and SGTL-2i treatment enhanced calorie-intake compared to controls ($p<0.03$), whereas LCHD unexpectedly increased body weight (LC*,**: 27.0±0.9, Ipra: 23.9±0.5, LC+Ipra: 22.9±0.7, C: 23.6±0.4 g, *$p<0.005$ vs C, **$p<0.005$ vs Ipra) and epididymal fat mass (LC*,**: 0.90±0.09, Ipra: 0.34±0.01, LC+Ipra: 0.43±0.01, C: 0.38±0.02 g, *$p<0.0001$ vs C, **$p<0.0001$ vs Ipra). Furthermore, insulin tolerance test indicated that LCHD induced insulin resistance in mice treated with SGLT-2i. (LC+Ipra vs Ipra, $p<0.05$). In contrast, glucose-stimulated insulin secretion was increased in LC to compensate glucose excursions. Finally, pair-feeding ameliorates LCHD-induced body weight gain, fat deposition and insulin resistance by calorie-adjustment to ND

Conclusion: Collectively, our results suggest that LCHD without calorie restriction may unexpectedly induce insulin resistance and obesity in non-diabetic mice.

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Hypersensitivity events with dapagliflozin: a pooled analysis

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Background and aims: In patients with type 2 diabetes mellitus dapagliflozin (DAPA) improves glycaemic control, is generally well tolerated, and has an adverse event (AE) profile typically related to its mechanism of action. Hypersensitivity events have been reported in some patients with sodium-glucose co-transporter 2 inhibitors, including a report of dermatological AEs in Japan.

Materials and methods: We therefore investigated the frequency and characteristics of hypersensitivity AEs pooled across 21 phase 2/3 comparator-controlled DAPA trials, including a subanalysis of Asian patients.

Results: In the total population, AEs and serious AEs (SAEs) of hypersensitivity were infrequent and reported in a similar proportion of patients with DAPA or comparator (Table); the most common events were rash, eczema, dermatitis, and urticaria. Few patients discontinued as a result of hypersensitivity AEs. In patients of Asian descent, a lower frequency of hypersensitivity AEs was observed with DAPA vs comparator. In the placebo (PBO)-controlled pools hypersensitivity AEs were slightly more frequent with DAPA vs PBO across the overall population and less frequent with DAPA vs PBO in Asian patients.

Conclusion: In conclusion, hypersensitivity events with DAPA were infrequent across the clinical programme and were similar between PBO and comparator. These events rarely led to discontinuation of DAPA and were not more frequent in Asian patients.

TABLE

Hypersensitivity events*		
	DAPA	**COMPARATOR**
Study Pool: All phase 2b and 3	**Total DAPA** N=5936	**All control** N=3403
Total AEs, n (%)	270 (4.5)	148 (4.3)
AEs in Asian pts, n/N (%)	21/1050 (2.0)	23/513 (4.5)
SAEs, n (%)	11 (0.2)	3 (0.1)
Discontinuations, n (%)	9 (0.2)	5 (0.1)
Most common events (≥ 0.5% in any group), n (%)		
Rash	63 (1.1)	36 (1.1)
Eczema	38 (0.6)	26 (0.8)
Dermatitis	29 (0.5)	13 (0.4)
Urticaria	27 (0.5)	6 (0.2)
PBO-controlled	**DAPA 10 mg**	**PBO**
Short-term PBO	N=2360	N=2295
Total AEs, n (%)	61 (2.6)	50 (2.2)
AEs in Asian pts, n/N (%)	2/209 (1.0)	7/206 (3.4)
SAEs, n (%)	2 (0.1)	2 (0.1)
Discontinuations, n (%)	4 (0.2)	3 (0.1)
Short-term plus long-term PBO	N=2026	N=1956
Total AEs, n(%)	96 (4.7)	75 (3.8)
AEs in Asian pts, n (%)	2/131 (1.5)	6/120 (5.0)
SAEs, n (%)	3 (0.1)	2 (0.1)
Discontinuations, n (%)	3 (0.1)	4 (0.2)
*Categorised using the Medical Dictionary for Regulatory Activities (MedDRA) 17.0 preferred terms and using the standardised MedDRA queries (SMQ) hypersensitivity narrow terms.		

Supported by: AstraZeneca

PS 066 SGLT-2 inhibitors: new insights, new uses

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Urinary glucose excretion and insulin to carbohydrate ratio to assess insulin dose adjustments in type 1 diabetes mellitus patients treated with dapagliflozin

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Background and aims: Dapagliflozin is a selective sodium-glucose co-transporter 2 inhibitor that lowers HbA1c, body weight and systolic blood pressure in patients with type 2 diabetes mellitus (T2DM). Dapagliflozin has previously been shown to be safe and well tolerated in a 14-day Phase 2 study in patients with type 1 diabetes mellitus (T1DM).
Materials and methods: We hypothesised that carbohydrate (CHO) loss, counted as 24 hour urinary glucose excretion (UGE), after 7 days of dapagliflozin treatment in patients with T1DM (Phase 2 data) or as UGE estimated from a dapagliflozin dose-response model, could be used with the calculated insulin to CHO ratio (I/C) to assess insulin dose adjustments needed upon initiation of dapagliflozin treatment. Data on total daily insulin dose (TDD) were obtained from the Phase 2 study, or arbitrarily chosen to represent "low" or "high" insulin dose. The '450 rule' i.e. I/C (for adults on short acting insulin)=450 / TDD was applied to derive I/C. TDD adjustment was calculated as UGE:I/C.
Results: Empirically, there was agreement between observed mean TDD adjustments (Phase 2 data) and I/C-calculated mean TDD adjustments using either observed mean UGE (Phase 2 data) or estimated mean UGE from the dapagliflozin dose-response model (Table).
Conclusion: UGE and I/C can be used to assess mean reductions in TDD upon initiation of dapagliflozin. Further assessments are needed to evaluate the impact of patient characteristics such as eGFR and average plasma glucose on individual TDD adjustments.

Method of assessment	Mean 24 hr UGE (g) after DAPA T/t	Mean BL TDD (IU)	I/C = 450/TDD (g/IU)	Mean daily INS dose reduction (%)
Phase 2 study – observed*				Observed
DAPA 5 mg (n=14)	71.25	39.36		19
DAPA 10 mg (n=13)	88.02	55.14		17
Phase 2 study – calculated from UGE*				Calculated
DAPA 5 mg (n=14)	71.25	39.36	11.43	16
DAPA 10 mg (n=13)	88.02	55.14	8.16	20
Model estimated UGE				Modelled
DAPA 10 mg	80.00	18 (low)	25.00	18
DAPA 10 mg	80.00	63 (high)	7.14	18

*From a Phase 2 study of DAPA in patients with type 1 diabetes; BL=baseline; DAPA=dapagliflozin; I/C=insulin to carbohydrate ratio; TDD=total daily insulin dose; T/t=treatment; UGE=urinary glucose excretion.

Clinical Trial Registration Number: NCT01498185
Supported by: AstraZeneca

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Empagliflozin decreases glucose exposure and variability in patients with type 1 diabetes: continuous glucose monitoring data (EASE-1)

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Background and aims: Empagliflozin (EMPA) lowers blood glucose by reducing renal glucose reabsorption via a mechanism independent of the action of insulin. In the EASE-1 study, we characterised the effects of EMPA as adjunct to insulin on glucose exposure in patients with type 1 diabetes, by analysing glucose patterns via continuous glucose monitoring (CGM).
Materials and methods: In this Phase II, double-blind trial, patients with type 1 diabetes were randomised to placebo (PBO; n=19), EMPA 2.5 mg (n=19), EMPA 10 mg(n=19) or EMPA 25 mg (n=18) as adjunct to insulin for 4 weeks. Insulin dose was to be kept as stable as possible for the first 7 days and was freely adjustable thereafter. Markers of glucose exposure and variability were assessed from 7-day blinded CGM periods.
Results: At week 1, all EMPA doses decreased total glucose exposure (AUC) and increased time in the glucose range of >70 mg/dL to ≤180 mg/dL vs PBO; results were sustained to week 4 with EMPA 25 mg (Table). A similar pattern was observed in nocturnal glucose exposure (00:00-05:59 h). Hours/day with glucose ≤70 mg/dL increased with EMPA 10 mg and 25 mg vs PBO at week 1 but there were no significant differences with EMPA vs PBO at week 4. Hours/day with glucose ≤54 mg/dL were not increased with EMPA vs PBO. All EMPA doses significantly reduced glucose variability, as measured by interquartile range (IQR [Table]; ambulatory glucose profiling) and mean amplitude of glucose excursions (MAGE), vs PBO at weeks 1 and 4.
Conclusion: These CGM data show that EMPA as adjunct to insulin therapy decreased glucose exposure and variability and increased time in glucose target range over 4 weeks in patients with type 1 diabetes. EMPA is not yet approved for use in patients with type 1 diabetes.

		PBO	EMPA 2.5 mg	EMPA 10 mg	EMPA 25 mg
Glucose exposure: hourly mean area under median curve over 24 hours (mg/dL·hr)	Baseline (week -1)	173.4 (6.2)	177.9 (7.6)	161.8 (5.1)	166.3 (4.4)
	Change from baseline at week 1	-0.9 (4.2)	-13.1 (4.1)*	-31.1 (4.2)***	-33.8 (4.2)***
	Change from baseline at week 4	-3.1 (5.1)	-7.2 (5.0)	-6.6 (5.3)	-19.0 (5.1)*
Glucose variability: interquartile range (mg/dL) (derived from weekly ambulatory glucose profiles)	Baseline (week -1)	99.1 (4.9)	95.2 (4.5)	92.6 (4.5)	92.0 (4.7)
	Change from baseline at week 1	-21.6 (2.6)	-35.6 (2.5)***	-41.6 (2.6)***	-42.7 (2.6)***
	Change from baseline at week 4	6.5 (3.7)	-15.4 (3.6)***	-15.0 (3.8)***	-20.7 (3.7)***
Time in glucose range of >70 mg/dL to ≤180 mg/dL (hours/day)	Baseline (week -1)	11.8 (0.7)	10.8 (0.7)	12.7 (0.5)	12.7 (0.5)
	Change from baseline at week 1	0.9 (0.6)	3.5 (0.6)**	4.6 (0.6)***	5.2 (0.6)***
	Change from baseline at week 4	0.2 (0.5)	1.5 (0.5)	1.5 (0.5)	2.9 (0.5)***

Baseline values are mean (SE). Changes are adjusted mean (SE) based on ANCOVA in subjects treated with ≥1 dose of study drug who had a 24-hour UGE measurement at baseline and at week 1 and/or week 4 (observed cases). *p<0.05; **p<0.01; ***p<0.001 for difference vs placebo.

Clinical Trial Registration Number: NCT01969747
Supported by: Boehringer Ingelheim and Eli Lilly and Company.

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Sotagliflozin, a dual SGLT1 and SGLT2 inhibitor, reduces glucose variability in type 1 diabetes mellitus in a randomised, placebo-controlled, double-blind study

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Background and aims: Sotagliflozin (LX4211) is a dual inhibitor of sodium-glucose cotransporters SGLT1 and SGLT2. Inhibition of SGLT1, the major intestinal glucose transporter, reduces glucose absorption in the gastrointestinal tract, and SGLT2 inhibition reduces renal glucose reabsorption. We hypothesized that sotagliflozin would increase "time in glucose range" by decreasing postprandial hyperglycemia and glycemic variability.

Materials and methods: This 29-day study evaluated 33 subjects with T1D (age 21-57 years, diabetes duration 3-42 years) with screening A1C 7.0%-9.0%, randomized to double-blind treatment with sotagliflozin 400 mg or placebo 1×/day. All subjects wore masked continuous glucose monitors (CGM).

Results: All 33 patients completed the study. Raw means are displayed; p-values for change from baseline and between group effects are based on least squares means.

Conclusion: Sotagliflozin, a next generation SGLT inhibitor, significantly improved time in glucose range as measured by the following CGM glucose indices: Mean daily glucose, % time spent between 70 and 180 mg/dL, >180 mg/dL, and >250 mg/dL, and glucose variability (mean standard deviation, MAGE, and HBGI). Sotagliflozin as an adjunctive treatment to insulin showed improved glucose control and glycemic variability. Larger studies of longer duration are needed to confirm these findings.

Results	Sotagliflozin N= 16		Placebo N=17		p-Value Sotagliflozin vs. Placebo
	Baseline (Days -6 to -2)	Treatment (Days 3 to 27)	Baseline (Days -6 to -2)	Treatment (Days 3 to 27)	
CGM Mean Daily Glucose (mg/dL)	163.6 (38.7)	148.8 (18.0)*	160.6 (25.9)	170.3 (24.0)	**0.010**
CGM Hypoglycemia events/pt/day (≥10 continuous min <70 mg/dL)	1.06 (0.59)	0.95 (0.41)	1.09 (1.01)	0.90 (0.47)	0.75
CGM Percent Time in Ranges					
% Time <70 mg/dL	7.9 (7.3)	6.7 (5.0)	8.5 (9.5)	5.8 (4.7)	0.80
% Time 70 – 180 mg/dL	56.4 (15.6)	68.2 (12.1)*	55.9 (12.1)	54.0 (12.0)	**0.003**
% Time >180 mg/dL	35.7 (18.3)	25.0 (11.2)*	35.6 (14.4)	40.2 (13.7)	**0.002**
% Time >250 mg/dL	15.3 (14.8)	6.7 (6.6)*	12.0 (9.3)	14.1 (7.9)	**0.008**
CGM Variability Measures					
Standard Deviation (mg/dL)	60.5 (16.5)	50.0 (12.2)*	57.2 (13.9)	58.8 (9.6)	**0.022**
Coefficient of Variation	37.4 (5.2)	33.7 (6.0)	35.6 (8.8)	35.4 (5.2)	0.41
Mean Amplitude of Glucose Excursion (MAGE)	145.5 (39.5)	120.8 (30.5)*	135.5 (34.9)	145.5 (25.6)	**0.041**
High Blood Glucose Index (HBGI)	9.2 (6.5)	6.2 (3.1)*	8.7 (3.7)	9.7 (3.7)	**0.006**
Low Blood Glucose Index (LBGI)	1.9 (1.5)	1.8 (1.2)	2.2 (2.2)	1.5 (1.1)*	0.61

Arithmetic change from Baseline shown; p-values are from least squares mean analyses of change from Baseline scores (absolute and % change).
Data are reported as the mean (SD), unless otherwise indicated.
The Baseline analysis period consists of Days -6 to -2, the Treatment analysis period consists of Days 3 to 27.
* p<0.05 Change from baseline

Clinical Trial Registration Number: NCT01742208

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Efficacy and safety of dapagliflozin in patients with type 2 diabetes mellitus and concomitant heart failure

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Background and aims: Over 40% of patients with heart failure (HF) have type 2 diabetes mellitus (T2DM); a number that is expected to increase due to rising prevalence of both conditions. Yet, remarkably little is known about the optimal glycaemic management in this patient group. Dapagliflozin (DAPA), a sodium-glucose co-transporter 2 inhibitor, promotes renal glucose excretion, causing osmotic diuresis, weight loss and decreased blood pressure (BP). These effects may provide a unique benefit to patients with coexisting T2DM and HF, but have not been formally evaluated in this group.

Materials and methods: We pooled data from 5 clinical trials, selecting patients randomised to DAPA 10 mg or placebo that had a documented history of HF. Using longitudinal repeated-measures models, we examined the effects of DAPA vs placebo on HbA1c, weight and BP for up to 1 year in patients with T2DM and HF. Safety was also assessed.

Results: In total, 171 patients received DAPA 10 mg and 149 patients received placebo across the 5 studies (age 64 years, T2DM duration 13.2 years, HbA1c 8.18%, ~50% with New York Heart Association [NYHA] class ≥2 HF). Patients receiving DAPA experienced clinically meaningful placebo-adjusted declines in HbA1c (−0.55%; 95% CI −0.80, −0.30), weight (−2.67 kg; 95% CI −3.88, −1.47) and systolic BP (−2.1 mmHg; 95% CI −5.68, 1.57) over 52 weeks; there was no change in heart rate in either group. The rates of orthostatic hypotension, syncope and hypoglycaemia were similar between the two groups. More patients on DAPA vs placebo had a decrease in creatinine clearance (11 [6.4%] vs 2 [1.3%]) and an increase in creatinine (7 [4.1%] vs 2 [1.3%]); however, absolute difference in estimated GFR from baseline to 50 weeks was minimal (−2.6 vs −1.4 mL/min/1.73 m^2 with DAPA vs placebo).

Conclusion: Treatment with DAPA 10 mg vs placebo produced clinically meaningful reductions in HbA1c, weight and systolic BP in patients with T2DM and HF, and was well tolerated. The weight and BP effects of DAPA may lead to improvement in HF-related symptoms in this patient population, which should be further investigated in prospective studies.

Clinical Trial Registration Number: NCT00663260, NCT00680745, NCT00673231, NCT01031680, *NCT01042977*

Supported by: AstraZeneca

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Consistent weight changes irrespective of baseline HbA_{1c} with the combination of empagliflozin/linagliptin in subjects with type 2 diabetes

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Background and aims: Two Phase III studies evaluated the efficacy and safety of once daily combinations of empagliflozin/linagliptin (EMPA/LINA) as initial therapy or add-on to metformin (MET) in subjects with type 2 diabetes (T2DM) for 52 weeks. Change from baseline in weight at week 24 was a key secondary endpoint in both studies. In both studies, EMPA/LINA led to significant reductions in weight vs LINA but not vs EMPA at week 24. In pre-specified subgroup analyses, we assessed the influence of baseline HbA1c on weight changes at week 24 with EMPA/LINA vs the individual components.

Materials and methods: Changes from baseline in weight at week 24 were analysed by subgroups of baseline HbA1c (<8.5% and ≥8.5%) in subjects randomised to EMPA 25 mg/LINA 5 mg, EMPA 10 mg/LINA 5 mg, EMPA 25 mg, EMPA 10 mg, or LINA 5 mg as initial therapy or as add-on to stable-dose MET.

Results: EMPA/LINA significantly reduced weight vs LINA in both HbA1c subgroups as add-on to MET but only in subjects with HbA1c <8.5% as initial therapy (Table). There were no significant differences in changes in weight with EMPA/LINA vs EMPA as initial therapy or add-

on to MET in either HbA1c subgroup. The treatment and baseline HbA1c interaction was not significant, suggesting no differential treatment effect across HbA1c subgroups (p=0.308 and p=0.649 for interaction when EMPA/LINA was given as initial therapy and add-on to MET, respectively).

Conclusion: The degree of glycemic control at baseline had no effect on weight changes with EMPA/LINA given as initial therapy or add-on to MET for 24 weeks in subjects with T2DM.

	EMPA 25 mg/ LINA 5 mg	EMPA 10 mg/ LINA 5 mg	EMPA 25 mg	EMPA 10 mg	LINA 5 mg
Changes in weight					
Initial therapy					
HbA1c <8.5% at baseline, n	**88**	**95**	**97**	**94**	**99**
Baseline weight, kg	88.3 (1.9)	87.5 (1.8)	87.4 (2.1)	88.9 (2.5)	89.2 (2.0)
Change from baseline	-2.5 (0.5)	-3.2 (0.4)	-2.9 (0.4)	-2.4 (0.4)	-1.0 (0.4)
Difference vs EMPA 25 mg/10 mg (95% CI)	0.4 (-0.9, 1.6)	-0.8 (-2.0, 0.4)			
Difference vs LINA 5 mg (95% CI)	-1.6 (-2.8, -0.4)**	-2.2 (-3.4, -1.0)***			
HbA1c ≥8.5% at baseline, n	**46**	**40**	**36**	**38**	**34**
Baseline weight, kg	87.1 (2.9)	86.9 (3.3)	84.9 (2.7)	85.2 (3.8)	90.5 (3.6)
Change from baseline	1.0 (0.6)	1.7 (0.7)	0.2 (0.7)	2.0 (0.7)	0.2 (0.7)
Difference vs EMPA 25 mg/10 mg (95% CI)	-0.9 (-2.7, 1.0)	0.3 (-1.6, 2.1)			
Difference vs LINA 5 mg (95% CI)	-0.8 (-2.7, 1.1)	-1.5 (-3.4, 0.4)			
Add-on to MET					
HbA1c <8.5% at baseline, n	**102**	**105**	**104**	**102**	**95**
Baseline weight, kg	85.6 (2.1)	86.8 (1.9)	89.0 (1.7)	86.2 (1.8)	86.6 (2.0)
Change from baseline	3.1 (0.4)	2.8 (0.4)	3.4 (0.4)	2.4 (0.4)	0.9 (0.4)
Difference vs EMPA 25 mg/10 mg (95% CI)	0.3 (-0.7, 1.2)	-0.4 (-1.3, 0.6)			
Difference vs LINA 5 mg (95% CI)	-2.2 (-3.1, -1.2)***	-1.8 (-2.8, -0.9)***			
HbA1c ≥8.5% at baseline, n	**32**	**30**	**36**	**35**	**33**
Baseline weight, kg	85.0 (3.1)	86.0 (3.5)	84.0 (3.1)	86.1 (3.2)	80.4 (2.7)
Change from baseline	-2.8 (0.6)	-2.0 (0.7)	-2.6 (0.6)	-2.8 (0.6)	0.1 (0.6)
Difference vs EMPA 25 mg/10 mg (95% CI)	-0.1 (-1.8, 1.6)	0.8 (-0.9, 2.6)			
Difference vs LINA 5 mg (95% CI)	-2.8 (-4.6, -1.1)**	-2.1 (-3.8, -0.3)*			

Baselines are mean (SE), changes from baseline are adjusted mean (SE) based on ANCOVA in patients who received ≥1 dose of study medication who had a baseline and an on-treatment HbA1c measurement, with last observation carried forward imputation.
*p<0.05; **p<0.01; ***p<0.001 for difference.

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Efficacy of SGLT2 inhibitors on bone mineral density in Japanese patients with type 2 diabetes

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Background and aims: Sodium-glucose co-transporter-2 (SGLT2) inhibitor enhances glucose excretion in urine, and reduces blood glucose in type 2 diabetic patients. In addition to glycemic control effects, it is reported that SGLT2 inhibitor lowers serum lipid profile and body weight. However, few studies have examined the effects of SGTL2 inhibitor on bone density. In the present study, we evaluated change of bone mineral density in Japanese patients with type 2 diabetes treated with SGLT2 inhibitors.

Materials and methods: The subjects were 115 type 2 diabetic outpatients, consisting of 77 men and 38 women (52±9 and 54±12 years old, respectively; mean ± SD) who were orally treated with any one of three SGLT2 inhibitors (luseogliflozin 2.5 mg qd, dapagliflozin 5 mg qd or tofogliflozin 20 mg qd) for 6 months. We measured BMI, hemoglobin and HbA_{1c} levels, serum uric acid (UA) level, renal function parameter (serum creatinine and urine creatinine and albumin levels), and bone mineral density (BMD) before and after the drug treatment.

Results: After the 6-month treatment, SGLT2 inhibitors significantly reduced BMI and HbA_{1c} level in both male and female patients (BMI in male: from 29.2±4.9 to 28.4±4.8 kg/m^2, p<0.01; in female: from 28.4 ±5.1 to 27.6±5.0 kg/m^2, p<0.01; HbA_{1c} in male: from 7.1±1.2 to 6.9± 0.9%, p<0.05; in female: from 6.8±0.9 to 6.7±0.7%, p<0.05). Hemoglobin level was significantly elevated (in male: from 14.6±1.1 to 15.6± 1.2 g/dL, p<0.01; in female: from 12.8±1.1 to 13.8±1.1 g/dL, p<0.01). Although significant changes were not detected in serum creatinine and urine creatinine and albumin levels, UA level was lowered (in male: from 5.6±1.3 to 5.0±1.3 mg/dL, p<0.01; in female: from 5.0±1.4 to 4.1± 1.2 mg/dL, p<0.01). BMD compared to that of a young adult mean was raised (in male: from 87±15 to 90±15%, p<0.01; in female: from 94±15 to 97±14%, p<0.01).

Conclusion: The treatment of SGLT2 inhibitors reduced BMI and HbA_{1c} level. Interestingly, SGLT2 inhibitors raised BMD compared to that of a young adult mean. Since it was reported that SGLT2 inhibitor preserves renal function in diabetic patients, the improvement of BMD seen in this study may be attributed to alteration of the renal function including production of active vitamin D. In addition, SGLT2 inhibitors decreased UA level although the detailed mechanisms were not cleared in this study. Some of antihypertensive agents elevate UA level and reduce BMD. Thus, SGLT2 inhibitors could also prevent such adverse effects. On the other hand, SGLT2 inhibitors increased hemoglobin level. These changes may derived from diuretic effects of SGLT2 inhibitors and consequent body fluid reduction. In the present study, it is suggested that SGLT2 inhibitors are potentially useful to reduce fracture risk while the treatment require attention to dehydration in type 2 diabetic patients.

Clinical Trial Registration Number: UMIN000016921

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Comparative persistency with newer agents to treat type 2 diabetes in the US: canagliflozin vs dipeptidyl peptidase-4 inhibitors and glucagon-like peptide-1 agonists

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Background and aims: Canagliflozin is an agent approved for the treatment of type 2 diabetes mellitus (T2DM) that inhibits sodium glucose cotransporter 2 (SGLT2), and as such, has been shown to reduce not only HbA1c, but also weight and blood pressure with an acceptable tolerability profile. In contrast, dipeptidyl peptidase-4 (DPP-4) inhibitors do not impact weight and blood pressure, and tolerability issues have been documented with glucagon-like peptide-1 (GLP-1) agonists. As these different treatment profiles may impact treatment persistency in the real world, retrospective claims data were used to estimate the time to discontinuation of canagliflozin versus these alternatives.

Materials and methods: Patients with T2DM who received a first prescription for a DPP-4 inhibitor (sitagliptin, saxagliptin, linagliptin), GLP-1 agonist (liraglutide, exenatide, exenatide long-acting), or canagliflozin in 2013 were extracted from two United States claims databases of commercially insured patients (Truven, Optum). The analytical sample included only patients with ≥6 months of retrospective data prior to their first paid claim. Discontinuation was defined as an observed refill gap ≥90 days (sensitivity analysis for 30/60 days) between two subsequent prescriptions. Time to discontinuation was analysed using Kaplan-Meier and Cox proportional hazards regression, including demographics, treatment background, and diabetes-related complications/comorbidities as covariates.

Results: 66,206 patients (mean age, 52.6 years; 50% male; median/ maximum follow-up, 10.1/19.0 months) were identified in the Truven database. After one year, the percentage of patients still on treatment was significantly higher with canagliflozin 100 mg (n=7,445; 64.0%) and 300 mg (n=4,486; 65.0%) versus DPP-4 inhibitors (30.2% [linagliptin] to 50.1% [sitagliptin]) and GLP-1 agonists (24.3% [exenatide] to 43.0% [liraglutide]) (P <0.0001 for all comparisons). The adjusted hazard ratio (HR) for time to discontinuation for canagliflozin 100 mg (reference) and 300 mg (HR=0.92 [0.86; 0.99]) was significantly lower versus DPP-4 inhibitors and GLP-1 agonists: sitagliptin (n=29, 426; HR=1.28 [1.22; 1.34]); saxagliptin (n=1,566; HR=2.01 [1.86; 2.16]); linagliptin (n=1,432; HR=2.08 [1.92; 2.24]); exenatide (n=2, 376; HR=2.59 [2.41; 2.77]); exenatide long-acting (n=5,922; HR=1.46 [1.40; 1.52]); liraglutide (n=17,690; HR=1.23 [1.20; 1.27]). Being younger, male, and being on monotherapy were associated with higher discontinuation risk. HRs were stable across sensitivity analyses using

alternative discontinuation definitions. Analyses from Optum were generally consistent with these results.
Conclusion: These analyses indicate that patients who received canagliflozin versus DPP-4 inhibitors or GLP-1 agonists remained on their therapy longer, which may reflect better effectiveness and/or tolerability.
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Sodium-glucose cotransporter 2 inhibitor increases circulating zinc-α2-glycoprotein levels in patients with type 2 diabetes
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Background and aims: Zinc-α2-Glycoprotein (ZAG) has recently been characterized as a potent metabolic regulator, but the effect of antidiabetic agents on circulating ZAG in humans remains unknown. Our aim was to study the effects of Sodium-glucose cotransporter 2 inhibitors (SGLT2) inhibitors on circulating ZAG and adiponectin (ADI) in newly diagnosed type 2 diabetes mellitus (nT2DM).
Materials and methods: 171 subjects with nT2DM were assigned to receive placebo or SGLT2 inhibitors for 3 months. Before and after treatment, ZAG and ADI concentrations were measured by ELISA.
Results: Circulating ZAG and ADI levels in nT2DM patients were significantly lower than in the controls ($P<0.01$). After 3-months of SGLT2 inhibitors therapy, HbA1c, fasting blood glucose (FBG), postprandial glucose (2 h-PBG), free fatty acides (FFA), triglyceride (TG), blood pressure, body mass index (BMI), waist-to-hip ratio (WHR), body weight, the percentage of fat in vivo (FAT%), fasting insulin (FINS), and homeostasis model assessment of insulin resistance (HOMA-IR) in T2DM patients decreased significantly ($P<0.05$ or $P<0.01$), whereas high- density lipoprotein cholesterol (HDL-C) was significantly increased ($P<0.05$). Importantly, circulating ZAG and ADI levels in nT2DM patients were also significantly increased after SGLT2 inhibitors therapy (both $P<0.01$). The change of circulating ZAG (ΔZAG) was associated with ΔTG, Δ total cholesterol (TC), ΔHDL-C and ΔFFA, whereas ΔADI associated Δ HDL-C, Δ2-h insulin after glucose load (2 h-INS) and ΔLDL-C.
Conclusion: These findings suggest that ZAG and ADI can be regulated by SGLT2 inhibitors, and SGLT2 inhibitors may play a physiologic role in enhancing insulin sensitivity.
Clinical Trial Registration Number: ChiCTR-OCS-13003185
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PS 067 GLP-1 receptor agonists: exenatide and liraglutide

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Three-year efficacy and safety of exenatide once weekly: a pooled analysis of three trials
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Background and aims: Long-term data for glucose-lowering therapies (eg, glucagon-like peptide-1 receptor agonists) help to inform drug selection for treatment strategies.
Materials and methods: This *post hoc* analysis examined the efficacy and safety of exenatide once weekly (QW) in pooled completer data (N= 329) from extensions (2.5-3 years) of three trials (DURATION-1, -2, -3); 3 year data for insulin glargine (IG) was included for reference (N=158), as IG was a long-term comparator in one trial (DURATION-3).
Results: Baseline mean age, weight, and HbA1c were 56/58 years, 93.7/ 90.3 kg, and 8.3/ 8.3%, in the exenatide QW/IG groups, respectively. After 3 years, patients receiving exenatide QW had improvements from baseline in HbA1c, fasting glucose, body weight, and some cardiovascular risk markers (Table). A total of 101 (30.7%) exenatide-treated patients and 26 (16.5%) IG-treated patients achieved the stringent goal of HbA1c ≤6.5%, and 95 (28.9%) and 11 (7.0%), respectively, achieved the Composite goal of HbA1c <7%, no hypoglycaemia, and no weight gain. Diarrhoea, nausea, vomiting, and injection-site pruritus occurred in 24.0%, 22.2%, 10.6%, and 8.2% of patients, respectively, receiving exenatide QW and in 8.2%, 2.5%, 3.2%, and 0% of patients, respectively, receiving IG. Major and minor hypoglycaemia were more frequent with sulphonylurea than without (exenatide QW: 32.6% vs 10.0%; IG: 70.2% vs 45.9%, respectively).
Conclusion: Long-term exenatide QW treatment improved multiple outcomes in a large, pooled cohort of patients. Exenatide QW was associated with weight loss and a lower risk of hypoglycaemia than the reference IG treatment, but had a higher incidence of gastrointestinal and injection-site events.

Table. Changes from Baseline in Outcomes at 156 Weeks

Parameter	Exenatide QW (N=329)	Insulin Glargine (N=158)
HbA1c (%), mean ± SEM	−1.06 ± 0.07*	−0.82 ± 0.08*
HbA1c goal <7%, n (%)	159 (48.3)	59 (37.3)
Fasting glucose (mmol/L), mean ± SEM	−1.7 ± 0.16*	−2.8 ± 0.27*
Body weight (kg), mean ± SEM	−2.4 ± 0.3*	2.0 ± 0.4*
Systolic BP (mmHg), mean ± SEM	−1.3 ± 0.9	1.5 ± 1.2
LDL cholesterol (µmol/L), mean ± SEM	−0.19 ± 0.06*	−0.17 ± 0.08*
HDL cholesterol (µmol/L), mean ± SEM	0.11 ± 0.01*	0.04 ± 0.02

* $P<0.05$ vs baseline; QW, once weekly.

Clinical Trial Registration Number: NCT00308139, NCT00637273, NCT00641056
Supported by: AstraZeneca

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Treatment of patients with type 2 diabetes mellitus and baseline HbA_{1c} ≥10% with exenatide twice daily, exenatide once weekly, or basal insulin: a pooled analysis of 20 randomised controlled trials

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Background and aims: Guidelines recommend insulin-based injectable therapy for type 2 diabetes mellitus (T2DM) patients presenting with HbA1c ≥10%-12%, to rapidly minimise glucotoxicity. We explored the effects of a glucagon-like peptide-1 receptor agonist, exenatide, on glycaemic control in patients with very high HbA1c, to determine whether options other than basal insulin may be shown to be appropriate for such patients. In individual randomised controlled trials (RCTs) with a mean baseline HbA1c of 8.1%-8.5%, HbA1c reduction with exenatide twice daily (BID) was noninferior to basal insulin and with exenatide once weekly (QW) was superior to basal insulin; however, no previous assessment of these agents has been conducted in the subset of patients with HbA1c ≥10%.

Materials and methods: Using pooled intention-to-treat (ITT) data from 4,340 patients treated for ≥24 weeks in 20 RCTs, we evaluated glycemic parameters for exenatide BID, exenatide QW and basal insulin, in a subpopulation of patients with baseline HbA1c ≥10%. Most patients in this subgroup (>80%) also used metformin-and/or sulfonylurea (SU) as background therapy.

Results: Reductions from baseline in HbA1c were significant from Week 2 (earliest timepoint; $P<0.001$) with exenatide BID, Week 4 ($P<0.001$) with exenatide QW, and Weeks 12 and 10 (earliest timepoints with available patient data; both $P<0.001$) for the corresponding basal insulin groups. Endpoint reductions in HbA1c (Table) were comparable between exenatide BID and basal insulin, and numerically greater with exenatide QW than basal insulin. Significant reductions from baseline in fasting glucose were observed at endpoint (Table) that were greater with basal insulin. A higher proportion of patients achieved an HbA1c of <7% or ≤6.5% with exenatide QW than with basal insulin. Weight significantly decreased with exenatide and increased with basal insulin. Major and minor hypoglycaemia incidence was numerically higher in SU vs non-SU users and with basal insulin.

Conclusion: In this post-hoc analysis, exenatide BID and QW achieved marked reductions in HbA1c in patients with T2DM and baseline HbA1c ≥10%. Exenatide BID and QW may be appropriate treatment alternatives to basal insulin for patients with HbA1c ≥10%.

Table. Endpoint parameters in patients with baseline HbA1c ≥10%.

Parameter	Exenatide BID Studies		Exenatide QW Studies	
	Exenatide BID (N=85)	Insulin (N=38)	Exenatide QW (N=168)	Insulin (N=21)
Mean ± SD baseline HbA1c (%)	10.4 ± 0.4	10.5 ± 0.6	10.5 ± 0.4	10.4 ± 0.4
Mean ± SEM change in HbA1c (%)	−2.0 ± 0.2*	−2.1 ± 0.2*	−2.6 ± 0.1*	−2.1 ± 0.2*
Achieved HbA1c <7% / ≤6.5% (%)	16.5 / 5.9	13.2 / 5.3	29.8 / 15.5	4.8 / 0.0
Mean ± SD baseline FG (mmol/L)	12.7 ± 2.4	14.7 ± 3.1	12.3 ± 3.0	12.6 ± 2.8
Mean ± SEM change in FG (mmol/L)	−2.0 ± 0.4*	−5.0 ± 0.7*	−3.7 ± 0.3*	−4.8 ± 3.0*
Minor + major hypoglycaemia (n/N [%])				
With SU	18/60 (30.0)	12/35 (34.3)	3/84 (3.6)	2/9 (22.2)
Without SU	3/25 (12.0)	0/3 (0.0)	1/84 (1.2)	2/12 (16.7)

*$P<0.001$ vs baseline. BID, twice daily; FG, fasting glucose; QW, once weekly; SU, sulfonylurea.

Clinical Trial Registration Number: NCT00039013, NCT00039026, NCT00035984, NCT00360334, NCT00359762, NCT00577824, NCT00082381, NCT00082407, NCT00381342, NCT00603239, NCT00434954, NCT00375492, NCT00308139, NCT00637273, NCT00877890, NCT01003184, NCT00641056, NCT00676338, NCT00917267
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Responses to exenatide twice daily, basal insulin or placebo with oral medications over different durations of type 2 diabetes mellitus: Does diabetes duration matter?

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Background and aims: Type 2 diabetes is a progressive disease; thus, patients with longer disease durations are usually more difficult to treat. Using the exenatide clinical development database, we investigated whether patients with longer durations of type 2 diabetes had lesser responses to therapy, and whether certain therapy combinations appeared more effective at certain type 2 diabetes durations.

Materials and methods: We compared the efficacy of exenatide twice daily (BID) with basal insulin therapy or placebo in patients with longer type 2 diabetes durations, and examined whether use of concomitant metformin, sulfonylurea, or both, affected response after 16-30 weeks of treatment, based on the duration of type 2 diabetes. We analysed intention-to-treat data from 14 studies of ≥16 weeks' duration with exenatide BID (N=2792) compared with basal insulin(N=935) or placebo (N=1092) in patients with type 2 diabetes treated with diet and exercise with or without ≥1 oral drug(s) (mean HbA1c 8.3%, type 2 diabetes duration 7.7 years). Patients continued existing treatment (metformin, n=1020; sulfonylurea, n=464; metformin + sulfonylurea, n=2433).

Results: Overall results from the metformin population showed that baseline HbA1c increased with type 2 diabetes duration (≤2 years, 7.83%; >2-≤5 years, 8.05%; >5-≤10 years, 8.14%; >10-≤15 years, 8.45%; >15 years, insufficient sample size), supporting concerns for loss of efficacy of metformin. However, correlations between improvement in efficacy variables (HbA1c, fasting glucose and weight) and type 2 diabetes duration were weak for all drug combinations studied. The table shows HbA1c changes and goal achievement by type 2 diabetes duration categories and concomitant oral drug(s). Responses with exenatide BID and basal insulin were similar within each type 2 diabetes duration category, which was particularly evident among patients on concomitant metformin + sulfonylurea (the largest concomitant treatment subgroup).

Conclusion: Our analysis indicated that a longer type 2 diabetes duration was not associated with a reduced response to exenatide BID compared with basal insulin; however, the majority of patients with longer durations of type 2 diabetes needed multiple therapies to achieve an HbA1c of 7%.

Table. HbA1c mean change from baseline / % achieving HbA1c <7%						
Oral drug	Study treatment	≤2 y	>2–≤5 y	>5–≤10 y	>10–≤15 y	>15 y
Metformin only	Exenatide BID	–0.8%* / 44.9% [n=154]	–0.8%* / 38.7% [n=175]	–0.6%* / 31.3% [n=135]	IS [n=49]	IS [n=17]
	Basal insulin	–0.8%* / 50.8% [n=60]	–0.9%* / 45.3% [n=75]	–1.1%* / 41.1% [n=69]	IS [n=29]	IS [n=10]
	Placebo	–0.0% / 27.8% [n=52]	–0.2% / 25.0% [n=62]	IS [n=49]	IS [n=14]	IS [n=8]
Sulfonylurea only**	Exenatide BID	–0.7%* / 43.1% [n=54]	–0.8%* / 20.7% [n=81]	–0.8%* / 23.8% [n=93]	IS [n=27]	IS [n=24]
Metformin + sulfonylurea	Exenatide BID	–0.5%* / 26.1% [n=78]	–0.9%* / 22.8% [n=288]	–1.0%* / 24.7% [n=496]	–1.1%* / 29.7% [n=228]	–1.0%* / 21.7% [n=174]
	Basal insulin	IS [n=41]	–1.0%* / 18.0% [n=117]	–1.1%* / 25.4% [n=201]	–1.1%* / 15.7% [n=125]	–1.1%* / 22.0% [n=79]
	Placebo	IS [n=34]	+0.2% / 15.4% [n=109]	+0.1% / 9.8% [n=157]	–0.1% / 10.5% [n=86]	–0.1% / 15.5% [n=51]
*P<0.05 vs baseline; **Basal insulin and PBO data not shown due to IS in each category; BID, twice daily; IS=insufficient sample size (<50); y, years.						

NCT00039013, NCT00039026, NCT00035984, NCT00360334, NCT00577824, NCT00082381, NCT00082407, NCT00099619, NCT00099320, NCT00324363, NCT00381342, NCT00603239, NCT00434954, NCT00375492

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The effect of added exenatide twice daily or bolus insulin on insulin glargine and total insulin dose with improved HbA_{1c}: subgroup analysis of the 4B study

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Background and aims: Persistent hyperglycaemia after insulin glargine (IG) up-titration necessitates treatment intensification. With multiple intensification options available for patients failing basal insulin, individualization of therapy becomes possible. A subgroup of particular interest is patients on high IG doses, who may respond less to additional therapies.

Materials and methods: This *post hoc* analysis examined responses to exenatide twice daily (BID) or insulin lispro (IL) added to IG + metformin in patients with type 2 diabetes mellitus (T2DM) according to IG dose tertile at randomisation. Baseline values in the exenatide BID/IL arms for total insulin were 33/33 U for tertile 1, 53/52 U for tertile 2 and 94/96 U for tertile 3. Postrandomisation, the daily IG dose was reduced by 33-50% and converted to IL in the IL arm, and reduced by ≥10% in patients with HbA1c ≤8.0% in the exenatide BID arm.

Results: The analysis included 627 patients. Baseline body weight with exenatide BID and IL was 81/82 kg for tertile 1, 90/87 kg for tertile 2 and 99/98 kg for tertile 3; baseline HbA1c was 8.2/8.0% for tertile 1, 8.2/8.1% for tertile 2 and 8.4/8.5% for tertile 3. At Week 30, HbA1c reductions with exenatide BID and IL were similar across tertiles (Table); fasting glucose decreased with exenatide BID in tertiles 1 and 3. Exenatide BID reduced body weight, while IL increased body weight. Proportions of patients achieving HbA1c <7.0% with exenatide BID and IL were generally similar across tertiles. In each tertile, greater proportions of patients treated with exenatide BID compared with IL achieved the composite goal of HbA1c <7.0%, no weight gain, and no major or minor hypoglycaemia. Tertile 3 had the numerically greatest IG dose reductions, but still had the highest total insulin dose at endpoint. Adverse events (AEs) were generally similar across tertiles within treatment groups. Gastrointestinal AEs were more common with exenatide BID compared with IL (tertile 1: 46% vs 17%; tertile 2: 48% vs 13%; tertile 3: 46% vs 9%). The major and minor hypoglycaemia event rate/patient-year was lower with exenatide BID than with IL (tertile 1: 1.7 vs 5.2; tertile 2: 2.3 vs 6.0; tertile 3: 2.6 vs 4.8).

Conclusion: Patients with the highest baseline IG doses were able to sustain the largest reductions in the IG dose in both treatment groups, but only those treated with exenatide BID used less total insulin, with similar improvement in HbA1c. Adding exenatide BID or IL to patients on low or high insulin doses improved glycaemic control to a similar extent, with weight loss and less hypoglycaemia in the exenatide BID group, regardless of baseline IG dose. There was a higher incidence of gastrointestinal AEs with exenatide BID compared with IL.

Table. Outcomes Across IG Dose Tertiles After 30 Weeks'

Treatment With Exenatide BID or IL Added to IG + Metformin

Parameter	Tertile 1		Tertile 2		Tertile 3	
	Exenatide BID (N=108)	IL (N=102)	Exenatide BID (N=101)	IL (N=107)	Exenatide BID (N=106)	IL (N=103)
Mean HbA1c (%), endpoint / change	7.3 / –0.9	7.1 / –0.9	7.2 / –1.0	7.2 / –1.0	7.3 / –1.2	7.3 / –1.2
Mean HbA1c (mmol/mol), endpoint	56.4	54.4	54.8	54.9	56.7	56.3
HbA1c goal <7.0%, n (%)	48 (44)	47 (46)	45 (45)	42 (39)	41 (39)	36 (35)
HbA1c <7.0%, no weight gain, and no hypoglycaemia, n (%)	30 (28)	12 (12)	18 (18)	6 (6)	17 (16)	8 (8)
Mean FSG (mmol/L), endpoint / change	6.7 / –0.4*	7.3 / +0.5	6.7 / –0.2	6.9 / +0.1	6.7 / –0.8*	7.6 / 0.0
Mean change in body weight (kg)	–2.4**	+1.4	–2.6**	+1.9	–2.4**	+2.2
Mean change in IG dose (U)	–0.7	–3.2	–4.2	–5.2	–10.4*	–19.9
Mean total insulin (IG + IL) dose at endpoint (U)†	32.5	58.2	48.3	84.4	84.3	136.6
*P<0.05, **P<0.001 vs IL. BID, twice daily; FSG, fasting serum glucose; IG, insulin glargine; IL, insulin lispro. †No inferential statistical comparison was conducted.						

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Safety and tolerability of liraglutide versus sulphonylurea in people with type 2 diabetes before, during and after Ramadan fasting: a randomised trial (LIRA-Ramadan)

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Background and aims: Fasting during Ramadan is challenging for people with diabetes due to 5- and 7.5-fold increased risks of severe hyper- and hypoglycaemia, respectively. Data are needed to guide planning and treatment. We report safety and tolerability data from the LIRA-Ramadan trial, which compared liraglutide (lira) with sulphonylurea (SU) in people with T2D during Ramadan.
Materials and methods: In this open-label trial, adults (HbA1c 7-10%; BMI >20 kg/m^2; stable SU + metformin [Met]) intending to fast during Ramadan were randomised to switch to once daily lira 1.8 mg (N=172) or continue pretrial SU (N=171), both + Met, then followed for ≤32 weeks. Lira was dose-escalated for 3-4-weeks then administered at ≤1.8 mg for 6-19-weeks before Ramadan (4 weeks). Changes in SU dose and/or timing using ADA recommendations were allowed during Ramadan. Primary endpoint was change in fructosamine (FA) from start to end of Ramadan (lira, N=151; SU, N=165). Treatment continued for 4 weeks post Ramadan.
Results: There was a significantly greater reduction in FA from baseline to end of Ramadan with lira vs SU (estimated treatment difference [ETD] [95% CI]: -10.3 [-18.7, -1.89] μmol/L; p=0.0165). Despite lower FA and HbA1c at start of Ramadan in the lira arm, similar FA reductions occurred with lira and SU during Ramadan (ETD: 3.51 [-5.26, 12.28] μmol/L; p=0.4311). Results were similar for HbA1c (data not shown). Nonetheless, significantly fewer confirmed hypoglycaemic events (ADA defined) occurred with lira vs SU over the study period (estimated rate ratio for lira/SU: 0.2 [0.1, 0.5]; p=0.0027). No nocturnal hypoglycaemic events occurred with lira (vs 3 with SU [42 events/1000 subject years of exposure]). During the trial, 77% of people switching to lira and 57% continuing on SU experienced adverse events (AEs). Few serious AEs were observed (lira, 2.9%; SU, 1.2%). More people had GI AEs with lira than SU, commonly nausea (generally transient; 33% vs 0%), diarrhoea (22% vs 4%) and vomiting (21% vs 1%). There were no cases of acute pancreatitis. The proportion of withdrawals was similar for lira and SU (15.1% and 14.0%, respectively). With lira, 20/26 withdrawals occurred before Ramadan. The most common reason for withdrawal with lira was an AE: 11 participants in the lira arm (6%) withdrew due to an AE (n=10 before Ramadan) vs 0 in the SU arm. With SU, 11/24 withdrawals occurred during Ramadan, and the most common withdrawal reasons were fasting plasma glucose exceeding the protocol limit (n=7), or not fasting in Ramadan (n=3). No withdrawals during Ramadan were due to AEs. No significant differences between lira and SU were observed for change in overall treatment satisfaction (Diabetes Treatment Satisfaction Questionnaire [DTSQ]) or Short Form-36 Health status questionnaire scores during Ramadan. A non-significant difference in perceived frequency of hypoglycaemia was observed for lira vs SU (DTSQ ETD -0.297 [-0.701, 0.106]; p=0.1480).
Conclusion: A significantly lower risk of hypoglycaemic events was seen with lira vs SU, despite lower levels of FA and HbA1c. No new safety concerns were identified with lira during the trial, which included a physiologically challenging fasting period during Ramadan.
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The addition of liraglutide 1.8 mg to insulin in type 1 diabetes does not improve patient-reported outcome measures

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Background and aims: In insulin treated type 2 diabetes (T2D) the addition of a glucagon-like peptide-1 receptor agonist (GLP-1RA) has improved both metabolic control, and patient satisfaction with treatment. In patients with type 1 diabetes (T1D) studies have reported positive effects on weight and glycaemic control by adding a GLP-1 RA to insulin treatment. However, as a potential future treatment of T1D it is important to evaluate the patient-reported outcomes.
Materials and methods: A total of 100 patients with T1D, poor glycaemic control, and overweight, were randomised to liraglutide 1.8 mg once daily or placebo as an add-on to intensive insulin therapy in a double-blind, parallel design (The Lira-1 Study). To evaluate patient-reported outcomes, we used the Diabetes Treatment and Satisfaction (DTSQs) and Problem Areas in Diabetes (PAID) questionnaires at baseline, after 3 weeks of treatment, and at the end of treatment (26 weeks). The DTSQs was divided into a treatment satisfaction section and a hypoglycaemia section.
Results: Mean baseline characteristics were similar between the liraglutide and placebo groups (liraglutide;placebo, mean ± SD) age 46 ±13;50±13 years, HbA1c 73±8;73±7 mmol/mol, total daily insulin dose 60±23;61±21 IU/day and bodyweight 93.0±14.3;93.7±13.2 kg except diabetes duration 20±12;27±12 years (p=0.009). Patient-reported outcomes were assessed in 73 patients (38 and 35 patients in the liraglutide and placebo groups, respectively). The overall satisfaction score for the DTSQs in the liraglutide group did not differ significantly from baseline 23.0±3.5 to 23.7±3.2 (p=0.18) after 3 weeks or at the end of treatment 24.1±3.4 (p=0.051). In the placebo group, the mean baseline score 23.7±4.1 showed no significant change after 3 weeks of treatment 24.3±3.1 (p=0.31), but increased to 25.5±3.3 (p=0.013) at the end of treatment. No significant differences were observed between the groups in changes or the overall satisfaction score at any time point (p>0.46). For the DTSQs hypoglycaemia section, the score in the liraglutide group increased from 4.7±1.4 to 5.4±1.0 (p=0.004) after 3 weeks of treatment, but showed no significant change 5.3±1.3 (p=0.055) at the end of treatment compared with baseline. In the placebo group, baseline 4.5±1.6, and 3 weeks score did not differ significantly 5.1±1.2 (p=0.075), but increased significantly to 5.3±0.9 (p=0.003) at the end of treatment compared with baseline. No significant differences were observed in the changes in the hypoglycaemia score between the groups at any time point (p>0.59). In the PAID questionnaire, the mean score in the liraglutide group decreased from 24.9±15.9 to 18.8±15.4 (*p< 0.0001*) after 3 weeks, and was 20.4±17.2 (p=0.029) at the end of treatment. In the placebo group, the mean baseline score of 22.6±14.4 showed no significant change at 3 weeks 19.3±13.3 (p=0.074) or at 26 weeks 20.7±15.8 (p=0.21). No significant differences were observed between the groups at any time point (p>0.21).
Conclusion: The addition of 1.8 mg liraglutide once-daily to insulin in overweight and poorly regulated patients with T1D did not improve the patient reported outcome measures DTSQs and PAID, during or after 26 weeks of treatment. These findings are in contrast to the improved treatment satisfaction reported in obese insulin treated T2D patients after add-on of the GLP-1 RA liraglutide to insulin.
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Liraglutide as add-on to insulin in normal-weight and poorly controlled patients with type 1 diabetes: the T1DMLIRA study

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Background and aims: Combination of a GLP-1 receptor agonist (GLP-1RA) with insulin is a novel, potential treatment strategy in patients with type 1 diabetes (T1D). Open-label and mechanistic studies indicate that GLP-1RA treatment induces weight loss, improves PPG excursions and reduces insulin requirements with improved or unaltered glycemic control in patients with T1D. This is the first randomized, double-blinded, placebo-controlled study to evaluate efficacy and safety of liraglutide as add-on to insulin treatment in normal-weight T1D patients without residual β-cell function

Materials and methods: In total 40 patients with T1D were randomized to once-daily liraglutide 1.2 mg or placebo adjunct to pre-existing insulin treatment for 12 weeks. Baseline characteristics were similar between groups (liraglutide; placebo): HbA1c 73±2; 72±2 mmol/mol, BMI 24.2±0.6; 22.8±0.4 kg/m2, diabetes duration 18±2 20±2 years, basal insulin 34±2; 34±4 IU/day, bolus insulin 28±3; 24±2 IU/day. Insulin dose was adjusted according to a treat-to-target algorithm. Primary endpoint was mean change in HbA1c from baseline to week 12

Results: After 12 weeks, changes in HbA1c from baseline were similar with liraglutide and placebo (table). Patients taking liraglutide had greater reductions in body weight and bolus insulin requirements compared with placebo (table). Changes in glycemic variability and frequency of hypoglycemia were similar between groups (table).Heart rate (HR) increased with liraglutide compared with placebo (between-group difference 2.44 beats/min (CI: -1.92 to 6.81), p=0.04). Simultaneously, systolic blood pressure (SBP) decreased with liraglutide compared with placebo-treated patients (between-group difference 3.21 mmHg (CI: -8.31 to 1.90), p=0.04). Gastrointestinal (GI) AEs were significantly more frequent with liraglutide than placebo (% pts): nausea 33; 23, dyspepsia 15; 5

Conclusion: In conclusion, liraglutide added to insulin significantly reduces body weight and bolus insulin requirements, but has no additional effect on HbA1c in T1D patients inadequately controlled on insulin alone. Frequency of hypoglycemia did not differ between groups. GI AEs were more frequent with liraglutide than placebo. HR increases and SBP decreases significantly with liraglutide compared with placebo

	Liraglutide (n=18)	Placebo (n=18)	P-value
Change in HbA1c (mmol/mol)$^{+}$	-6.2±1.8	-5.6±1.6	0.78
Change in weight, kg^{+}	-3.1±0.6	+1.2±0.4	<0.0001
Change in bolus insulin, IU^{+}	-4.0±1.3	0.0±1.0	0.02
Change in basal insulin, IU^{+}	-0.1±1.6	-0.1±1.5	0.99
Glycemic Variability$^{*,+,\Phi}$			
ΔTime in hypoglycemia, h	0.40±0.53	-0.14±0.40	0.42
ΔTime in normoglycemia, h	+0.21±0.90	0.70±1.04	0.72
ΔTime in hyperglycemia, h	-0.61±1.20	-0.56±1.17	0.98

* Assessed by blinded continuous glucose monitoring
$^{+}$ Values represent differences from week 0 to week 12.
$^{\Phi}$ Hypoglycemia: <3.9 mmol/L, normoglycemia: 3.9 – 10 mmol/L, Hyperglycemia > 10 mmol/L
Data are presented as mean±SEM, h=hours/day

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Effects of liraglutide vs lifestyle changes on subcutaneous and visceral fat, liver steatosis, insulin sensitivity and beta cell function after comparable weight loss

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Background and aims: Obesity, insulin resistance and beta cell deterioration are key issues in the development and progression of type 2 diabetes (T2DM). Given the concurrent effects acknowledged for GLP-1 agonists on body weight, fat mass, insulin resistance and beta cell preservation, we hypothesized that this class of drugs may exert additional actions on top of those anticipated for lifestyle intervention-mediated weight loss.

Materials and methods: Twenty-nine metformin-treated obese subjects with impaired glucose tolerance (IGT), impaired fasting glucose (IFG) or newly diagnosed T2DM, were randomized to liraglutide treatment (1.8 mg/d) or lifestyle counseling to assess whether changes in subcutaneous (SAT) and visceral (VAT) adipose tissue distribution and in degree of non-alcoholic fatty liver disease (NAFLD) (all assessed by MRI) after a modest and comparable weight loss (7% of initial body weight), might affect insulin sensitivity (Matsuda Index) and β-cell performance (by Insulin Secretion-Sensitivity Index-2 (ISSI-2)) during multiple sampling oral glucose tolerance test.

Results: At baseline, VAT was correlated directly with HbA1c (Rho= 0.374, p=0.045) and ISSI-2 (Rho=-0.378, p=0.043), and inversely with adiponectin (Rho=-0.470, p=0.010), whereas SAT was significantly and directly correlated with C-reactive protein (Rho=0.466, p=0.013), leptin (Rho=0.811, p<0.0001) and NAFLD degree (Rho=0.379, p=0.042). VAT, SAT and NAFLD grade were significantly and comparably reduced in both treatment groups, whereas insulin sensitivity was not significantly affected by any intervention. In contrast, the liraglutide group showed a significantly greater reduction in median VAT (p=0.001), as compared to the lifestyle group (-15.3% vs. -7.3% median decrease) and a greater improvement in beta cell function (ISSI-2) (96.3% vs. 29.8%, p= 0.006), which translated into a significantly more pronounced reduction in both fasting, 1-hour and 2-hour postprandial plasma glucose, despite comparably reduced HbA1c in both groups (by 7.0%). In the liraglutide arm, but not in the lifestyle arm, VAT percent decrease throughout the intervention period was significantly related to improvement in beta-cell function (ISSI-2, Rho=-0.60, p=0.023) and increase in adiponectin levels (Rho=-0-589, p=0.021). SAT and leptin percent changes were instead associated with improvement in insulin sensitivity, as reflected by Matsuda index (Rho=-0.722,p=0.002, and Rho=-0.708, p=0.003).

Conclusion: This pilot study may help establishing a cause-and-effect relationship between VAT inflammation, adipokine secretion, beta cell performance and development or progression of T2DM, unraveling as well potential mechanisms by which liraglutide may favorably impact T2DM pathogenesis.

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PS 068 GLP-1 receptor agonists: dulaglutide and albiglutide

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The efficacy and safety of once-weekly, subcutaneous dulaglutide monotherapy compared to glimepiride in Asian patients with type 2 diabetes mellitus

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Background and aims: This Phase 3, randomized, double-blind, parallel-arm 26-week (wk) monotherapy study compared the efficacy and safety of once weekly dulaglutide (DU), a long-acting glucagon-like peptide-1 (GLP-1) receptor agonist, with glimepiride in Asian patients with type 2 diabetes mellitus who had discontinued oral antihyperglycemic medication (OAM) monotherapy or were OAM-naïve.
Materials and methods: A total of 807 patients (all Asian; mean baseline age, 52.8 years; mean duration of T2DM, 3.7 years; HbA1c, 7.94%; weight, 70.0 kg) were randomly assigned to once-weekly DU 1.5 mg, DU 0.75 mg or glimepiride (1-3 mg/day) in a 1:1:1 ratio. The primary and key secondary objectives were to test the noninferiority (margin 0.4%) and superiority of DU 1.5 and 0.75 mg versus glimepiride using a prespecified gatekeeping method, as measured by A1C change from baseline at Week 26.
Results: Both DU 1.5 mg and DU 0.75 mg demonstrated superiority to glimepiride (Least-squares [LS] mean difference [%][95% CI]: -0.60% [-0.78%, -0.41%] and -0.31% [-0.49%, -0.13%], respectively). More patients achieved HbA1c target <7.0% in both DU 1.5 mg and DU 0.75 mg than glimepiride. LS mean decrease from baseline in fasting blood glucose was significantly greater for both DU 1.5 mg and DU 0.75 mg compared with glimepiride. Total hypoglycemia incidence was significantly lower in both DU 1.5 mg and DU 0.75 mg than glimepiride. No severe hypoglycemic events were reported. Weight decreased with DU and increased with glimepiride. The most common treatment-emergent gastrointestinal adverse events for DU 1.5 mg, DU 0.75 mg were diarrhea (14.9%, 6.3%), nausea (8.6%, 4.1%), abdominal distension (6.0%, 2.6%) and vomiting (6.3%, 1.1%).
Conclusion: Superiority in change from baseline HbA1c of both dulaglutide 1.5 mg versus glimepiride and dulaglutide 0.75 mg versus glimepiride were statistically demonstrated in this study. These results, combined with the overall efficacy, safety, and tolerability data, support the use of dulaglutide as one of monotherapy options in Asian patients with T2DM.

Efficacy Measures (26wk, mITT)	**Dula 1.5 mg (N=263)**	**Dula 0.75 mg (N=259)**	**Glimepiride (N=268)**
HbA_{1c} change, %, LS mean (SE)	-1.48 (0.069)†	-1.19 (0.069)†	-0.88 (0.069)
% of patients with HbA_{1c} <7.0%	73.8*	62.9	54.5
Fasting blood glucose change (mmol/L), LS mean (SE)	-2.66 (0.173)*	-2.09 (0.165)#	-1.68 (0.171)
Safety Measures (26wk, safety analysis population)	**Dula 1.5 mg (N=268)**	**Dula 0.75 mg (N=268)**	**Glimepiride (N=269)**
Weight change (kg), LS mean (SE)	-1.46 (0.185)*	-0.82 (0.186)*	0.96 (0.182)
Total hypoglycemia incidence (%)	4.9*	3.4*	14.9

Abbreviations: mITT=modified intent-to-treat; SE=standard error

†2-sided p<.05 for superiority vs glimepiride for HbA1c change

#2-sided p<.05 vs glimepiride

*2-sided p<.001 vs glimepiride

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Relationship between weight change and glycaemic control with once weekly dulaglutide treatment in patients with type 2 diabetes

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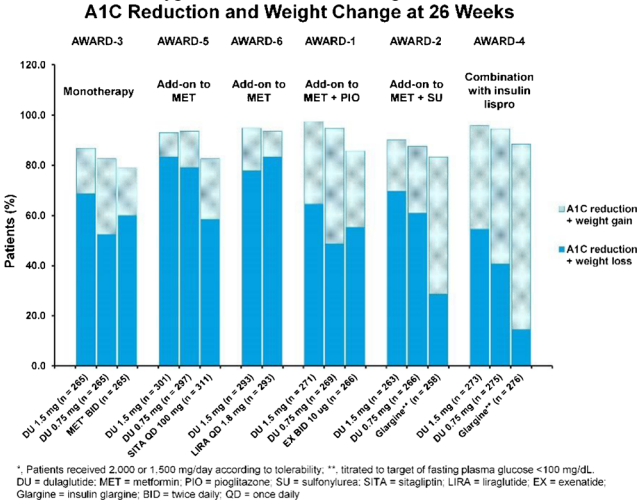
Background and aims: Dulaglutide, a once weekly GLP-1 receptor agonist, was studied in the AWARD clinical trial program in adult patients with type 2 diabetes. In 6 head-to-head phase 3 trials, dulaglutide demonstrated significant HbA1c reduction and weight control effects. To assess the relationship between weight change and glycaemic control in dulaglutide-treated patients, HbA1c and body weight data from the 6 trials were analysed.
Materials and methods: Due to differences in design, background therapy, and baseline characteristics, analyses on HbA1c and body weight data at 26 weeks were conducted by trial rather than by pooling.
Results: Across the studies 87%-97% and 83%-95% of patients treated with dulaglutide 1.5 mg and 0.75 mg, respectively, demonstrated HbA1c reduction. Among the patients with HbA1c reduction, the majority of patients receiving dulaglutide 1.5 mg experienced weight loss (55%-83%) while 41%-79% in the dulaglutide 0.75 mg arm lost weight. Minimal correlation was observed between the changes in HbA1c and weight (IRI <0.3, all). Baseline characteristics of gender, age, duration of diabetes, HbA1c, body weight and BMI did not correlate with different weight responses.
Conclusion: In summary, dulaglutide demonstrated a dose-dependent effect on both weight loss and HbA1c reduction. Dulaglutide is an effective treatment option with both HbA1c reduction and weight loss observed across the type 2 diabetes treatment spectrum.



*, Patients received 2,000 or 1,500 mg/day according to tolerability; **, titrated to target of fasting plasma glucose <100 mg/dL. DU = dulaglutide; MET = metformin; PIO = pioglitazone; SU = sulfonylurea; SITA = sitagliptin; LIRA = liraglutide; EX = exenatide; Glargine = insulin glargine; BID = twice daily; QD = once daily

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Baseline factors associated with the glycaemic response to treatment with once weekly dulaglutide in patients with type 2 diabetes

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Background and aims: Dulaglutide (DU) is a once weekly glucagon-like peptide-1 (GLP-1) receptor agonist that has been recently approved

in the US and EU for the treatment of type 2 diabetes. Glycaemic efficacy of DU has been demonstrated in six Phase 3 studies. The objective of this analysis is to determine major baseline factors that are associated with the reduction in glycosylated haemoglobin A_{1c} (HbA1c) in response to treatment with DU.

Materials and methods: DU patient (pt) covariates from across the 6 DU Phase 3 clinical trials were analysed using gradient boosting analysis to assess their relative contribution to the change in HbA1c at 26 weeks of treatment. Baseline variables which were associated with the greatest amount of influence on HbA1c change were further evaluated in univariate and multivariable modeling to assess the magnitude and direction of impact. Analysed baseline factors included age, race, ethnicity, gender, duration of diabetes, serum glucose measures, fasting serum C-peptide and insulin (FSI), weight, BMI, cardiovascular disease history, estimated glomerular filtration rate (eGFR), and albuminuria.

Results: This analysis included 2806 DU treated pt with mean age 56.3± 9.8 years, duration of diabetes 7.8±6.0 years, baseline HbA1c 8.0±1.0% (64±11 mmol/mol), BMI 32.4±5.2 kg/m^2,and eGFR 89.5±16.9 mL/min/1.73 m^2. Based on gradient boosting, the top 5 baseline factors associated with reduction in HbA1c (and their relative influence) are baseline HbA1c(48.8%), age (9.1%), fasting serum glucose (FSG) (8.2%), FSI (6.7%), and eGFR (5.4%). The role of baseline glycaemic factors (HbA1c, FSG, FSI) was evaluated. Univariate regression demonstrated that higher baseline HbA1c and FSG values and lower FSI values were associated with greater reduction in HbA1c (coefficient estimates of HbA1c change: -0.598%, -0.101%, and 0.002%, respectively, p< 0.0001 for all). Multivariable linear regression showed similar results for baseline HbA1c (-0.594±0.027%) and FSI (-0.197±0.045% for FSI ≤55 pmol/L), but greater baseline FSG was associated with smaller decrease in HbA1c when adjusted for the other 4 factors (0.047±0.010%, p <0.0001), indicating that pts with smaller baseline FSG, while having similar baseline HbA1c and other variables, are likely to have a greater decrease in HbA1c in response to dulaglutide. Subgroup analysis by baseline HbA1c (9 quantiles) confirmed that higher FSG is associated with smaller decreases in HbA1c for pts within the same category of baseline HbA1c. Multivariable linear regression also showed that greater reduction in HbA1c was observed in associated pts with baseline eGFR≤ 100 mL/min/1.73 m^2 (coefficient estimate of HbA1c change: -0.143± 0.047%, p=0.002) and age≤65 years (coefficient estimates of HbA1c change: -0.175±0.058%, p=0.003).

Conclusion: These data indicate that higher baseline HbA1c, reflecting poor glycaemic status, is the major factor associated with greater reduction in HbA1c in response to treatment with DU. Other baseline factors associated with greater decrease in HbA1c in response to dulaglutide, albeit to a limited extent, include baseline FSG, FSI, age, and eGFR.

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Improvement in HbA_{1c} in patients with type 2 diabetes mellitus treated with once weekly dulaglutide across baseline body mass index (BMI) subgroups at 26 or 52 weeks

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Background and aims: This post-hoc analysis investigated the efficacy of dulaglutide and active comparators across baseline BMI categories (BMI <30, ≥30-<35 or ≥35 kg/m^2) in patients with T2DM using data from the phase 3 randomised trials AWARD-1 to -6.

Materials and methods: Patients with T2DM received dulaglutide [1.5 mg, n=1719 (AWARD-1 to -6); 0.75 mg, n=1417 (AWARD-1 to -5)], or exenatide (n=276), insulin glargine (n=558), metformin (n=268), sitagliptin (n=315) or liraglutide (n=300), in addition to other concomitant background treatments (Table). Analysis of covariance models (AWARD-1 to -5) or mixed-effects model for repeat measures (AWARD-6), including treatment-by-BMI subgroup interaction terms, were applied by study to estimate the effect of each treatment on HbA1c at 52 weeks (AWARD-1 to -5) or 26 weeks (AWARD-6) and to compare dulaglutide and corresponding active comparators for patients with baseline BMI <30, ≥30-<35 or ≥35 kg/m^2 (intention-to-treat population).

Results: Baseline mean BMI in each study ranged from 31.2-33.6 kg/m^2. HbA1c reductions from baseline according to BMI subgroup are shown for each study (Table). In all studies, dulaglutide 1.5 mg, dulaglutide 0.75 mg and all active comparators achieved statistically significant HbA1c reductions from baseline overall and in all BMI subgroups. No statistically significant treatment-by-BMI subgroup interactions were found for reductions in HbA1c (Table).

Conclusion: Dulaglutide (1.5 mg or 0.75 mg) is an effective treatment for patients with T2DM, regardless of baseline BMI. There was no evidence of any treatment-by-BMI subgroup interaction for HbA1c change, suggesting that baseline BMI had no effect on the relative antihyperglycaemic efficacy associated with dulaglutide versus comparator antidiabetes agents.

Table 1

Least squares mean change in HbA1c from baseline [%] to 52 weeks (AWARD-1 to -5) or 26 weeks (AWARD-6)

Study and comparator (concomitant background treatment)	BMI subgroup	Dulaglutide 1.5mg	Dulaglutide 0.75mg	Comparator	Treatment-by-BMI subgroup interaction (p-value)
AWARD-3: Metformin	<30; ≥30-<35; ≥35kg/m²	-0.64; -0.80; -0.64	-0.72; -0.49; -0.44	-0.54; -0.57; -0.40	0.406
AWARD-5: Sitagliptin (metformin)	<30; ≥30-<35; ≥35kg/m²	-1.24; -1.21; -0.96	-0.97; -0.79; -0.89	-0.54; -0.42; -0.29	0.598
AWARD-6: Liraglutide (metformin)	<30; ≥30-<35; ≥35kg/m²	-1.39; -1.43; -1.45	NA	-1.32; -1.36; -1.40	0.898
AWARD-1: Exenatide (metformin + pioglitazone)	<30; ≥30-<35; ≥35kg/m²	-1.33; -1.32; -1.44	-1.13; -1.01; -1.09	-0.72; -0.76; -0.91	0.871
AWARD-2: Insulin glargine (metformin + glimepiride)	<30; ≥30-<35; ≥35kg/m²	-1.03; -1.24; -0.92	-0.76; -0.77; -0.75	-0.47; -0.72; -0.77	0.159
AWARD-4: Insulin glargine (insulin lispro ± metformin)	<30; ≥30-<35; ≥35kg/m²	-1.38; -1.53; -1.54	-1.46; -1.46; -1.36	-1.10; -1.30; -1.31	0.668

NA, not applicable as there was no DU 0.75mg dose group in this study.

Clinical Trial Registration Number: H9X-MC-GBDA; H9X-MC-GBDB; H9X-MC-GBDC; H9X-MC-GBDD; H9X-MC-GBCF; H9X-MC-GBDE
Supported by: Eli Lilly and Company, Ltd.

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Achieving the composite endpoint of HbA_{1c} <7.0% (53 mmol/mol), no hypoglycaemia, and no weight gain in the once weekly dulaglutide AWARD programme

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Background and aims: The 6 completed Phase 3 trials from the dulaglutide AWARD program, a once weekly glucagon-like peptide-1 receptor agonist, demonstrated significant improvements in HbA1c and weight, with a low risk for hypoglycaemia. Post-hoc analyses from these data compare dulaglutide 1.5 mg and 0.75 mg with active comparator therapies and placebo on the composite endpoint of HbA1c <7% (53 mmol/mol), and no weight gain or hypoglycaemia.

Materials and methods: A logistic regression analysis was performed on the intent-to-treat population, last observation carried forward patient data on the composite endpoint of glycated haemoglobin HbA1c <7.0% (53 mmol/mol), no weight gain (≤0 kg) and no hypoglycaemia (glucose <3.0 mmol/L or any report of severe hypoglycaemia) after 26 weeks of treatment.

Results: At 26 weeks, within each study, 37 to 58% of patients on DU 1.5 mg, 27 to 49% of patients on DU 0.75 mg, and 9 to 61% on AC

achieved the composite endpoint. Significantly more patients reached the composite endpoint with DU 1.5 mg than with metformin, sitagliptin, exenatide BID, and insulin glargine (odds ratio [95% CI]: 1.5 [1.0, 2.2; p<.05], 4.5 [3.0, 6.6; p<.001], 2.6 [1.8, 3.7; p<.001], 7.4 [4.4, 12.6; p <.001], respectively); with no difference between dulaglutide 1.5 mg and liraglutide 1.8 mg (Figure). In addition, significantly more patients reached the composite endpoint with DU 0.75 mg compared to sitagliptin and insulin glargine (3.3 [2.2, 4.8; p<.001], 4.5 [2.7, 7.8; p<.001], respectively).

Conclusion: Dulaglutide is an effective treatment option, resulting in a similar or greater proportion of patients who reached the HbA1c target of <7.0% (53 mmol/mol), without weight gain or hypoglycaemia compared to active comparators.

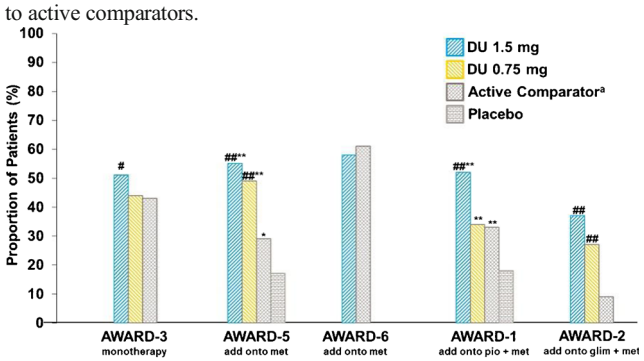

Figure. Proportion of patients achieving the composite outcome of A1C <7.0%, no hypoglycemia, and no weight gain at Week 26. #p <.05 and ##p <.001 vs active comparator; *p <.05 and **p<.001 vs PL. [a]AWARD-3, -5, -6, -1, and -2 active comparators: metformin (1500-2000 mg), sitagliptin 100 mg, liraglutide 1.8 mg, exenatide 10 µg twice daily, titrated insulin glargine, respectively.

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Effects of once weekly dulaglutide on kidney function in clinical trials

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Background and aims: Dulaglutide is a once weekly glucagon-like peptide 1 receptor agonist approved for the treatment of type 2 diabetes (T2D). Postmarketing cases of acute decreases in kidney function related to other incretin-based therapies have been reported. However, experimental models of diabetes have not suggested harm to the kidney from these therapies. The aim of this study was to determine effects of dulaglutide (1.5 mg and 0.75 mg) on kidney function and adverse events (AEs) in post hoc analyses of clinical trials with placebo (PL) and active comparators.

Materials and methods: Serum creatinine (sCr), estimated glomerular filtration rate (eGFR), and urine albumin-to-creatinine ratio (UACR) were evaluated using integrated data from 9 completed Phase 2 and 3 trials. Dulaglutide was compared to PL in 3 studies (treatment duration 16-26 weeks) and to a combined active comparator group (sitagliptin, exenatide [twice daily], insulin glargine, and metformin) in 5 studies (Treatment duration 52-104 weeks). Dulaglutide was also compared to insulin glargine alone in 2 studies (at 52 weeks).

Results: Treatment durations were comparable between dulaglutide and comparator arms for all studies included in this analysis. No significant differences in sCr or eGFR were observed at baseline or during treatment between dulaglutide and PL, the combined active comparator group, or insulin glargine alone. UACR values were slightly but significantly lower for dulaglutide than for PL, the combined active comparators, and insulin glargine alone during the treatment period. AE terms reflecting potential acute kidney injury were reported at rates of 3.4, 1.7, and 7.0 events per 1000 patient years of exposure for dulaglutide (n=12; N=4006), all active comparators (n=3; N=1541), and PL (n=2; N=703), respectively.

Conclusion: Treatment of T2D patients with dulaglutide in clinical trials did not appear to alter kidney function when compared to PL, a group of active comparators, or insulin glargine alone. Rates of reported AEs related to kidney disease were comparable for all groups.

Measures of kidney function and albuminuria during treatment with dulaglutide (DU) versus placebo (PL), combined active comparators[a] (AC), or insulin glargine (IG) alone

Time Point	Treatment	Median (Interquartile Range) [p-Value[b]]					
		sCr		eGFR[c]		UACR	
		n	mg/dL	n	mL/min/1.73m²	n	mg/g
Baseline	PL	568	0.84 (0.74-0.99)	568	90.5 (77.7-101.7)	557	8.9 (5.5-21.2)
	DU	1669	0.84 (0.71-1.00)	1669	91.6 (77.6-101.7)	1631	8.9 (4.4-23.9)
16-26 weeks	PL	551	0.84 (0.72-1.00)	551	90.4 (75.9-101.6)	527	8.0 (4.4-23.9)
	DU	1622	0.85 (0.72-1.00) [0.096]	1622	90.3 (75.5-100.4) [0.075]	1548	8.0 (4.4-20.4) [0.023]
Baseline	AC	1417	0.83 (0.71-0.96)	1417	92.4 (78.2-101.6)	1409	10.6 (5.3-29.2)
	DU	2836	0.84 (0.71-0.98)	2836	91.3 (77.6-101.5)	2807	8.9 (4.4-27.4)
26 weeks	AC	1365	0.84 (0.72-0.98)	1365	90.2 (76.3-100.0)	1337	8.9 (4.4-27.4)
	DU	2735	0.85 (0.72-1.00) [0.133]	2735	89.7 (75.3-100.2) [0.234]	2659	8.0 (4.4-21.2) [0.013]
52 weeks	AC	1204	0.84 (0.72-0.98)	1204	89.7 (75.6-98.8)	1185	8.9 (4.4-23.9)
	DU	2445	0.85 (0.72-1.00) [0.561]	2445	89.4 (75.2-99.3) [0.513]	2390	8.0 (4.0-21.2) [0.035]
78-104 weeks	AC	477	0.83 (0.70-0.96)	477	91.4 (77.8-99.7)	460	8.9 (4.4-27.9)
	DU	965	0.81 (0.69-0.95) [0.542]	965	92.5 (79.7-101.4) [0.223]	918	8.9 (4.4-25.7) [0.029]
Baseline	IG	558	0.83 (0.74-0.98)	558	90.3 (75.0-100.0)	558	14.2 (6.2-46.9)
	DU	1133	0.84 (0.70-0.98)	1133	90.6 (77.4-100.5)	1133	10.6 (5.3-36.3)
52 weeks	IG	485	0.86 (0.74-1.01)	485	86.8 (72.2-96.1)	482	13.3 (5.3-45.1)
	DU	987	0.85 (0.72-1.00) [0.428]	987	88.6 (74.2-98.5) [0.423]	978	9.7 (4.4-31.0) [0.031]

[a]Active comparators includes sitagliptin. exenatide (twice daily), insulin glargine, and metformin groups combined.
[b]p-values from a mixed-effects model for repeated measures of the logarithm of the response variable, with terms adjusting for study, treatment, time, and baseline values of serum creatinine, urinary albumin-to-creatinine ratio (UACR), and hemoglobin A1c.
[c]eGFR was calculated using Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) formula and serum creatinine value.

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Low incidence of anti-drug antibody in type 2 diabetes patients treated with once weekly dulaglutide

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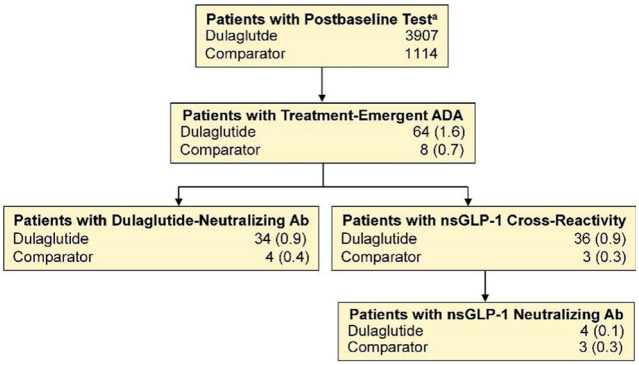
Background and aims: Dulaglutide, a once weekly glucagon-like peptide-1 (GLP-1) IgG4-Fc fusion protein for type 2 diabetes treatment, was structurally modified to reduce immunogenic potential by eliminating T-cell epitopes based on the results of the EpiVax algorithm. Immunogenicity of dulaglutide was assessed in 9 clinical studies.

Materials and methods: Blood samples collected serially were assayed for dulaglutide anti-drug antibody (ADA) using validated ELISAs. Samples with treatment-emergent ADA were tested for dulaglutide - neutralizing antibody and native sequence GLP-1 (nsGLP-1) cross-reactivity, and then neutralization of nsGLP-1. Associations between ADA and hypersensitivity adverse events, injection site reactions, and HbA_{1c} changes were assessed.

Results: A total of 3907 dulaglutide and 1114 non-GLP-1 receptor agonist comparator patients were tested. The incidence of treatment-emergent ADA with dulaglutide was 1.6% vs 0.7% with non-GLP-1 receptor agonist comparator. Dulaglutide-neutralizing antibody, nsGLP-1 cross-reactivity, and nsGLP-1 neutralizing antibody were observed in 0.9%, 0.9%, and 0.1% of dulaglutide patients, respectively. There was no evidence of an effect of ADA on HbA_{1c} change after 26 or 52 weeks of treatment. Only 19 (0.5%) of dulaglutide patients had a hypersensitivity adverse event, none had treatment-emergent ADA. There were 20 (0.5%) dulaglutide-exposed patients with potentially immune-mediated injection site reactions; 2 of them (0.05% of 3907) had treatment-emergent ADA.

Conclusion: Incidence of ADA in patients exposed to dulaglutide was low. No association between dulaglutide ADA and hypersensitivity adverse events, injection site reactions, or HbA_{1c} changes was observed.



Data presented as n (%) unless otherwise noted
Abbreviations: Ab = antibody; ADA = anti-drug antibody; n = number of patients in specified category; nsGLP-1 = native sequence glucagon-like peptide-1
[a]9 patients tested positive for ADA prior to dulaglutide exposure

Supported by: Eli Lilly and Company

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Albiglutide provides effective glycaemic lowering across diabetes duration subgroups

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Background and aims: Incretin therapies work in part by stimulating insulin secretion, so it is important to characterize their efficacy in people with longer-standing diabetes and less residual β-cell function. Here we test the hypothesis that albiglutide will have a similar efficacy and safety profile independent of diabetes duration.
Materials and methods: Pooled phase-3 data were from 1391 participants in placebo comparator studies, randomized 923 to albiglutide and 468 to placebo, ± oral agents. Efficacy analyses use the primary outcome variable (HbA1c) and other measures pooling data 6 months from randomization
Results: Baseline characteristics were generally well balanced vs placebo and typical of people with T2DM (age 54 yr, HbA1c 8.1% (65.02 mmol/mol), BMI 33.1 kg/m2). Change in HbA1c for albiglutide vs placebo varied from a difference of -0.93 (-1.08,-0.79) %-units (-10.17 [-11.81, -8.64] mmol/mol) in the <5-yr group to -0.70 (-0.90, -0.50) %-units (-7.65 [-9.84, -5.47] mmol/mol) in the ≥10-yr group (Table). The fasting plasma glucose difference was -1.65 (-2.03, -1.27) mmol/L at short duration, -1.30 (-1.75, -0.86) mmol/L in the middle group, and -1.44 (-1.97, -0.92) mmol/L at ≥10-yr duration. Adverse events did not differ between albiglutide groups except for injection site reactions (fewer at longer duration). Serious adverse events were similar by duration of diabetes for the albiglutide group.
Conclusion: Albiglutide had good efficacy independent of diabetes duration with no deterioration of adverse event profile.

Duration of diabetes		albiglutide n	albiglutide HbA1c (%-units)	albiglutide HbA1c (mmol/mol)	placebo n	placebo HbA1c (%-units)	placebo HbA1c (mmol/mol)
<5 yr	change (SD)	403	-0.91 ± 0.85	-10.0 ± 9.3	205	-0.01 ± 0.90	-0.1 ± 9.8
	difference, (95% CI)						
	%-units			-0.93 (-1.08,-0.79)			
	mmol/mol			-10.2 (-11.8,-8.6)			
≥5 – 10 yr	change (SD)	301	-0.79 ± 0.83	-8.6 ± 9.1	145	-0.06 ± 1.17	-0.7 ± 12.8
	difference, (95% CI)						
	%-units			-0.75 (-0.92,-0.58)			
	mmol/mol			-8.2 (-10.16,-6.3)			
≥10 yr	change (SD)	200	-0.73 ± 0.74	-8.0 ± 8.1	109	-0.06 ± 0.92	-0.7 ± 10.1
	difference, (95% CI)						
	%-units			-0.70 (-0.90,-0.50)			
	mmol/mol			-7.7 (-9.8, -5.5)			

Supported by: GSK

PS 069 GLP-1 receptor agonists: approaching approvability

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Efficacy and tolerability of ITCA 650 (continuous subcutaneous exenatide) for 39 weeks in patients with poorly controlled type 2 diabetes mellitus and high baseline HbA_{1c} (>10%)

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Background and aims: ITCA 650 is an injection-free glucagon-like peptide-1 (GLP-1) receptor agonist that provides continuous subcutaneous (SC) exenatide for up to 12 months from a single sub-dermal placement. Open-label treatment with ITCA 650 was offered to type 2 diabetes (T2DM) patients ineligible for a double-blind, placebo-controlled trial because of HbA1c >10%.
Materials and methods: Patients were 18-80 years with HbA1c >10% to ≤12%, BMI 25-45 kg/m2, on stable (≥3 months) diet and exercise or oral antidiabetics (OADs). ITCA 650 was initiated at 20 mcg/d for 13 weeks, then 60 mcg/d for 26 weeks. Baseline (BL) OADs were continued; sulfonylureas (SUs) were adjusted if medically necessary. Endpoints were change from BL for HbA1c and body weight (BW) and % achieving HbA1c <7% at 39 weeks.
Results: Sixty patients were enrolled. Six (10.0%) patients discontinued for adverse events (AEs). At BL, HbA1c was 10.8%, age 51.9 years, BMI 32.0 kg/m2, diabetes duration 9 years, OAD use 69%. At Week 39, mean reduction of HbA1c from BL to Week 39 (last observation carried forward) was -2.8% ($p<0.001$), and 22% achieved HbA1c <7%. Significant A1C reductions were seen by Week 6 (Figure). The most common AEs were nausea, vomiting, diarrhea, headache, and urinary tract infection. Nausea, vomiting, and diarrhea rapidly decreased after Week 1. There were no reports of pancreatitis or major hypoglycemic events; 6.7% reported minor hypoglycemia, and all serious AEs were considered unrelated to ITCA 650.
Conclusion: ITCA 650 for 39 weeks produced significant reductions in HbA1c, improved BW and goal attainment and was well tolerated in these poorly controlled T2DM patients with high HbA1c.
Clinical Trial Registration Number: NCT01785771
Supported by: Intarcia Therapeutics, Inc.

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Absorption, metabolism and excretion of [^{3}H]-semaglutide following a single, subcutaneous dose in healthy male subjects

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Background and aims: Semaglutide is a human GLP-1 analogue in clinical development for once-weekly treatment of T2D. Semaglutide has minor modifications versus native human GLP-1 that aim to reduce renal clearance and protect against dipeptidyl peptidase-4 degradation, resulting in a prolonged half-life. This open-label study investigated the absorption, metabolism and excretion of radiolabelled semaglutide in humans.
Materials and methods: Healthy male subjects (n=7; aged 48-64 years; body mass index 21.7-29.7 kg/m^2) received a single subcutaneous dose of

radioactively labelled semaglutide (0.5 mg/450 μCi/16.5 MBq [^{3}H]-semaglutide). The [^{3}H] label, used previously in non-clinical semaglutide studies, was attached to the semaglutide fatty acid side chain. Radioactivity was determined in the blood, plasma, urine, faeces and expired air up to 56 days after administration. Metabolites were quantified in plasma, urine and faeces using HPLC followed by radio detection. In addition, routes and rates of excretion, blood-to-plasma ratio, pharmacokinetics (PK) of [^{3}H]-semaglutide-related material and standard PK were assessed.

Results: Total recovery of [^{3}H]-semaglutide-related material was 75.1% (coefficient of variation in percentage [CV%] 5.2) of the administered dose: urine 53.0% (8.2); faeces 18.6% (19.9); and expired air 3.2% (9.0) (Figure). Intact [^{3}H]-semaglutide was the major component in plasma at all time points analysed, accounting for 83% of the total radioactivity, based on the area under the concentration-time curve (AUC). A total of 6 metabolites were detected in plasma, each accounting for between 0.4% and 7.7% of the total amount of radioactivity. Intact semaglutide accounted for ~3% of the administered dose in urine. In addition, 21 metabolites were detected in urine; the 2 most abundant each comprised ~14% of the administered dose (all other metabolites each ≤1.8%). Intact semaglutide was not detected in faeces. In total, 7 metabolites were detected in faeces, each ≤1.5% of the administered dose. The blood-to-plasma ratio of radioactivity showed that [^{3}H]-semaglutide-related material was primarily distributed in the plasma compartment, and was relatively constant over time. The PK properties of [^{3}H]-semaglutide in this study were comparable with results from previous trials with unlabelled semaglutide. No new or unexpected safety or tolerability issues were identified.

Conclusion: This study shows that, following treatment with [^{3}H]-semaglutide, intact semaglutide is the main component in plasma. Semaglutide is extensively metabolised prior to elimination, and semaglutide-related material is primarily excreted in the urine. Semaglutide has limited renal excretion in its intact form. This supports that semaglutide may be used without dose adjustment in subjects with renal impairment.

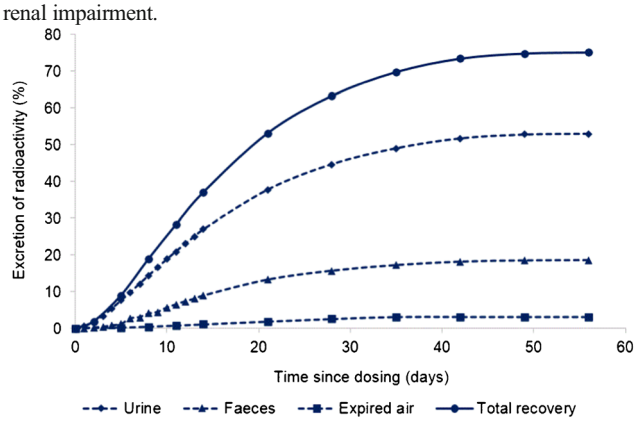

Figure. Cumulative excretion of radioactivity in urine, faeces and expired air (mean for all subjects; n=7) after a single, subcutaneous dose of 0.5 mg/450 μCi/16.5 MBq [^{3}H]-semaglutide. Subjects who met the end criterion prior to Day 56 had their missing cumulative values imputed with the last available cumulative value.

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Superagonistic mechanism of increased glucodynamic and weight loss effects of ^{LAPS}*CA*-exendin-4 (efpeglenatide)

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Background and aims: The novel long-acting GLP-1 receptor agonist, efpeglenatide, was developed for the treatment of type 2 diabetes and is currently in phase II clinical trials as a weekly or monthly GLP-1 receptor agonist (GLP-1RA). It consists of an exendin-4 analog (*CA*-Exendin-4) conjugated via non-peptidyl linker to a non-glycosylated human Fc fragment. Previously, *CA*-Exendin-4 showed greater blood glucose lowering efficacy in *db/db* mice compared to native exendin-4 and it was hypothesized that such a superagonistic action of *CA*-Exendin-4 is due to its fast dissociation from human GLP-1 receptor. In this study, we investigated further the mechanism using *CA*-Exendin-4 and efpeglenatide.

Materials and methods: Affinity to GLP-1 receptor (GLP-1R) was measured by Surface Plasmon resonance (SPR) analysis. Internalization of GLP-1R was assessed in GLP-1R/U2OS cells using the PathHunter™ eXpress Kit (DiscoveRx Corp. Ltd., UK). Surface GLP-1R level was determined by [^{125}I]GLP-1 binding after acid-wash of the cells. *In vitro* activity after chronic treatment of GLP-1RAs was determined by measuring intracellular cyclic AMP in GLP-1R/CHO and insulin release in RINm5F cells. The cells were pre-treated with 10 nM of GLP-1RAs for 4 to 24 hours and followed by cAMP or insulinotropic induction by re-treatment with 0-5,000 nM of GLP-1RAs. In order to compare *in vivo* efficacy between efpeglenatide and other GLP-1RAs, change of blood glucose and HbA1c in *db/db* mice by subcutaneous injection was monitored during 4 weeks and body weight loss was compared in DIO rats for 4 weeks.

Results: *CA*-Exendin-4 elicited less internalization of GLP-1 receptors compared to exendin-4 in human GLP-1R overexpressed U2OS, and this was also confirmed in hGLP-1R/CHO cells by measuring the residual surface receptors. Further, GLP-1R internalization was compared between efpeglenatide and other long-acting GLP-1RAs. Efpeglenatide led to less GLP-1R internalization (e.g. 50% or less) than liraglutide and dulaglutide in both hGLP-1R/U2OS and hGLP-1R/CHO cells. Efpeglenatide also led to less receptor desensitization under chronic treatment conditions in cAMP accumulation and insulinotropic assays. Finally, in *db/db* mice, efpeglenatide showed a greater HbA1c decrease (-3.8% in 4 weeks) than liraglutide and dulaglutide (-2.6% and -2.8%, respectively) at doses to corresponding to human use. In addition, efpeglenatide revealed greater efficacy in body weight loss of -20.9% in DIO mice for 4 weeks,compared with -18.6% by liraglutide and -7.1% by dulaglutide.

Conclusion: Following conjugation to Fc the fast receptor dissociation kinetics of *CA*-Exendin-4 were maintained in efpeglenatide (k_d 4.2×10^{-3} s^{-1} vs. 1.0×10^{-3} s^{-1} for efpeglenatide). We also demonstrated that efpeglenatide lead to significantly less GLP-1R internalization than liraglutide and dulaglutide in human GLP-1R transformed U2OS cells. This translated into more potent glucose lowering in *db/db* mice and greater body weight loss in DIO mice in comparison to liraglutide and dulaglutide. Taken together, *CA*-Exendin-4 even after conjugation to the human Fc-fragment seems to achieve more pronounced GLP-1R activation due to reduced receptor internalization and consequently leads to more potent effects. These findings may explain the observed efficacy of efpeglenatide in clinical trials relative to liraglutide.

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CCK and GLP-1 receptor agonist co-administration ameliorates diabetes in mice

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Background and aims: GLP-1 and CCK are hormones secreted from gut endocrine cells in response to meals that regulate metabolism. Co-administration of CCK and GLP-1 analogs induce synergistic weight loss in diet-induced obese rats.
Materials and methods: We investigated the impact of selective CCK1R (A1) or CCK2R (A2) agonists alone or in combination with a GLP-1R agonist (AC3174) on acute inhibition of food intake in lean mice, insulin secretion from a rat beta cell line (Ins-1 cells), and on body weight and glycemia in obese and diabetic *db/db* mice.
Results: Combined AC3174 (0.3 nmol/kg, sc) and A1 (300 nmol/kg, ip) administration had greater impact on inhibiting food intake vs. monotherapy, whereas addition of A2 to AC3174 had no further impact on food intake. In Ins-1 cells, glucose-dependent insulin secretion was enhanced by incubation with AC3174, and to a lesser extent A1. Incubating Ins-1 cells with AC3174 and A1, but not A2, resulted in additive effects on insulin secretion. These findings suggest synergy between CCK and GLP-1 requires activation of CCK1R and not CCK2R. Diabetic *db/db* mice administered AC3174 (0.3 nmol/kg, sc, qd) and A1 (300 nmol/kg, ip, qd) for 14 days exhibited a robust reduction in body weight (-25.8%) compared to vehicle (+8%), AC3174 alone (-4.9%) or A1 alone (-13.4%). Weight loss was associated with reduced food intake, and combination treatment was associated with lower glucose and a trend towards lower HbA_{1C} levels vs. monotherapy- or vehicle-treated groups. AC3174 did not affect liver weight, however a significant reduction in liver weight was observed with A1 alone and in combination with AC3174, and was associated with reduced plasma alanine aminotransferase and triglyceride levels.
Conclusion: In summary, our results demonstrate that co-administration of CCK1R and GLP-1R agonists elicits synergistic weight loss and improvement in glucose control, potentially via improved liver function and/or co-operative actions on pancreatic function.

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Potent weight loss mechanism and improvement of NASH by the long-acting GLP-1/glucagon receptor dual agonist HM12525A

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Background and aims: Dual agonists activating the GLP-1 receptor (GLP-1R) and the glucagon receptor (GCG-R) may represent a new therapeutic approach for obesity with the potential for enhanced weight loss beyond those of GLP-1R agonists. Oxyntomodulin, a human gut hormone with agonism to the GLP-1R and the GCG-R, causes a significant reduction in weight by regulating both, appetite and energy expenditure, however its clinical application is limited due to a short half-life. HM12525A is a long-acting GLP-1/glucagon receptor dual agonist for once-weekly administration. It consists of a GLP-1/glucagon receptor dual agonist conjugated via non-peptidyl linker to a non-glycosylated human Fc fragment. The aim of this development was to achieve increased body weight loss (BWL) compared to GLP-1R agonists while extending the half-life. This study investigated the mechanism responsible for increased BWL and the effect on nonalcoholic steatohepatitis (NASH) by HM12525A by assessing energy expenditure (EE) and lipid metabolism.
Materials and methods: To evaluate the effect on FGF21 related EE increase, firstly, the secretion of FGF21 was investigated in primary hepatocytes and DIO (diet induced obesity) rats after HM12525A treatment. Next, the expression of UCP-1, a well known mediator of EE, was investigated in differentiated 3 T3-L1 adipocytes by co-treatment with FGF21. In vivo energy intake and expenditure as well as locomotor activity were assessed by using a combined indirect calorimetry system in DIO mice. To evaluate the effect on lipid metabolism in the liver, liver histology and CPT-1 expression, a marker of β-oxidation, were investigated in DIO rats following chronic administration of HM12525A. To evaluate the effect on NASH, hepatic levels of inflammation, fibrosis marker, and histological scoring were investigated in ALIOS- (American lifestyle induced obesity syndrome) and methionine-choline deficient (MCD) diet-mice as NASH models.
Results: The secretion of FGF21 was significantly increased in primary hepatocytes (2-fold vs. vehicle) as well as in DIO rats after HM12525A treatment. HM12525A treatment induced the expression of UCP-1 (4.5-fold vs. vehicle) which was further enhanced by co-treatment with FGF21 (13.4-fold vs. vehicle). Consistent with these in vitro results, HM12525A increased EE without affecting locomotor activity in DIO mice. These results suggest that the HM12525A-FGF21 axis plays an essential role in BWL by enhancing EE. In DIO rats, the chronically administered HM12525A ameliorated fatty livers together with the increase of CPT-1 expression (1.8-fold vs. vehicle). In ALIOS mice, HM12525A resulted in greater effects on body weight and hepatic TG reduction compared to liraglutide. Hepatic levels of inflammation, fibrosis marker, and histological scoring for NASH were decreased consistently after HM12525A administration in MCD mice.
Conclusion: Taken together, our results demonstrate that that the novel GLP-1/glucagon receptor dual agonist HM12525A may have clinical potential for the treatment of obesity and obesity-related liver disease.

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The ultra-long acting ^{LAPS}GLP/GCG dual agonist, HM12525A, demonstrated safety and prolonged pharmacokinetics in healthy volunteers: a phase 1 first-in-human study

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Background and aims: Glucagon-like peptide-1 (GLP-1) /glucagon (GCG) dual agonists have potential to treat obesity and diabetes by activating both GLP-1 and GCG receptors. Once-weekly ^{LAPS}GLP/GCG dual agonist, HM12525A, is site-specifically conjugated by LAPSCOVERY technology for sustained duration. Combining the synergetic effects with this long-acting platform, HM12525A may improve patient compliance and thereby treatment outcomes further. This First-in-Human, Phase I, randomized, double-blinded, placebo-controlled single ascending dose study evaluated the safety, tolerability, PK and PD of HM12525A in healthy volunteers.
Materials and methods: Forty subjects (age: 48.3±10.4 years; male: 70%; BMI: 29.7±3.3 kg/m^2) were randomly assigned to 5 cohorts (0.25, 0.5, 1.0, 2.0, and 4.0 nmol/kg), and received a single subcutaneous injection of either HM12525A or placebo (ratio, 3:1). The total duration of the study was 56 days, including follow up.
Results: HM12525A demonstrated prolonged PK profiles with tolerance. The observed C_{max} in each cohort was ranged from 0.759 to 18.5 nmol/L at 113.1±55.3 hr post-dose. $AUC_{0\text{-last}}$ (120 to 6560 hr·nmol/L) and $T_{1/2}$ (112.5 to 276.2 hr) was also observed. Among the 5 cohorts, the maximum tolerated single dose was determined at a dose level of 2.0 nmol/kg. Overall, none of the subjects exhibited any clinically significant alterations in vital signs, laboratory values, and 24 hr ambulatory ECG. During 24 hr ambulatory blood pressure assessments, no significant change

in heart rate (HR) was observed up to 1.0 nmol/kg dose. At 2.0 and 4.0 nmol/kg doses, mean HR was elevated by 5.77±2.30 and 8.06± 2.56 (p-value: 0.017 and 0.004) compared to the placebo, while mean systolic and diastolic BPs had no significant changes. Anti-drug antibodies were detected in 7 subjects; 3 subjects already had ADA at baseline, while the other 4 were newly detected during the study. None of these 7 subjects had neutralizing antibodies. The most frequent treatment emergent adverse events (TEAEs) were gastrointestinal disorders, such as nausea and vomiting. No serious TEAEs were occurred.

Conclusion: In summation, this single ascending dose study demonstrates that HM12525A was well tolerated with prolonged PK profile, which may confirm the potential for once-weekly injections. Further investigation in T2DM patients is expected to explore further synergetic potential of this ^{LAPS}GLP/GCG dual agonist to provide beneficial and effective therapeutic regimens.

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LAPS-Exendin-4 (HM11260C) enhances insulin secretion and beta cell responsiveness in subjects with type 2 diabetes

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Background and aims: LAPSExendin-4 (HM11260C) is a glucagon like peptide -1 (GLP-1) receptor agonist being developed for the treatment of type 2 diabetes mellitus (T2DM). In this phase 1B study the effects of different HM11260C regimens on insulin secretion rate (ISR), β-cell responsiveness, and gastric emptying (GE) compared to placebo and to liraglutide were investigated.

Materials and methods: Subjects with T2DM received 6 mg HM11260C weekly (cohort A; age: 52.8 years, HbA1c: 8.4, n=13), 16 mg HM11260C monthly (cohort B; age: 50.1 yr, HbA1c: 8.1, n= 13), 1.8 mg liraglutide daily (cohort C; age: 54.9 yr, HbA1c: 8.02 n= 13), or placebo (age: 54.1 yr, HbA1c: 8.6, n=8). Subjects in cohorts A and C were evaluated at baseline and steady state, and cohort B was evaluated at trough (day [d] 82) and peak (d89) drug concentration. Islet β-cell function was assessed during a graded IV glucose infusion (GGI); infusion steps: 2, 4, 6, 8, 12 mg/kg/min of 20% IV glucose for 30 min each. Insulin C-peptide levels, and plasma glucose response to each GGI step were measured. ISR were determined using a population based C-peptide de-convolution model. AUC(insulin) and AUC(C-peptide) as measures of ISR were determined for each treatment. β -cell responsiveness was assessed as the ratio of ISR/ blood glucose (BG) over the duration of the GGI, and the slope of the ISR/BG was compared between the treatments. The rate of GE was determined for 1 g of liquid acetaminophen following a mixed meal and a mixed meal tolerance test (MMTT) was performed. For GE, non-inferiority to liraglutide was tested using a margin of 0.8 for the least squares (LS) mean ratio.

Results: Insulin secretion for all active treatments was increased compared to placebo and for HM11260C 6 mg and 16 mg was greater than for liraglutide (point estimates LS mean ratio: 1.765 mU/L, 90% CI: 1.331, 2.342 mU/L, one-sided p=0.0008, and LS mean ratio: 1.440 mU/L, 90% CI: 1.080, 1.920 mU/L, one-sided p=0.0196). Comparisons of the relationship between ISR and plasma glucose using mixed effects modelling showed an improvement in beta cell responsiveness relative to placebo/ baseline which was not different among treatments. In the monthly treatment group, there was a diminution of effect at PK trough (d82). For postprandial glucose following the MMTT the LS means of the Cmax, AUC0-180, and AUC0-360 parameters were significantly (two-sided p 0.8) to liraglutide in terms of acetaminophen Cmax, AUC0-120 min, and AUC0-180 min. While for Cohort B the effect on gastric emptying appeared to be similar or even less when compared to liraglutide, formal non-inferiority could not be established for all parameters. GE was delayed in cohorts A and B compared to placebo.

Conclusion: HM11260C improved measures of β-cell function significantly compared to placebo, and for the weekly treatment regimen (cohort A), significantly compared to liraglutide. The results of this trial suggest that HM11260C is a safe and efficacious GLP-1 receptor agonist and that with regard to gastric emptying it inhibited less or similarly compared to other known GLP-1 receptor agonists.

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Dose-response improvements in glycaemic control and body weight reduction with HM11260C, a once weekly GLP-1 receptor agonist with liraglutide as reference, in type 2 diabetes

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Background and aims: HM11260C (HM) is a novel long acting GLP-1R agonist with a $T_{1/2}$ of~158 hrs resulting in a flat PK profile. This 12-week, randomized, placebo (PBO) controlled, double-blind parallel group study with an open label active control liraglutide (LIRA) arm, was designed to investigate the dose ranging efficacy, safety, and tolerability of once weekly (QW) doses of HM in subjects with Type 2 Diabetes (T2DM).

Materials and methods: Stratified by metformin use 254 patients (mean age 55.3 yrs, BMI 31.76 kg/m^2, diabetes duration 73.08 months) were randomized to one of five HM QW doses, PBO or to LIRA QD uptitrated to 1.8 mg. All HM doses were not titrated.

Results: HM 0.3 to 4 mg produced dose-dependent reduction in HbA1c, fasting plasma glucose, and 7-point daily glucose (Table 1). In addition, HM 4 mg reached statistical significance in demonstration for non-inferiority to LIRA by mean % change of HbA1c from baseline (Difference=-0.23, 95.1% CI=[-0.56, 0.10]). Attainment of target HbA1c<7% with HM was 89.7% (3 mg, p<0.0001) and 89.3% (4 mg, p<0.0001) vs 26.5% (PBO), and 73.3% (LIRA). The reduction in body weight with HM 3 mg (2.732 kg) and 4 mg (3.309 kg) was significantly greater than that with PBO (1.290 kg, p<0.049, both). The most frequent AEs were gastrointestinal side effects of mild or moderate severity: nausea and vomit that subsided over time.

Conclusion: In summary, all doses of HM demonstrated clinically meaningful improvements in blood glucose and body weight, with higher doses being more effective. Safety profiles were comparable to LIRA. The current results warrant further studies to assess the long-term efficacy and safety of HM11260C in T2DM.

Table 1. Summary of efficacy and safety measures after 12 weeks (Final analysis)

	Placebo (n=37)	HM11260C 0.3 mg/wk (n=37)	HM11260C 1 mg/wk (n=37)	HM11260C 2 mg/wk (n=35)	HM11260C 3 mg/wk (n=36)	HM11260C 4 mg/wk (n=36)	Liraglutide 1.8 mg/day (n=36)
Baseline characteristics (Safety set)							
HbA1c, %	7.99 (0.831)	7.86 (0.745)	7.76 (0.567)	7.73 (0.698)	7.82 (0.871)	7.99 (0.816)	7.97 (0.787)
Body weight, kg	85.519 (18.449)	95.066 (19.783)	89.505 (18.296)	89.850 (17.157)	94.380 (18.704)	86.908 (18.078)	90.014 (20.224)
Efficacy measures (Full Analysis Set)							
HbA1c change, % [95.1% CI] [a, †]	-0.40 (0.111)	-0.56 (0.114)	-0.95 (0.111)	-1.19* (0.121) (p<0.0001)	-1.41* (0.119) (p<0.0001)	-1.61* (0.118) (p<0.0001) [-0.56, 0.10]†	-1.38* (0.120) (p<0.0001)
% of subjects with HbA1c < 7.0% [b]	26.5	37.5	72.7* (p=0.0002)	71.4* (p=0.0003)	89.7* (p<0.0001)	89.3* (p<0.0001)	73.3
Fasting plasma glucose change, mmol/L [a]	-0.49 (0.283)	-0.48 (0.295)	-1.31* (0.282) (p=0.0335)	-1.27 (0.307)	-2.19* (0.305) (p<0.0001)	-2.44* (0.303) (p<0.0001)	-1.46 (0.306)
Mean daily glucose change (7-point SMBG), mmol/l [a]	-0.582 (0.267)	-0.637 (0.274)	-1.519* (0.257) (p=0.0060)	-2.008* (0.295) (p=0.0001)	-2.348* (0.288) (p<0.0001)	-2.628* (0.295) (p<0.0001)	-2.107 (0.286)
Body weight change, kg [a]	-1.290 (0.511)	-1.209 (0.526)	-2.014 (0.508)	-1.522 (0.553)	-2.732* (0.550) (p=0.0453)	-3.309* (0.543) (p=0.0050)	-3.212 (0.558)
Safety measures (Safety Set)							
Incidence of Nausea, %	16.2	10.8	8.1	27.3	22.2	33.3	33.3
Incidence of Vomit, %	0	0	2.7	12.1	11.1	22.2	11.1
Heart rate change, BPM [c]	0.7 (10.49)	0.3 (7.39)	-0.5 (7.02)	1.8 (8.40)	3.9 (9.08)	3.0 (10.14)	5.6 (8.50)

[a] MMRM, statistics indicate LS Mean (SE), MMRM includes treatment, visit and their interaction as factors to explain change in HbA1c and Metformin use as a covariate with unstructured covariance matrix across all visits. Type I error rate was adjusted using O'Brien-Fleming boundary for final that is 0.049.

[b] Cochran-Mantel-Haenszel Test, controlling for Metformin use

[c] Mean (SD)

* *p*-value <0.049 vs. placebo

† Non-inferiority in HbA1c reduction was concluded if the upper limit of the two-sided 95.1% confidence interval (CI) for the difference in HbA1c at Visit 7 (Visit 7/Week 13) was smaller than 0.3.

Clinical Trial Registration Number: NCT02057172
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PS 070 GLP-1 receptor agonists: emerging concepts

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Once weekly glucagon-like peptide 1 receptor agonists for type 2 diabetes: systematic review and meta-analysis

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Background and aims: Albiglutide, dulaglutide and exenatide extended release are injectable glucagon-like peptide 1 receptor agonists (GLP-1 RAs) administered once weekly that have recently been approved for type 2 diabetes. We performed a systematic review and meta-analysis to assess their efficacy and safety.
Materials and methods: We searched for randomised controlled trials with at least 12 weeks duration of treatment in Medline, Embase, the Cochrane Library, ClinicalTrials.gov, and abstracts of major scientific meetings through December 2014. We synthesised results regarding change in HbA_{1c} and body weight, and incidence of hypoglycemia, injection site reactions, nausea, vomiting, serious adverse events (SAEs) and all cause mortality. We conducted separate analyses for studies against placebo or active comparator. We quantified heterogeneity with the I^2 statistic.
Results: We included 33 trials (16,003 participants) with a duration between 12 and 156 weeks in our systematic review. Participants' mean age ranged from 52 to 63 years and baseline HbA_{1c} from 7.1% to 8.7%. The majority of albiglutide and dulaglutide trials utilised a double blind design, whilst most exenatide extended release studies were open label. Compared with placebo, change in HbA_{1c} was -0.66% (six studies; 95% CI -1.14 to -0.19; I^2=88%) with albiglutide, -1.18% (seven studies; 95% CI -1.34 to -1.02; I^2=65%) with dulaglutide, and -1.48% (two studies; 95% CI -2.61 to -0.36; I^2=80%) with exenatide extended release. Once weekly GLP-1 RAs outperformed sitagliptin, daily exenatide and basal insulin in terms of HbA_{1c} lowering, and were equally effective with liraglutide (Table). Based on placebo controlled trials, treatment with once weekly GLP-1 RAs did not result in significant body weight reduction (weighted mean difference -0.61 kg; 95% CI -1.31 to 0.09; I^2=84%), although weight sparing benefits were evident relative to sitagliptin and basal insulin, but not versus liraglutide (Table). Risk for any hypoglycaemia was higher compared to sitagliptin, similar to daily GLP-1 RAs, and lower in comparison with basal insulin (Table). Main adverse effects included gastrointestinal and injection site reactions (Table). Incidence of SAEs and all cause mortality did not differ between once weekly GLP-1 RAs and across all comparator arms (ORs 0.93; 95% CI 0.82 to 1.04; I^2=13% and 0.67; 95% CI 0.40 to 1.11; I^2=0% respectively).
Conclusion: Given their overall efficacy and safety profile and convenient dosing scheme, once weekly GLP-1 RAs are an attractive therapeutic alternative to oral antidiabetic agents, basal insulin, or twice daily exenatide in patients inadequately controlled with metformin alone.

Comparison	No of studies/ patients	WMD (95% CI); I^2	No of studies/ patients	OR (95% CI); I^2	No of studies/ patients	OR (95% CI); I^2
Weekly GLP-1 RA vs	**Change in HbA_{1c} (%)**		**Incidence of hypoglycaemia**		**Incidence of injection site reactions**	
Placebo	15 / 2583	−1.01 (−1.20, −0.81); 82%	11 / 2460	1.59 (1.15, 2.19); 0%	8 / 1901	4.72 (2.69, 8.27); 27%
Sitagliptin	5 / 1783	−0.40 (−0.66, −0.14); 85%	5 / 2455	1.54 (1.15, 2.07); 0%	3 / 1718	3.42 (1.95, 5.99); 70%
Daily exenatide	5 / 1833	−0.44 (−0.58, −0.29); 40%	3 / 1102	0.70 (0.46, 1.07); 0%	2 / 744	8.26 (4.37, 15.64); 0%
Liraglutide	4 / 2591	0.05 (−0.14, 0.23); 84%	4 / 2739	1.02 (0.80, 1.31); 44%	3 / 1828	4.42 (1.97, 9.94); 44%
Basal insulin	7 / 2793	−0.30 (−0.45, −0.14); 78%	6 / 2875	0.52 (0.42, 0.64); 0%	4 / 1923	11.98 (6.86, 20.91); 84%
Weekly GLP-1 RA vs	**Body weight (kg)**		**Incidence of nausea**		**Incidence of vomiting**	
Placebo	15 / 2600	−0.61 (−1.31, 0.09); 84%	14 / 3209	2.24 (1.75, 2.86); 52%	12 / 3108	3.33 (2.24, 4.95); 59%
Sitagliptin	5 / 1782	−1.17 (−1.53, −0.81); 0%	5 / 2455	2.44 (1.83, 3.27); 0%	5 / 2455	2.87 (1.93 to 4.26); 34%
Daily exenatide	5 / 1735	0.12 (−0.66, 0.90); 68%	5 / 1844	0.58 (0.47, 0.72); 81%	5 / 1844	0.83 (0.63, 1.10); 57%
Liraglutide	4 / 2619	0.80 (0.16, 1.45); 82%	4 / 2739	0.49 (0.40, 0.61); 90%	4 / 2739	0.52 (0.38, 0.72); 58%
Basal insulin	7 / 2796	−3.11 (−4.02, −2.20); 91%	7 / 3331	5.91 (4.28, 8.15); 74%	7 / 3331	2.82 (1.98, 4.00); 70%

WMD: weighted mean difference, GLP-1 RA: glucagon-like peptide 1 receptor agonist.

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Adherence with GLP-1RA therapy in Germany: exenatide once weekly vs liraglutide once daily

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Background and aims: The aim was to estimate the adherence of primary care patients initiating therapy with GLP-1 receptor agonist (GLP-1 RA) once weekly (exenatide) or once daily (liraglutide).

Materials and methods: Initiation of GLP-1 RA therapy documented in statutory health insurance patients in Germany between January 1, 2011 and September 30, 2014 (date of initiation=index) were studied. The database covers 60% of all prescriptions nationwide. A minimum 6-month prior to and at least 6-month post-index pharmacy benefits enrollment was required for eligibility of inclusion. Adherence was measured using the 'proportion of days covered' (PDC) with index GLP-1RA during the 6-month post-index period. PDC was calculated by taking the total number of days supply per patient during the post-index 6-month period and dividing that by 180 days. Days supplied was calculated as follows: pack size * pack count * 7 for exenatide; and strength * pack size * pack count / 1.3 (median dose) for liraglutide.

Results: Overall, 5,449 patients (mean (sd) age: 59.7 (11.8) years, men 51.4%) initiating exenatide, and 24,648 patients (mean (SD) age: 59.4 (11.4) years, men 49.7%) initiating liraglutide therapy were included in the data analysis. The median PDC was 0.88 for exenatide and 0.77 for liraglutide ($p<0.001$). 53.4% of the patients receiving exenatide, and 47.7% of those receiving liraglutide had an adherence of at least 80% ($p<0.001$) during the 6 months after initiating the drugs. There was no significant gender difference in the median PDC (exenatide: male 0.85 and female 0.86; liraglutide: male 0.77 and female 0.77). The medians of the PDC in age groups of ≤50, 51-60, 61-70 and >70 years were 0.78, 0.92, 0.92 and 0.81 for exenatide, and 0.77, 0.79, 0.79 and 0.77 for liraglutide, respectively.

Conclusion: Over a 6-month period after initiation of GLP-1RA therapy, patients treated with exenatide once weekly had significantly better adherence compared with patients on liraglutide once daily, particularly among patients aged 50 years or older.

Supported by: Astrazeneca

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Achieving postprandial glucose control with lixisenatide improves glycaemic control in patients with type 2 diabetes mellitus on basal insulin

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Background and aims: Lixisenatide is a prandial glucagon-like peptide-1 receptor agonist. Given the rationale for combining complementary therapies to improve glycaemic control, we examined the impact of lixisenatide on postprandial plasma glucose (PPG) target achievement (measured 2 hours after a standard liquid breakfast), and subsequent efficacy and safety in patients with type 2 diabetes mellitus (T2DM) on basal insulin. The hypothesis was that achieving PPG targets with lixisenatide increases the likelihood of reaching HbA_{1c} goals.

Materials and methods: Data were analysed from the GetGoal-L, GetGoal-Duo1, and GetGoal-L Asia studies in which patients with T2DM were treated with lixisenatide plus basal insulin. Patients (lixisenatide: n=587, 47% male, mean weight 82 kg, mean HbA_{1c} 8.1%; and placebo: n=484, 50% male, mean weight 81 kg, mean HbA_{1c} 8.1%) were categorized by baseline fasting plasma glucose (FPG) levels of<126 or≥126 mg/dL (<7.0 or≥7.0 mmol/L, respectively). Lixisenatide-treated patients were grouped as PPG responders (<180 mg/dL [<10.0 mmol/L]) or non-responders (≥180 mg/dL [≥10.0 mmol/L]). Data were derived from a fixed effect meta-analysis with inverse variance weights. Changes in HbA_{1c} and body weight were analysed using an analysis of variance using last observation carried forward at Week 24.

Results: In both FPG categories, more lixisenatide-treated patients with T2DM reached target PPG versus placebo (<126 mg/dL [<7.0 mmol/L]: 55% vs 18%; and≥126 mg/dL [≥10.0 mmol/L]: 48% vs 10%). Greater reductions in HbA_{1c} and weight were seen in lixisenatide-treated patients; efficacy was superior in patients meeting the PPG targets for both FPG categories (Table). Lixisenatide-treated patients were more likely to achieve HbA_{1c}<7.0% and had no increased risk of symptomatic hypoglycaemia (Table).

Conclusion: These results support the hypothesis that achieving PPG targets with lixisenatide increases HbA_{1c} goal achievement in patients with T2DM and uncontrolled HbA_{1c} on basal insulin, and highlights the importance of PPG control.

Table. Efficacy and safety outcomes in patients with T2DM treated with lixisenatide.

	Baseline FPG < 126 mg/dL (< 7.0 mmol/L)			Baseline FPG ≥ 126 mg/dL (≥ 7.0 mmol/L)		
	PPG responders (PPG < 180 mg/dL [< 10.0 mmol/L])	PPG non-responders (PPG ≥ 180 mg/dL [≥ 10.0 mmol/L])	Difference between groups (SE), P-value	PPG responders (PPG < 180 mg/dL [< 10.0 mmol/L])	PPG non-responders (PPG ≥ 180 mg/dL [≥ 10.0 mmol/L])	Difference between groups (SE), P-value
	Lixisenatide n = 156 Placebo n = 47	Lixisenatide n = 327 Placebo n = 210		Lixisenatide n = 147 Placebo n = 23	Lixisenatide n = 157 Placebo n = 204	
Patients with HbA_{1c} < 7.0% at Week 24, %						
Lixisenatide	61.8	30.9	-30.7 (5.4), < 0.001	47.3	18.1	-33.7 (5.9), < 0.001
Placebo	55.3	12.9	-27.2 (9.2), < 0.001	35.0	7.2	-17.1 (10.7), 0.107
Change in HbA_{1c} from baseline to Week 24, mean (SE), %						
Lixisenatide	-0.56 (0.07)	-0.37 (0.07)	-0.55 (0.12), < 0.001	-1.07 (0.08)	-0.60 (0.08)	-0.65 (0.11), < 0.001
Placebo	+0.40 (0.12)	+0.11 (0.09)	-0.34 (0.12), 0.006	+0.73 (0.19)	+0.44 (0.07)	-0.43 (0.21), 0.035
Rate of symptomatic hypoglycaemia during the 24 week study period, mean (SE), rates per patient year						
Lixisenatide	0.40 (0.04)	0.36 (0.01)	0.03 (0.05), 0.609	0.26 (0.04)	0.19 (0.03)	0.06 (0.05), 0.219
Placebo	0.15 (0.05)	0.23 (0.03)	-0.05 (0.06), 0.418	0.12 (0.07)	0.15 (0.03)	-0.01 (0.08), 0.895
Body weight change from baseline to Week 24, mean (SE), kg						
Lixisenatide	1.37 (0.29)	0.15 (0.17)	1.35 (0.34), < 0.001	-0.79 (0.19)	-0.17 (0.15)	-0.83 (0.24), 0.634
Placebo	0.95 (0.47)	0.94 (0.19)	0.29 (0.47), 0.532	0.36 (0.54)	-0.01 (0.14)	0.26 (0.57), 0.654

SE, standard error.

Supported by: Sanofi US, Inc.

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Glucagon-like peptide 1 receptor agonist or rapid-acting insulin as add-on to basal insulin therapy in patients with type 2 diabetes mellitus: a meta-analysis

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Background and aims: When patients with type 2 diabetes mellitus (T2DM) are suboptimally controlled on oral antidiabetes agents plus basal insulin, clinicians must choose from a variety of next-step treatments. Glucagon-like peptide 1 receptor agonists (GLP) have been used as add-on to basal insulin (BI) in patients with T2DM due to their advantage in improving glycaemic control, with a lower risk of hypoglycaemia and less weight gain compared with intensified insulin regimens. This meta-analysis was conducted to compare add-on GLP (BI+GLP) with add-on rapid-acting insulin (BI+RAI) in patients with T2DM whose glycaemic control remained unoptimised with basal insulin therapy alone.
Materials and methods: Peer-reviewed literature was searched using PubMed and EMBASE, as well as abstracts presented at the American Diabetes Association and European Association for the Study of Diabetes meetings from 2009-2013. Endpoints included HbA_{1c} levels and symptomatic hypoglycaemia (defined as blood glucose level <56 mg/dL); and change in body weight and fasting plasma glucose (FPG). Three randomised clinical trials (duration 26-44 weeks) involving 1,370 patients with T2DM (28.4-54.0% of which were female) were included. Patients were aged 54.8-61.1 years, had baseline HbA_{1c} levels of 7.7-8.5%, and a BMI of 32.0-32.7 kg/m^2.
Results: Compared with patients treated with BI+RAI, patients treated with BI+GLP had significantly greater reductions in HbA_{1c} and body weight, and a significantly lower risk of symptomatic hypoglycaemia. Furthermore, these patients had numerically greater FPG reductions and, although not significant, higher odds of achieving HbA_{1c} <7.0% than patients treated with BI+RAI (Table).
Conclusion: GLP-1 RA therapy is a viable option to complement insulin therapy as the addition of a GLP-1 RA to basal insulin therapy improved glycaemic control with a lower risk of symptomatic hypoglycaemia, and without weight gain compared with the addition of RAI to basal insulin therapy. Therefore, this combined approach can be particularly valuable for patients with T2DM who are treated with basal insulin and have poor glycaemic control or issues with weight gain.

Table. Summary of meta-analysis outcomes.

Outcome	Trials	GLP-1 RA vs RAI Added to Basal Insulin	95% CI	*P* value
		Point estimate		
Change in HbA_{1c}, %	3	−0.16	−0.31, −0.01	0.0383*
Change in weight, kg	3	−3.27	−5.36, −1.18	0.0021*
Change in FPG, mmol/L	2	−0.43	−0.97, 0.12	0.1293
mg/dL		−7.7	−13.9, −2.7	
		Odds ratio		
Endpoint HbA_{1c} < 7.0%	3	1.22	0.95, 1.56	0.1203
Symptomatic hypoglycaemia	2	0.53	0.38, 0.73	0.0001*
*Indicates a significant *P* value.				

Supported by: Sanofi US, Inc.

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Real world glucose and weight control in patients treated with GLP-1 receptor agonists, with addition or treatment change to insulin

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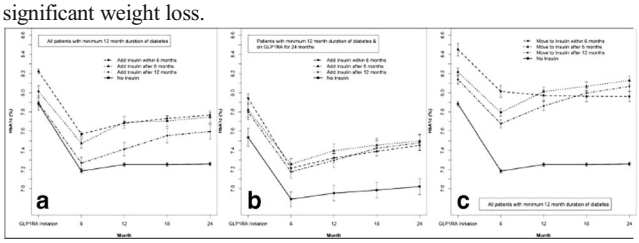
Background and aims: Glucose control and weight change in patients (pts) treated with GLP-1 receptor agonists (GLP-1 RA), and the patterns of addition or change to insulin from GLP-1 RA are not well studied in a real world setting. The aims were to (1) evaluate the change in HbA1c and weight over 6 months, 1- and 2-years post initiation of GLP-1 RA and (2) evaluate the time and possible benefits of adding or change to insulin treatment post GLP-1 RA.
Materials and methods: A cohort of 75964 T2DM pts, who initiated GLP-1 RA treatment from May 2005, were selected for this longitudinal cohort study from the Centricity EMR Database of USA, with follow-up data available till September 2014. Longitudinal data on demographics, HbA1c, weight and anti-diabetes medication before/after the GLP-RA initiation were available. These pts did not receive insulin before GLP-1 RA initiation.
Results: At baseline, the 75964 pts were mean (SD) 61 (11) yrs old, 43% male, with mean(SD) HbA1c, weight and BMI of 8.2 (1.5)%, 109 (26) kg and 38 (8) kg/m2 respectively. Of these, 46353 and 29611 initiated Exenatide and Liraglutide respectively with concomitant medications of 86% / 59% metformin/sulfonylurea (SU), while 13%/12% stopped metformin/SU. The proportions of pts with HbA1c>7.5% and 8% at GLP-1 RA initiation were 69% and 51% respectively. Mean (min, max) and median (IQR) duration of treatment with GLP-1 RA were 11 (1, 121) months and 5 (1, 14) months respectively. After GLP-1 RA treatment for at least 6-months (n=35267), 1-year (n=23414) and 2-years (n=10834), 30%, 32% and 33% pts reduced their HbA1c below 7% respectively. The adjusted changes in body weight at these time intervals were -2.3 (-2.4, -2.2) kg, -3.0 (-3.1, -2.9) kg and - 3.7 (-3.9, -3.5) kg respectively. In the cohort, 44% (n=33341) and 22% (n=16726) pts added and changed to insulin, with mean (min, max) 3 (0, 106) months and 16 (0, 111) months to adding and changing to insulin respectively. The mean / median HbA1c levels at the time of adding and changing to insulin were 8.3/7.9% and 8.8/8.5% respectively. Pts who added insulin within 1 year of GLP-1 RA initiation reduced the HbA1c by an additional 0.60% at 1 year, starting with a baseline HbA1c of 8.3% (Fig 1). Compared to pts who changed to insulin within a year, those who added insulin showed an additional HbA1c reduction by 0.34%. Compared to pts who moved to insulin, those who added insulin within 1 year were 2.4 times (95% CI of odds ratio: 2.2, 2.6) more likely to reduce HbA1c below 7% within 1 year.
Conclusion: Among pts with HbA1c above 8%, more than a third were likely to achieve a guideline recommended glucose control within one year of initiation of GLP-1 RA treatment with accompanying weight loss during this period. In poorly controlled pts, early addition of insulin treatment with GLP-1 RA, rather than changing to insulin, offers clinically significant glycaemic benefits, while maintaining the statistically significant weight loss.



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Non-adherence and non-persistence related to GLP-1 therapy in patients with type 2 diabetes mellitus: analysis of a large UK CPRD dataset

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Background and aims: This study describes the extent of non-adherence (NA) and non-persistence (NP) with Glucagon-like peptide 1 agonists (GLP-1) and oral anti-diabetic (OAD) therapy in T2DM patients in clinical practice in the UK.
Materials and methods: Electronic primary health care data collected in the CPRD (Clinical Practice Research Datalink) database from 2010-2012 were used to identify patients with ≥1 diagnosis code for T2DM and also at least one antidiabetic medication prescription. NA and NP were measured for patients initiating new T2DM medication (no prescription of respective medication in prior 6 months). A patient was classified as NP when a medication gap≥90 days was observed. Patient-specific estimates of adherence were calculated via medication possession ratios (MPR) for those patients maintaining their therapy (without gaps≥ 90 days) during a one-year observational period. The maximum adherence possible was based on the assumption that adherence to therapy is equivalent to the drug-specific DDD (defined daily dosage). NA was defined as MPR<80%. Descriptive analyses with percentage of patients classified as NP and/or NA were conducted.
Results: The persistence analysis included 1,905 and 13,387 T2DM patients initiating GLP-1 and OAD-therapy, respectively. Among GLP-1 patients, 1,083 received once-daily (OD) and 545 received twice daily (BID) therapies, the remaining patients switched between regimens. Mean age for GLP-1 users was 55.5 (SD 10.6) and 60.6 (SD 13.2) years in the OAD cohort. After 12 months, the percentage of patients with NP was 29.6% (all GLP-1 s), 27.2% (OD-GLP-1), 36.0% (BID-GLP-1), and 23.2% (OAD). The NA analysis included 1,744 GLP-1 and 11,722 OAD patients initiating therapy with ≥1 follow-up prescription. Mean age was 55.2 (SD 10.4) and 60.2 (SD 12.9) years for the GLP and OAD cohorts, respectively. Mean MPR was 88.6% (all GLP-1 s), 88.2% (OD-GLP-1), 89.3% (BID-GLP-1) and 64.7% (OAD). Percentage of patients affected by NA was 20.2% (all GLP-1 s), 20.0% (OD-GLP-1), 20.5% (BID-GLP-1) and 68.4% (OAD).
Conclusion: In this UK dataset, we observed several patterns of patient behaviour. Persistence with OAD therapy was numerically higher than with GLP-1 therapy. Adherence to therapy within the GLP-1 cohort was high, and appears to be similar between dosing frequency regimens, while persistence rates may be higher with less frequently dosed GLP-1 s and should be investigated further.
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Co-administration of a lipidated GIPR agonist with a GLP1 analogue provides no additional benefit on HbA_{1c}% over GLP1 analogue in db/db mice

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Background and aims: The incretin hormone glucose-dependent insulinotrophic polypeptide (GIP) has well established actions in the β cell to enhance insulin secretion in humans. In addition, variants at the *GIP receptor* (*GIPR*) gene locus are associated with elevated glucose and decreased insulin secretion. However, there has been controversy regarding the anti-diabetic potential for GIPR agonists as many patients with diabetes exhibit greatly reduced insulin responses to GIP. Further studies have suggested that normalisation of glycemia in diabetics can restore GIP sensitivity. In this study we set out to examine the anti-diabetic potential for GIPR agonism alone, or in combination with a GLP1R agonist in *db/db* mice.
Materials and methods: A total of 56 male db/db mice 9 weeks of age were randomised into seven groups based on baseline HbA1c%. Mice received either vehicle, low dose (LD) GLP1R agonist alone, LD GIPR agonist alone, combination of LD GLP1R agonist plus LD GIPR agonist, high dose (HD) GLP1R agonist alone, HD GIPR agonist alone or combination of HD GLP1R agonist plus HD GIPR agonist. LD and HD were determined by acute dose response glucose tolerance tests carried out in lean C57BL/6J mice. The GLP1 analogue was dosed at 10 nmol/Kg and 30 nmol/Kg for LD and HD respectively. The GIPR agonist was a lipidated GIP molecule dosed at 1 nmol/Kg and 10 nmol/Kg for LD and HD respectively. Mice received daily s.c. injections≈3 hours before the dark cycle for three weeks. HbA1c% was measured at the beginning and end of the study, 6 hour fasted blood glucose was measured at weekly intervals. Data was analysed by a one way ANOVA with a Fisher's LSD post hoc test.
Results: Over the duration of the study the HbA1c% in vehicle group increased by 2.49%, treatment with a GLP1 analogue significantly attenuated the increase in HbA1c% by 1.2% in HD group and by 0.79% in the LD group, (vehicle vs HD GLP1R $p<0.0001$, vehicle vs LD GLP1R $P<0.001$). Treatment with lipidated GIP attenuated the increase in HbA1c% in the HD group by 0.52% (vehicle vs HD GIP1R $p<0.05$) and had no significant effect in the LD group. In combination with a GLP1 analogue the addition of GIPR agonism provided no additional benefit over GLP1R agonism to lower HbAc1% at either dose. Initially, weekly fasted glucose readings suggested dual GLP1R/GIPR agonism did improve glucose control to a greater extent than either single agent alone in the HD groups (Δfasted glucose 7 days: HD combination -11.96±0.91 vs HD GLP1R -4.10±3.44 $p=0.046$, 14 days: HD combination -8.71±1.46 vs HD GLP1R 0.03±3.64 $p=0.054$). Any differential effect between GLP1 single agent HD and the combination with GIP was lost by the end of the study (21 days: -0.40±3.72 vs -0.53±2.24 p=n.s). These results are consistent with a parallel study in which an Fc conjugated GIP molecule had only modest effects on HbA1c% as a single agent and provided no additional benefit in combination with GLP1R agonism in *db/db* mice.
Conclusion: The glucose lowering actions of GIP are greatly blunted in the diabetic *db/db* mouse model. In combination with a GLP1 analogue, GIPR agonism did provide an initial additional benefit in glucose control; however, these effects were not sustained and did not translate to an overall improvement in HbA1c%. In summary, these data do not support the hypothesis that combinations of GIPR/GLP1R agonists would provide a useful strategy to treat T2D.

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Do we need another consideration during incretin based therapy? Aberrant expression of glucagon-like peptide-1 receptor in papillary thyroid carcinomas

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Background and aims: Incretin-based therapies are rapidly becoming one of the main glucose lowering agents in diabetes. Considering the large numbers of papillary thyroid carcinomas (PTC) and possible effects of Glucagon-like peptide-1 (GLP-1) on cell proliferation, the expression of GLP-1 receptor (GLP-1R) in PTC is likely to have clinical

significance. We performed this study to evaluate the expression of GLP-1R in PTC.

Materials and methods: Eighty-three cases of PTC and fifty-five cases of normal thyroid tissue were selected for immunostaining for GLP-1R by polyclonal antibody. Twenty-eight cases of PTC and twelve cases of normal thyroid tissue were selected for immunostaining for GLP-1R by monoclonal antibody. Total RNA was isolated from twenty-one cases of PTC and twelve cases of normal thyroid tissue for evaluation of GLP-1R mRNA RT-PCR.

Results: Immunohistochemical staining for GLP-1R by polyclonal antibody exhibited immunoreactivity in thirty-seven of eighty-three cases (44.6%) in PTC but, none of fifty-five cases of normal thyroid follicular cells. Eight of twenty-one cases (38.1%) of PTC and none of twelve normal thyroid tissues expressed GLP-1R mRNA.

Conclusion: Some of PTC tissues express GLP-1R, although normal thyroid follicular tissues do not express GLP-1R. The clinical significance of GLP-1R expression in PTC and long-term influence of pharmacologically increased GLP-1 to development and progression of GLP-1R expressed PTC associated with incretin based therapy should be evaluated.

PS 071 DPP-4 inhibitors: antihyperglycaemic efficacy

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A randomised, double-blind trial of saxagliptin add-on to dapagliflozin + metformin

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Background and aims: DPP-4 and SGLT2 inhibitors have complementary mechanisms of action that can potentially improve glucose control with a low risk of hypoglycemia. We compared the efficacy and safety of saxagliptin (SAXA) vs placebo (PBO) add on to dapagliflozin (DAPA) + metformin IR (MET) in adults with type 2 diabetes (T2D).

Materials and methods: Patients on stable MET (≥1500 mg/d) for ≥8 weeks with baseline A1C 8.0%-11.5% (mean A1C at screening 9.3%) received open-label DAPA 10 mg/d + MET for 16 weeks. At the end of the open-label period, patients with inadequate glycemic control (A1C 7%-10.5%) were randomized to PBO (n=153) or SAXA 5 mg/d (n=162) in addition to background DAPA+MET. Primary end point was change in A1C from baseline to week 24. Secondary end points included fasting plasma glucose (FPG), 2-hour postprandial glucose (PPG), and the proportion of patients achieving A1C<7%.

Results: There was a significantly greater reduction in A1C at 24 weeks with SAXA+DAPA+MET vs PBO+DAPA+MET **(Table)**. Reductions in FPG and PPG were similar between treatment arms. A greater proportion of patients achieved A1C <7% with SAXA+DAPA+MET vs PBO+DAPA+MET. AEs were similar across treatment groups, although urinary tract infections and nasopharyngitis were higher with SAXA+DAPA+MET. Episodes of hypoglycemia were rare.

Conclusion: Addition of SAXA to DAPA+MET is well tolerated and produces significant improvements in glycemic control in patients with T2D inadequately controlled with DAPA+MET.

	SAXA+DAPA+MET n=153	PBO+DAPA+MET n=162
Mean (SD) age, y	55 (9.8)	55 (9.3)
Women, n (%)	80 (52)	86 (53)
Mean (SD)T2D duration, y	8.1 (7.0)	7.4 (5.8)
End point at 24 weeks		
A1C,%		
n	150	160
Baseline mean (SD)	7.95 (0.83)	7.85 (0.92)
Adjusted mean change from baseline at 24 weeks (95% CI)	−0.51 (−0.63, −0.39)	−0.16 (−0.28, −0.04)
Mean difference (95% CI) vs PBO	−0.35 (−0.52, −0.18), *P*<0.0001	
2-h PPG, mg/dL		
n	135	144
Baseline mean (SD)	208 (50.6)	204 (52.0)
Adjusted mean change from baseline at 24 weeks (95% CI)	−37 (−43.6, −30.7)	−31 (−37.5, −25.0)
Mean difference (95% CI) vs PBO	−6 (−14.9, 3.1), *P*=0.20	
FPG, mg/dL		
n	151	160
Baseline mean (SD)	164 (34.3)	157 (33.9)
Adjusted mean change from baseline at 24 weeks (95% CI)	−9 (−14.3, −3.9)	−5 (−10.4, −0.2)
Mean difference (95% CI) vs PBO	−4 (−11.0, 3.6), *P*=0.32	
Patients with A1C<7%		
x/n	51/150	39/160
Adjusted % (95% CI)	35 (28.2, 42.4)	23 (16.9, 29.3)
Mean difference % (95% CI) vs PBO	12 (3.4, 21.0), *P*=0.0068	
Adverse events, n (%)		
Hypoglycemia	2 (1.3)	4 (2.5)
Urinary tract infection	8 (5.2)	6 (3.7)
Nasopharyngitis	8 (5.2)	3 (1.9)
Vulvovaginal mycotic infection	0	4 (2.5)

Clinical Trial Registration Number: NCT01619059
Supported by: AstraZeneca

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Effects of gemigliptin versus sitagliptin or glimepiride on glycaemic variability as initial combination therapy with metformin in drug-naïve patients with type 2 diabetes

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Background and aims: Glycemic variability and chronic sustained hyperglycemia are the main components of dysglycemia in diabetes. The purpose of this study was to assess whether there is a difference between- or within drug classes on glycemic variability and glucose control as initial combination therapy with metformin in drug-naive patients with type 2 diabetes.
Materials and methods: A multi-center, randomized, active controlled, open-label, parallel design study was performed in 69 patients with HbA1c greater than 7.5%. Subjects were randomized (1:1:1) to receive gemigliptin 50 mg qd, sitagliptin 100 mg qd, or glimepiride 2 mg qd for 12 weeks. The mean amplitude of glycemic excursions (MAGE) and standard deviation (SD) were used for assessing glucose fluctuations at baseline and after 12 weeks. Glycosylated hemoglobin (HbA1c), glycated albumin (GA), fructosamine, C-reactive protein and other metabolic parameters were also measured. Safety and tolerability based on adverse events (AEs) were assessed. To strengthen the reliability of the study results, data from continuous glucose monitoring system (CGMS) was evaluated independently by a blinded central evaluator.
Results: A total of 69 subjects were randomized to receive gemigliptin 50 mg (n=24), sitagliptin 100 mg (n=23) or glimepiride 2 mg (n=22). Mean baseline characteristics were similar across the groups (age, 50.0 years; HbA1c, 9.4%; GA, 26.4%; fructosamine, 412.8 μmol/L). At 12 weeks, MAGE was significantly lower in the DPP-4 inhibitor groups, gemigliptin and sitagliptin, than in the glimepiride group (-43.1, -38.3, and -21.7 mg/dl, respectively). Furthermore, the SD of mean glucose was significantly lower in patients with gemigliptin (vs sitagliptin p=0.01; vs glimepiride p=0.007) when compared with sitagliptin and glimepiride. Mean HbA1c was reduced from baseline by 2.75%, 2.24% and 2.75% for gemigliptin, sitagliptin and glimepiride, respectively. A similar profile was also observed in other glycemic control parameters (FPG, glycated albumin, and fructosamine). A greater decrease in total- and LDL-cholesterol and nitrotyrosine was observed for DPP-4 inhibitor groups versus glimepiride, although there were no significant differences between the groups. In addition, only gemigliptin significantly decreased C-reactive protein levels from baseline. Drug-related AEs including symptomatic hypoglycemia were reported more frequently in glimepiride group than in other groups.
Conclusion: In summary, gemigliptin was more effective than glimepiride and sitagliptin in reducing glucose variability as initial combination therapy with metformin in drug-naïve patients with T2DM.
Clinical Trial Registration Number: NCT01787396
Supported by: LG Life Sciences

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Efficacy and safety of gemigliptin in type 2 diabetes patients with moderate to severe renal impairment

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Background and aims: Renal impairment in type 2 diabetes mellitus limits the available glucose-lowering medication and requires frequent monitoring of renal function. Gemigliptin, a potent and selective DPP-4 inhibitor, can be used without dose reduction in renal impairment. This randomized, double blind, parallel group Phase 3b study comprised a 12-week, placebo-controlled phase followed by a 40-week, double blind active-controlled extension phase (placebo switched to linagliptin). This 12-week randomized trial investigated the efficacy and safety of gemigliptin in type 2 diabetic patients with moderate to severe renal impairment. It was also evaluated whether gemigliptin has renoprotective effects in these patients.
Materials and methods: A total of 132 patients were randomized to gemigliptin (n=66) or placebo (n=66). Primary endpoint was HbA1c change from baseline at Week 12. Baseline demographics were similar between treatment groups (mean HbA1c 8.4%; age 62.0 years; BMI 26.2 kg/m2, duration of T2DM 16.3 years; eGFR 33.3 mL/min/1.73 m2). The predominant background therapy was insulin (63.1%).
Results: At Week 12, adjusted mean±SE change HbA1c with gemigliptin was -0.83±0.14%(change with placebo 0.38±0.14%; difference -1.21, 95% CI -1.54 to -0.89; p<0.0001). A similar profile was also observed in other glycemic control parameters (FPG, glycated albumin, and fructosamine). After 12 weeks of gemigliptin treatment, albuminuria (measured by urinary albumin creatinine ratio [UACR]) significantly decreased in the patients with micro- and macro-albuminuria. Furthermore, urinary nephrin and type IV collagen in the gemigliptin were also significantly reduced compared to placebo. Drug-related AEs including hypoglycemia for gemigliptin was similar to placebo (15.2% and 12.1%, respectively). There was no meaningful change from baseline in body weight (gemigliptin, -0.3 kg; placebo -0.2 kg).
Conclusion: Gemigliptin improved glycemic control and provided additional renoprotection in T2DM patients with moderate to severe renal impairment. There was no additional risk of hypoglycemia and no weight gain.
Clinical Trial Registration Number: NCT01968044
Supported by: LG Life Sciences

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Reduced incidence of hypoglycaemia with sitagliptin used with intensively titrated insulin may be due to factors other than the difference in insulin dose

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Background and aims: Addition of sitagliptin (SITA) relative to placebo (PBO) in patients (pts) (N=658) with type 2 diabetes who intensively titrate insulin glargine±metformin has been shown to result in a lower incremental insulin dose requirement (mean Δ=-4.7 units), better

glycemic control (mean ΔA1C=-0.45%), and a lower incidence of adverse events of symptomatic hypoglycemia (HYPO) (mean Δ=-11.6%).
Materials and methods: To determine if the reduced insulin dose requirement with SITA accounted for the lower HYPO incidence, we conducted a post hoc analysis of the incidence of HYPO in subgroups based on quartiles (Q) of change in insulin dose from baseline for the entire study cohort (Table).
Results: PBO-adjusted ΔA1C in Q 1-4 were -0.33%, -0.62%, -0.47% and -0.47%, respectively. For pts with the lowest insulin dose increment (<=6 units), no difference in incidence of HYPO was seen. For pts in the remaining 3 Qs, the incidence of HYPO was lower with SITA. Additionally, an analysis of the incidence of asymptomatic hypoglycemia showed similar between-group results in Q1 and Q2 but lower incidences in the SITA group in Q3 and Q4 (7.7% vs 17.9% and 1.5% vs 12.5% for SITA and PBO, respectively).
Conclusion: These analyses suggest that the reduced incidence of hypoglycemia seen when sitagliptin is used in conjunction with intensively titrated insulin may be due to factors other than the difference in insulin dose.

	Within-quartile Change from baseline in Insulin Dose	Mean Δ Insulin Dose: Sitagliptin Group	Mean Δ Insulin Dose: Placebo Group	HYPO: Sitagliptin Group Incidence (n/N)	HYPO: Placebo Group Incidence (n/N)	Difference in % HYPO (95% CI)
1st quartile	<=6 IU/Day	0.2	-0.8	37.0% (37/100)	30.8% (24/78)	6.2 (-7.9, 19.9)
2nd quartile	6, 14.3 IU/Day	11.1	10.6	15.7% (13/83)	31.8% (21/66)	-16.2 (-30.0, -2.5)
3rd quartile	14.3, 32 IU/Day	22.7	22.6	25.6% (20/78)	45.3% (43/95)	-19.6 (-33.0, -5.3)
4th quartile	>32 IU/Day	49.1	53.7	20.0% (13/65)	37.5% (33/88)	-17.5 (-31.0, -2.8)

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Type 2 diabetes patients on metformin and well-controlled basal: Can supplementary vildagliptin control residual prandial hyperglycaemia? VIBE study results

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Background and aims: In patients with type-2 diabetes (DT2) treated with metformin and well-titrated basal insulin whose HbA1c remains above 7%, failure to optimize glycemic control is mainly due to a persistent elevation in postprandial glycaemia (PPG). Can supplementary vildagliptin reduce HbA1c to target levels (<7.0%) vs. placebo?
Materials and methods: In the study, 34 DT2 patients receiving metformin at the maximum tolerated dose together with well-titrated insulin glargin (fasting blood glucose: <1.20 g/l), but with HbA1c persistently between 7 and 9%, were randomised to double-blind cross-over treatment with either vildagliptin (50 mg b.i.d.) or placebo, with the 3-month treatment periods being separated by a 3-month wash-out period. Immediately prior to the end of these two periods, patients wore a continuous glucose monitoring device for 5 days. The amount of carbohydrates was collected at the same time for each of the 5-day meals.
Results: 31 patients were randomised (4 F/27H) and 3 dropped out. Baseline data were as follows: HbA1c: 7.65.±0.5%; diabetes duration: 6.1±7.9 years; age: 59.4±7.6 years; BMI: 28.6±4.3; insulin glargin dose: 39.3±36.8 u/d; metformin dose: 2.8±0.29 g/d. The proportion of patients acheiving an HbA1c <7% with vildagliptin was 4 times as high as that of patients treated with placebo (28.6% vs. 7.4%, p=0.007). Moreover, the addition of vildagliptin allowed a mean HbA1c reduction of 1% in the vildagliptin group vs placebo (vildagliptin: -0.7 (±0.9)%; placebo=+0.3 (±0.9)%; p=0.002). Glucose excursions on CGM curves at mealtime, estimated through AUCs, were significantly lower with vildagliptin vs. placebo. Significantly more time was spent in the range [70-180 mg/dl] with vildagliptin vs placebo [74.8 (±18.0)% vs. 61.1 (±22.9)%]. Conversely the time spent above 180 mg/dl was significantly lower for vildagliptin vs placebo (21.2 (±17.9)) vs 37.4 (±23.2). Predictors for patient response to treatment were also studied.
Conclusion: In patients treated with metformin and well-titrated basal insulin, uncontrolled postprandial glycemic excursions are significantly improved by the addition of vildagliptin. By that way, 4 times more patients achieved the HbA1c target below 7%. This improved response involved a better control of post-prandial glycaemia, as attested by CGM data.
Clinical Trial Registration Number: NCT01757587
Supported by: Novartis

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Efficacy and safety of linagliptin/metformin fixed-dose combination as initial therapy in drug-naïve Asian patients with type 2 diabetes

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Background and aims: A 24-week study investigated the efficacy and safety of initial fixed-dose combination (FDC) therapy with linagliptin (L) and metformin (M) immediate release in drug-naïve patients from Asia (83.4% from China) with type 2 diabetes (T2D) and inadequate glycemic control.
Materials and methods: In total 733 patients (initial HbA1c [%] ≥7.5-< 11) were randomized to one of five regimens (L 5 mg QD, M 500 mg BID, M 1000 mg BID, L 2.5 mg/M 500 mg BID, L 2.5 mg/M 1000 mg BID). The primary endpoint was HbA1c change from baseline to week 24.
Results: Overall mean baseline (SD) age, body mass index, HbA1c and fasting plasma glucose (FPG) were 51.3 (10.0) years, 26.0 (3.6) kg/m^2, 8.7 (1.0) % and 169.0 (39.5) mg/dL, respectively. At week 24, the FDCs showed clinically meaningful reductions from baseline in HbA1c (Table) and FPG. The first test of the primary HbA1c analysis (L 2.5 mg /M 1000 mg BID vs M 1000 mg BID) was borderline non-significant; however, all but one pre-defined sensitivity analysis showed that the FDCs had significantly greater reductions in HbA1c from baseline vs their respective monotherapy components. This was also shown for a predefined subgroup analysis in Chinese patients. Target HbA1c (<7%, week 24) was reached in the following % of patients: L 5 mg QD (44%), M 500 mg BID (50%), M 1000 mg BID (69%), L 2.5 mg/M 500 mg BID (72%), L 2.5 mg/M 1000 mg BID (77%). Adverse event (AE) rates were similar across groups except for a small but expected increase in gastrointestinal AEs with M and FDCs particularly with M 1000 mg. Hypoglycemic AEs were low across groups.
Conclusion: FDCs significantly improved glycemic control and were well tolerated.

Table. *Adjusted change from baseline (BL) to week (wk) 24 for HbA1c by treatment groups**

Primary endpoint**	**L 5 mg QD (*n*=141)**	**M 500 mg BID (*n*=144)**	**M 1000 mg BID (*n*=133)**	**L 2.5 mg/ M 500 mg BID (*n*=142)**	**L 2.5 mg/ M 1000 mg BID (*n*=141)**
BL, mean (SE)	8.66 (0.08)	8.70 (0.09)	8.63 (0.09)	8.65 (0.08)	8.74 (0.09)
Change from BL to wk 24, adjusted mean (SE)	−1.29 (0.08)	−1.64 (0.08)	−2.07 (0.08)	−2.15 (0.08)	−2.29 (0.08)
Comparison with L2.5/M500 (L2.5/M500 – comparator), adjusted mean (95% CI)	−0.87 (−1.09, −0.64; p<0.0001)	−0.51(−0.73, −0.29; p<0.0001)	–	–	–
Comparison with L2.5/M1000 (L2.5/M1000 – comparator), adjusted mean (95% CI)	−1.00 (−1.23, −0.78; p<0.0001)	–	−0.22 (−0.45, 0.01; p=0.0587)	–	–
Pre-defined sensitivity analysis***	**L 5 mg QD (*n*=138)**	**M 500 mg BID (*n*=144)**	**M 1000 mg BID (*n*=133)**	**L 2.5 mg / M 500 mg BID (*n*=142)**	**L 2.5 mg / M 1000 mg BID (*n*=139)**
BL, mean (SE)	8.65 (0.08)	8.70 (0.09)	8.63 (0.09)	8.65 (0.08)	8.74 (0.09)
Change from BL to wk 24, adjusted mean (SE)	−1.34 (0.08)	−1.68 (0.08)	−2.08 (0.08)	−2.16 (0.08)	−2.38 (0.08)
Comparison with L2.5/M500 (L2.5/M500 – comparator), adjusted mean (95% CI)	−0.81 (−1.04, −0.58; p<0.0001)	−0.47 (−0.70, −0.24; p<0.0001)	–	–	–
Comparison with L2.5/M1000 (L2.5/M1000 – comparator), adjusted mean (95% CI)	−1.04 (−1.27, −0.81; p<0.0001)	–	−0.30 (−0.53, −0.07; p=0.0109)	–	–

*Full analysis set (FAS, all randomized pts with a BL HbA1c value, treated with ≥1 dose of the study drug, and with ≥1 on-treatment HbA1c value); **FAS using last observation carried forward method – model includes continuous BL HbA1c and treatment group; ***FAS (observed cases) using mixed model repeated measurements − model includes continuous BL HbA1c, treatment, week and treatment*week (significant treatment differences were seen in favor of the 2 FDCs for all 4 comparisons vs individual components at wks 6, 12, 18 and 24)

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Use of the Japanese health insurance claims database to assess durability of DPP-4 inhibitors in patients with diabetes: comparison with other anti-diabetic drugs

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Background and aims: Dipeptidyl peptidase-4 inhibitors (DPP-4i) have been widely used to manage type 2 diabetes (T2DM), especially in Japanese who show greater HbA1c-lowering effects compared to other ethnicities. However, its long-term therapeutic durability remains to be shown in actual clinical practice. The current study was initiated to evaluate durability of DPP-4i, GLP-1 receptor agonists (GLP-1RA) and other oral anti-diabetic drugs (OADs) among Japanese T2DM patients.
Materials and methods: The Japan Medical Data Center Claims Database, a comprehensive medical and pharmacy claims database, was used to compare average days before prescription change (DBPC) of anti-diabetic drugs in 1) untreated patients who received DPP-4i, GLP-1RA or other OADs as monotherapy (n=1,918) and 2) patients who had been on one OAD or GLP-1RA and received DPP-4i, GLP-1RA or other OADs as either switch (n=662) or add-on therapy (n=1,113). Patients aged 30-74 years with pharmacy and medical claims data for a continuous period of at least 32 months were included. This allowed a 6-month period for baseline observations and 26 months of observation after initiation of the index medication. Patients with at least one ICD-10 code of E11 or E14 during the observational period were subjected to further analyses, while those with E10, E12 and E13 were excluded. Patients on insulin injection and/or those on more than 2 OADs were excluded. The index date was defined as the prescription date of the first claim for a new OAD during the target period from December 1, 2009 through May 31, 2012. An anti-diabetic drug was considered new if there were no claims for the medication during the prior 6 months. The observation period started on the index date and ended on the initiation of another new OAD, GLP-1RA or insulin. The same patients were included multiple times into different index drug groups if they met the criteria.
Results: Among untreated patients who received OAD as monotherapy, DPP-4i (n=795) showed significantly longer DBPC compared to other OADs (n=1,117) (DPP4i 546.9 days; and Others 433.9 days; $p<0.0001$). Kaplan Meier analysis confirmed that DPP-4i showed longer DBPC than other OADs (Log-rank test, $p<0.0001$). Among patients who had been on one OAD or GLP-1RA and received OAD as switch, DPP-4i (n=397) showed significantly longer DBPC compared to other OADs (n=261) (DPP4i 554.5 days; and Others 367.7 days; $p=0.0002$). Among patients who had been on one OAD or GLP-1RA and received OAD as add-on, DPP-4i (n=431) showed significantly longer DBPC compared to other OADs (n=682) (DPP4i 564.9 days; and Others 442.4 days; $p<0.0001$). Among various OADs, DPP-4i add-on showed longer DBPC with α-glycosidase inhibitors (AGI) (AGI 650.0 days; Others 555.7 days; $p=0.0004$). Cox proportional hazard models revealed lower chance for prescription change with DPP-4i compared to other OADs as monotherapy or dual therapies. Limited or no patients receiving GLP-1RA as monotherapy (n=10) or dual therapy (n=0) did not allow meaningful analysis.
Conclusion: Despite numerous limitations, our findings indicate that DPP-4i, as first-line or second-line therapy, has long-term therapeutic durability superior to other OADs for Japanese T2DM in clinical practice.
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PS 072 DPP-4 inhibitors: non-glycaemic effects

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Dipeptidly peptide-4 (DPP-4) inhibitors did not increase the incidence of infection in immune-compromised patients under chemotherapy

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Background and aims: It is still unknown whether suppression of DPP-4 activity, which is known as T-cell activation antigen CD26, increases risk of infection. To clarify this issue, we compared the infection rate between the patients with malignant lymphoma with and without administration of DPP-4 inhibitor (DPP-4I) under chemotherapy.
Materials and methods: We analyzed 815 exposure chemotherapies, performed in 162 patients from September 2006 to December 2014 [mean (±SD) age: 66.8±13.2 years, men: 43.1%, range: 1-8 times/person]. Infection was defined as a body temperature higher than 38 degrees C except for infusion reactions of anticancer drug, or diagnosed by physician within 28 days after chemotherapy. We compared incidence of infection between 2 groups, classified by user of DPP-4I or non-user.
Results: The numbers of infection case/total care were 11/54 (20.4%) in user of DPP-4I, 215/761 (28.3%) in non-user. No significant increased risk of infection was observed in DPP-4I user adjusting for age, sex, Ann Arbor staging, performance status and serum levels of albumin [OR: 1.62 (95%CI: 0.78 - 3.33), $p=0.193$]. In contrast, serum levels of albumin and hemoglobin were significant preventive factor for infection [OR: 0.37 (95%CI: 0.25 - 0.53) and OR: 0.88 (95%CI: 0.79 - 0.99), respectively].
Conclusion: This study suggested that DPP-4I treatment did not affect the immune system even in immune-compromised patients under chemotherapy.

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Linagliptin acutely increases endothelial progenitor cells and anti-inflammatory monocytes in a randomised, placebo-controlled cross-over study

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Background and aims: Circulating cells, including endothelial progenitor cells (EPCs) and monocyte subtypes are likely involved in the pathogenesis of diabetic complications. We herein assessed whether the dipeptidyl peptidase-4 (DPP-4) inhibitor linagliptin acutely modifies EPCs and monocyte subsets in patients with type 2 diabetes.
Materials and methods: This was a randomized cross-over placebo-controlled trial in which 46 type 2 diabetic patients with (n=18) or without (n=28) chronic kidney disease (CKD) underwent a 4 day treatment with linagliptin or placebo in random order, in 2 periods separated by a washout of 2 weeks. Before and the day after each period of 4-day treatment with linagliptin or placebo, blood samples were collected for the determination of EPC phenotypes and monocyte subsets.
Results: Linagliptin reduced DPP-4 activity by about 70%. Compared to placebo, linagliptin increased CD34+CD133+ progenitor cells (placebo-subtracted effect +40.4±18.7/10^6; $p=0.036$), CD34+KDR+ EPCs (placebo-subtracted effect +22.1±10.2/10^6; $p=0.036$), and CX3CR1^bright monocytes (placebo-subtracted effect +1.7±0.8%; $p=0.027$). No effect was seen on other EPC phenotypes, M1 and M2 monocyte-macrophages, as well as traditional monocyte subsets (classical, intermediate and non-classical). In patients with CKD, as compared to those without, CD133+ and CD34+CD133+ cells were significantly reduced, whereas CD34+

and CD34+KDR+ cells were reduced with borderline significance (p= 0.06 for both).
Conclusion: DPP-4 inhibition with linagliptin acutely increases vasculoregenerative and anti-inflammatory cells. These results highlight the direct, glucose-independent, effect of DPP-4 inhibition and may be important to lower vascular risk in diabetic patients, especially in the presence of CKD.
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Gemigliptin, a dipeptidyl peptidase-4 inhibitor, inhibits retinal neovascularisation in oxygen-induced ischaemic retinopathy and retinal vascular leakage in db/db mice
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Background and aims: Retinal vascular leakage and neovascularization are features of diabetic retinopathy. Gemigliptin, a dipeptidyl peptidase-4 (DPP-4) inhibitor, has shown robust blood-glucose lowering effect in type 2 diabetic patients, but its effects on diabetic retinopathy have not been reported. This study evaluated the efficacy of gemigliptin on retinal vascular leakage in db/db mice, an animal model for type 2 diabetes and neovascularization in oxygen-induced retinopathy (OIR) mice, an animal model for ischemic proliferative retinopathy.
Materials and methods: Gemigliptin (100 mg/kg/day) was orally administered to db/db mice for 12 weeks. In OIR models, neonatal mice at postnatal day 7 (P7) were exposed to 75% concentration of oxygen for 5 days (P7~P12), and then returned to room air from P12 to P17 to induce retinal neovascularization. Gemigliptin (50 mg/kg/day) were administered once per day for 5 consecutive days (P12~P16) by intraperitoneal injection. Retinal neovascularization was measured at P17.
Results: Oral administration of gemigliptin for 12 weeks significantly ameliorated retinal vascular leakage and tight junction protein loss in db/db mice. Gemigliptin also ameliorated retinal neovascularization in OIR mice. Gemigliptin also attenuated overexpression of plasminogen activator inhibitor-1(PAI-1), monocyte chemoattractant protein-1 (MCP-1) and vascular endothelial growth factor (VEGF) in the retinas of diabetic and OIR mice.
Conclusion: These results suggest that gemigliptin has a potent anti-angiogenic activity via its ability to inhibit the pro-angiogenic PAI-1-related signaling pathway and support the direct retinoprotective action of gemigliptin.
Supported by: LG Life Sciences

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DPP-IV inhibitor linagliptin restores insulin-mediated vasorelaxation and attenuates vascular remodelling in middle cerebral arteries from type 2 diabetic GK rats
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Background and aims: Diabetes is associated with macrovascular and microvascular complications leading to cerebrovascular disease. Insulin resistance in type-2 diabetes leads to impaired endothelial cell function and vascular dysfunction characterized by decreased vasodilation and increased vascular remodeling. It has been shown that insulin signaling in middle cerebral arteries (MCAs) induces vasorelaxation through production of nitric oxide in a PI3K/Akt dependent manner. The enzyme dipeptidyl peptidase-IV (DPP-IV) degrades glucagon-like peptide 1 (GLP-1), thereby reducing insulin secretion. DPPIV inhibitors such as linagliptin have been shown to improve insulin secretion and endothelium dependent vascular function through PI3K/Akt signaling in rat aorta and mesentery. We hypothesized that linagliptin reverses cerebrovascular remodeling and restores vascular function via its direct and glucose lowering properties in type-2 diabetic GK rats.
Materials and methods: Diabetic and non-diabetic male GK rats, aged 24 weeks were used in this study, and were fed either normal chow or linagliptin mixed with normal chow for 4 weeks at a concentration of 166 mg/kg of chow (n=5-6/group). MCA segments were mounted in an arteriograph chamber and vascular relaxation was determined by performing concentration response to insulin (Ins, 10^{-13}-10^{-6} M), and measurements of vascular structure were recorded. Statistical significance was determined for multiple comparisons by ANOVA with Tukey post hoc correction or 2-Way ANOVA with Bonferroni post hoc correction at $p<0.05$.
Results: Linagliptin treatment does not alter hyperglycemia levels (Hemoglobin A1C%, Non-diabetic Vehicle (NDV): 5.68±0.086, Diabetic Vehicle (DV): 8.02±0.273***, Non-diabetic Linagliptin (NDL): 5.85± 0.077, Diabetic Linagliptin (DL): 8.20±0.327***, ***=p<0.001) or body weight (grams, NDV: 404.5±13.35, DV: 411.3±16.12, NDL: 421.9±14.98, 398.8±8.656). Linagliptin restores insulin-mediated vascular relaxation in MCAs from diabetic rats (Maximum Relaxation RMax%: NDV: 46.04±2.803, DV: 12.69±0.9657***, DL: 45.89± 2.632###, ***=p<0.001 vs. NDV, ###=p<0.001 vs. DV). Linagliptin ameliorates pathological vascular remodeling in MCAs from diabetic rats. Improvement was observed in cross-sectional area(μm at max intraluminal pressure 120 mmHg: NDV: 21673±569.8, DV: 25765± 1530*, DL: 21319±664.8#, *=p<0.05 vs. NDV, #=p<0.05 vs. DV), media thickness (μm at max intraluminal pressure 120 mmHg: NDV: 40.00±1.027, DV: 51.43±3.139*, DL: 40.50±1.067#, *=p<0.05 vs. NDV, #=p<0.05 vs. DV) and wall:lumen ratio (at max intraluminal pressure 120 mmHg: NDV: 0.1217±0.0062, DV: 0.1750±0.0184*, DL: 0.1244±0.0070##, *=p<0.05 vs. NDV, ##=p<0.01 vs. DV).
Conclusion: There was no reduction in hyperglycemia level in the diabetic rats. This allowed us to examine the glucose-independent effects of DPP-IV inhibition with linagliptin. Type-2 diabetic rats have impaired cerebrovascular response to insulin-mediated relaxation and pathological vascular remodeling remodeling, both of which were improved following linagliptin treatment. DPP-IV inhibition with linagliptin holds potential as a possible therapy for diabetic vascular disease in addition to its clinical anti-hyperglycemic use.
Supported by: Boehringer Ingelheim Pharm., Inc.

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A randomised, active- and placebo-controlled, three-period cross-over trial to investigate short-term effects of linagliptin on endothelial function in type 2 diabetes
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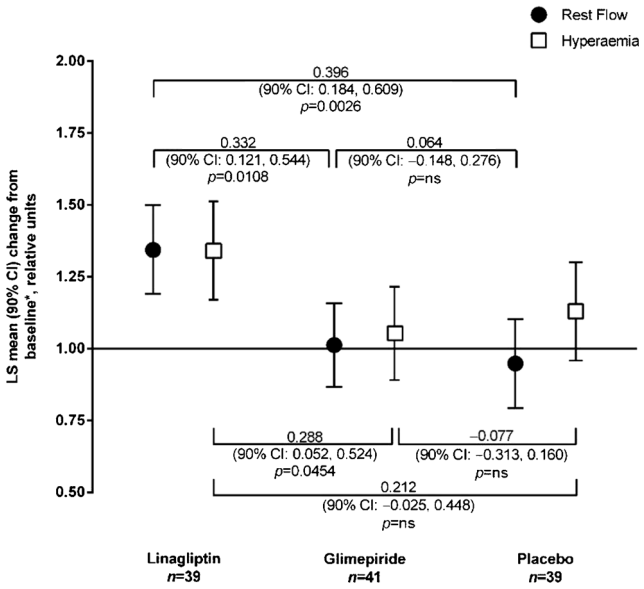
Background and aims: Studies of dipeptidyl peptidase (DPP)-4 inhibitors report heterogeneous effects on endothelial function in patients with type 2 diabetes. This study assessed the effects of the DPP-4 inhibitor linagliptin versus the sulphonylurea glimepiride and placebo on measures of macro- and microvascular function.
Materials and methods: This study randomised type 2 diabetes patients (n=42) with HbA_{1c} ≤7.5%, no micro- or macrovascular disease and on stable metformin background to linagliptin 5 mg qd, glimepiride 1-4 mg qd or placebo for 28 days. Fasting and postprandial macrovascular

endothelial function, measured by brachial flow-mediated vasodilation (FMD), and microvascular function, measured by laser-Doppler, were analysed after 28 days.

Results: Baseline mean (SD) age and body-mass index were 60.3 (6.0) years and 30.3 (3.0) kg/m^2, respectively. After 28 days, changes in fasting FMD were similar between the 3 study arms [treatment ratio, gmean (90% CI): linagliptin vs glimepiride, 0.884 (0.633, 1.235); linagliptin vs placebo, 0.884 (0.632, 1.235); glimepiride vs placebo, 1.000 (0.715, 1.397); all p= not significant]. Similarly, no differences were seen in postprandial FMD. Linagliptin significantly improved fasting, but not postprandial, microcirculation (figure). Linagliptin had no effect on heart rate or blood pressure. Rates of overall adverse events with linagliptin, glimepiride and placebo were 27.5%, 61.0% and 35.0%. Fewer hypoglycaemic events were seen with linagliptin (5.0%) than glimepiride (39.0%).

Conclusion: Linagliptin had no effect on macrocirculation in type 2 diabetes, but significantly improved microcirculatory function in the fasting state.

Figure. *Change from baseline after 28 days in fasting microcirculatory function using laser-Doppler method*



*Data are expressed as a ratio of day 28 value to baseline value
LS = least squares; ns = not significant

Clinical Trial Registration Number: NCT01703286
Supported by: Boehringer Ingelheim

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Safety and efficacy of linagliptin in patients with type 2 diabetes and coronary artery disease: analysis of pooled incident investigator-reported events from phase 3 trials

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Background and aims: Patients with type 2 diabetes (T2D) continue to have increased coronary artery disease (CAD) morbidity and mortality. This analysis evaluated safety and efficacy data of the dipeptidyl peptidase-4 inhibitor linagliptin in patients with CAD.

Materials and methods: Data from T2D patients with CAD (identified using standardised MedDRA query "embolic and thrombotic events") were pooled from a large clinical trial programme that included randomised placebo-controlled trials of linagliptin 5 mg. The safety set included patients with ≥12 weeks of treatment (19 trials: linagliptin, n=451; placebo, n=272). The efficacy set included patients with ≥24 weeks of treatment (12 trials: linagliptin, n=328; placebo, n=198).

Results: In the safety set, baseline mean (SD) age, body-mass index, and HbA_{1c} were 64.7 (9.2) years, 30.6 (4.9) kg/m^2, and 8.1 (0.9)%, respectively. Approximately 50% of patients in each group received ≥2 additional background antidiabetes drugs. Use of cardiovascular (CV) non-study drugs was similar between the linagliptin vs placebo groups: aspirin, 74.9 vs 77.6%; beta-blockers, 60.1 vs 59.9%; angiotensin-converting enzyme inhibitors, 46.3 vs 44.5%; statins, 62.3 vs 67.6%. Baseline mean (SD) vital signs and lipid levels were as follows: systolic BP, 135.5 (16.1) mmHg; pulse, 70.1 (10.5) bpm; total cholesterol, 176.6 (46.5) mg/dl. Median (range) exposure to linagliptin and placebo was 169 (6-707) days and 170 (1-706) days, respectively. Baseline CV risk factors/history were well matched between the groups (Table). The overall incidence of adverse events (AEs) was numerically lower with linagliptin than with placebo (Table). Cardiac AEs were reported by 9.1% of linagliptin patients and 9.2% of placebo patients (Table). In the efficacy set [baseline mean (SD) HbA_{1c} was 8.2 (0.9%)], the placebo-adjusted mean HbA_{1c} change from baseline at week 24 with linagliptin was -0.57% (95% CI -0.70, -0.43; p<0.0001). Although patients were more likely to achieve HbA_{1c} <7% with linagliptin vs placebo (OR 3.70; p<0.0001), the incidence of hypoglycaemia was 20.8% with linagliptin and 24.6% with placebo.

Conclusion: Linagliptin was well tolerated and efficacious in T2D patients with known risk factors for CAD. Ongoing CV outcomes trials will provide further insights into the long-term safety and efficacy of linagliptin.

	Linagliptin 5 mg n=451	Placebo n=272
Baseline cardiovascular risk factors/history, %		
Diabetes duration >5 years	71.0	77.6
Previous / current smoker	35.0 / 13.3	38.2 / 14.0
Hypertension*	87.6	84.6
Peripheral artery disease	11.3	13.6
Cerebrovascular disease	11.1	12.5
Myocardial infarction	30.4	32.4
Overall adverse events (AE) and cardiac AEs†, %		
Any AE	65.2	75.4
Drug-related AE	16.2	19.9
AE leading to discontinuation	2.9	5.1
Serious AE	10.9	13.2
Requiring hospitalisation	10.4	11.0
Fatal	0.7	1.5
Cardiac AEs (frequency ≥0.5%)‡	9.1	9.2
Acute myocardial infarction	0.7	0.4
Angina pectoris	2.0	2.6
Atrial fibrillation	1.3	0.0
Cardiac failure	0.4	0.7
Congestive cardiac failure	0.7	0.0
Coronary artery disease	0.9	0.7
Left ventricular failure	0.7	0.0
Myocardial ischaemia	0.9	0.7
Palpitations	0.4	0.7
Tachycardia	0.7	0.0

*Missing: linagliptin, n=14; placebo, n=15.
†MedDRA v16.0 used for reporting.
‡Data are exposure adjusted and unadjudicated; defined by the cardiac disorders MedDRA System Organ Class.

Supported by: Boehringer Ingelheim

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Hepatic fat deposition is improved more with SGLT2 inhibitor luseogliflozin compared with sitagliptin: a randomised, crossover, controlled study using computed tomography

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Background and aims: There are few drug therapies approved for non-alcoholic fatty liver disease (NAFLD) based on evidence-based medicine. Recently, incretin-related drugs with multiple pharmacological effects are expected to improve NAFLD. In addition, sodium-glucose co-transporter 2 (SGLT2) inhibitors are a new class of oral hypoglycemic agents that improve hyperglycemia and reduce body weight due to increased urinary glucose excretion. We hypothesized that SGLT2 inhibitors have the potential to improve NAFLD owing to this new mechanism. We aimed to compare the effect of the SGLT2 inhibitor luseogliflozin with that of the dipeptidyl peptidase-4 inhibitor sitagliptin on hepatic fat deposition.
Materials and methods: This study was conducted in 16 Japanese type 2 diabetic outpatients (male: 9, female: 7) treated with only oral hypoglycemic agents, including sitagliptin (50 mg/day). Participants were randomly assigned to groups that either continued with sitagliptin (S) or switched to luseogliflozin (L). After 4 weeks, participants then switched to the other drug for another 4 weeks. Characteristics of participants were as follows: age, 62 (57.3-66.5) years; diabetes duration, 9.5 (5-13.5) years; BMI, 27.7 (24.3-29.7) kg/m2; and HbA1c, 7.6% (7.2-7.9%). Other oral agent(s) received by participants were glimepiride (4), biguanide (12), thiazolidine (2), α-glucosidase inhibitor (2), statin (11), and angiotensin receptor blocker (7). The dosages of oral agents did not change in this study. Outcomes were as follows: evaluation of liver attenuation, liver-to-spleen attenuation ratio (L/S), and visceral fat mass area (VFA) measured by computed tomography; body weight (BW); and fasting plasma levels of alanine aminotransferase, aspartate aminotransferase, γ-glutamyl transferase, glycosylated hemoglobin, glucose, glycoalbumin, ketone bodies, immunoreactive insulin, and immunoreactive C-peptide. These data were evaluated at the end of administration of each drug.
Results: Liver attenuation and L/S in the L group were significantly higher than in the S group (liver attenuation: 50.6 (40.6-57.8) Hounsfield units (HU) vs. 44.2 (36.5-58.1) HU, P=0.034; L/S: 0.98 (0.73-1.04) vs. 0.90 (0.82-1.14), P=0.013, respectively). Moreover, both VFA and BW in the L group were significant lower than in the S group (VFA: 123.0 (105.3-151.6) vs. 135.4 (104.7-156.6) cm2, P=0.007; BW: 68.5 (58.8-76.2) vs. 69.3 (59.6-76.3) kg, P=0.049, respectively), although plasma levels of ketone bodies in the L group were significant higher (P=0.0007) than in the S group. There was no significant difference among other outcomes measures, including glycemic control.
Conclusion: Based on both the reduced liver attenuation as well as L/S, this study suggests that luseogliflozin decreased hepatic fat deposition compared with sitagliptin. It is conceivable that increasing urinary glucose excretion with luseogliflozin, independently of glycemic control improvement, induced consumption of visceral fat and hepatic fat deposition.

PS 073 DPP-4 inhibitors: mechanisms and implications

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Exogenous glucagon-like peptide 1 is not fully protected by acute dipeptidyl peptidase 1 inhibition

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Background and aims: Dipeptidyl peptidase 4 (DPP-4) inhibitors limit glucagon-like peptide-1 (GLP-1) degradation and are widely used to treat type 2 diabetes. DPP-4 exists in soluble and membrane-bound forms. It is unclear if plasma DPP-4 activity levels reflect the full extent of DPP-4 inhibition in all compartments. We used the selective inhibitor sitagliptin to explore the relationship between DPP-4 activity and protection of GLP-1.
Materials and methods: On four separate days, eight patients with type 2 diabetes (BMI: 28.8±1.4 kg/m2 [mean±SEM]; HbA1c: 43.1±2.4 mmol/mol) and eight matched healthy controls (BMI: 28.1±1.2 kg/m2; HbA1c: 34.4±1.2 mmol/mol) received continuous i.v. GLP-1 (1.0 pmol/kg/min) and oral sitagliptin (0 [placebo], 25, 100 or 200 mg) administered in a double-blinded randomised order. Plasma (soluble) DPP-4 activity was measured by chromogenic assay, and the degree of protection of GLP-1 (assessed with specific RIAs) was used as a surrogate index of inhibition of 'total' DPP-4 activity in all compartments of the body.
Results: At steady-state (180-360 minutes), plasma DPP-4 activity decreased dose-dependently (Table), but with differences between 'total' and plasma DPP-4 compartments. A difference between the groups was also explored, possibly due to differences in absorption (Table). Despite relatively high inhibition of plasma DPP-4 activity, intact GLP-1 levels (N-terminal RIA) remained lower than total GLP-1 levels (C-terminal RIA).
Conclusion: Our results suggest that membrane-bound DPP-4 was not fully inhibited by single-dose sitagliptin. Furthermore, other enzymes, not inhibitable by sitagliptin, may also have been responsible.

	Type 2 diabetes (n=8)		Control (n=8)	
Sitagliptin dose (mg)	Plasma DPP-4 activity (% of placebo) ±SEM	'Total' DPP-4 activity (% of placebo) ±SEM	Plasma DPP-4 activity (% of placebo) ±SEM	'Total' DPP-4 activity (% of placebo) ±SEM
0	100 (±0.0)	100 (±0.0)	100 (±0.0)	100 (±0.0)
25	70.0 (±6.9)	46.3 (±15.8)	78.3 (±6.2)	64.4 (±11.0)
100	39.9 (±7.7)	22.4 (±18.8)	66.7 (±7.4)	56.3 (±12.3)
200	33.4 (±8.8)	35.0 (±11.5)	59.8 (±9.2)	58.6 (±11.8)
P values	*p<0.0001**	*p<0.0001**	*p<0.0001**	*p<0.0001**
	p=0.34 (plasma *vs* 'total')		*p=0.58* (plasma *vs* 'total')	
	p=0.21 (type 2 diabetes *vs* respective control, 'total' DPP-4 activity)			
	*p=0.01*** (type 2 diabetes *vs* respective control, plasma DPP-4 activity)			

*Total and plasma dipeptidyl peptidase 4 (DPP-4) activity during different doses of sitagliptin (mg) in patients with type 2 diabetes and matched controls. 'Total'- DPP-4 activity being calculated as surrogate measure of the DPP-4 activity in all compartments from the total and intact levels of GLP-1 and the plasma DPP-4 activity being a direct measure. Data are expressed in percentage of the corresponding placebo day (%) ±SEM. *p>0.0001 for the difference between the placebo day and the days with sitagliptin in both groups. **p=0.01 for the difference in plasma DPP-4 activity between type 2 diabetic patients and controls.*

Clinical Trial Registration Number: H-2-2012-149

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Islet-independent mechanisms contribute to the glucose-lowering effect of acute DPP-4 inhibition in healthy non diabetic and type 2 diabetes subjects after mixed meal intake

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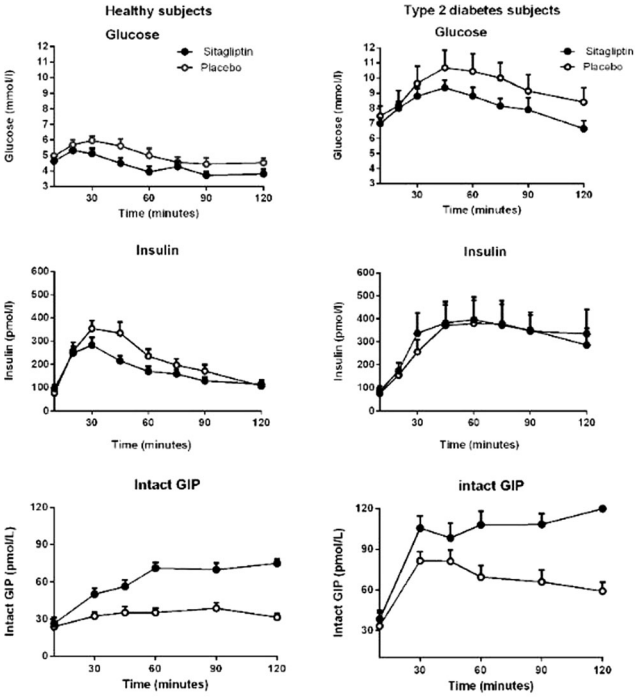
Background and aims: Chronic use of DPP-4 inhibitors has been shown to improve glycemic control in type 2 diabetic subjects (T2D) by increasing levels of incretin hormones, glucagon-like peptide-1 (GLP-1) and glucose dependent insulinotropic polypeptide (GIP), resulting in stimulation of insulin secretion. It has been suggested that the insulinotropic effect of GIP is reduced in T2D subjects with insufficient glycemic control, and therefore, that GIP may be of less importance for the early glucose-lowering effect of DPP-4 inhibition. We therefore explored whether an increase in intact GIP by an acute single dose of the DPP-4 inhibitor sitagliptin is associated with effects on the islet hormones after intake of mixed meal in healthy and drug-naïve T2D subjects.
Materials and methods: After an overnight fast, twelve healthy male (mean age 22 yrs, BMI 22 kg/m2) and twelve well-controlled and drug-naïve T2D male subjects (mean age 64 yrs, BMI 27 kg/m2, HbA1c 43 mmol/mol=6.0%, diabetes duration 4 yrs) underwent two tests in random order and ingested a standardized mixed meal with sitagliptin (SITA; 100 mg) or placebo (PBO). Blood samples were taken for analyses of glucose, insulin, glucagon and intact GIP and their suprabasal 120 min areas under the curve (AUC) were calculated. β-cell function was estimated as insulinogenic index [IGI=AUC insulin/ AUC glucose] calculated from the glucose and insulin levels.
Results: Plasma intact GIP levels increased after meal intake with augmented effects of sitagliptin in healthy subjects (SITA vs PBO AUC GIP 5835±409, 3059±294 pmol/l x min; P<0.001) and in T2D subjects (SITA vs PBO AUC GIP 9227±1157 vs 5891±553 pmol/l x min; p=0.003). Sitagliptin almost completely suppressed the rise in glucose in healthy (AUC 46±16 vs 70±15 mmol/l x min; P=0.03) and in T2D subjects (AUC 146±28 vs 239±49 mmol/l x min; P=0.04). This was associated with significant reduction in insulin levels in healthy (AUC 16±1 vs 21±2 nmol/l x min, P=0.02) but not in T2D subjects (AUC 29.2 ±7.1 vs 29.8±6.9 nmol/l x min; P=0.3). There were increases in IGI, however, sitagliptin treatment showed no significant increase in IGI compared to the placebo both in healthy (AUC 4.6±0.4 vs 4.8±0.4 nmol/mmol, p=0.7) and T2D subjects (AUC 3.7±1.1 vs 3.6±1.1 nmol/mmol; P=0.4). Sitagliptin did not suppress glucagon in healthy (AUC 382±102 vs 294±88 pmo/l x min; p=0.2) or T2D subjects (AUC 643±98 vs 569±143 pmol/l min, P=0.2).
Conclusion: The increased glycemia after meal ingestion is prevented by sitagliptin without preceding increase in insulin or reduction in glucagon in well controlled and drug-naïve type 2 diabetes and healthy non-diabetic subjects. Furthermore, the β-cell function is not increased by sitagliptin in spite of augmentation of intact GIP levels in T2D. Our results therefore suggest that extra-islet effects contribute to the glucose-lowering effect of a single dose of sitagliptin after standardized mixed meal ingestion in healthy and in T2D individuals but increases in intact GIP do not appear to contribute.



Levels of plasma glucose, insulin and intact GIP after ingestion of mixed meal in healthy non-diabetic and T2D subjects with concomitant administration of sitagliptin (•-•) or placebo (o-o). Mean±SEM are shown.

Clinical Trial Registration Number: 2012-005660-98
Supported by: VR, Region Skane

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Sitagliptin effect on lipid and glucose metabolism (SLIM) study

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Background and aims: DPP4-inhibitors are expected to improve the post-prandial hyperlipidemia in addition to improving the glycemic control. This study was designed to investigate the "Sitagliptin Effect on Lipid and Glucose Metabolism" (SLIM Study) in the type 2 diabetic patients.
Materials and methods: The SLIM study was a multicenter, prospective, open-labeled, randomized study from April 2010 to September 2014. The type 2 diabetic outpatients treated with sulfonylureas (SU), who accepted the informed consent, were recruited. The inclusion criteria were HbA1c 6.9-8.5% and serum triglyceride (TG) concentraton 120-400 mg/dl. They were randomized to the sitagliptin group (Sita, 50 mg/day of sitagliptin addition) and the non-sitagliptin group (non-Sita, SU multiplication), and the goal after 6 months treatment was HbA1c<6.9%. This study conformed to the principles out-lined in the Declaration of Helsinki, and was approved by the ethics committees of all participating hospitals. We monitored the fasting levels of blood glucose, HbA1c, glycated albumin (GA), lipids (TC, TG, HDL-C), and apolipoproteins (AI, AII, B, CII, CIII, E, and B48) before and after 3 and 6 months treatment.
Results: There were no significant differences in the sex and age distribution (n=81, 45 men, 62±13 years old and 36 women, 68±10 years old in the sitagliptin group, and n=83, 45 men, 62±11 years old, and 38

women, 66±9 years old in the non-sitagliptin group, mean ± SD), and HbA1c 7.5±0.8 vs. 7.5±0.7%, GA 20.2±4.0 vs. 20.5±3.6%, TC 5.4±1.0 vs. 5.4±0.9 mmol/L, TG 2.1±1.4 vs. 1.8±1.3 mmol/L, HDL-C 1.3±0.3 vs. 1.4±0.3 mmol/L, and Apo B48 6.6±5.2 vs. 5.7±4.0 μg/ml, sitagliptin group vs. non-sitagliptin group, respectively. There were no differences in the Apo CII (5.1±2.5 and 4.6±2.0) or CIII (11.8±4.7 and 10.4±3.6) levels between the sitagliptin and non-sitagliptin group. The reduction of HbA1c (-0.5±0.8%) and GA (-2±3.1%) in the sitagliptin group were significant, but not in the non-sitagliptin group (0.1±0.9 and -0.1±3.5%, respectively). The fasting lipids-profile was not significantly changed in the both groups, but TG levels slightly decreased in the sitagliptin group (-0.2±1.0 mmol/L). Apo B48, CII and CIII levels were significantly decreased in the sitagliptin group (-1.2±4.5 μg/ml, -0.4±1.3 mg/dl, and -1.1±2.7 mg/dl, respectively), but not in the non-sitagliptin group.

Conclusion: The SLIM Study demonstrated that the sitagliptin treatment effectively reduced the fasting levels of Apo B48, CII and CIII, which are related to the chylomicron and remnant metabolism, with the improved glucose metabolism. DPP4-inhibitor, sitagliptin, will effectuate the reduction of atherogenic lipoproteins in diabetes. The further study should elucidate the mechanism of DPP4-inhibitor effect on the lipid metabolism through the direct effect on lipid metabolism by this agent, the inhibitory effect on DPP4, or the improved glucose metabolism.

Clinical Trial Registration Number: UMIN000006511

Supported by: The Kidney Foundation, Japan

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Incidence of microvascular outcomes in type 2 diabetes patients treated with vildagliptin vs sulphonylurea: a retrospective study using German electronic medical records

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Background and aims: Preliminary data suggest that dipeptidyl peptidase-4 (DPP-4) inhibitors may reduce microvascular events, but there is little evidence to support this from adequate real-world studies. This study aimed to compare the microvascular outcomes between patients prescribed vildagliptin and those prescribed sulphonylurea (SU).

Materials and methods: This retrospective cohort study was conducted on a large sample from the German electronic medical records database IMS Lifelink Disease Analyzer. We used propensity score matched samples of patients prescribed either vildagliptin or SU. Exposure was defined as therapy (SU or vildagliptin); primary outcomes were a diagnosis of retinopathy, nephropathy, neuropathy or diabetic foot ulcer over the observation period in patients with no previous record of these outcomes. Secondary outcome was a composite of any primary outcome occurring during the observation period.

Results: 16321 patients prescribed SU and 4481 prescribed vildagliptin met the inclusion criteria. After propensity score matching each sample comprised 3015 patients. Mean age was 63.7/64.6 years for SU/vildagliptin, respectively, with mean disease duration of 3.2/3.1 years and mean treatment duration of 2.5/2.3 years. Treatment with vildagliptin was associated with a significantly lower incidence of retinopathy (OR=0.55, p=0.0004), neuropathy (OR=0.71, p=0.001) and the composite outcome (OR=0.70, p<0.0001). Incidences of nephropathy and diabetic foot ulcer were lower for vildagliptin, but not significantly so (OR=0.90, p=0.3920; OR=0.76, p=0.0742, respectively). There were no significant differences in incident rate ratios (all p>0.05).

Conclusion: Treatment with vildagliptin was associated with a reduced incidence of microvascular complications, especially neuropathy and retinopathy, compared to treatment with SU.

Supported by: Novartis

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Linagliptin, independently of blood glucose control, ameliorates cognitive impairment and brain atrophy induced by transient cerebral ischaemia in type 2 diabetic mice

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Background and aims: It is well established that type 2 diabetes is significantly associated with cognitive decline and stroke. It remains to be clarified whether dipeptidylpeptidase-4 (DPP-4) inhibition can counteract the impairment of cognitive function and brain atrophy caused by transient cerebral ischemia in type 2 diabetes. The present study was performed to test our hypothesis that linagliptin, a DPP-4 inhibitor, administration following transient cerebral ischemia can ameliorate cognitive impairment and brain atrophy in diabetic mice.

Materials and methods: Eight-week-old male db/db mice, a model of obese type 2 diabetes, were subjected to transient cerebral ischemia by 17 minutes of bilateral common carotid artery occlusion (BCCAO), and were administered (1) vehicle or (2) linagliptin (0.083 g/kg in chow) for 8 weeks or 1 week.

Results: Linagliptin administration almost completely suppressed the blood DPP-4 activity in db/db mice ($P<0.01$). However, linagliptin did not significantly reduce blood glucose or not improve glucose tolerance in old db/db mice. Linagliptin administration following transient cerebral ischemia significantly counteracted cognitive impairment in diabetic mice subjected to BCCAO, as estimated by water maze test (Figure (A)) and passive avoidance test. Linagliptin administration ameliorated the decrease in brain weight (Figure (B)), and also alleviated the decrease in cerebral volume (Figure (C)) and neuron cell number (Figure (D)) in hippocampus and cortex of diabetic mice subjected to BCCAO. Linagliptin administration significantly reduced the increase in cerebral IgG extravasation (blood brain barrier disruption) and the increase in reactive microglia caused by BCCAO in diabetic mice. Linagliptin significantly suppressed the increase in cerebral oxidative stress in BCCAO-subjected diabetic mice. Furthermore, linagliptin significantly increased cerebral claudin-5, a main cerebral endothelial tight junction protein, and significantly decreased gp91phox, a major subunit of NADPH oxidase, in diabetic mice subjected to BCCAO.

Conclusion: DPP-4 inhibition with linagliptin counteracted cognitive impairment and brain atrophy induced by transient cerebral ischemia in type 2 diabetic mice, independently of blood glucose-lowering effect. This cerebroprotective effect of linagliptin was associated with the suppression of blood brain barrier disruption and the attenuation of cerebral oxidative stress. We propose that DPP-4 inhibition seems to be a promising therapeutic strategy for cognitive impairment and cerebral vascular complications in type 2 diabetes.

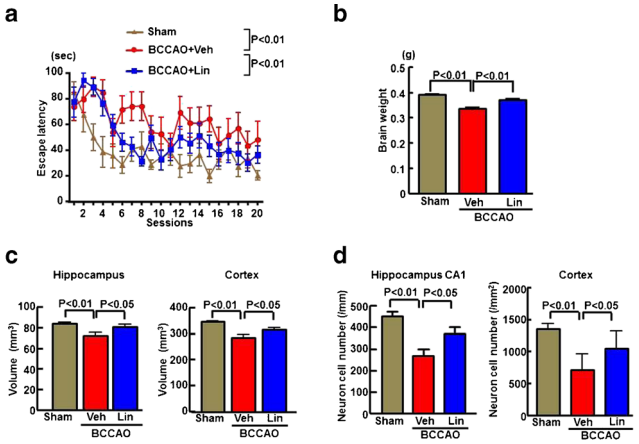

Figure legends: Effect of linagliptin administration following transient cerebral ischemia on cognitive function (escape latency on the water maze test) (A), brain weight (B), volume of hippocampus and cortex (C), and hippocampal or cortical neuron cell number (D) in type 2 diabetic mice

Abbreviations used: BCCAO, transient cerebral ischemia by 17 minutes of bilateral common carotid artery occlusion; Sham, sham-operated group; BCCAO+Veh, group subjected to transient BCCAO followed by vehicle administration; BCCAO+Lin, group subjected to transient BCCAO followed by linagliptin administration. Each value represents mean±SEM (n=10 in Sham, n=10 in BCCAO+Veh, n=11 in BCCAO+Lin, except for cortical neuron cell number; in cortical neuron cell number, n=7 in Sham, n=6 in BCCAO+Veh, n=7 in BCCAO+Lin). In (A), statistical analysis was performed by two-way ANOVA with repeated measurements. In (B)-(D), statistical analysis was performed by one-way ANOVA followed by the Tukey's multiple comparisons test.

Supported by: Boehringer Ingelheim

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Incidence of hypoglycaemia with the dipeptidyl peptidase-4 inhibitor linagliptin in type 2 diabetes patients with renal impairment

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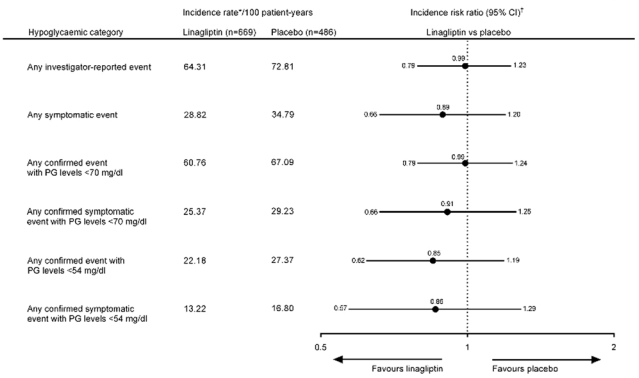
Background and aims: Patients with type 2 diabetes (T2D) and renal impairment (RI; eGFR <60 ml/min/1.73 m^2) have a significantly increased risk for hypoglycaemia. This can lead to therapeutic inertia, a reluctance to intensify management and the compromise of less stringent targets for glycaemic control in patients with RI. In this context, the addition of a dipeptidyl peptidase (DPP)-4 inhibitor is a potentially attractive alternative. Therefore, we evaluated the incidence of hypoglycaemic events in placebo-controlled trials with the DPP-4 inhibitor linagliptin specifically in participants with RI.
Materials and methods: Participants with T2D and RI were identified from 20 randomised placebo-controlled trials (duration 12–52 weeks) of linagliptin 5 mg once daily as monotherapy or add-on to other glucose-lowering drugs. The incidence of pre-defined overall and confirmed hypoglycaemic events was determined.
Results: 1155 participants with T2D and RI were identified (linagliptin, n=669; placebo, n=486). 80% of participants had an eGFR between 30 and <60 ml/min/1.73 m^2 and 20% had an eGFR <30 ml/min/1.73 m^2. Overall, participants with T2D and RI were 66±9 years of age, had a prolonged duration of diabetes and frequent vascular comorbidities. 44% of participants were treated with insulin. Mean baseline HbA_{1c} was 8.2% and mean exposure was >6 months in both groups. In these participants, linagliptin significantly lowered HbA_{1c} versus placebo (−0.53%, −0.57% and −0.53% at 12, 24 and 52 weeks, respectively; all p<0.0001). Although the background incidence of hypoglycaemia was high (>30%), lowering of glucose levels with linagliptin was not associated with an increase in any of the pre-defined hypoglycaemic event categories (Figure). The incidence risk ratio of any confirmed hypoglycaemic event for linagliptin versus placebo was 0.99 (95% CI 0.79–1.24). Severe hypoglycaemia occurred in 1.3% of linagliptin participants and 1.0% of placebo participants (p=not significant).
Conclusion: These findings support the addition of linagliptin for improving glycaemic control in patients with T2D and RI without increasing the risk for hypoglycaemia.

Figure. Forest plot of incidence risk ratio for hypoglycaemic events in T2D patients with RI (eGFR <60 ml/min/1.73m^2)



*Patients with adverse event (AE): time at risk = start of first AE – start of treatment + 1; patients without AE: time at risk = end of time at risk – start of treatment + 1
†The estimates and 95% CIs are based on a Cochran-Mantel-Haenszel test stratified by study
eGFR = estimated glomerular filtration rate (based on Modification of Diet in Renal Disease equation); PG = plasma glucose

Supported by: Boehringer Ingelheim

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Major cardiovascular outcomes in the EXAMINE trial according to ACE inhibitor use

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Background and aims: Activation of the sympathetic nervous system through substance P occurs when there is DPP-4 inhibition (DPP-4i) in the presence of high-dose ACE inhibition (ACEi). This has led to concerns of potential increases in cardiovascular (CV) events when the 2 classes of drugs are used together; hence, we evaluated CV outcomes from the large CV outcomes trial EXAMINE according to ACEi use.
Materials and methods: Patients with type 2 diabetes mellitus (T2DM) with a recent acute coronary syndrome (ACS) were randomly assigned to receive alogliptin or placebo added to existing antihyperglycemic and CV prophylactic therapies. Major CV events were adjudicated by a committee blinded to treatment assignment. Risks of CV death, nonfatal MI and stroke, and hospitalized HF (HHF) were analyzed using a Cox proportional hazards model in patients with and without baseline ACEi.
Results: There were 5380 patients randomized for a median follow-up of 18 months. At baseline, 3323 (62%) EXAMINE patients were using an ACE inhibitor (1681 on alogliptin; 1642 on placebo). The composite rates of CV mortality, nonfatal MI, and nonfatal stroke were similar for alogliptin vs placebo with ACEi (11.4% vs 11.8%; HR=0.97; 95% CI, 0.79-1.19) and without ACEi use at baseline (11.2% vs 11.9%; HR=0.94; 95% CI, 0.72-1.21). CV death or HHF in patients on ACEi use at baseline occurred in 6.8%of patients on alogliptin vs 7.2% on placebo (HR=0.93; 95% CI, 0.72-1.20). In addition, alogliptin showed no effect on HHF alone in ACEi-treated patients (3.3% vs 3.1%; HR=1.09; 95% CI, 0.81-1.48) for alogliptin vs placebo, respectively. There were also no significant differences for these endpoints in patients without ACEi use at baseline. Subgroup analyses according to any prerandomization history of HF and ACEi use at baseline showed the primary endpoint (CV death, MI, and stroke) occurring in 13.9% and 16.5% of patients on alogliptin vs placebo, respectively (HR=0.87; 95% CI, 0.63-1.19) and CV death or

HHF in 12% and 13.2% of patients on alogliptin vs placebo, respectively (HR=1.01; 95% CI, 0.69-1.49).
Conclusion: Cardiovascular outcomes were not different for alogliptin compared with placebo in patients with T2DM and coronary disease treated with ACE inhibitors.
Clinical Trial Registration Number: NCT00968708
Supported by: Takeda Development Center Americas, Deerfield, IL

PS 074 The future of diabetes pharmacology

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RG-125 (AZD4076): clinical candidate with a novel modality to treat insulin resistance through inhibiting miR-103/107
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Background and aims: Anti-miRs targeting the miR-103/107 family have been shown to improve insulin sensitivity and glucose homeostasis in diet induced obese (DIO) and db/db mice. In vitro and in vivo screening of anti-miR-103/107 oligonucleotides (anti-miRs) led to the identification of RG-125, a GalNAc conjugated anti-miR. The goal of these studies was to evaluate RG-125 potency and efficacy in insulin resistant DIO and db/db mice. In addition a hyperinsulinemic-euglycemic clamp was performed in DIO mice to examine the mechanism by which miR-103/107 inhibition improves insulin sensitivity. RG-125 was also evaluated in mouse and non-human primate (NHP) toxicology studies.
Materials and methods: DIO and db/db mice were dosed s.c. at dose levels noted, once weekly for 3-9 weeks. Oral glucose tolerance tests and weekly fasting glucose and insulin were performed after 6 hour fasting. Clamp studies were performed on conscious, overnight-fasted mice using an indwelling intravenous catheter with operator blinded to dosing group. Exploratory toxicology studies were performed in CD-1 mice and cynomolgus monkeys, dosed s.c. once weekly for 5-6 weeks.
Results: RG-125 induces dose-dependent reductions in weekly fasting blood glucose and plasma insulin in DIO mice, with significant improvements in the homeostatic assessment of insulin resistance (HOMA-IR) at doses as low as 5 mg/kg. There were no effects on body weight. RG-125 normalized glucose tolerance to that of lean controls. Clamp studies in DIO mice confirm the insulin sensitizing effects of miR-103/107 inhibition with RG-125 administration giving clear dose-responsive improvements in both glucose infusion rate and whole body glucose turnover. Decreases in clamped hepatic glucose production along with increased glucose uptake in brown adipose tissue and skeletal muscle indicates that RG-125 improves hepatic and peripheral insulin sensitivity. RG-125 also reversed the extreme hyperglycemia that develops with age in db/db mice. Exploratory toxicology studies in mice and NHP show RG-125 was well tolerated across multiple dose levels and provided insight into GLP study design.
Conclusion: RG-125 (AZD4076), via inhibition of miR-103/107, is a novel insulin sensitizer with proven efficacy across a number of mouse disease models, and good tolerability in both mouse and NHP. These data support clinical development of RG-125 for treatment of type 2 diabetes and associated metabolic disorders.

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Preclinical pharmacokinetics-pharmacodynamics modelling to guide first-time-in-human studies with the anti-miR-103/107, RG-125 (AZD4076)
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Background and aims: MicroRNAs (miR) are short, non-coding RNA sequences controlling the expression of multiple genes by mRNA

degradation or post-transcriptional regulation. In humans, hepatic expression of miR-103/107 is increased in patients with fatty liver disease and correlates with insulin resistance. In rodent models of insulin resistance and diabetes, inhibition of miR-103/107 alleviates insulin resistance and reduces hyperglycemia. RG-125, a novel anti-miR-103/107 GalNAc-conjugated oligonucleotide, increases insulin sensitivity in the diet-induced obesity mouse model (DIO). By using pharmacokinetic-pharmacodynamic modelling to integrate all preclinical data we predicted human dose and time-course of response.

Materials and methods: RG-125 was administered s.c. once weekly at multiple dose levels to mouse (50-450 mg/kg) and non-human primate (NHP; 5-150 mg/kg) in combined pharmacokinetic and toxicology studies. A mathematical pharmacokinetic model was fitted, describing both plasma and liver exposure for parent drug and its metabolites in mouse and NHP. In a separate series of studies, RG-125 was dosed once weekly s.c. in DIO mice (1.7-45 mg/kg) and plasma glucose and insulin were measured over time to assess potency and efficacy. The homeostatic model assessment of insulin resistance (HOMA-IR) response in DIO mice was calculated from glucose and insulin, and fit to a turnover model, taking the animal disease progression into account.

Results: A pharmacokinetic model including saturated uptake into liver, predicted observed plasma and liver concentrations of the combined RG-125 active metabolites in the evaluated animal species well, justifying an allometric approach to predict human pharmacokinetic parameters. The pharmacodynamic model liver IC_{80} parameter estimate was 8.4 μM (95% confidence interval: 6-12 μM) in DIO mice and assumed applicable to humans. Allometric scaling was further implemented on the pharmacodynamic model to predict human response over time. The observed time to achieve 50% maximal inhibitory response in RG-125 treated DIO mice (~5 days) was extrapolated to humans (~30 days), based on reported glucose turnover half-life values in mouse (~3 days) and human (~19 days) studies with thiazolidinediones. The model predicted human therapeutic s.c. dose of RG-125 was predicted to be 70 mg once weekly and which will need to be confirmed in clinical testing.

Conclusion: RG-125 (AZD4076) causes robust insulin sensitization in DIO mice and is an attractive candidate to investigate effects on insulin sensitization in man. The human PKPD model, albeit assumption-rich, could be further used to guide the design of safety studies as well as early clinical trials, impacting both duration and dosing regimen.

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GMC-252 Lysine Salt, a conjugate of the anti-inflammatory diflunisal and the antioxidant N-acetylcysteine, shows anti-diabetic effects in db/db mice

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Background and aims: Chronic inflammation is seen to be involved in the pathogenesis of insulin resistance and type 2 diabetes. Oxidative stress is the common factor underlying insulin resistance, type 2 diabetes and cardiovascular disease, which helps to explain the presence of inflammation in all these conditions. Here we investigate the anti-diabetic effects of GMC-252, a conjugate, which allows the sustained release of the anti-inflammatory Diflunisal and the antioxidant N-acetylcysteine, in db/db mice.

Materials and methods: 5-weeks old BKS(D)-Lepr^{db}/JOrlRj mice (db/db) were administered orally daily with GMC-252 Lysine Salt (0.5 mmol/kg/day) or vehicle (PBS-Tween 20 0.1%) for 28 days. Non-fasting glycemia, body weight, food and fluid intake were measured every 2-3 days. GTT and ITT test were performed before the end of the treatment. C-Peptide, insulin, HbA1c, plasma lipids, pancreatic insulin content and pancreatic morphology were analyzed at the end of the treatment.

Results: After 4 weeks of treatment, db/db mice treated with GMC-252 Lysine Salt showed an increase in glycemic control (reduction in non-fasting glycemia and HbA1c). There was also an improvement in pancreatic function (increases of plasma insulin, plasma c-peptide and pancreatic insulin content). Pancreata morphology analysis revealed increased insulin immunostaining signal (1.75 fold; $P<0.05$) and islet size (1.62 fold; $P<0.05$) due to GMC-252 treatment. There was also a decrease in plasma Free Fatty Acids and plasma Triglycerides but plasma total cholesterol was not modified (see Table 1). Moreover, GMC-252 oral chronic treatment does not affect animal weight. We observed in GMC-252 treated mice an improvement in glucose tolerance in the glucose tolerance test ($P<0.05$) and the action of insulin lasted longer during the insulin tolerance test ($P<0.05$).

Conclusion: In conclusion, GMC-252, a conjugate formed by the anti-inflammatory Diflunisal and the antioxidant N-acetylcysteine, showed anti-diabetic effects in db/db mice. This indicates that co-targeting inflammation and oxidative stress could be a new terapeutic approach for the treatment of type 2 diabetes.

	db/db + vehicle (n = 10)	db/db + GMC-252 (n =9)	P-Value
Non-Fasting Blood Glucose (mg/dl)	397.9 ±19.9	325.3 ±29.3	**0.0259**
Plasma Insulin (ng/ml)	7.3 ±1.1	15.3 ± 2.0	**0.0026**
Plasma C-Peptide (ng/ml)	14.7 ± 2.6	25.7 ± 5.4	**0.0384**
HbA1c (%)	8.8 ± 0.4	7.3 ± 0.7	**0.0397**
Plasma TG (mmol/l)	4.1 ± 0.2	2.3 ± 0.2	**0.0001**
Plasma FFA (mmol/l)	397.9 ±19.9	397.9 ±19.9	**0.0017**
Plasma Total Cholesterol (mmol/l)	4.0 ± 0.1	3.9 ± 0.1	0.3439
Pancreas Insulin (ng/mg tissue)	6.3 ±1.0	17.6 ±3.6	**0.0077**

Data were analyzed using t-Test. Data are Mean ± SEM

Supported by: ENISA, CDTI

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Metabolic effects of dietary supplementation with Lactobacillus reuteri DSM 17938: a randomised proof-of-concept study in type 2 diabetes

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Background and aims: Growing clinical evidence bridges composition of the human gut microbiota to metabolic diseases. However, very few randomised controlled trials (RCT) have addressed the effect of probiotics on metabolic outcomes in type 2 diabetes (T2D) patients. We conducted a RCT in T2D patients and evaluated metabolic effects after oral supplementation with *Lactobacillus reuteri* DSM 17938 (LR).

Materials and methods: Forty-six insulin treated T2D patients participated in a double-blind and placebo-controlled trial and were randomised to one of three parallel groups receiving daily either placebo (n=15, 11 M/4 F, Age: 65±5 years, BMI: 31.2±4.0 kg/m^2, HbA1c: 60.4±1.5 mmol/mol and total insulin dose: 67±52 U/day, Mean ± SD), a low dose of LR (10^8 Colony Forming Unit (CFU), n=16, 12 M/4 F, Age: 65±6, BMI: 31.3±4.0, HbA1c: 61.5±1.5 and total insulin dose: 41±25) or a high dose of LR (10^{10} CFU, n=15, 12 M/3 F, Age: 64±6, BMI: 32.5±3.0, HbA1c: 66.4±2.2 and total insulin dose: 51±37). We assessed metabolic control by HbA1c, fat distribution by CT scan, liver steatosis by MRI, insulin sensitivity by a euglycemic hyperinsulinemic glucose clamp (120 mU/m^2/min), fat cell size by subcutaneous needle biopsy, dietary

registration by a validated questionnaire and fecal microbiota composition by sequencing of the 16S rRNA gene at the baseline and 12 weeks after treatment in an intention to treat analysis.
Results: The study groups differed in HbA1c at baseline but showed similar anthropometry and pharmacological treatment (oral hypoglycemic agents in addition to insulin were metformin≈80% and sulfonylurea≈20%). We observed no difference in HbA1c, BMI, fat distribution, liver steatosis, calorie intake and microbiota composition when comparing baseline and follow-up after 12 weeks within or between the placebo (P), low dose (L) or high dose (H) LR groups. However, patients treated with the high dose LR increased the insulin sensitivity index (1.41±0.16 vs 1.76±0.24 $100\times mg\times L\times kg^{-1}\times min^{-1}\times mU^{-1}$, mean±SEM, $p<0.05$, Wilcoxon signed rank test). While fat cell size increased in the placebo group (81±2 vs 88±1 μm, mean±SEM, $p<0.05$, n=11), we observed a tendency to decreased fat cell size in the high LR dose group (84±3 vs 81 ±4 μm, $p=0.09$, n=9). Finally, we observed a trend for an increased serum adiponectin at follow-up in the high LR dose group (7.6±1.5 vs 8.4±1.6 mg/L, mean±SEM, $p=0.06$, n=14).
Conclusion: Dietary supplementation with Lactobacillus reuteri DSM 17938 for 12 weeks did not improve HbA1c in insulin treated T2D patients. However, insulin sensitivity in skeletal muscle and adipose tissue significantly increased in patients treated with the highest dose of Lactobacillus reuteri.
Clinical Trial Registration Number: NCT01836796
Supported by: BioGaia, Per-Anders Jansson (SRC and ALF)

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Effect of Gymnema sylvestre administration on metabolic syndrome, insulin sensitivity and insulin secretion
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Background and aims: The metabolic síndrome(MetS) is a cluster of most dangerous heart attack risk factors: diabetes mellitus type 2 and prediabetes, abdominal obesity, high cholesterol and high blood pressure. A quarter of the world´s adults have MetS in Mexico 49.8%. Gymnema sylvestre indigenous herb, belonging to the class dicotyledonous of Ascleapidaceae. Although the herb is widely used as a naturopathic treatment for diabetes, it also demonstrates promising effects in the treatment of obesity, dyslipidemia, hypertension, insulin secretion, among others. The above-mentioned findings show that Gymnema sylvestre has an excellent potential for the prevention and treatment of MetS. The aim of this study was to evaluate the effect of Gymnema sylvestre administration on MetS, insulin sensitivity, and insulin secretion.
Materials and methods: A randomized, double-blind, placebo-controlled clinical trial was carried out in 24 patients with a diagnosis of MetS in accordance with the modify International Diabetes Federation criteria. After simple random allocation using a random number list, 12 patients received Gymnema sylvestre capsules (300 mg), two times per day before breakfast and dinner for 90 days. The remaining 12 patients received placebo at the same dose and the same pharmacological presentation. Before and after intervention we evaluated: the components of MetS, body weight, body mass index, total cholesterol, low-density lipoprotein, very-low-density lipoprotein. We calculated: area under the curve of glucose and insulin, Insulinogenic index, Stumvoll index, and Matsuda index.
Results: The placebo group showed significant increase in body weight (78.8±16.1 vs. 80.3±18.2 kg, p=0.01), body mass index (30.3±3.2 vs. 30.7±3.8 kg/m2, p=0.03), waist circumference (98.3±13.6 vs. 100.5± 14.7 cm, p=0.01), low density lipoprotein (82±23 vs. 107±21 mg/dl, p= 0.01), area under the curve of insulin (10719±5819 vs. 15136±7556 μU/ min, p=0.01), Insulinogenic index (0.61±0.33 vs. 0.95±0.66 p=0.01), as well as an decrease in the Matsuda index (3.3±1.9 vs. 2.5±1.4, p=0.02). After Gymnema sylvestre administration, there were significant decrease in body weight (81.3±10.6 vs. 77.9±8.4 kg, p=0.02), body mass index (31.24±2.55 vs. 30.43±2.23 kg/m2, p=0.02) and very low density lipoprotein (40±13 vs. 31±13 mg/dl, p=0.05).
Conclusion: Gymnema sylvestre decreased body weight, body mass index and the low density lipoprotein in patients with MetS, likewise, in counterpart with placebo, Gymnema sylvestre prevented decrease in insulin sensitivity and compensatory hyperinsulinemia in this population.
Clinical Trial Registration Number: NCT02370121

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Insulin-sensitising effects of methysulfonlymethane (MSM), an organosulfur compound, in insulin-resistant obese mice
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Background and aims: Methysulfonlymethane (MSM) is an organosulfur compound present in plants. MSM has been used as a supplement to improve various metabolic-related diseases, including joint inflammation, osteoarthritis, rheumatoid arthritis, osteoporosis, musculoskeletal pain, and chronic pain. However, the effects of MSM treatment on obesity-linked metabolic disorders remaims unclear. The aim of this work was to determine whether MSM therapy ameliorates glucose metabolism and insulin resistance.
Materials and methods: Male leptin receptor-deficient (db/db, 6 weeks of age) and control mice were treated with MSM (3-5% m/v) in drinking water for 4 weeks. Male C57 mice (5 weeks of age) fed a high-fat diet (HFD) were treated with MSM (2.5-5% m/v) for 10 weeks. Insulin sensitivity and glucose tolerance were assessed; body weight and glucose levels were monitored. Hepatic and serum content in triglycerides and cholesterol and gene expression of key molecules involved in lipid metabolism and inflammation were determined. Hematoxylin and eosin stain (H&E stain) of liver sections from control, db/db and HFD mice was performed.
Results: Four weeks of MSM treatment decreased blood glucose levels from 516±18.79 mg/dl (before treatment) to 242.3±41.78 mg/dl (after treatment) in db/db mice ($p<0.001$), suggesting effects for glucose-lowering action. Interestingly, this effect occurred in the presence of hyperinsulinemia (6.349±1.121 ng/mL for untreated versus 12.59± 1.204 ng/mL for treated db/db mice, $p<0.001$). Histological analysis indicated that hepatic steatosis was decreased in MSM-treated db/db mice. This was consistent with a reduction in hepatic triglyceride (263.8±34.46 mg/dL for untreated versus 137.6±10.96 mg/dL for treated db/db mice, $p<0.01$) and cholesterol (231.2±29.45 mg/dL for untreated versus 144.4±9.131 mg/dL for treated db/db mice, $p<0.01$) levels. MSM treatment also promoted a decrease in serum triglycerides (171.1± 12.84 mg/dL for untreated versus 105.6±4.975 mg/dL for treated db/db mice, $p<0.01$) and cholesterol (109.0±3.591 mg/dL for untreated versus 79.97±15.89 mg/dL for treated db/db mice, $p<0.05$). In mice fed a high-fat diet (HFD), 10 weeks of MSM treatment led to a decrease in insulin (10.40±1.183 ng/mL for HFD versus 3.285±1.168 ng/mL for treated HFD mice, $p<0.01$) and glucose 152.4±6.743 mg/dL for HFD versus 128.2±3.364 mg/dL for treated HFD mice, $p<0.01$) levels. Importantly, MSM treated HFD-fed mice displayed hypersensitivity to insulin, indicating that MSM has insulin-sensitizing effect. In response to MSM, hepatic triglyceride (21.19±0.9239 mg/g of tissue for HFD versus 16.76±0.3724 mg/g of tissue for treated HFD mice, $p<0.01$) and cholesterol (6.780±0.3642 mg/g of tissue for HFD versus 4.906±0.5015 mg/g of tissue for treated HFD mice, $p<0.05$) contents were decreased in HFD fed mice. This was associated with a reduction in lipogenic gene expression of FAS and ACC. In addition, gene expression of key molecules

involved in inflammation, MCP1, TNF-alpha, and IL-6, were greatly suppressed by MSM.
Conclusion: These data suggest that MSM has beneficial metabolic effects on hyperglycemia, insulin resistance, and hepatic steatosis, all of which are often found in obesity and type 2 diabetes. Thus, MSM could be the therapeutic option for the treatment of metabolic disorders.
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TAZ modulator, TM-25659-induced activation of FGF21 levels decreases insulin resistance

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Background and aims: Transcriptional co-activator with PDZ binding motif (TAZ) plays a key role in regulating myogenic differentiation and muscle regeneration. In this study, we investigated the effects of TAZ on palmitate-induced insulin resistance.
Materials and methods: The effects of TAZ on palmitate-induced insulin resistance were investigated using TM-25659 to modulate TAZ, C2 myotubes, and C57BL/6J mice. And, we investigated the relationship between the expressions of FGF21 and TM-25659 using immunoblotting and quantitative real-time PCR. To elucidate the mechanism of action of TM-25659, we measured the levels of the pro-inflammatory cytokines TNF-α, IL-1-β, IL-6, and MCP-1, as well as FGF21. We also measured the secretion of FGF21 from cells into the cytosol after treatment with TM-25659. To determine whether TM-25659 exerted the same effects *in vivo*, C57BL/6J mice were injected intraperitoneally with either vehicle or TM-25659 every other day for 1 week. Finally skeletal muscle cells were transfected with FGF21 siRNA, and FGF21 levels and the effects of TM-25659 on palmitate-induced insulin resistance were measured.
Results: Palmitate reduced the phosphorylation of Akt, thereby impairing insulin signaling and eventually leading to reduced glucose uptake in C2 myotubes. However, treatment with TM-25659, which stimulates TAZ, blocked palmitate-inhibited glucose uptake and insulin signaling significantly. Different doses of TM-25659 inhibited the production of pro-inflammatory cytokines and induced FGF21 mRNA and protein levels. TM-25659 increased FGF21 RNA and protein levels significantly in the skeletal muscle. TM-25659 could not rescue cells from palmitate-induced insulin resistance when FGF21 had been knocked down. Therefore, the TAZ activator TM25659 protected palmitate-induced insulin resistance via FGF21.
Conclusion: The present study suggests that TM-25659 might have therapeutic potential as a treatment for insulin resistance and diabetes by inducing FGF21 expression.

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Effect of lobeglitazone, a new PPAR-gamma agonist, on restenosis after balloon injury in diabetic rats

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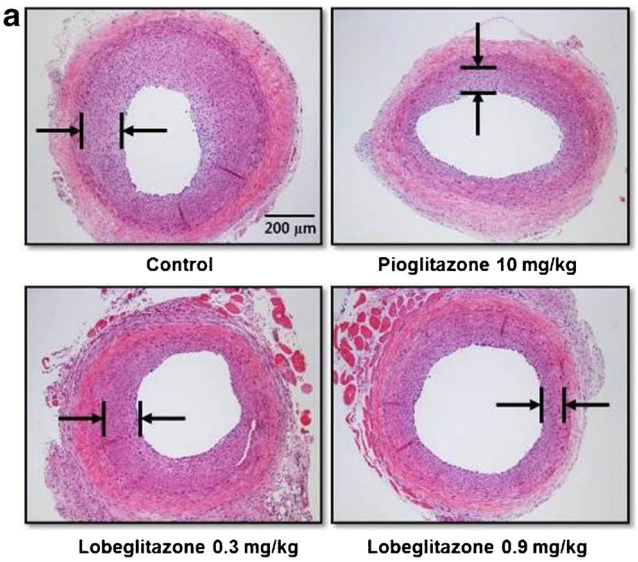
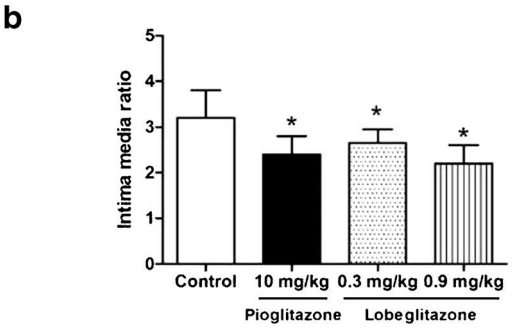
Background and aims: The ligand-activated transcription factor peroxisome proliferator-activated receptor gamma (PPARγ) is a key factor in adipogenesis, insulin sensitivity, and cell cycle regulation. Activated PPARγ might also have anti-inflammatory and antiatherogenic properties. We tested whether lobeglitazone, a PPARγ agonist, might protect against atherosclerosis.
Materials and methods: A rat model of balloon injury to the carotid artery, and high-fat, high-cholesterol diet-fed apolipoprotein E gene double knockout (ApoE-/-) mice were studied.
Results: After the balloon injury, lobeglitazone treatment (0.3 and 0.9 mg/kg) caused a significant decrease in the intima-media ratio compared with control rats (2.2±0.9, 1.8±0.8, vs. 3.3±1.2, P<.01) (figure 1). Consistent with this, in ApoE-/- mice fed a high-fat diet, lobeglitazone treatment (1, 3, and 10 mg/kg) for 8 weeks reduced atherosclerotic lesion sizes in the aorta compared with the control mice in a dose-dependent manner. Treatment of vascular smooth muscle cells with lobeglitazone inhibited proliferation and migration and blocked the cell cycle G0/G1 to S phase progression dose-dependently. In response to lobeglitazone, tumor necrosis factor alpha (TNFα)-induced monocyte-endothelial cell adhesion was decreased by downregulating the levels of adhesion molecules. TNFα-induced nuclear factor kappa-B (NF-κB) p65 translocation into the nucleus was also blocked in endothelial cells. Insulin resistance was decreased by lobeglitazone treatment. Circulating levels of high sensitivity C-reactive protein and monocyte chemoattractant protein-1 were decreased while adiponectin levels were increased by lobeglitazone in the high-fat diet-fed ApoE-/- mice.
Conclusion: Lobeglitazone has antiatherosclerotic properties and has potential for treating patients with diabetes and cardiovascular risk.





Supported by: the research center of CKD

PS 075 Investigational injectable glucose-lowering therapy

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Insulin degludec/liraglutide (IDegLira) improves patient-reported outcomes in subjects with type 2 diabetes uncontrolled on insulin glargine + metformin: DUAL V

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Background and aims: This 26-week, open-label trial compared the efficacy and safety of IDegLira vs insulin glargine (IGlar) in subjects with type 2 diabetes inadequately controlled on IGlar (20–50U).
Materials and methods: Adults (n=557, HbA_{1c} 7–10%) were randomised to either once-daily IDegLira or continued IGlar uptitration, both + metformin. Initial doses were 16 dose steps (16U insulin degludec + 0.6 mg Liraglutide; maximum 50 dose steps) for IDegLira and pre-trial dose for IGlar (baseline mean 32U; no maximum). Fasting self-measured blood glucose titration target was 4.0–5.0 mmol/L for both arms. Patient-reported impact of treatment on functioning and well-being were assessed by TRIM-D and SF-36 v2. Scores were analysed using an ANCOVA method with treatment and region as fixed factors and baseline value as a covariate. Missing data were imputed using last observation carried forward.
Results: TRIM-D scores were summed from 5 subdomains and weighed together to give total score. Change from baseline was higher with IDegLira vs IGlar for total score (p=0.003) as well as the treatment burden (p=0.017) and diabetes management (p<0.001) subdomains (Table). The SF-36 validated multi-purpose questionnaire is grouped into 8 domains, including a physical component summary (PCS) score and mental component summary (MCS) score. The improvements in PCS (p<0.001) and 3 of the physical domain scores were significantly greater with IDegLira vs IGlar. The MCS and the mental domain scores were similar for both arms.
Conclusion: The statistical significant changes in the TRIM-D total score and SF-36 PCS score, indicate that patients with type 2 diabetes treated with IDegLira versus IGlar observed more improvement in their treatment-related impact as well as physical functioning.

	IDegLira Baseline (SD)	IGlar Baseline (SD)	IDegLira Change from baseline (SD)	IGlar Change from baseline (SD)	Treatment difference IDegLira – IGlar [95% CI]	p-value
TRIM-D total score	**74.6 (13.2)**	**73.6 (12.5)**	**7.5 (13.0)**	**5.1 (12.6)**	**2.8 [0.9; 4.7]**	**0.003**
Treatment burden	66.0 (21.4)	64.4 (18.6)	10.1 (22.0)	7.0 (21.2)	3.7 [0.7; 6.8]	0.017
Daily life	82.9 (17.0)	81.4 (16.9)	2.6 (18.0)	1.9 (18.5)	1.3 [–1.3; 4.0]	0.332
Diabetes management	57.5 (19.7)	56.3 (20.8)	13.8 (24.0)	7.2 (21.1)	7.2 [4.2; 10.2]	<0.001
Compliance	82.0 (17.9)	81.4 (16.9)	6.4 (17.8)	5.7 (18.0)	1.1 [–1.2; 3.5]	0.342
Psychological health	83.1 (16.4)	82.6 (16.4)	5.2 (15.7)	3.9 (15.8)	1.5 [–0.7; 3.6]	0.176
SF-36 PCS score	**47.4 (9.0)**	**47.7 (8.4)**	**1.6 (7.2)**	**–0.6 (7.8)**	**1.9 [0.8; 3.1]**	**<0.001**
Physical functioning	47.0 (10.0)	47.5 (9.1)	0.7 (8.9)	–1.1 (8.9)	1.4 [0.0; 2.7]	0.045
Role – Physical	46.6 (10.1)	47.2 (10.1)	1.2 (8.8)	–0.5 (9.3)	1.3 [–0.0; 2.6]	0.051
Bodily pain	49.4 (11.2)	50.0 (11.0)	1.8 (10.4)	–0.7 (11.4)	2.0 [0.4; 3.6]	0.012
General Health	42.9 (9.0)	43.6 (9.3)	3.3 (8.6)	1.3 (8.3)	1.7 [0.4; 2.9]	0.008
SF-36 MCS score	**46.7 (11.4)**	**48.1 (9.9)**	**1.7 (10.2)**	**1.0 (9.4)**	**–0.1 [–1.5; 1.3]**	**0.928**
Vitality	50.8 (10.3)	51.2 (9.8)	2.1 (8.7)	1.5 (8.3)	0.4 [–0.8; 1.7]	0.498
Social functioning	47.2 (10.4)	48.8 (8.9)	1.5 (10.4)	0.1 (8.9)	0.4 [–0.9; 1.8]	0.546
Role – Emotional	45.3 (11.6)	46.1 (10.8)	0.8 (11.8)	–0.6 (11.2)	0.9 [–0.7; 2.6]	0.250
Mental health	45.9 (11.4)	47.6 (10.8)	1.9 (9.9)	1.0 (10.1)	–0.0 [–1.5; 1.4]	0.949

TRIM-D and SF-36 scores represent number of points. The higher the number of points the better in terms of the scores. Scores were analysed using an ANCOVA method with treatment and region as fixed factors and baseline value as a covariate. Missing data were imputed using last observation carried forward. Role – Physical: Related to work or other daily activities as a result of physical health. Role – Emotional: Related to work or other daily activities as a result of emotional problems. CI: confidence interval; MCS: mental component summary; PCS: physical component summary; SD: standard deviation; SF-36: Short-Form 36 Health Survey; TRIM-D: Treatment related impact measure for diabetes

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Supported by: Novo Nordisk

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Assessment of glycaemic control by continuous glucose monitoring in patients with type 2 diabetes treated with IDegLira

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Background and aims: This post-hoc analysis of the DUAL I trial examined glycaemic fluctuations and day-to-day variability with a novel, once-daily combination of insulin degludec (IDeg) and liraglutide (Lira) compared with IDeg or Lira alone.

Materials and methods: Seventy-two hour continuous glucose monitoring (CGM) was performed in a subset of patients (type 2 diabetes [T2D] uncontrolled on metformin±pioglitazone; oral anti-diabetic drug use balanced across treatment groups; N=260) in DUAL I, a 52-week trial in which IDegLira reduced HbA1c (1.8%) more than IDeg (1.4%, p< 0.0001) or Lira alone (1.2%, p<0.0001).

Results: Mean interstitial glucose (IG) decreased more with IDegLira vs. Lira (p<0.0001), while IDeg produced a similar reduction (Table). Significantly lower IG fluctuations (adjusted integrated absolute distance from the mean profile, i.e. flatness of IG profile, p=0.0018) and postprandial IG increments across all meals (p=0.0288) were observed with IDegLira vs. IDeg. Time outside the IG target range was lower with IDegLira vs. Lira (p=0.0072). Day-to-day IG variability (SD of daily [24 h] mean) was similar with IDegLira vs. IDeg or Lira.

Conclusion: IDegLira shifted the glucose profile downwards compared with Lira and flattened the profile compared with IDeg. Day-to-day glycaemic variability was low and similar between IDegLira, Lira and IDeg. These complementary actions on fasting and postprandial glucose narrow the overall range of glucose excursion and produce HbA1c levels lower than those routinely achieved in the management of T2D.

Table: Glycaemic fluctuation and variability in patients with type 2 diabetes treated for 52 weeks

CGM parameter		IDegLira (N=131)	IDeg (N=64)	Estimated treatment difference IDegLira vs IDeg	Lira (N=65)	Estimated treatment difference IDegLira vs Lira
Mean IG (mmol/l)	Mean at baseline (N) Mean Δ, w52 (N)	10.2 (104) –3.5 (72)	10.2 (53) –3.6 (34)	0.0 *p*=NS	10.0 (52) -2.5 (37)	–1.0 *p*<0.0001
IG fluctuation (mmol/l)	Geometric mean at baseline (N) Geometric mean at w52 (N)	1.5 (104) 1.0 (83)	1.6 (53) 1.3 (35)	Ratio: 0.8 *p*=0.0018	1.5 (52) 1.1 (42)	Ratio: 1.0 *p*=NS
Postprandial IG increment (90 min, mmol/l)	Mean at baseline (N) Mean Δ, w52 (N)	1.4 (101) –0.5 (68)	1.4 (50) 0.2 (31)	–0.5 *p*=0.0288	1.5 (50) –0.2 (34)	–0.2 *p*=NS
Time (hours/day) outside IG target range (<3.9 to ≥9.0 mmol/l)	Mean at baseline (N) Mean at w52 (N)	14.7 (104) 3.2 (83)	14.6 (53) 3.8 (35)	–0.7 *p*=NS	13.2(52) 5.6 (42)	–2.2 *p*=0.0072
Day-to-day IG variability (SD of daily [24 hours] mean, mmol/l)	Mean at baseline (N) Mean at w52 (N)	0.8 (104) 0.6 (83)	1.0 (53) 0.6 (35)	–0.1 *p*=NS	0.8 (52) 0.5 (42)	0.1 *p*=NS

CGM parameters are analysed based on observed data using an ANOVA method with treatment, region, baseline HbA_{1c} stratum (≤8.3%, >8.3%) and previous OAD treatment as fixed effects. Fluctuation is log-transformed before analysis. ANOVA, analysis of variance; CGM, continuous glucose monitoring; IDeg, insulin degludec; IDegLira, combination of insulin degludec and liraglutide; IG, interstitial glucose; Lira, liraglutide; OAD, oral anti-diabetic drug; SD, standard deviation; w, week.

Clinical Trial Registration Number: NCT01336023
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Similar incidence of gastrointestinal side effects between IDegLira and non-glucagon-like peptide-1 receptor agonist comparators

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Background and aims: IDegLira is a novel, fixed-ratio combination of insulin degludec (IDeg), a basal insulin with an ultra-long duration of action, and liraglutide (Lira), a glucagon-like peptide-1 (GLP-1) receptor agonist (GLP-1RA). This combination has previously demonstrated advantages in glycaemic control compared with basal insulin and GLP-1 therapy alone. In this post-hoc analysis, gastrointestinal side effects, often associated with GLP-1RAs during the first 12 weeks of treatment (Figure 1), were compared between IDegLira and non-GLP-1RA comparators.

Materials and methods: This is a post-hoc analysis of two double-blind, 26-week, phase 3 trials: (DUAL IV: IDegLira vs. placebo in patients inadequately controlled on oral antidiabetic drugs [OADs]; and DUAL II: IDegLira vs. IDeg in patients inadequately controlled on basal insulin and OADs) in which IDegLira was initiated at 10 and 16 dose steps, respectively (1 dose step=1 unit of IDeg and 0.036 mg Lira). We compared the proportion of patients experiencing gastrointestinal (GI) side effects.

Results: The GI side effects are presented in Figure 1. There was no statistically significant difference in the odds of experiencing GI side effects for subjects on IDegLira vs. pooled comparators at weeks 4, 8, 12 or during the entire trial period (odds ratios, DUAL II: 2.0, 1.1, 1.1, 2.0; DUAL IV: 1.0, 2.0, 0.4, 0.9, respectively).

Conclusion: In double-blinded studies, a similar proportion of patients treated with IDegLira experienced GI side effects compared with IDeg or placebo. This may be explained by the slow and steady titration of IDegLira which appears to improve tolerability vs. GLP-1RA therapy, while also improving glycaemic control.

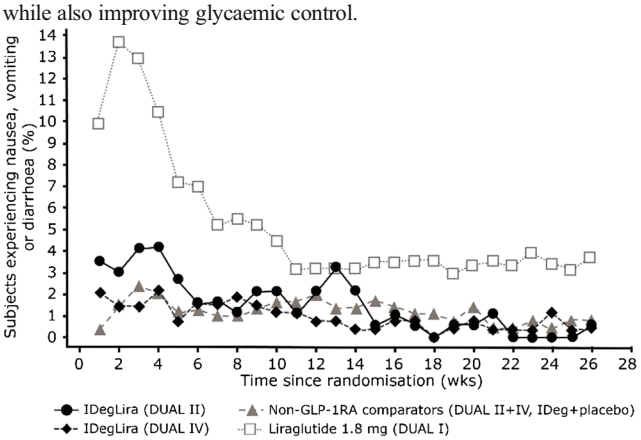

Figure 1. Incidence of nausea, vomiting or diarrhoea in DUAL II and IV. Lira arm from DUAL I (unblind 26 wk trial of IDegLira vs IDeg and Lira) included for comparison only.

Clinical Trial Registration Number: NCT01618162 / NCT01392573
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Efficacy and safety of IDegLira (combination of insulin degludec + liraglutide), in insulin-naïve patients with type 2 diabetes uncontrolled on GLP-1 receptor agonist (GLP-1RA) therapy

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Background and aims: Due to the progressive nature of type 2 diabetes (T2D) most patients will require treatment intensification. This phase 3 trial aimed to investigate the efficacy of IDegLira (a combination of insulin degludec and liraglutide) in controlling glycaemia in adults with T2D who were inadequately controlled on a glucagon-like peptide-1 receptor agonist (GLP-1RA) and oral anti-diabetic drugs (OADs).

Materials and methods: In this 26-week open-label trial, adults with T2D uncontrolled on maximum dose GLP-1RA therapy (liraglutide once daily [OD] or exenatide twice daily) + metformin ± pioglitazone ± sulphonylurea were randomised 2:1 to IDegLira OD (n=292) or continue unchanged GLP-1RA therapy (n=146); previous OADs were continued.
Results: Mean HbA1c decreased from baseline (7.8%/7.7%) to 6.4% (IDegLira) and 7.4% (unchanged GLP-1RA) (estimated treatment difference –0.94%, p<0.001). 75% of patients on IDegLira achieved HbA1c <7% vs. 36% on unchanged GLP-1RA (p<0.001); 63% on IDegLira vs. 23% on unchanged GLP-1RA attained HbA1c ≤6.5% (p<0.001). Fasting plasma glucose and 9-point self-measured blood glucose profiles improved significantly more with IDegLira than unchanged GLP-1RA (Table). Weight change was +2.0 kg with IDegLira and –0.8 kg with unchanged GLP-1RA. Confirmed hypoglycaemia rates were low (Table) but higher with IDegLira vs. unchanged GLP-1RA. Mean IDegLira dose at 26 weeks was 43 dose steps (i.e. 43 U insulin degludec, 1.55 mg liraglutide). Safety profile of IDegLira was consistent with previous findings; both treatments were well tolerated.
Conclusion: Consistent with previous findings on GLP-1RA intensification with insulin-containing therapy, IDegLira provided superior glycaemic control vs. unchanged GLP-1RA and represents an efficacious approach to intensifying therapy in patients with T2D uncontrolled on GLP-1RAs.

Observed changes or rates, and estimated treatment differences or rate ratio			
	Observed change from baseline to 26 weeks IDegLira	Observed change from baseline to 26 weeks Unchanged GLP-1RA	Estimated treatment difference [95% CI], ANCOVA analysis IDegLira – Unchanged GLP-1RA
HbA1c (%), mean (SD)	−1.3 (0.8)	−0.3 (0.9)	−0.94 [−1.11; −0.78] p<0.001
FPG (mmol/L), mean (SD)	−2.98 (2.28)	−0.60 (2.74)	−2.64 [−3.03; −2.25] p<0.001
Mean of 9-point SMBG profile (mmol/L), mean (SD)	−2.2 (1.9)	−0.6 (2.4)	−1.78 [−2.13; −1.43] p<0.001
Weight (kg), mean (SD)	+2.0 (3.9)	−0.8 (3.0)	2.89 [2.17; 3.62] p<0.001
Hypoglycaemia (PG <3.1 mmol/L or severe) during treatment period [a]	Observed rate/PYE 2.82	Observed rate/PYE 0.12	Estimated rate ratio [95% CI], negative binomial regression 25.4 [10.6; 60.5] p<0.001
Values based on last observation carried forward. [a]Severe hypoglycaemia was reported by one patient in the IDegLira group. ANCOVA, analysis of covariance; FPG, fasting plasma glucose; PYE, patient-year of exposure; SMBG, self-measured blood glucose			

Clinical Trial Registration Number: NCT01676116
Supported by: Novo Nordisk

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IDegLira in insulin-naïve patients with type 2 diabetes (T2D) inadequately controlled on sulphonylureas (SU) alone or combined with metformin: The DUAL IV Study

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Background and aims: This 26-week, multinational, double-blind study assessed the efficacy and safety of an insulin degludec/liraglutide combination (IDegLira) in adults (n=435) with T2D (HbA_{1c} 7.0–9.0%).
Materials and methods: Patients were randomised 2:1 to receive once-daily IDegLira or placebo added to SU±metformin, and started at 10 dose steps (10 units IDeg/0.36 mg Lira), with titration to a fasting glycaemic target of 4.0–6.0 mmol/L.
Results: Mean HbA_{1c} decreased from 7.9% (both groups) to 6.4% with IDegLira and to 7.4% with placebo, (estimated difference –1.02%, p< 0.001). HbA_{1c} <7% was achieved by 79.2% of IDegLira patients vs. 28.8% receiving placebo, odds ratio 11.95, p<0.001 (for HbA_{1c} ≤6.5%: 64.0% vs. 12.3%, odds ratio 16.36, p<0.001). Mean fasting plasma glucose decreased from 9.1 mmol/L (both groups) to 6.5 mmol/L with IDegLira and to 8.8 mmol/L with placebo, (estimated difference –2.3 mmol/L, p<0.001). At 26 weeks, a self-monitored 9-point blood glucose profile showed reductions in the profile mean of 2.2 vs. 0.7 mmol/L with IDegLira and placebo, respectively (estimated difference in mean glucose of –1.6 mmol/L, p<0.001). Blood glucose was significantly lower with IDegLira at all 9 time-points (post-hoc analysis). Mean IDegLira dose at 26 weeks was 28 dose steps (28 units IDeg/1.0 mg Lira). Confirmed hypoglycaemia (severe or plasma glucose <3.1 mmol/L) occurred in 41.7% and 17.1% of IDegLira- and placebo-treated patients, respectively, with rates of 3.5 vs. 1.4 events/patient year (estimated rate ratio: 3.74, p<0.001). Mean weight change was +0.5 kg with IDegLira vs. –1.0 kg with placebo (estimated difference 1.48 kg, p< 0.001). The rate of serious adverse events was higher in the IDegLira group than in the placebo group (20.3 vs. 8.0 per 100 patient-years of exposure) without any obvious patterns in the type of events.
Conclusion: IDegLira significantly improved glycaemic control in combination with SU. Hypoglycaemia rates were low, but given the use of SUs, more common than in previous trials where IDegLira was used in combination with other oral anti-diabetic agents. Treatment was well tolerated, with overall adverse event rates comparable to previous IDegLira trials.
Clinical Trial Registration Number: NCT01618162
Supported by: Novo Nordisk

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Insulin degludec/liraglutide (IDegLira) is superior to insulin glargine (IGlar) in HbA_{1c} reduction, risk of hypoglycaemia and weight change: DUAL V study

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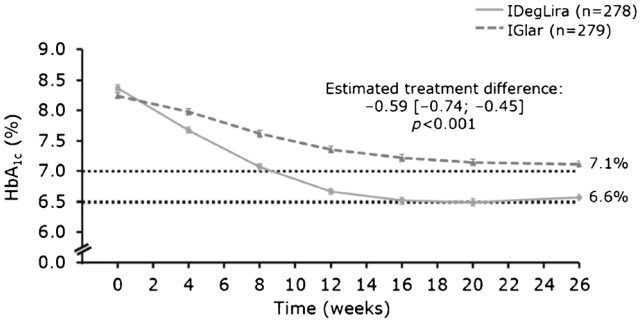
Background and aims: This 26-week, open-label trial compared the efficacy and safety of IDegLira vs IGlar in subjects with type 2 diabetes uncontrolled on IGlar (20–50U) and metformin.
Materials and methods: Adults (n=557, HbA_{1c} 7–10%) were randomised to either once-daily IDegLira or continued IGlar uptitration, both plus metformin. Initial doses were 16 dose steps (16U IDeg + 0.6 mg Lira; maximum 50 dose steps) for IDegLira and pre-trial dose for IGlar (mean 32U; no maximum). Fasting self-measured blood glucose titration target was 4.0–5.0 mmol/L for both arms.
Results: Mean HbA_{1c} decreased from 8.4 to 6.6% with IDegLira (change from baseline [SD] –1.81% [1.08]) and from 8.2 to 7.1% with IGlar (change from baseline [SD] –1.13% [0.98]); estimated treatment difference (ETD) IDegLira–IGlar [95% CI] –0.59 [–0.74; –0.45], p<0.001 (Figure). Mean fasting plasma glucose decreased similarly in both arms from 8.9 to 6.1 mmol/L with a change from baseline [SD] of –2.83 mmol/L [2.80] with IDegLira and –2.77 mmol/L [3.02] with IGlar; ETD [95% CI] –0.01 mmol/L [–0.35; 0.33], NS. Weight decreased from 88.3 to 86.9 kg with IDegLira (change from baseline [SD] –1.4 kg [3.5]) and increased from 87.3 to 89.1 kg with IGlar (change from baseline [SD] 1.8 kg [3.6]); ETD [95% CI] for weight change –3.20 kg [–3.77; –2.64], p

<0.001. Significantly more subjects achieved the ADA HbA_{1c} target and pre-specified composite endpoints with IDegLira vs IGlar: HbA_{1c} <7%, 71.6 vs 47.0%, estimated odds ratio (EOR) [95% CI] 3.45 [2.36; 5.05]; HbA_{1c} <7% without hypoglycaemia, 54.3 vs 29.4%, EOR [95% CI] 3.24 [2.24; 4.70]; HbA_{1c} <7% without hypoglycaemia and no weight gain, 38.8 vs 12.2%, EOR [95% CI] 5.53 [3.49; 8.77], respectively, all p< 0.001. IDegLira was insulin sparing; mean 26-week dose was 41 dose steps (IDegLira) and 66U (IGlar), p<0.001.

Conclusion: IDegLira was superior to IGlar in terms of HbA_{1c} reduction, risk of hypoglycaemia and weight change and decreased insulin dose requirements. Through a greater reduction in HbA_{1c} combined with a lower rate of hypoglycaemia, IDegLira offers meaningful clinical advantages over IGlar in intensifying therapy, in subjects with type 2 diabetes uncontrolled on IGlar.



Mean observed values with error bars (standard error mean) based on full analysis set and LOCF imputed data. --- ADA/EASD HbA_{1c} target <7.0%; AACE HbA_{1c} target ≤6.5% AACE, American Association of Clinical Endocrinologists; ADA, American Diabetes Association, EASD, European Association for the Study of Diabetes

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Supported by: Novo Nordisk

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Higher early insulin exposure and greater early glucose-lowering effect with faster-acting insulin aspart vs insulin aspart in Japanese patients with type 1 diabetes

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Background and aims: Faster-acting insulin aspart (faster aspart) is a new formulation of insulin aspart (IAsp), with faster initial absorption following subcutaneous (s.c.) injection. This trial aimed to investigate the pharmacokinetic (PK) and pharmacodynamic (PD) properties of faster aspart vs IAsp following s.c. injection in Japanese patients with type 1 diabetes.

Materials and methods: Patients (N=43; mean age±SD: 39.4± 9.4 yrs) received a single dose (0.2 U/kg s.c.) of faster aspart or IAsp under glucose-clamp conditions (STG-22; blood glucose target 100 mg/dL; duration 12 h post-dose) in a double-blind, randomised, crossover design.

Results: Onset of appearance and $t_{50\%Cmax}$ with faster aspart occurred 58% and 35% earlier than with IAsp, and faster aspart had greater early insulin exposure during the first 2 h (2-fold greater exposure than IAsp in the first 30 min post-dose); total exposure was similar (Table). Faster aspart had a greater glucose-lowering effect within 2 h post-dose vs IAsp (greatest difference in the first 30 min), and earlier onset of glucose-lowering effect (Table). Earlier onset of action with faster aspart vs IAsp was supported by ~10 min shorter $t_{50\%GIRmax}$ (37.5 vs 47.4 min; treatment diff. [95% CI]: -9.97 min [-12.78; -7.15]). Both treatments were well tolerated.

Conclusion: Earlier onset and greater early insulin exposure with faster aspart led to a greater early glucose-lowering effect vs IAsp in Japanese patients with type 1 diabetes.

Table: PK and PD results for faster aspart vs IAsp

PK endpoints (insulin exposure†)	Treatment ratio: faster aspart/IAsp [95% CI]*	PD endpoints (glucose-lowering effect)	Treatment ratio: faster aspart/IAsp [95% CI]
Onset		*Onset*	
Onset of appearance#	0.42 [0.32; 0.53]	Onset of action#	0.79 [0.69; 0.91]
$t_{50\%Cmax}$§#	0.65 [0.59; 0.72]	$t_{50\%GIRmax}$#	0.79 [0.74; 0.84]
t_{max}§#	0.78 [0.67; 0.90]	t_{GIRmax}#	0.87 [0.77; 0.97]
Early		*Early*	
$AUC_{0-15min}$	3.26 [2.65; 4.02]	–	–
$AUC_{0-30min}$	1.94 [1.68; 2.25]	$AUC_{GIR,0-30min}$#	2.10 [1.32; 4.08]
AUC_{0-1h}‡	1.31 [1.17; 1.47]	$AUC_{GIR,0-1h}$	1.36 [1.20; 1.54]
AUC_{0-2h}	1.10 [1.01; 1.19]	$AUC_{GIR,0-2h}$	1.09 [1.01; 1.17]
Total		*Total*	
AUC_{0-12h}	0.99 [0.96; 1.02]	$AUC_{GIR,0-12h}$	0.93 [0.87; 0.99]
C_{max}	1.07 [0.96; 1.19]	GIR_{max}	0.95 [0.89; 1.02]

*90% CI for AUC_{0-12h}; †Based on free serum insulin aspart; ‡Primary endpoint; §*Post hoc* analysis; #Treatment ratios and 95% CI estimated using Fieller's method; AUC=area under the curve; C_{max}=maximum observed concentration; GIR_{max}=maximum glucose infusion rate; onset of appearance=time from dosing until the first time serum IAsp concentration ≥ lower limit of quantification; t_{max}=time to maximum observed concentration; $t_{50\%Cmax}$=time to reach 50% of maximum serum insulin aspart concentration; $t_{50\%GIRmax}$=time to 50% of maximum glucose infusion rate; t_{GIRmax}=time to maximum glucose infusion rate.

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A novel GIP receptor agonist enhances the body weight lowering effect of liraglutide in diet-induced obese mice and has the potential for once-weekly administration in humans

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Background and aims: Analogs of the incretin hormone glucagon-like peptide-1 (GLP-1) are used for the management of type 2 diabetes and are often accompanied by modest weight loss. One approach to enhance the efficacy of GLP-1 receptor (GLP-1R) agonists includes co-administration with other endogenous hormones e.g. glucagon. Recently, it has been demonstrated in animals that the incretin hormone glucose-dependent insulinotropic peptide (GIP) enhances the weight loss induced by GLP-1. Here we report the characterization of a novel long-acting GIP receptor (GIP-R) incretin (I) agonist, ZP-I-98.

Materials and methods: In vitro receptor activation by ZP-I-98 was measured as cAMP formation in HEK293 cells stably expressing human or mouse GIP receptors or human GLP-1 or glucagon receptors. Pharmacokinetic (PK) characterization of ZP-I-98 was performed in mice and monkeys and the acute pharmacodynamic effect of ZP-I-98 was investigated in mice by measuring blood glucose levels during an oral glucose tolerance test (OGTT). Sub-chronic effects of ZP-I-98, with or without liraglutide (GLP-1 analog) co-administration, on body weight and food intake were investigated in diet-induced obese (DIO) mice.

Results: ZP-I-98 activated the human and mouse GIP-R with EC50 values of 9 and 52 pM, respectively, while it displayed selectivity >300 fold over the GLP-1 and glucagon receptors. The terminal half-life of ZP-I-98 in mice and monkeys, following i.v. administration, was estimated to approximately 15 h and 57 h, respectively. Simple two species allometric scaling of the PK indicated that the compound is suitable for once-weekly dosing in humans.

Compared to vehicle treatment, ZP-I-98 dosed s.c. 4 h prior to an OGTT, dose-dependently (3 and 30 nmol/kg) and significantly reduced blood glucose levels in lean mice (p<0.001 at 30 nmol/kg, 0-180 min after glucose administration), thus indirectly confirming the incretin effect of the compound. Administration with liraglutide (20 nmol/kg, once-daily) induced a transient reduction in food intake in DIO mice and 3 weeks of treatment resulted in a mean weight loss ± SEM of 3.0±1.3%. DIO mice treated with vehicle gained 4.3±0.95% body weight during the study. In contrast, co-administration of liraglutide (20 nmol/kg, once-daily) and ZP-I-98 (30 nmol/kg, once every third day) resulted in a weight loss of 9.0±1.9% over the three weeks of treatment. This weight loss was significantly greater than the liraglutide-induced weight loss (p<0.05). Co-treatment of liraglutide and ZP-I-98 transiently suppressed food intake to

a similar extent as liraglutide alone. Finally, ZP-I-98 (30 nmol/kg, once every third day) did not significantly affect the body weights of the mice compared to vehicle.

Conclusion: Our results suggest that a combination therapy consisting of ZP-I-98 and a GLP-1R agonist can improve the management of type 2 diabetes by inducing body weight loss superior to GLP-1R agonist alone. The in vivo profile of ZP-I-98 further suggests that ZP-I-98 can be used as a convenient once-weekly treatment in humans.

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Patient characteristics and predictors of a future termination of basal insulin supported oral therapy in the DIVE registry

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Background and aims: The addition of a single daily injection of long-acting insulin to oral therapy is a well-established method for treating type-2 diabetes patients. Termed basal supported oral therapy (BOT), this approach can greatly improve glycaemic control. However, it is not an efficacious long term solution for every individual. The identification of patient-related characteristics that may predict failure of BOT would be beneficial in a clinical setting when assessing potential treatment regimens.

Materials and methods: Data taken from the DIabetes Versorgungs-Evaluation (DIVE) registry was analysed for patients who were younger than 90 years and had been treated with BOT for at least 3 months. Time origin was set to BOT-initiation. The event of interest 'BOT failure' was defined as the cessation of oral therapy, the cessation of insulin therapy, or the addition of short-acting insulin to the treatment regimen during the observational period. Risk quantification for demographic, glycaemic, and pharmacotherapeutic characteristics of patients was based on univariate and multivariate Cox proportional hazards regression.

Results: BOT failure occurred in 2,021 patients (35.7%) of the 5,663 that fulfilled the inclusion criteria for the study. Of these, 46.7% discontinued oral therapy, 32.7% discontinued insulin, and 20.6% had short-acting insulin added to their treatment. Multivariate analysis revealed that body mass index (BMI; HR: 1.012; 95% CI: 1.001, 1.023), diabetes duration (HR: 0.982; 95% CI: 0.976, 0.989), and HbA1c level (HR: 1.102; 95% CI: 1.022, 1.188) were associated with BOT failure. Age, gender, fasting plasma glucose (FPG), presence of micro- or macrovascular disease, number of oral antidiabetic drugs (OADs), and number of concomitant drugs were not found to be predictive of BOT failure.

Conclusion: The identification of factors that may be predictive of the failure of BOT could be highly useful in a clinical setting when assessing the most appropriate treatment strategy for type-2 diabetes patients.

Predictors for a termination of BOT	Univariate HR (95%CI)	Multivariate HR (95%CI)
Age (years)	**0.996** (0.991,1.000)	1.003 (0.999,1.008)
BMI (kg/m²)	**1.014** (1.005,1.023)	**1.012** (1.001,1.023)
Diabetes duration (years)	**0.984** (0.978,0.989)	**0.982** (0.976,0.989)
HbA1c (%)	**1.111** (1.047,1.178)	**1.102** (1.022,1.188)
FPG (mmol/l)	1.033 (0.999,1.068)	1.006 (0.963,1.051)
Gender (female)	1.006 (0.922,1.098)	0.990 (0.904,1.084)
Anamnestic microvascular diseases (yes vs. no)	0.978 (0.850,1.013)	0.940 (0.858,1.030)
Anamnestic macrovascular diseases (yes vs. no)	0.998 (0.911,1.094)	1.051 (0.955,1.159)
No. OADs at baseline 2 vs. 1	0.928 (0.839,1.028)	0.918 (0.828,1.019)
No. OADs at baseline 3 vs. 1	0.903 (0.734,1.110)	0.876 (0.711,1.079)
Concomitant medication yes vs. no	0.935 (0.854,1.025)	0.926 (0.843,1.017)
Age (years)	**0.996** (0.991,1.000)	1.003 (0.999,1.008)

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A composite measure to define insulin responders: HbA_{1c} <7% and ≥1% absolute decrease in HbA_{1c} from baseline

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Background and aims: A majority of patients with type 2 diabetes (T2D) treated with insulin do not reach the hemoglobin A1c (HbA_{1c}) goal of <7% suggested by guidelines, yet they can have clinically relevant HbA_{1c} reductions with insulin use. Using an integrated database of 53 insulin lispro clinical trials, we propose a composite HbA_{1c} measure to define patients with T2D responding to therapy and report their baseline (BL) characteristics.

Materials and methods: The analysis population included 9869 patients with T2D treated with any insulin regimen with a BL and ≥1 post-BL HbA_{1c} value. Responders were defined as patients with an endpoint HbA1c <7% and/or a ≥1% absolute (AA) decrease from BL in HbA1c. The percentage of responders was assessed at 12 and 24 weeks, along with the BL demographics of responders versus non-responders.

Results: Overall, only 29.6% and 40.6% of patients reached HbA_{1c} <7% at 12 and 24 weeks respectively, whereas the AA model identified greater proportions of patients as responders (62.3% at 12 weeks and 72.6% at 24 weeks). Responders at both 12 and 24 weeks had a significantly lower duration of diabetes and higher BL HbA_{1c} compared to non-responders. Compared to whites, significantly higher proportions of Hispanic patients were responders at 12 weeks (odds ratio [OR]: 1.81; 95% confidence interval [CI]: 1.45, 2.25) and 24 weeks (OR: 1.45; 95% CI: 1.11, 1.90). Conversely, significantly lower proportions of Asians compared to whites were responders at 12 (OR: 0.81; 95% CI: 0.71, 0.92) and 24 weeks (OR: 0.70; 95% CI: 0.60, 0.82). A limitation of this integrated database was that the clinical trial population is different from a real-world treatment population.

Conclusion: The composite HbA_{1c} measure (AA model), which has a ≥1% absolute decrease in HbA_{1c} from BL added to HbA_{1c} <7%, identified more patients with clinically meaningful responses to insulin therapy than an HbA_{1c} target alone. Characteristics of responders by the AA model (higher BL HbA_{1c}, fewer years of diabetes duration) were consistent with other reports. Proportions of responders by the AA model may be significantly higher among Hispanics and lower among Asians compared with Whites. Although additional validation analyses in real-world populations are necessary, this composite model of defining insulin therapy response may be useful in population management and quality measures.

Characteristics	12 Weeks			24 Weeks		
	N	HbA_{1c} <7%	AA	N	HbA_{1c} <7%	AA
Responders, n (%) in overall population	6506	1923 (29.6%)	4050 (62.3%)	4908	1991 (40.6%)	3561 (72.6%)
Baseline Characteristics of Responders and Non-Responders by the AA Model						
	Responders	**Non-Responders**		**Responders**	**Non-Responders**	
Age, years, mean (SD)*	57.9 (9.7)	58.5 (9.9)		57.9 (9.6)	57.8 (10.0)	
Gender (% male)*†	2125 (52.5%)	1215 (49.5%)		1880 (52.8%)	654 (48.6%)	
Race, n (%)*†						
White	2656 (65.6%)	1628 (66.3%)		2379 (66.8%)	858 (63.7%)	
African American/Black	176 (4.3%)	86 (3.5%)		155 (4.4%)	58 (4.3%)	
Hispanic	342 (8.4%)	116 (4.7%)		298 (8.4%)	74 (5.5%)	
Asian	693 (17.1%)	525 (21.4%)		600 (16.8%)	309 (22.9%)	
Other	181 (4.5%)	100 (4.1%)		127 (3.6%)	48 (3.6%)	
Duration of diabetes, years, mean (SD)*†	10.8 (6.9)	12.3 (7.3)		10.9 (6.7)	11.4 (7.0)	
BL HbA_{1c}, %, mean (SD) *†	9.0 (1.4)	8.4 (1.0)		9.0 (1.3)	8.3 (0.9)	

SD = standard deviation.
*P<0.05 between responders and non-responders at 12 weeks; †P<0.05 between responders and non-responders at 24 weeks.

Supported by: Eli Lilly and Company

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Patient characteristics are associated with treatment response to second line glucose lowering therapy: a MASTERMIND study

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Background and aims: The most common second-line glucose-lowering agent in type 2 diabetes is a sulfonylurea (SU). Draft NICE (UK) guidelines, currently out for consultation, propose using a thiazolidinedione (TZD), pioglitazone, as second-line. There is no guidance regarding which treatment is most effective in which patients. We assessed whether patient clinical characteristics could be used to predict response and aid treatment decisions.
Materials and methods: 12-month glycaemic response (HbA1c change from baseline) was calculated for patients in the CPRD (UK primary care) dataset treated with SUs (n=8748) or TZDs (n=8876). Relationships between clinical phenotype and glycaemic response were assessed using linear regression, with adjustment for baseline HbA1c. This analysis was repeated for the ADOPT randomised controlled trial (SUs n=1441; TZDs n=1456).
Results: In CPRD, patients diagnosed younger had a smaller glycaemic response to both SUs and TZDs (~2 mmol/mol greater response per 10-year increase in age at diagnosis, p<0.0001). Female patients responded better to TZDs (1.9 mmol/mol greater HbA1c reduction compared with males, p<0.0001), but worse to SUs (2.4 mmol/mol smaller response, p<0.0001). Obese patients (BMI≥30 kg/m2) responded better to TZDs (2.1 mmol/mol greater HbA1c reduction compared with non-obese patients, p<0.0001), but non-obese patients responded better to SUs (1.9 mmol/mol greater HbA1c response, p<0.0001). Combining categories led to greater differences (obese females: 4.4 mmol/mol greater response to TZDs; non-obese males: 3.3 mmol/mol greater response to SUs). Effect sizes were similar in ADOPT.
Conclusion: Clinical phenotype helps determine likely initial response to second-line glucose lowering therapies, and could influence therapeutic choices. Obese females have the greatest HbA1c reduction with TZDs, and non-obese males with SUs.
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Factors associated with type 2 diabetes mellitus treatment choice across Europe

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Background and aims: Demographic and clinical factors associated with choice of type 2 diabetes mellitus (T2DM) treatment at treatment intensifications were explored using electronic health care databases in four European countries.
Materials and methods: Antidiabetic drug prescription records were obtained from electronic health care record databases for a 5-year study period (2007-2011/2008-2012) in The Netherlands (NL), Italy (IT), Spain (ES) and the United Kingdom (UK). A standardized analytical tool assessed treatment patterns in each database. Oral monotherapy was defined as first line, oral dual therapy as second line, >2 oral treatments or oral combined with an injectable as third line and injectables only as fourth line therapy. Treatment intensification was defined as starting a higher line of treatment, including start of treatment. Factors associated with the choice of treatment were identified using multivariate logistic regression. Potential associated factors included general characteristics, comedication, comorbidities and clinical parameters. Missing values were categorized as a separate category in order to avoid major loss of data.
Results: A total of 617,346 T2DM patients were included; 48,479 from NL, 67,751 from IT, 348,572 from ES and 152,544 from UK. Prescription of a sulfonylurea (SU) as a first line treatment was associated with age >75 years (RR ranged from 2.05 in ES to 3.66 in IT) and renal comorbidity (RR ranged from 1.36 in NL to 2.65 in UK) in all countries, while there was an inverse association with BMI ≥30 kg/m2 (RR 0.22 and 0.46, respectively) in UK and ES. For second line treatment, across all countries, age >75 years was associated with receiving metformin + SU (RR ranged from 1.05 in ES to 1.34 in IT) and renal comorbidity with receiving SU + dipeptidyl peptidase-4 inhibitors (DPP4) (RR ranged from 1.26 in UK to 2.31 in NL). In UK and ES, BMI ≥30 kg/m2 increased the probability of receiving metformin + thiazolidinedione (TZD) (RR 1.44 and 1.25, respectively) and, in UK only, also the probability of receiving metformin + DPP4 (RR 2.38). For third line treatment, age >75 years (RR ranged from 2.49 in ES to 3.56 in UK) and renal comorbidity (RR ranged from 1.61 in ES to 3.45 in UK) were associated with SU + insulin. BMI ≥30 kg/m2 decreased the probability of receiving metformin + SU + TZD as third line treatment in UK (RR 0.90), but increased the risk in ES (RR 1.51). Furthermore, a BMI ≥25 kg/m2 decreased the probability to receive any third line treatment including insulin in these two countries. Analyses of associations with fourth line treatments were not performed for all countries because there were few treatment options. Year of index or intensification date and prior treatment class also played a significant role in choosing treatment when intensifying.
Conclusion: The results from this study in four different EU countries suggest that age and renal comorbidity were the predominant factors significantly associated with T2DM treatment choice across treatment intensifications. Our findings might be used to investigate on appropriateness of diabetes pharmacotherapy in Europe.
Supported by: BMS/AZ

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Real-life intensification of pharmacotherapy in type 2 diabetes. ARETAEUS2 Study results

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Background and aims: Type 2 diabetes (DM2) is a progressive disease, subsequently its pharmacotherapy requires constant intensification. Patients are also often treated for hypertension or dyslipidaemia, and these therapies are not seen as requiring a step up approach over time. In 2012 a cross-sectional nationwide study assessing DM2 treatment efficacy was conducted.

Materials and methods: Patients with DM2 (n=15,643) were enrolled at primary care (68%) and specialist (32%) outpatient clinics, and were analysed in three groups according to diabetes duration: up to 2, 2-10, more than 10 years.

Results: The patients with longer DM2 duration received more intensified treatment, with almost 50% taking insulin after 10 years of diabetes (Table). In the whole group HbA1c less than 7% was achieved in 30% of those treated with oral monotherapy, in 46% - dual oral therapy, in 62% - insulin only and in 69% - metformin + insulin. Among cardiovascular risk related therapies only statins and ACE inhibitors were used with increased frequency over time. Less than 10% of the patients achieved all main therapeutic targets (HbA1c lower than 7%, total cholesterol less than 175 mg/dl, HDL cholesterol over 40 (men) or 50 mg/dl (women), LDL cholesterol less than 100 mg or 70 mg/dl in patients with CVD, triglycerides less than 150 mg/dl, blood pressure less than 140/90 mmHg), while 20-25% failed to achieve any of them.

Conclusion: Despite widely accepted need for progressive and multifactorial treatment of DM2, still many patients are not treated intensively enough, even with older and inexpensive therapies.

	Total	*Diabetes duration (years)*		
		<2 (n=2,574)	2-10 (n=7,689)	>10 (n=5,380)
Age (years)	63.1	58.6	62.0	66.7
Ever Smokers (%)	52.8	54.9	53.6	50.7
BMI (kg/m^2)	30.2	29.9	30.1	30.5
HbA1c (%)	7.2	7.1	7.1	7.4
Past acute coronary syndrome (%)	14.8	10.4	12.3	20.4
Stable angina (%)	38.1	23.3	34.2	50.8
Past stroke (%)	6.9	3.5	5.2	11.0
Any retinopathy (%)	23.8	7.7	16.9	41.9
Any nephropathy (%)	12.8	4.4	7.9	24.0
Diabetic foot syndrome (%)	4.5	0.9	2.7	8.8
Hypertension (%)	86.4	77.5	86.7	91.0
Dyslipidaemia (%)	78.8	71.6	79.0	82.5
Metformin monotherapy (%)	23.8	48.5	26	8.9
Sulfonylurea monotherapy (%)	6.2	7.8	7.4	3.7
Insulin monotherapy (%)	4.8	3.7	5.5	13.7
Metformin + Sulfonylurea (%)	25.7	20.6	29.8	22.1
Metformin + insulin (%)	17.7	6.5	13.7	28.8
Acetylsalicylic acid (%)*	89.4	87.7	88.7	90.4
Beta blockers (%)*	85.7	85.4	86.4	84.8
Statins (%)	75.2	66.4	75.2	80.0
Fibrates (%)	14.6	13.2	14.2	15.8
ACE inhibitors (%)**	79.2	68.1	78.6	86.0
All therapeutic targets (blood glucose, lipids, blood pressure) achieved (%)	6.2	8.2#	7.3#	4.2
None of therapeutic targets (blood glucose, lipids, blood pressure) achieved (%)	22.2	18.0#	20.1#	26.5

*Data are means or %; * only patients with CHD; ** only patients with hypertension*
#p<0.05 vs patients with diabetes duration >10 years

Supported by: TEVA Pharmaceuticals

844

Quality of diabetes care and gender differences in the Netherlands (ZODIAC-45)

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Background and aims: The risk of cardiovascular disease and mortality increases by about two times in men and about four times in women with type 2 diabetes mellitus (T2DM) compared to men and women without T2DM. Poorer control of cardiovascular risk factors in women has been described as a possible explanation for this difference. Aim of the current study was to investigate whether trends in quality of diabetes care differ between sexes in the Netherlands from 1998 till 2013.

Materials and methods: This is a prospective observational cohort study (ZODIAC) using data from patients with T2DM treated in primary care. Information about HbA1c, blood pressure, BMI, lipid profile, renal function, foot and eye examination and medication use was collected annually. Target values for clinical parameters were defined according to national guidelines. Linear time trends form 1998 to 2013 were estimated using linear mixed models, adjusted for gender and age. Differences in trends between genders were investigated by adding sex as an interaction term to the model and by performing stratified analyses. Absolute differences between gender were visually investigated and by adding gender as a confounder to the model for the whole study group.

Results: The number of participating patients increased in the period 1998 to 2013 from 2644 to 62,230. Trend differences between sexes over time were observed for systolic blood pressure and smoking (figure 1). Throughout the whole study period the target value for systolic blood pressure was achieved more often in men and the cut-off value for cholesterol-HDL ratio was achieved more often in women. The difference between sexes concerning the percentage of smokers decreased to a 5% difference, still in favor of women. The prevalence of albuminuria decreased from 48% to 24% in men and from 38% to 16% in women. In the group of patients with a BMI >25 kg/m2, men were more overweight and women were more obese. Antihypertensive drugs and insulin were prescribed more often in women and lipid lowering drugs more often in men. No relevant absolute gender differences were found for HbA1c, neuropathy and diabetic retinopathy.

Conclusion: The quality of care for patients with T2DM has improved considerably in the period 1998-2013 in both sexes. In all years, absolute gender differences were observed for systolic blood pressure, cholesterol-HDL ratio, smoking and BMI. Relevant trends differences in the improvement of the quality of care between sexes were only observed for systolic blood pressure and smoking, with women reaching target values of blood pressure almost as much as men and with men smoking less every year while smoking in women has plateaued.

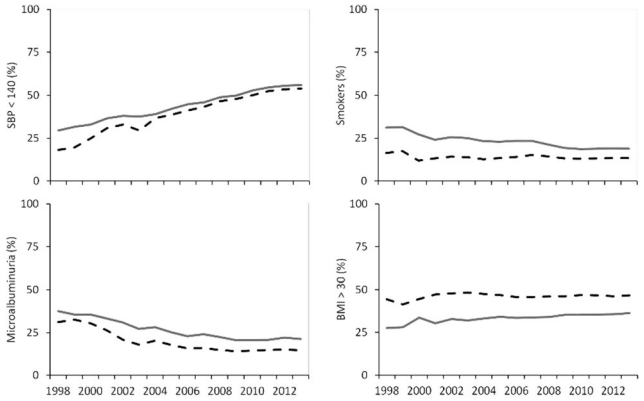

Figure 1: Trends in achieving target values stratified according to gender (Men: gray line, Women: black striped line). SPB: Systolic blood pressure; BMI: Body Mass Index.

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Treatment of patients with type 2 diabetes mellitus with HbA_{1c} levels above target; the follow up of the Diabetes Care System (DCS) cohort

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Background and aims: Because of the progressive nature of the type 2 diabetes (T2DM), many patients show a pattern of so-called 'serial failure' as a result of sequential addition of glucose lowering drugs including insulin as the final step. The determinants and the sustainability of good glycemic control following the initiation of insulin therapy have not been described. The aim of this study was to assess the patient characteristics and the treatment responses to the initiation of insulin therapy in a large cohort of T2DM patients.
Materials and methods: The study was performed in the population based diabetes cohort of the Diabetes Care System (DCS) responsible for the quality of T2DM care and uses a managed care plan for almost all T2DM patients in the region, in collaboration with contracted general practices (GP's). The managed care plan encompasses the care provided by patient's GP, according to the Dutch College of GPs' treatment guidelines for T2DM and a standardised annual assessment organised centrally since 1998 by the DCS in the Netherlands. Of a total of 9849 type 2 diabetes patients, 1203 T2DM patients initiated insulin. 'Serial failure', compared to 'good response', was defined as HbA1c levels of 53 mmol/mol or higher (7%) at more than 1/3 of the time points during the mean 5.6 year (SD 2.8) follow-up. Differences of the demographic characteristics, HbA1c, BMI, and diabetes duration at baseline between the 'serial failure' group and 'good response' group were tested with ANOVA and post hoc Bonferroni tests for mean levels, with Chi-square tests for proportions and Kruskal - Wallis test for median levels.
Results: Of the 1203 patients initiating insulin therapy 294 patients (24.4%) showed a 'good response', whereas 909 patients (75.6%) showed a 'serial failure' response with an equal follow-up of 5.5 year. Patients in the 'serial failure' group were significantly younger, and had higher HbA1c and glucose levels at the initiation of insulin. More patients in the 'serial failure' group were using a combination of metformin and sulfonylureas (SU) (table 1). There was no difference in total mortality between serial failure (14.0%) and good response (16.0%).
Conclusion: Less than a quarter of the patients showed sustained glucose control after the start of insulin treatment. Those with 'good response' were characterised by lower HbA1c level and higher age at the start of insulin therapy. The 'good responders' may represent a subgroup of patients with a less progressive disease. Alternatively, it may be speculated that initiating insulin earlier, increases the likelihood of achieving and sustaining glycemic control by preserving beta-cell function.

Table 1. Baseline characteristics of the 'good response' and 'serial failure' patients.

	Good response	Serial failure
N (%)	294 (24.4)	909 (75.6)
Baseline characteristics		
Age (yr)	67.3 ± 9.9*	64.8 ± 10.1*
Male (%)	52.4	50.8
Diabetes duration (yr)	8.2 (4.8 – 12.9)	8.4 (5.3– 12.4)
HbA1c (mmol/mol)	50 ± 0.8*	63 ± 1.2*
BMI (kg/ m²)	31.0 ± 6.2	30.8 ± 5.6
Fasting glucose (mmol/L)	7.5 ± 2.1*	8.8 ± 2.7*
Metformin use only (%)	22.8	21.7
SU use only (%)	15.3	14.3
Metformin + SU use (%)	40.5*	46.6*
Other combination use (%)	6.1*	3.5*
Total mortality (%)	16.0	14.0
Follow-up duration (yr)	5.4	5.5

Data represent mean ± standard deviation, proportions, or median (interquartile range). Between-cluster differences were tested with ANOVA and post hoc Bonferroni for mean levels, with χ^2 tests for proportions and Kruskal-Wallis test for median levels. Abbreviation: BMI: body mass index, SBP: systolic blood pressure, SU: sulfonylureas. * statistically significantly ($p < 0.05$) different.

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Identification of key success factors in type 2 diabetes care in Sweden

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Background and aims: In Sweden, the National Diabetes Register (NDR) has shown differences in treatment outcomes for diabetes patients in primary care, both between and within the 21 county counties (CCs). This study of the structure and provision of diabetes care was conducted to identify factors associated with high performance in type 2 diabetes (T2D) primary care, based on glycemic (HbA1c) and blood pressure control, and use of lipid-lowering medication.
Materials and methods: 4 CCs with high performance, and 4 with low performance, as well as 4 high performing and 6 low performing primary care centers (PCCs) were selected based on 2011 NDR outcome data. Differences in the provision of T2D care were explored using a combination of quantitative and qualitative analysis. Semi-structured in-depth interviews were conducted with 66 selected individuals with professional key roles in diabetes care, either as PCC managers, members of the commissioning organisation or political leadership, including 22 in-depth interviews with PCC clinical staff. In addition, steering documents, guidelines, staffing levels and diabetic medication use were analysed.
Results: Comparing high and low performing CCs no systematic differences were identified in tender documents or reimbursement models. Different reimbursement models and care requirements were used, but no apparent pattern distinguishing high and low performing CCs could be identified. The number of listed patients per full-time specialist ranged from 1955 to 2275 (median=2048) and 1755 to 2181 (median=1840), in high and low performing CCs respectively. No clear differences were seen regarding insulin use. The average number of defined daily doses of insulin per diabetes patient ranged from 212 to 281 (median=227) and 209 to 244 (median=219), and the proportion of the total number of diabetes patients using insulin ranged from 45 to 53% (median=47%) and 45 to 51% (median=46%) in high and low performing CCs respectively. The interview analysis did however show large differences in the structure and provision of diabetes care between high and low performing CCs and PCCs. Systematic differences, where CCs and PCCs with better outcomes stand out, were described in terms of seven success factors relating to PCC design and delivery of healthcare (1. Focus on quickly achieving target values for each patient; 2. Systematic targeted efforts to help patients with suboptimal outcomes; 3 Own results always on the agenda of both clinical staff and management), CC leadership and

management of care (4. Easily accessible evidence-based guidelines, and expectation of adherence to recommendations; 5. Follow-up and feedback on results; 6. Continuous improvement efforts in diabetes care), and the culture that permeates the organisation as a whole (7. Ownership of results and focus on secondary prevention).
Conclusion: Comparison of high and low performers, based on national register data from Sweden, yielded insight into key success factors in T2D care. The factors can serve as a basis for discussion and improvement initiatives striving toward evidence-based and equal care.
Supported by: Swedish Association of Local Authorities and Regions

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Real-life practice glycaemic control in patients with latent autoimmune diabetes of the adult and type 2 diabetes
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Background and aims: The objective of our study was to compare the glycemic control before and after initiation of insulin therapy between type 2 diabetes patients and patients with latent autoimmune diabetes in adults (LADA).
Materials and methods: We restrospectively assessed previous glycemic control in a case-control study on cardiovascular risk in 189 patients with type 2 diabetes and 66 patients with LADA. All the HbA1c determinations from primary and specialized care during the period 2004-2014 and time of insulin initiation were recorded. Differences in raw Area Under the Curve (AUC) of HbA1c and HbA1c-weighted by the follow-up time were assessed with a Mann-Whitney test. All analyses were obtained using R, setting a threshold for significance at 5% (α=0.05)
Results: Patients with type 2 diabetes and LADA were similar in age (58.4±10.1 vs. 58.3±12.1 yr), gender distribution (55% vs. 48% male participants), prevalence of retinopathy (19% vs.18%) and albumin-to-creatinine ratio (14.5±32.9 vs. 19.7±71.6 mg/g) respectively. Type 2 diabetic patients exhibited higher body mass index (31.2±5 vs. 27.5± 5.6 Kg/m2) and waist circumference (104.5±11.7 vs. 95.5±14.9 cm), and shorter diabetes duration (8.8±7.9 vs. 12±8.8 years), respectively (P≤ 0.01 for all comparisons). Frequency of insulin treatment was 19% in type 2 diabetes and 86% in LADA (P <0.001). Mean day-weighted HbA1c AUC was higher in LADA than in patients with type 2 diabetes (7.9%±1 vs 7.3%±1, P &lt 0.001). Patients with type 2 diabetes were exposed for a longer period to values of HbA1c &gt 7.5% than LADA patients before insulin iniciation (527.9±699.6 vs 197.7±361, P &lt 0.001). However, LADA patients were exposed to HbA1c &gt 7.5% for an overall longer period than type 2 diabetic patients (1232±834 vs 735±821 days, days, p <0.001)
Conclusion: Despite earlier initiation of insulin therapy for poor glycemic in patients with LADA,they were exposed to higher burden of hyperglycemia than type 2 diabetes patients.

PS 077 Burden of diabetes: economic and otherwise

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Humanistic and economic outcomes of people with type 1 diabetes in Europe with various degrees of complications
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Background and aims: Following the limited research on Type 1 diabetes (T1DM) regarding burden of the disease, aim of this study was to estimate the impact of complications on health-related quality of life, work productivity, and healthcare resource use.
Materials and methods: The 5EU NHWS is a proprietary survey of adults (≥18 years) where respondents complete a suite of demographic, clinical and healthcare utilization questions, as well as standardized PROs to assess health-related quality of life (SF-36) and work impairment (WPAI) approximately every 18 months. Data from the SF-36, WPAI and healthcare resource use items from the 2013 survey were evaluated for patients indicating a diagnosis of T1DM. Within this sample, data was compared between those with no diabetes-related micro or macrovascular complications, 1 complication or 2 or more complications using one-way ANOVAs with a significance level of $p<0.05$.
Results: The T1DM sample comprised 402 people. Mean time since T1DM diagnosis was 21.7 years. 67.2% of patients had no complications, 18.9% had one complication, and 13.9% had 2 or more complications. Groups were similar in terms of age, gender, marital status, working status, education, smoking status and income. Significant differences were observed in BMI, such that the proportion of those who were obese was higher in the ≥2 complications group relative to the other two groups, 19.6% vs. 5.6% and 5.3%, respectively. This group was also less likely to report that they had exercised in the past month, 42.9% vs. 65.6% and 61.8%. In general, as the number of complications increased (0, 1, ≥2) overall health-related quality of life and health utility scores, as reported by the SF-36 decreased; see Table. On the WPAI, there were significant differences such that those with 1 complication and those with ≥2 complications were significantly more likely to report impairment in the form of Presenteeism, Work Productivity, and Activity Impairment than those with no complications. Those with 1 complication did not differ significantly from those with ≥2 complications on all work productivity and activity impairment measures; see Table. Patients with ≥2 complications reported significantly more ER Visits, number of hospitalizations, and visits to healthcare providers than those patients with no complications. Those with 1 complication did not differ significantly from either those with no complications or those with ≥2 complications.
Conclusion: Diabetes-related complications can have a significant humanistic burden on patients with T1DM, and a significant economic impact on the healthcare system. Patients should be educated beyond the incidence of complications to understand the humanistic and economic burden.

	Type 1 Diabetes by Complications					
	None (n = 270)		1 (n = 76)		≥2 (n = 56)	
Health-related Quality of Life (mean, sd)						
Mental Component Summary	44.57_a	11.28	$42.93_{a,b}$	9.79	40.51_b	12.10
Physical Component Summary	48.47_a	8.91	43.95_b	8.93	39.22_c	10.13
Health Utilities	$.69_a$	.14	$.63_b$	.12	$.60_b$	.14
Work Productivity and Activity Impairment(mean, sd)*						
Absenteeism	6.10_a	18.51	6.58_a	11.62	15.28_a	22.34
Presenteeism	21.51_a	25.60	38.60_b	27.31	44.40_b	30.83
Work Productivity Loss	24.71_a	29.34	41.53_b	28.20	51.08_b	32.72
Activity Impairment	30.56_a	29.05	43.82_b	29.26	53.75_b	33.33
Healthcare Resource Use (mean, sd)						
ER Visits (number in last 6 months)	$.29_a$	.79	$.42_{a,b}$	.93	$.73_b$	1.52
Hospitalizations (number in last 6 months)	$.24_a$	1.07	$.25_{a,b}$	.52	$.66_b$	1.64
HCP Visits (number in last 6 months)	6.28_a	7.45	7.96_a	7.65	15.20_b	14.87

Note: P value represents significance of overall ANOVA. Subscripts represent pairwise comparisons. Values in the same row not sharing the same subscript (e.g., "a" and "b") are significantly different from each other at p< .05. Those that are not (e.g., "a" and "a", "b" and "b") are not significantly different from each other at p<.05. Cells with no subscript are not included in the test. Tests assume equal variances.[1]

1. Tests are adjusted for all pairwise comparisons within a row of each innermost subtable using the Bonferroni correction.

**Absenteeism, presenteeism, and work productivity loss were only assessed for those respondents who were employed at the time of the survey.*

Supported by: AstraZeneca

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The humanistic and economic burden of type 1 diabetes in Europe

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Background and aims: Following the limited research on Type 1 diabetes mellitus (T1DM) regarding burden of the disease, aim of this study was to estimate the humanistic and economic burden of T1DM regarding health-related quality of life, work productivity, and healthcare resource use.

Materials and methods: The 5EU NHWS is a proprietary survey of adults (≥18 years) where respondents complete a suite of demographic, clinical and healthcare utilization questions, as well as standardized PROs to assess health-related quality of life (SF-36) and work impairment (WPAI) approximately every 18 months. Data from the SF-36, WPAI and healthcare resource use items from the 2013 survey were compared between patients indicating a diagnosis of Type 1 diabetes and those who indicated no diabetes diagnosis (thereby excluding type 2 diabetes from the comparator group) via one-way ANOVAs with a significance level of $p<0.05$.

Results: The sample comprised 402 patients with T1DM and 57,912 people without diabetes (non-diabetes). Mean time since T1DM diagnosis was 21.7 years. Two thirds of the T1DM respondents had no diabetes-related micro or macro-vascular complications. T1DM respondents reported lower income, lower BMI scores, and higher Charlson comorbidity index scores relative to non-diabetes respondents. With regard to demographics, a higher proportion of T1DM patients were male and a higher proportion were current smokers when compared with non-diabetes respondents. Age, marital status, working status, education and exercise frequency was similar between the cohorts. 83.1% of T1DM respondents reported experiencing hypoglycaemia; 62.7% in the past month. T1DM respondents reported significantly worse health-related quality of life, with lower SF-36 scores on all domains as well as mental component and physical component scores compared to non-diabetes respondents; see Table. The utility values were also lower for the T1DM patients and they reported significantly more Presenteeism (27.94 vs. 16.35, p<.001), work productivity loss (30.91 vs. 19.74, p <.001), and activity impairment (36.29 vs. 23.95, p<.001) on the WPAI compared to non-diabetes respondents, but no more absenteeism (7.22 vs. 5.45, p .154). Furthermore, T1DM patients reported significantly more healthcare resource use than non-diabetes respondents: ER Visits 0.38 vs. 0.19 (p<.001); Days of hospitalization 0.30 vs. 0.12 (p .001); HCP visits 7.86 vs. 4.43 (p<.001).

Conclusion: There is a significant humanistic and economic burden of T1DM. Treatment strategies for T1DM should seek to balance the clinical, humanistic and economic outcomes to achieve the greatest value to the healthcare system and to individual patients.

SF-36 (norm based scores)	Non-Diabetic		T1DM		
	M	SD	M	SD	*p*
Bodily Pain	49.63	10.27	46.35	11.03	< .001
General Health	49.34	9.58	40.92	10.20	< .001
Mental Health	45.96	10.39	43.66	11.31	< .001
Physical Functioning	51.68	8.41	47.88	10.11	< .001
Role Emotional	47.88	10.57	44.33	12.03	< .001
Role Physical	49.74	9.21	44.98	10.79	< .001
Social Functioning	48.13	9.85	44.64	11.14	< .001
Vitality	49.46	9.32	46.05	9.71	< .001
Physical Component Summary	51.63	8.70	46.33	9.67	< .001
Mental Component Summary	46.18	10.60	43.70	11.20	< .001
Health Utilities	.72	.13	.67	.14	< .001

Supported by: AstraZeneca

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Post-prandial hyperglycaemic episodes (PPH): impact on healthcare resource use among people with type 1 and type 2 diabetes in the US, UK and Germany

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Background and aims: Post-prandial hyperglycaemic episodes (PPH) among people with diabetes are a well-known clinical challenge to diabetes management. While the impact of post-prandial hypoglycaemia on healthcare resource use has been well studied, little is known about the impacts of PPH episodes on healthcare resource use. The purpose of this study was to assess the impact of respondent-reported PPH on healthcare resource use among people with type 1 (T1DM) and type 2 (T2DM) diabetes treated with meal-time bolus insulin.

Materials and methods: Data were collected in a web-survey of 906 adults diagnosed with T1DM (39%) and T2DM (61%) and treated with self-administered bolus insulin therapy, but not using pre-mixed insulin or GLP-1 analogues (with or without OADs), in the US (40%), UK (26%), and Germany (34%). Respondent experience of PPH was self-reported.

Results: Analyses demonstrated that inadequate post-prandial glucose control was prevalent among people with diabetes. Sixty-six percent of respondents reported difficulty getting blood glucose (BG) stable after eating during the past week. Thirty-six percent of respondents indicated that they experienced post-prandial hypoglycaemia in the past week, and 62% reported experiencing PPH in the past week. A total of 11% of respondents experienced post-prandial hypoglycaemia 3 or more times in the previous week, and 30% experienced PPH episodes 3 or more times in the previous week. Further, respondents who experienced PPH episodes in the past week called or emailed their physicians or other healthcare professionals about their diabetes an average of 2.7 times in the previous year compared to an average of 1.4 times for those who did not experience PPH episodes in the previous week (p<.001). Respondents who experienced PPH in the past week made an average of 5.5 diabetes-related visits to their physicians or healthcare professionals in the past year, compared to an average of 4.4 visits for those who did not experience PPH episodes (p<.001). The associations between PPH and contact with healthcare professionals were significant among those with T2DM, but not for those with T1DM. Of those who experienced PPH episodes during the past week, 72% reported at least one medical complication related to diabetes, compared to 55% of those who did not experience PPH episodes (p<.001). More specifically, compared to those

without PPH in the past week, respondents who experienced PPH in the previous week were significantly more likely to report being diagnosed with high blood pressure (41% vs. 29%, p<.001), eye problems (33% vs. 21%, p<.001), and nerve damage (neuropathy) (28% vs. 21%, p<.05) related to their diabetes. Respondents who experienced PPH episodes during the previous week reported measuring their BG 1.9 additional times on days they experienced symptoms of PPH compared to their usual BG measurement on an average day.

Conclusion: These results indicate that PPH episodes among adults with diabetes are common and are significantly associated with greater use of healthcare resources, including increased communication and contact with physicians and other healthcare professionals and greater incidence of one or more medical complications related to diabetes, as well as more frequent BG measurement. The additional use of healthcare resources associated with PPH may increase the costs of diabetes management for both T1DM and T2DM.

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Economic impact and associations of recurrent diabetic ketoacidosis in type 1 diabetes

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Background and aims: For a small, but high risk sub-population of type 1 diabetes (T1D) patients, self-management is extremely difficult and characterised by recurrent admissions with diabetic ketoacidosis (rDKA). Economic studies demonstrate T1D patients experiencing DKA incur 1.4-2.2 times the cost of a non-DKA T1D patient, with further increases with rDKA. There are currently no UK studies that have analysed the healthcare cost burden of patients with rDKA admissions. To understand the reasons for rDKA and its economic impact we have reviewed data on all individuals with rDKA admissions over 8-years at two inner-city teaching hospitals.

Materials and methods: Retrospective analysis of combined biochemical (HbA1c), demographic (age, sex, ethnicity and socio-economic status) and health resource utilisation data (outpatient and inpatient activity) collected between 2006-2014 for T1D patients with rDKA in a defined geographical catchment area of London, UK. rDKA patients were identified by ≥3 DKA admissions and compared with the local T1D population. Costs were calculated using the UK National Schedule of Reference costs (2010-2011) and NHS best practice tariffs for DKA episodes post April 2013.

Results: 68 patients, mean age 27.8 (±12.5) years, had a total of 407 DKA admissions (mean 6.0 admissions/patient, range 3-24) with total bed day occupancy for DKA of 1,373 days (mean 3.4 days/admission). rDKA admission tended to 'cluster' with 43.2% occurring ≤3 months from the previous episode. The total cost of DKA admissions was £360,093 [€493,829] (£5,295/patient [€7,262]). Additionally, other diabetes-related and non-diabetes related inpatient visits cost £227,640 [€312,184] and £171,581 [€235,305] respectively for this group. These patients were also high users of diabetes outpatient services (mean 5.4 diabetes clinic appointments/year) but were poor attenders, with 34.8% of appointments being missed. Total annualised cost of 'routine' diabetes care was £317,565 [€435,507] (£584/patient/year [€801]). Use of non-diabetes appointments was too high totaling £264,595 [€362,864]. Compared to the local T1D cohort, rDKA was associated with greater socio-economic deprivation (mean index of multiple deprivation score 32.9± 10.5 vs. 29.5±9.8; p=0.01) and ethnic minority status (62% vs. 27% non-white ethnicity, p<0.01). For those with complete medical history data (n=37) glycaemic control was consistently poor, with mean HbA1c 101 (±30.6) mmol/mol and 92 (±20.7) mmol/mol (p=0.10) at the start and end of the 8-year period. Only 16.7% had attended structured diabetes education, 88.9% had noted issues with compliance and 30.6% had a clinical diagnosis of depression.

Conclusion: These data demonstrate that rDKA patients incur extremely high healthcare costs. DKA episodes have a tendency to 'cluster' but occur on a background of long-term non-compliance and outpatient non-attendance. This behaviour suggests a long-term relational psychological difficulty and would fit with acute admissions related to affect regulation. Therefore, specific psychological and social interventions capable of even a modest decrease in DKA frequency would have a significant economic impact.

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Risk factor and economic burden of hypoglycaemia in elderly patients with type 2 diabetes mellitus initiating Basal Insulin (BI) in a US medicare advantage population

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Background and aims: Insulin treatment is an essential tool in diabetes management and hypoglycaemic (HG) remains a barrier for optimal glycaemic control. Older patients are at increased risk of experiencing severe HG events compared with other age groups with Type 2 diabetes. We present a retrospective US health insurance claims database study to assess risk factors for HG and its cost burden in elderly patients with T2DM who initiated BI on top of OADs/GLP-1 s (2007-2013).

Materials and methods: The Clinformatics® Data Mart Medicare Advantage database was used to identify patients with T2DM who initiated Basal insulin (BI) between January 2007 and December 2013. All patients had a minimum of one year continuous medical and pharmacy coverage prior to (baseline period) and after (follow-up period) BI initialization. Logistic regression was used to identify risk factors of HG occurred during 1-year follow-up, adjusting baseline patient characteristics that include age, gender, comorbidities, prescriptions, HG and healthcare utilization. The economic burden of HG was evaluated accounting for baseline patient characteristics with a generalized linear model assuming gamma distribution with log link for healthcare costs to accommodate its skewed distribution.

Results: There were 31,035 patients included in the study (mean age: 72 years). Of these patients, during the one year follow up period, 3,066 (9.9%) patients experienced one or more episodes of medically attended HG (HG group) and 27,969 patients did not experience HG (non-HG group). At baseline, the HG group were more likely to have general comorbid conditions (Charlson Comorbidity Index excluding diabetes 2.6 *vs* 1.7, p<0.001) and incurred higher total annual healthcare costs ($54,057 *vs* $30,249, p<0.001) than the non HG group. The HG group were also more strongly associated with baseline macrovascular (OR= 1.23, 95%CI: 1.12-1.34) and microvascular (OR=1.50, 95%CI: 1.38-1.63) complications, mental illness (OR=1.24, 95%CI: 1.15-1.35) and foot disease (OR=2.32, 95%CI: 2.08-2.59). In the one year follow up period, percentage of patients with such conditions increased from baseline by 8.0-9.3% for HG group and was lower or stable in the non-HG group. After BI initiation, average annual healthcare costs slightly fell, from $32,601 at baseline to $32,460. However, there was a 8.3% decrease in non-HG while 39% increase in HG group. After adjusting for baseline characteristics, the HG group was associated with substantially higher annual healthcare expenditure ($53,375 vs $25,881) and diabetes-related costs ($19,938 vs $8539) compared with non-HG group, mainly driven by inpatient and outpatient services. The HG group also incurred $5337 HG-related annual costs.

Conclusion: Elderly T2DM patients with comorbidities are at increased risk of HG after BI initiation and patients experiencing HG are associated with substantially higher healthcare utilization and costs, highlighting the need for a BI with a reduced risk of HG that can further improve glycaemic control.
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Investigating sickness absence as a pathway behind labor market consequences of childhood onset type 1 diabetes mellitus

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Background and aims: Earlier studies from our research group show that childhood onset of type 1 diabetes mellitus (T1DM) negatively impacts employment and earnings from labor market entrance and onwards. Less is however known to what extent these differences are directly related to indicators of adult health and what is explained by other factors. This study analyzed sickness absence as a mediator explaining the negative impact on employment and earnings in adults with childhood onset of T1DM.
Materials and methods: We selected all individuals born 1962-1977 (n=3,507) with onset of T1DM before age 15 and registered in the national Swedish Childhood Diabetes Register (SCDR) which covers incident cases of T1DM since 1977. The impact of T1DM was investigated using a matched case-control design where Statistics Sweden identified controls matched for year of birth and residency (n=14,029). The SCDR has been linked to national health and labour market registers and we used information on sickness absence including sickness benefits (shorter duration) and sickness compensation (long duration or permanent; full or part time) as indicators of health in ages 30-33 and 40-43 years old, respectively. First, we investigated the absolute and relative risks of sickness absence in people with T1DM compared to controls. Secondly, we added the sickness absence as a control variable in regression analyses of employment and earnings to explore the robustness of the basic T1DM effect shown in previous work. The analyses used logistic and panel data regression methods, controlling for calendar year, demographic and socioeconomic background.
Results: Men and women with childhood onset of T1DM were more likely to have been on sickness benefits at least once in ages 30-33 years (men 31% vs 19% (OR 1.94, 1.71-2.19); women 64% vs 46% (OR 2.09, 95% CI 1.85-2.35)) and in ages 40-43 years (men 33% vs 22% (OR 1.80, 1.30-2.50); women 53% vs 33% (OR 2.40, 1.71-3.36)). Additionally, the length of the sickness period was longer, statistically significantly for women with T1DM (men 14 days more (p=0.209); women 21 days more (p=0.007) in age 33). The likelihood of long-term/permanent sickness compensation was also higher (≤ age 33; men 5% vs 3% (OR 1.98, 1.49-2.64), women 10% vs 4% (OR 2.84, 2.27-3.55) and≤age 43; men 12% vs 5% (OR 2.64, 1.53-4.56), women 28% vs 8% (OR 4.20, 2.71-6.50)). In ages 30-33 years, T1DM had a negative effect on employment (men OR 0.63, 0.51-0.77; women OR 0.56, 0.44-0.70) and earnings if employed (men -9.4%, p<0.001; women -7.3%, p=0.004) and remained or slightly increased in age 40-43 years. Adding sickness absence to the regression analyses slightly decreased the estimated effect of T1DM on employment and earnings but T1DM in itself remained negative and significant.
Conclusion: While childhood onset of T1DM significantly increased the risk of sickness absence early in adulthood, particularly among women, it accounted for only a small part of the diabetes related difference in employment and earnings we have shown earlier.
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Type 1 diabetes with early onset and educational field at upper secondary and university level: Is own experience an asset for a health care carrier?

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Background and aims: Earlier work from our group on long-term consequences of type 1 diabetes with early onset has shown small but significant negative impact on school grades, final educational level and earnings. Less is known about other aspects of consequences, such as choices of educational field. We analyzed the probability of having a health-related education at upper secondary and university level, comparing people with type 1 diabetes onset before age 15 and matched population controls.
Materials and methods: From the Swedish Childhood Diabetes Register we retrieved all incident cases of childhood-onset type 1-diabetes from 1977-1990 for people born 1962-1975 and their four population controls matched for year of birth and municipality of residence at the time of diabetes onset. The main outcome variable, health-oriented education was defined using the SUN-classification in the Swedish National Educational Register for vocational and theoretical programmes at the upper secondary level and at university level using the last available registration up to year 2010. These educations thus ranged from nursing assistant exam from the vocational programmes at upper secondary level to physician exam at the university level. The hypothesis of no systematic differences between people with diabetes and population controls was tested using regression analyses (multinomial and standard logit regression) for women and men separately, and controlling for demographic and socioeconomic background obtained from the Longitudinal Integration Database for Health Insurance and Labour Market Studies (LISA) at Statistics Sweden.
Results: From the selected years, 2,756 people had onset of diabetes in ages 2-15 years (mean age at diagnosis 10.9 (SD 2.9); 53% boys) and there were 11,020 population controls (51% boys). The majority of the included subjects completed upper secondary school (girls/ boys: 90.5/ 88.1%, with no significant differences between people with diabetes and population controls. Women with type 1 diabetes were more likely to complete health-oriented education at the upper secondary level (+5.3%, p<0.01 vocational health; +1.4%, p=0.08 theoretical health) compared to vocational non-health programme (reference category), but less likely to complete a theoretical non-health oriented programme (-6.4%, p<0.01). Men were more likely to complete a vocational health programme (+3.2%, p<0.01) but less likely to complete both types of theoretical programmes (health -0.7%, p=0.01; non-health -4.1%, p<0.01). People with type 1 diabetes were less likely to have a university exam (women 40% vs 46%; -6.4% p<0.01; men 30% vs 35%;-5.7% p<0.01), but among people with a university exam, women were more likely to have a health orientated exam (+7.4%, p<0.01), while no significant difference was found for men. The effect was consistent at all levels for women with diabetes, adding to the population based differences between men and women as regards field of education. The significant pro-health orientation was robust in sub-group analysis and remained when controlling for school grades at previous level.
Conclusion: We rejected the hypothesis of no systematic differences in choice of educational field between people with type 1 diabetes onset up to age 15 and population controls. People with diabetes were more likely to complete health-oriented educational programmes, both at upper secondary and university levels.
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Education level, occupational status and quality of life in adults with type 1 diabetes and in the general population: a comparative study

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Background and aims: Type 1 diabetes is a complex disease, demanding a high degree of self-care throughout life. Few studies have examined how adults with type 1 diabetes differ from the general popula-tion regarding education level, occupational status and self-rated quality of life. Research in this field has shown inconsistent results and is characterized by small samples and poor clas-sification of type 1 diabetes. Furthermore most studies are published before 2005. The aim of this study is to compare adults with type 1 diabetes and the general population in terms of education level, occupational status and self-rated quality of life.
Materials and methods: This study compared 2.415 adults with type 1 diabetes and 48.511 adults from the general population. Data were collected in 2010 and 2011 from two cross-sectional surveys of Danish adults living in the capital region. One survey from a specialist diabetes clinic targeting type 1 diabetes patients and one from the capital region of Denmark representing the general popu-lation. Validated questions measured education level, occupational status, working hours, sick leave per year and self-rated quality of life. Self-rated quality of life was assessed with eight domains of health scored with SF-12. Data were sex- and age standardized. Mean difference and risk difference (RD) were calculat-ed as outcome measures. For each outcome we used stepwise addition of covariates in a mul-tivariable linear regression model to identify potential confounding (partner, children living at home, education, occupational status and self-rated quality of life). Further, we analysed the difference between education level, occupational status and self-rated quality of life in subgroups by age and sex. A sensitivity analysis excluding individuals participating in both surveys from the general population was conducted.
Results: Compared to the general population, a larger proportion of the diabetes population were male (52% vs. 44%) and between 30-59 years old (58% vs. 52%). After taking age and sex into account, adults with type 1 diabetes, on average, reported a higher education level (RD between 0.2 - 1.6, $p<.0001$), lower level of employment (RD=7.69, $p<.0001$), more sick leave per year (mean difference=0.71 days, $P=0.0027$) and lower scores on all domains of self-rated quality of life (RD between 3 - 8, $p<.0001$) compared to the gen-eral population. No difference was found in working hours per week (mean difference=0.06 hours, $p=0.6$). Yet, after adjusting for education, the results suggested that adults with type 1 diabetes worked fewer hours a week (0.28 hours, P=0,0119) compared to the general population. Covariates explained only a small proportion of the overall difference. Results remained the same in the sensitivity analysis.
Conclusion: Our findings indicate differences between adults with type 1 diabetes and the general popula-tion in relation to education level, occupational status and self-rated quality of life. Further research into the mechanisms is needed in order to develop interventions supporting adults with type 1 diabetes.

PS 078 Double trouble: depression and diabetes

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A trial to decrease diabetes distress in diabetic persons with depression

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Background and aims: Screening for depression is recommended annually. We set up a programme to screen for diabetesdistress (DD), depressive symptoms (Depr), and hypoglycaemia fear burden (Hypo). If Depr was detected in combination with DD, DD was discussed with the patient in 2 structured meetings in order to reduce the burden and influence both DD and Depr.
Materials and methods: DD was measured bij PAID-5; cutoff value 8 for distress; Depr was measured by Extended Kessler -10 (EK-10) ; cutoff value 20; Hypo was determined by both dutch 33-item Hypoglycaemia Fear Survey (HFS-AHV) and 3-item questionnaire (3I-S); non-parametric statistics (Wilcoxon, spearman correlation and chi square).
Results: Out of 810 patients screened 73 (9%) had both Depr and DD. 61 patients were invited for structured discussing; 20 completed the intervention, 30 started the intervention but failed to complete. No show was 50% in the total group. In Completers PAID-5 score decreased from 11,2(SD 2,5) to 8,9(3,8)($p<0.05$) and EK-10 score decreased from 24,2(8,6) to 20,8 (6,9)($p<0.05$). In a subset of 410 insulin treated patients DD was present in 52% of those with Hypo (n=95) and in 9% without Hypo ($P<0.001$). Also, HbA1c was higher (71 mmol/mol) in those with Hypo than without (62 mmol/mol; $P<0.001$). HFS-AHV score correlated with 3I-S ($rs=0,65$, $p<0.001$); Sensitivity of 3I-S in detecting fear of hypoglycaemia was 90% and specificity was 98%. PPV was 57% and NPV was 95%
Conclusion: In patients with Depression and DiabetesDistress a structured intervention aimed at reducing DiabetesDistress reduces both. Hypoglycaemia fear burden is an important determinant of diabetes distress and can be measured by a 3- item questionnaire. Longer duration follow up is warranted.
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Association of anxiety, stress and depression with demographic, anthropometric, socioeconomic glycaemic and cardiometabolic risk factors in patients with type 2 diabetes

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Background and aims: Depression is a serious mental disease quite prevalent. Surveys have shown a strong association between depression, anxiety and stress with diabetes mellitus. These disorders can negatively affect disease control. The purpose of this study is to explore possible correlations between demographic, anthropometric, socioeconomic and cardiometabolic parameters with the presence and severity of depression, anxiety and stress in people with type 2 diabetes.
Materials and methods: 391 patients (193 males, aged 64.9±10.3 years) with type 2 diabetes were included in the study. Information regarding demographic characteristics, socioeconomic status, anthropometric and metabolic parameters, glycemic control, type of antidiabetic treatment

and the presence of complications of diabetes were collected from all patients. The questionnaire DASS-21 is widely used and it is a set of three self-report scales designed to measure the emotional states of depression, anxiety and stress. It consists of 21 questions each answer being scored on a scale value of 0 to 3. The higher total scores indicate more severe symptoms. Statistical analysis was performed using STATA 9.0 software.

Results: Severity of stress showed a statistically significant association with the following parameters: sex(p=0.020), age(p=0.002), marital status(p=0.045) and insulin treatment(p=0.032), regarding anxiety: sex(p=0.002), age(p=0.022) and insulin treatment (p<0.001) while depression was associated with: sex(p=0.004), age(p=0.007), educational level(p=0.043), marital status(p=0.004), BMI (p=0.012) and insulin treatment (p=0.01). On multivariate analysis, insulin treatment showed significant positive association with stress and anxiety (Coefficient=4.56, 95%CI:0.61-8.52, p=0.024 and Coefficient=6.46, 95%CI:3.02-9.9, p< 0.001, respectively), while BMI showed significant positive association with the severity of depression (Coefficient=0.27, 95%CI:0.03-0.51, p= 0.028). Moreover, further analysis regarding insulin treatment revealed that when the number of insulin injections per day was increased from two injections to three or four per day, a strong association with the severity of stress was observed (p=0.007 and p=0.019, respectively).

Conclusion: Obesity appears to act adversely to the presence and severity of depression, giving further emphasis on the importance of adopting a healthy lifestyle of living and weight loss in people with type 2 diabetes. Insulin treatment (especially when more than two injections per day are performed) is accompanied by increased stress and anxiety levels in people with type 2 diabetes.

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Glycaemic control and diabetes mellitus duration are associated with geriatric depressive scale in elderly male people with type 2 diabetes mellitus

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Background and aims: The prevalence of depression and Type 2 Diabetes Mellitus (T2DM) are increasing in the elderly population of Korea and these are known to be related with each other. We evaluated the relationship between T2DM related factors and the degree of depression using the Korean Geriatric Depressive Scale (K-GDS) in elderly Type 2 DM subjects.

Materials and methods: This study was based on data from seven hospitals in Korea. To assess the status of depressive symptoms, the K-GDS was used. High GDS meant high probability of depression. DM related factors were the duration of T2DM, HbA1c, and the existence of T2DM complications.

Results: A total of 154 subjects (55 male and 99 female) with a mean age of 71.3 years, and a mean HbA1c of 7.6% were included. In males, the well-controlled (HbA1c<7%) T2DM group showed low GDS compared to the less-controlled T2DM group (1.94±2.59 vs. 4.54±3.57, p=0.016). The mean GDS of well/less controlled T2DM groups did not show any significant difference in females. The mean GDS was higher in the group with a longer duration (10 years and more) of T2DM in both gender groups, but this trend showed significance only in the male group. Compared to the subjects with no complications or micro-vascular complications, the subjects with micro- and macro-vascular complications showed high GDS in males (p=0.029). In a multiple linear regression analysis, DM duration and HbA1c were independently associated with GDS in the male group.

Conclusion: Greater depression was associated with poorer glycemic control and longer diabetic duration in elderly male subjects with T2DM.

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The temporal relationship between diagnosis of diabetes and depression

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Background and aims: The purpose of this study was to investigate the temporal relationship between diagnosis with diabetes (Source: Danish National Diabetes Register) and first purchase of prescribed antidepressants (Source: Register Medicinal Product Statistics) in a working-age population.

Materials and methods: We included all Danish adults aged 18-59 who were diagnosed with diabetes in the study period (01/01/96 - 31/12/10) We ceased inclusion after 31/12/05 in order to ensure all subjects could be followed for five years after diagnosis. We excluded individuals who purchased antidepressants at any time within, and up to, a year prior to their inclusion in the data-set. Subjects were stratified according to the highest occupational category they attained in study period. For those who purchased antidepressants in the study period, we calculated the time elapsed from their diabetes diagnosis to the point at which they first purchased antidepressants. We calculated the percentage of people with diabetes who purchased antidepressants within one and five years post-diagnosis. Furthermore, we calculated the proportion who purchased antidepressants within one year post-diagnosis among all those who purchased antidepressants within five years. P-values for between-group differences were calculated using two sided Fisher's exact tests.

Results: Of those who develop depression within five years, 25.3% do so in the first year. Thus, more develop depression within the first year than if the likelihood was the same in all five years of follow-up. Of the individuals who go on to purchase antidepressants within five years of diagnosis, a greater proportion from the higher occupational groups did so within the first year (e.g. Employee at highest level 29.7%: Employee at basic level 24.2%). In contrast, overall incidence after five years is significantly greater in the lower occupational groups (e.g. Employee at highest level 9.7%: Employee at basic level 11.9%). Likewise, men as a whole were more liable to purchase antidepressants within one year of their diagnosis (Men: 27.1%: Women: 23.5%), whereas the incidence among men after five years is significantly lower than that of women (Men: 9.7%: Women: 13.5%).

Conclusion: The data indicate that diagnosis of diabetes precipitates depression across the workforce. Of those who purchase antidepressants within five years of diagnosis, both men and individuals with higher occupational status are more likely to do so within the first year. With previous research indicating purchase of antidepressants is more prevalent among women and lower occupational groups in the general population, our results suggest that it is in the period immediately after diagnosis that people with diabetes least resemble the general population in terms of their propensity to purchase antidepressants, with the impact dissipating over time.

	Depression within 1 year, %	Depression within 5 years, %	Share with depression within 1 year, % (1)/(2)	Different from employee at highest level, p-value	N	N with depression within 1 year	N with depression within 5 years
(a) Both men and women							
All	2,9%	11,3%	25,3%		32.863	942	3.728
1: Employee at highest level	2,9%	9,7%	29,7%		6.207	178	599
2: Executive management	2,3%	8,0%	28,3%	0,831	1.587	36	127
3: Employee at medium level	2,7%	10,7%	25,6%	0,129	5.089	139	542
4: Employee at basic level	2,9%	11,9%	24,2%	0,009	14.879	429	1.771
5: Self-employed	3,3%	11,4%	28,8%	0,864	1.991	65	226
6: Other employee	3,0%	14,0%	21,5%	0,006	2.665	80	372
7: Unemployed	3,4%	20,4%	16,5%	0,008	445	15	91
(b) Men							
All	2,6%	9,7%	27,1%		18.622	490	1.807
1: Employee at highest level	2,7%	8,3%	32,0%		3.281	87	272
2: Executive management	2,4%	7,5%	31,9%	1,000	1.249	30	94
3: Employee at medium level	2,5%	8,7%	28,2%	0,376	2.529	62	220
4: Employee at basic level	2,5%	10,2%	24,8%	0,023	8.664	219	883
5: Self-employed	3,2%	10,8%	29,9%	0,673	1.552	50	167
6: Other employee	3,0%	12,1%	24,8%	0,149	1.266	38	153
7: Unemployed	4,9%	22,2%	22,2%	0,446	81	4	18
(c) Women							
All	3,2%	13,5%	23,5%		14.241	452	1.921
1: Employee at highest level	3,1%	11,2%	27,8%		2.926	91	327
2: Executive management	1,8%	9,8%	18,2%	0,305	338	6	33
3: Employee at medium level	3,0%	12,6%	23,9%	0,282	2.560	77	322
4: Employee at basic level	3,4%	14,3%	23,6%	0,135	6.215	210	888
5: Self-employed	3,4%	13,4%	25,4%	0,754	439	15	59
6: Other employee	3,0%	15,7%	19,2%	0,025	1.399	42	219
7: Unemployed	3,0%	20,1%	15,1%	0,026	364	11	73

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Depression and anxiety among young adults with type 1 diabetes

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Background and aims: Data on prevalence of depression and anxiety among young adults with type 1 diabetes are heterogeneous. Most studies report a high rate of comorbidity. In a representative sample of young adults with type 1 diabetes in Lower-Saxony (Germany) the prevalence of both mental disorders and the association with critical life events and quality of metabolic control were assessed.
Materials and methods: In 26 specialized diabetes out-patient units all patients (19-30 yrs.) diagnosed with type 1 diabetes in childhood or adolescence were invited to complete a questionnaire on their psychosocial and diabetes status, diabetes specific distress (PAID), critical life-events, and depression and anxiety (HADS-D).
Results: 306 patients (47% female, mean age 24.1±3.5 yrs., mean diabetes duration 11.7±5.8 yrs.; 43% CSII; HbA1c: 8.3±1.6% (67.2± 6.0 mmol/mol)) participated (additional 12 patients refused). HADS-score >10 indicating clinical relevant anxiety was seen in 6.1% of the participants, symptoms of depression in 4.0% (HADS-D score >10). These prevalence data correspond to published prevalence data of the background population. On the other hand 25% of the participants reported a high level of diabetes specific distress (PAID score >39; mean score: 26.8±19.8). Diabetes distress was mainly connected to the items "worrying about future" and "feeling guilty". Sum scores of HADS-D and PAID were both associated with HbA1c (r=-0.14; r=0.23; each p<0.01). Critical life events during the last 12 months were reported by 33.2% of the patients with negative impact on metabolic control (HbA1c 8.8±1.8% vs. 8.0±1.5%; p<0.001)
Conclusion: Despite several challenges of diabetes the majority of young adults were able to cope successfully with age specific developmental tasks without suffering from mental disorder (anxiety and depression). In contrast they report of a high level of diabetes specific distress, mainly associated with fear of late complication and feelings of guilt. A regular assessment of diabetes specific burden and critical life events is recommended to suggest a personalized insulin therapy regimen and psychosocial support.
Supported by: Deutsche Diabetes Stiftung

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Undiagnosed cognitive impairment, health status and depressive symptoms in patients with type 2 diabetes

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Background and aims: Type 2 diabetes is associated with cognitive impairment. We aimed to examine whether undiagnosed cognitive impairment in patients with type 2 diabetes is associated with a reduced health status and depressive symptoms.
Materials and methods: 225 primary care patients aged ≥70 years with type 2 diabetes were examined, both at their homes and at a memory clinic, for undiagnosed cognitive impairment (dementia or mild cognitive impairment [MCI]). Dementia and MCI were defined according to internationally accepted criteria. Questionnaires assessing health status (SF-36, EQ-5D and EQ-VAS) and depressive symptoms (CES-D) were filled out. Health status and depressive symptoms were compared between patients with and without cognitive impairment.
Results: Patients with cognitive impairment (n=57) showed significantly lower scores on the SF-36 on six out of the eight domains, on the physical and mental summary scores, and on both the EQ-5D and the EQ-VAS scores. CES-D scores were significantly higher in patients with cognitive impairment (9.2±7.1 versus 12.7±8.5; p=0.009). Depression (CES-D≥ 16) occurred almost twice as often in patients with cognitive impairment (RR 1.8; 95%-CI: 1.1-3.0).
Conclusion: Undiagnosed cognitive impairment in patients with type 2 diabetes is associated with a reduced health status and more depressive symptoms. Detection of cognitive impairment in type 2 diabetes patients identifies a vulnerable group of patients that could benefit from integrated and tailored treatment.
Supported by: EFSD/Lilly

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Female sex, age (<50 and ≥70 years), northern German residency, high HbA_{1c} and insulin use predict depressed mood in 35,691 type 2 diabetes patients

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Background and aims: A bidirectional relationship between type 2 diabetes (T2D) and depressive symptoms has been reported. The primary aim was to analyze predictors of depressed mood in T2D. Secondly, the odds ratio of developing a clinically recognized depression in patients with conspicuous screening result was evaluated.
Materials and methods: 35,691 T2D patients aged ≥18 years (median [IQR]: 68.9 [59.2-76.5] years) from the German/Austrian multicenter prospective diabetes follow-up registry (DPV) were analyzed. All patients had completed the WHO-5 questionnaire, a reliable and validated 5-item screening tool for depression (score ≤7: likely depression). Logistic regression modeling (SAS 9.4) was applied to study potential predictors (e.g. demographics, regional aspect, diabetes therapy, glycemic

control) for depressed mood as well as the risk of developing clinically recognized depression.

Results: Depressed mood was present in 11.2% (n=4,000) of patients screened and thereby significantly more prevalent compared to the adult German population (DEGS study: 8.1%, p<0.001). Patients with likely depression had a later diabetes onset (60.5 [49.6-70.2] vs. 58.3 [49.1-67.7] years, p<0.001) and were more often female (54.0 vs. 48.0%, p< 0.001) compared to patients with inconspicuous results. Duration of diabetes did not differ significantly between groups (7.6 [2.4-12.9] vs. 7.0 [2.1-13.5] years, p=0.76). Young and very old age as well as female sex were associated with depressed mood (table 1, model 1). Moreover, living in northern federal states of Germany, poor glycemic control (HbA_{1c} ≥58 mmol/mol) and insulin treatment were significantly related to depressed mood in T2D (table 1). Overall, the odds of developing a clinical diagnosis of depression was 1.95 (95%CI: 1.66-2.29) times higher in patients scored ≤7 in the WHO-5 questionnaire.

Conclusion: Depressed mood is a frequent psychological comorbidity in adult T2D patients. In clinical care, routinely screening for psychological problems as recommended by guidelines is absolutely advisable, especially in high-risk patients.

Table 1. Sequential multiple logistic regression models for predicting depressed mood (WHO-5 score ≤7) in 35,691 T2D patients

	Model I: Demographics only	Model II: Region, corrected for demographics	Model III: Glycemic control, corrected for demographics and region	Model IV: Therapy, corrected for demographics and region	Model V: Full model
I. Demographics					
18-<50 y (vs. 50-<70 y)	**1.20 (1.06-1.35)**	**1.21 (1.07-1.36)**	**1.16 (1.02-1.31)**	**1.20 (1.07-1.36)**	**1.20 (1.06-1.36)**
≥70 y (vs. 50-<70 y)	**1.27 (1.18-1.36)**	**1.24 (1.15-1.33)**	**1.32 (1.23-1.42)**	**1.22 (1.13-1.31)**	**1.24 (1.15-1.33)**
Female sex	**1.24 (1.16-1.32)**	**1.24 (1.16-1.32)**	**1.26 (1.18-1.35)**	**1.25 (1.17-1.33)**	**1.27 (1.19-1.36)**
Diabetes duration ≥5 y	**1.13 (1.05-1.21)**	**1.13 (1.06-1.22)**	1.07 (0.99-1.15)	0.95 (0.88-1.02)	0.96 (0.89-1.03)
II. Region					
Northern vs. southern federal states of Germany		**1.26 (1.18-1.35)**	**1.22 (1.14-1.31)**	**1.25 (1.17-1.34)**	**1.20 (1.12-1.29)**
III. Glycemic control					
HbA_{1c} ≥58 mmol/mol			**1.74 (1.63-1.87)**	-	**1.58 (1.47-1.69)**
IV. Diabetes therapy					
Insulin treatment				**1.70 (1.58-1.82)**	**1.49 (1.38-1.61)**

Given are odds ratios with 95% confidence interval. Bold-typed figures indicate p<0.05.

Supported by: EFSD/AatraZeneca, German Competence Net Diabetes, DZD, DDG

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The synergistic effect of depression and diabetes on cognitive function in a community sample of older adults

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Background and aims: Diabetes and depression are independently associated with increased risk of cognitive decline. But the synergistic effect of depression and diabetes on cognitive function has yet to be studied in the general population. We used a nationally representative sample of community-dwelling individuals aged ≥50 years to investigate whether patients with diabetes and depression are at greater risk of cognitive decline compared with patients with either depression or diabetes.

Materials and methods: Our sample comprised 10,812 individuals (45.5% male) from the English Longitudinal Study of Ageing (mean age 65.0 years; SD±10.1). Diabetes was defined by self-reported diagnosis and depression by a score of ≥4 symptoms on the 8-item Centre for Epidemiological Studies-Depression scale. We measured two domains of cognitive function, memory and verbal fluency. We estimated OLS regression models of change in the summary score of immediate and delayed recall (range 0-20 words) over a 10-year follow-up (2002-2012) and change in the animal naming score (range at baseline 0-50) over an 8-year follow-up (2002-10). Age, sex, marital status, education, occupational class, BMI, physical activity levels, smoking, and alcohol use were assessed at baseline and used as covariates. We also used six repeated measurements of the word recall score and five of the animal naming score to estimate lagged GEE models of the average 2-year change in these two cognitive function domains.

Results: At baseline, 616 individuals had T2DM, 1,574 had depression, and 188 individuals both. In OLS regression models adjusted for baseline cognition, age, sex, and marital status, individuals with diabetes, depression, or both experienced a significant decline in both memory and verbal fluency compared with individuals free of both conditions. In the fully adjusted model, individuals with diabetes but without depression no longer scored significantly lower on the word recall score compared with individuals free of both diabetes and depression (β coeff -0.31; -0.70 to 0.85), but those with depression (-0.42; -0.68 to -0.14) or with depression and diabetes (-1.25; -2.1 to -0.46) did. Similarly, in the fully adjusted model, individuals with depression (β coeff -0.64; -1.11 to -1.81) and those with both diabetes and depression (-1.87; -3.27 to -0.46) scored significantly lower on the animal naming test compared with individuals free of both conditions; this did not apply to individuals with diabetes (-0.54; -1.27 to 0.18). The GEE models indicated that, on average, compared with individuals without diabetes and depression, those with either condition or both conditions experienced a significant decline on both domains of cognitive function on a 2-year basis.

Conclusion: Depression and diabetes are associated with an increased risk of cognitive decline in a large community cohort of older adults. The effect of diabetes and depression on cognitive function appears to be multiplicative. The effects are seen over a 10-year period, but are evident also over repeated 2-year assessments. In most cases, the risk of cognitive decline was independent of sociodemographic, lifestyle, and clinical risk factors. Cognitive decline was more pronounced in depressed individuals than in diabetes patients. The consequences of depression, especially in individuals with diabetes, are serious; regular monitoring of depression in clinical practice is required.

PS 079 Recognising and treating hypoglycaemia

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Nocturnal hypoglycaemia can be predicted by retrospective analysis of glucose variability in elderly type 2 diabetic patients treated by insulin

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Background and aims: Nocturnal hypoglycemia (NH) is underestimated complication of insulin therapy, especially in elderly subjects. We aimed to determine the applicability of glucose variability (GV) indices, calculated from continuous glucose monitoring (CGM) data, for prediction of NH in elderly type 2 diabetic patients treated with insulin.
Materials and methods: We analyzed blinded CGM data for 176 nights generated by 83 in-patients, aged 65-80 years, HbA1c 7.8, 6.9-8.2% (median, 25th-75th percentile). Patients were treated with basal insulin (n=17), premixed insulin (n=24), or basal-bolus insulin therapy (n=42). The values of standard deviation (SD), continuous overlapping net glycemic action (CONGA), lability index (LI), M-value, J-index, mean absolute glucose (MAG), high blood glucose index (HBGI), low blood glucose index (LBGI), mean amplitude of glucose excursions (MAGE) and average daily risk range (ADRR) were estimated. Most of GV parameters were computed for nocturnal hours, daytime hours and one-hour pre-midnight interval. The values of MAGE and ADRR were calculated for the whole recordings, in accordance with computational algorithms for these indices. Bedtime glucose trend was evaluated as difference between glucose values at 11 p.m. and at midnight (deltaG). Hypoglycemia was defined as glucose level ≤3.9 mmol/L.
Results: At least one episode of non-severe CGM-defined NH was recorded in 39 (47%) patients. The NH was present in 68 out of 176 analyzed nights (39%). Subjects with detected NH did not differ significantly from those without by age, body mass index, diabetes duration, total and basal insulin dose and HbA1c levels (all $p>0.05$). None of these parameters was significant predictor for NH in univariate logistic regression model. Lower daytime mean glucose, CONGA, J-index (all $p<0.02$) and higher MAG and LBGI (both $p<0.0001$) values were found in patients with NH as compared to those without. Bedtime mean glucose and J-index was lower ($p<0.0001$), while bedtime M-value, deltaG and LBGI was higher (all $p<0.01$) in patients with NH. The value of ADRR was higher in patients with NH as compared to those without ($p=0.003$), meanwhile the differences in MAGE did not reach statistical significance ($p=0.07$). Antecedent daytime hypoglycemia increased the risk of NH ($p<0.0001$). In multivariate logistic regression analysis the combination of three bedtime parameters (mean glucose, LBGI and deltaG) was the most reliable predictor of subsequent NH (accuracy 93.5%%, $p<0.0001$).
Conclusion: The retrospective analysis of CGM-derived GV indices could improve prediction of NH in elderly type 2 diabetic patients treated with insulin.
Supported by: RSF (14-15-00082)

865

Prevention of hypoglycaemia by the predictive low glucose management feature in a user evaluation study

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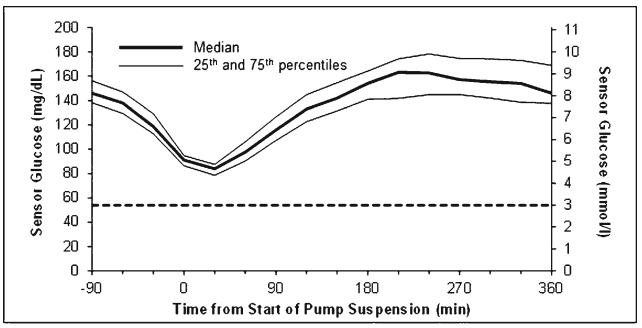
Background and aims: Automated suspension of insulin delivery in response to hypoglycaemia detected by continuous glucose monitoring (CGM) sensors has been shown to reduce severity and duration of hypoglycemic events. Suspending insulin delivery earlier, in response to predicted hypoglycaemia, may allow patients with type 1 diabetes to further reduce or avoid hypoglycaemia entirely. Performance of a predictive low glucose management feature in a commercially-available sensor-augmented pump system was evaluated in a user evaluation study.
Materials and methods: Three study sites in Europe enrolled 40 subjects (18 female, ages 9-65 years) with type 1 diabetes whose mean (±SD) A1C was 7.6±0.87%. The pumps were programmed to suspend insulin delivery if the sensor glucose (SG) value was predicted to be at or below the programmed threshold with a +20 mg/dL offset within 30 min and to restart when the SG value was at least 20 mg/dL above the threshold value and predicted to be at least 40 mg/dL above it within 30 min. Subjects wore the pumps and sensors for 4 weeks, after which data were downloaded and questionnaires were completed.
Results: The pump suspension feature was activated 2417 times, or 2.1 times per subject-day. The median duration of pump suspensions was 57.8 (interquartile range [IQR], 49.2-63.3) min. The median minimum SG value during the suspensions was 3.85 (IQR, 3.67-4.15) mmol/l (69.3 mg/dL), and the median SG value was 8.95 (IQR, 8.04-9.27) mmol/l (161.1 mg/dL) 2 h after restart. Most (70.5%) pump suspensions were terminated automatically; 29.5% were terminated manually. The Figure shows median and IQR of SG values surrounding the activations; the dotted line at 3 mmol/l represents the most-commonly chosen threshold value. The mean and median absolute relative differences (ARDs) between 5195 pairs of SG and contemporaneous blood glucose values were 9.5% and 5.6%, respectively. Of the 2401 evaluable activations, 2327 (96.9%) were not followed by any SG value(s) below the prespecified threshold value. Based on mean questionnaire responses, subjects found the system's low glucose management features easy to set up and beneficial with respect to managing hypoglycaemia, and almost all (97%) expressed a preference for it compared to the system they had been using before the study.
Conclusion: Insulin pump suspension in advance of predicted hypoglycaemia helped subjects with type 1 diabetes avoid hypoglycaemia in 97% of the occasions when it was predicted. These data show that predictive low glucose management was effective in preventing hypoglycaemia and well accepted by patients.



Sensor glucose values surrounding N=2401 evaluable activations of the predictive low glucose management feature. Time 0 represents the time of pump suspension. The dotted line at 3 mmol/l represents the most commonly-used threshold value.

Clinical Trial Registration Number: NCT01726621

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Detecting hypoglycaemia in breath-exhaled isoprene and acetone as biomarkers of blood glucose

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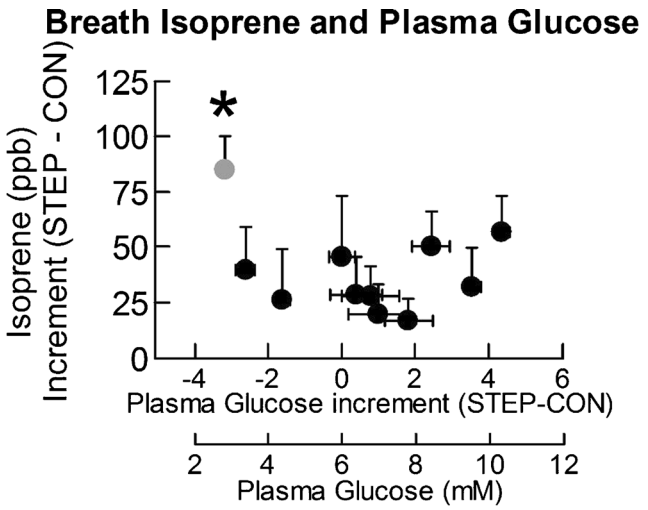
Background and aims: Despite decades of research, no viable non-invasive alternatives to capillary blood glucose testing currently exist. Given reports of rescue dogs responding to changes in glycaemia, particularly alerting of hypoglycaemia, we hypothesized that volatile compounds (VOCs) in exhaled breath might change with blood glucose offering biomarkers for non-invasively monitoring glycaemia

Materials and methods: 8 female non-smoking patients with type 1 diabetes (age 46±14, duration of diabetes 23±20 years) attended on 2 occasions. After an overnight fast, we used a 220 min stepped insulin clamp technique (actrapid 0.3 mU/kg/min for 2 h then increased to 1.5 mU/kg min) with variable 20% dextrose. On one occasion, arterialised plasma glucose was adjusted stepwise to 7.1+0.8, 8.7+0.4, 10.7+0.1, 4.3+0.3 and 2.8+0.1 mM (40 min steps). On the other day, glucose was maintained throughout at 6.2+0.1 mM (CON) with STEP and CON studies performed in random order at least 1 week apart (single blinded). Thus, the study design allowed us to examine the relationship between VOCs and glucose independent of insulin which was infused identically on both study days. At 20 minute intervals, 1.1 L of exhaled breath samples were collected in Fischer Analysen Instrumente GmbH bags and analysed using V&F Airsense Compact Ion-Molecule-Reaction Mass Spectrometer. VOCs (acetone, isoprene, methyl nitrate, ethyl benzene, ethanol and propane) were standardised for exhaled CO2. We subtracted CON from STEP values for VOCs and glucose and examined using Pearson Correlation with IBM SPSS Statistics 21. Looking specifically for a biomarker of hypoglycaemia, we also compared VOC values during final 2.8 mM step with those from non-hypoglycaemia range.

Results: (1) Exhaled breath isoprene increased significantly at glucose of 2.8 mM compared to non hypoglycaemia range (85±15 vs 34±13 parts per billion (ppb), $p<0.05$- see figure). (2) We found a linear correlation across study glucose values between exhaled acetone and plasma glucose with higher acetone at higher glucose values ($r=0.25$, $p<0.05$). (3) There was no obvious correlation for other measured VOCs.

Conclusion: Our data suggest that exhaled breath VOCs might offer an alternative non-invasive method for monitoring changes in blood glucose, including detecting hypoglycaemia, in diabetes.



Supported by: NIHR Cambridge Biomedical Research Centre

867

Needle-free nasal delivery of glucagon is superior to injectable delivery in simulated hypoglycaemia rescue

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Background and aims: Injecting glucagon to treat severe hypoglycemia (SH) can be difficult for caregivers of persons with diabetes (PWDs). This study evaluated the ability of people to use injectable glucagon (Glucagon Emergency Kit - GEK) and needle-free Glucagon Nasal Powder (GNP) devices to treat a simulated episode of SH.

Materials and methods: The Caregiver arm included 16 pairs each composed of an adult with diabetes (PWD) and their caregiver. PWDs, who were taught how to use 1 of the devices in random order, then instructed their caregivers on device use. Approx. 1 wk later, caregivers treated a manikin during a simulated episode of SH. The procedure was repeated with the other product. The Acquaintance arm had 15 adults, not associated with any particular PWD, who said they were willing to assist someone in distress. Acquaintances were not trained on device use but were shown the device prior to the simulation. Acquaintances treated 2 episodes of SH, in random order, with a delay of about 10 min between each. A fully clothed adult manikin represented a PWD in the simulation. Participants were told the manikin was in SH and that they had to administer the rescue glucagon as quickly as possible. They were told to find the PWD's glucagon rescue kit in the PWD's backpack. The backpack contained the glucagon rescue kit being evaluated in that episode and the PWD's diabetes supply pouch (glucometer and strips, alcohol swabs, lancing device, insulin syringe and a vial of insulin). Sound effects and distractions created a sense of urgency.

Results: Fifteen of 16 caregivers and 14 of 15 acquaintances administered a full dose of GNP (avg. time 0.27 and 0.44 min, respectively; see table below). The 2 participants who failed to fully depress the plunger on the GNP device also failed to inject GEK. With GEK, only 8 of 16 caregivers injected glucagon (avg. time 1.89 min) of which 2 gave a full dose. Failures included injection of diluent only (n=4) and bent needle (n=1). Three caregivers administered insulin instead of glucagon; when then prompted to use GEK, 2 injected only diluent. Only 3 of 15 acquaintances injected glucagon (avg. time 2.4 min); none gave a full dose. Failures included injection of diluent only (n=9), refusal to attempt to inject (n=1) and injection with an empty syringe (n=1). One acquaintance injected insulin instead of glucagon; when then prompted to use GEK, only diluent was injected.

Conclusion: Under conditions that simulate some of the stress associated with real episodes of SH, Caregivers and Acquaintances injected glucagon with a very low success rate and numerous errors; however, the same people used GNP successfully in less time. The data indicate that delivery of GNP is easier for non-medical third parties to administer successfully and suggest administering glucagon using a different route and dosage form than those used for insulin may also reduce the risk of accidental delivery of insulin.

Arm	Parameter	GNP	GEK
Caregivers	Number	16	
	Age - median (min, max)	53.5 yrs (20, 69)	
	Male:Female	6:10	
	Full dose delivered - number (%)[1]	15 (94)	2 (12.5)
	Partial dose delivered - number (%)[1] (% min, max)	0 (0)	6 (37.5) (3, 85)
	No dose delivered - number (%)	1 (6)	8 (50)
	Minutes to deliver dose - mean[2] (min,max)	0.27 (0.03, 0.93)	1.89 (1.27, 2.75)
Acquaintances	Number	15	
	Age - median (min, max)	40.0 yrs (22, 78)	
	Male:Female	6:9	
	Full dose delivered - number (%)[1]	14 (93)	0 (0)
	Partial dose delivered - number (%)[1] (% min, max)	0 (0)	3 (20) (36, 56)
	No dose delivered - number (%)	1 (7)	12 (80)
	Minutes to deliver dose - mean[2] (min,max)	0.44 (0.17, 0.78)	2.40 (1.30, 3.93)

[1] GNP delivers entire dose when plunger is fully depressed. For GEK, full dose defined as at least 90% of the targeted 1 mg dose.
[2] Includes only those who injected glucagon, regardless of % dose delivered. Failures to deliver not included.

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Subcutaneous low-dose glucagon bolus in the treatment of mild hypoglycaemia in insulin pump treated patients with type 1 diabetes

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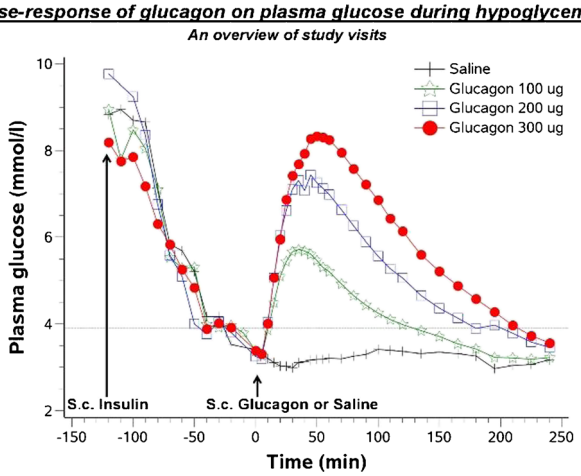
Background and aims: Hypoglycaemia is a common side effect to insulin treatment in patients with type 1 diabetes (T1D). Dual-hormone treatment with combined subcutaneous (s.c.) infusion of insulin and glucagon, has potential to improve glucose control without inducing hypoglycaemia. However, in order to use glucagon in a dual-hormone approach, the dose-response relationship of exogenous glucagon on plasma glucose (PG) is a prerequisite.
Materials and methods: We carried out a single-blinded, randomized, placebo-controlled four-way crossover study to determine the dose-response relationship of s.c. glucagon to increase PG during mild hypoglycaemia in 8 T1D insulin-pump treated patients (5 females, median (range) age 47 (19-64) years, HbA_{1c} 52 (43-57) mmol/mol, BMI 24 (20-25) kg/m^2, C-peptide 3 (3-30) ρmol/l, no autonomic neuropathy and no hypoglycaemia unawareness). Each patient underwent 4 study visits. In fasting state, an s.c. insulin bolus expected to reduce PG to 3.0 mmol/l was given, however, when PG reached 3.9 mmol/l (t=0 min), 1 of 3 different low-doses of s.c. glucagon or saline was given as a bolus. A repeated measurement linear mixed model was applied and results expressed as absolute change (95% CI).
Results: Mean (95% CI) PG peak increased with 2.3 (1.7-3.0), 4.2 (3.5-4.8) and 5.0 (4.3-5.6) mmol/l after 100, 200 and 300 µg glucagon, respectively, compared with saline (all p<0.001) (figure 1). Time to peak was 30 (27-39), 40 (32-44) and 50 (43-55) min after glucagon. Within 15 min each glucagon dose significantly increased PG with 1.4 (0.4-2.5), 2.1 (1.1-3.2), and 2.0 (1.0-3.0) mmol/l. The PG values were significantly higher compared with the saline day until 120 (83-156), 189 (152-225) and 195 (185-257) min after glucagon bolus. The incremental areas under the curve (iAUC) for glucagon were 894, 1148, and 1354 mmol/l x min and for saline 550 mmol/l x min (all p<0.001 compared to saline).There was no significant difference between 200 and 300 µg glucagon doses in regard to iAUC (p=0.13) or PG values at any time points. Mean peak plasma glucagon response above baseline occurred 10 min after glucagon administration and was 100 (47-152), 217 (118-317), and 358 (190-526) ρmol/l (p<0.001). However, 45 min after saline administration a PG of 3.2 (2.9-3.4) mmol/l increased plasma peak endogenous glucagon concentration with 4.5 (1.7-7.3) ρmol/L above baseline (p<0.01).
Conclusion: Low-dose s.c. glucagon bolus significantly elevates plasma glucose in case of relative insulin over-bolusing. Low dose s.c. glucagon is an alternative to oral glucose in the treatment of mild hypoglycaemia in insulin pump treated patients.

Dose-response of glucagon on plasma glucose during hypoglycemia



Clinical Trial Registration Number: NCT02232971
Supported by: Danish Diabetes Academy

869

Targeted photodynamic therapy of insulinomas and congenital hyperinsulinism by exendin-700DX

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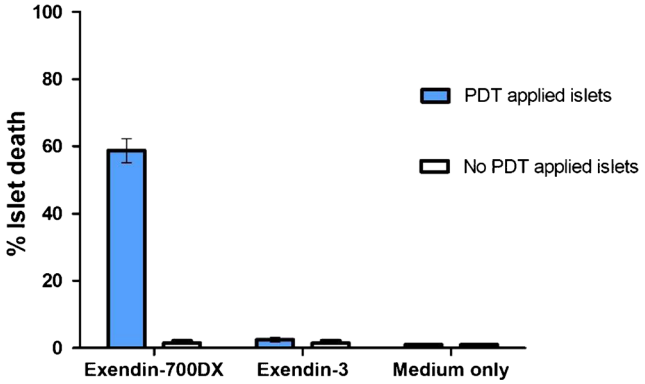
Background and aims: Insulinomas are tumors of the pancreas that are derived from beta cells. Because the tumor produces excess amounts of insulin, it can lead to hypoglycemia. Congenital hyperinsulinism is a disease that causes high levels of insulin. People with this condition have frequent episodes of hypoglycemia which can cause complications such as breathing difficulties, seizures, brain damage, and coma. Currently, treatment options are limited and there is an urgent need for novel therapeutic options. Exendin is a peptide which can specifically bind to the glucagon-like peptide 1 receptor (GLP-1R) which is expressed in the beta cells in the pancreas. Photosensitizing fluorescent dyes as 700DX can create free radicals and singlet oxygen that can be fatal to the cells, when they are subjected to laser irradiation. Labeling Exendin with 700DX can be a promising way to produce a new agent for laser induced photodynamic therapy (PDT) of *insulinomas* in adults and *congenital hyperinsulinism* in children, aimed at selective destruction of diseased beta cells. With this therapy, it would be possible to treat insulinomas or destroy diseased beta cells in a specific area of pancreas and leave a sufficient amount of beta cells to prevent diabetes. The aim of the study is development of a new agent and method for laser induced photodynamic therapy of insulinomas and congenital hyperinsulinism.
Materials and methods: Exendin-3 (Ex-3) and 700DX coupled Exendin(Ex-700DX) were used in the experiments. The therapeutic effect of the compound was examined on islets isolated from C3H mice, a rat insulinoma cell line (INS-1E) and GLP-1R expressing CHL cells. In vitro PDT experiments, were performed by incubating islets with 165 nM Ex-700DX, 165 nM Ex-3 and medium only (control) for 3 hours at 37°C. The islets and cells were irradiated for 30 min at 60 Watt, with a LED laser system that emits radiation in the 690 nm spectral band while the control plate was not laser irradiated. An hour after the PDT, cell titer glo assay was performed to detect the islet death in both plates. The effect of internalization of Ex-700DX was examined using CHL-GLP1R(+) cells by incubation at 0°C (no internalization) or 37°C (internalization).
Results: Nearly 60% islet death was detected after the targeted PDT, on the Ex-700DX treated group of islets. No significant islet death was

observed with Ex-3 and vehicle incubated groups after PDT, which proves that the compound and laser irradiation itself does not cause any islet death. (Figure) The percentage of cell death after PDT was calculated as 44% when Ex-700DX internalized into the cells, while 21% cell death was observed when Ex-700DX was only bound to GLP-1 receptors on the cells.

Conclusion: First trials on mice islets and GLP-1R positive tumor cells showed that targeted PDT with Ex-700DX efficiently destroys the target cells. For efficient PDT, Ex-700DX should be at least bound to GLP-1 receptor and the efficiency of the therapy increases nearly 2 times when Ex-700DX is internalized into the cells.



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Factors associated with post-prandial hypoglycaemia in patients with remission of type 2 diabetes after bariatric surgery

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Background and aims: Post-prandial hypoglycemia (P-HYPO) occurs in a significant proportion of patients who achieve remission of type 2 diabetes (DM2) after bariatric surgery (BS). Aim of this study is to evaluate the factors associated with P-HYPO in patients with remission of DM2 (HbA1c <6.5%, fasting glucose <125 mg / dl in the absence of hypoglycemic therapy) after gastric bypass (BPG) or sleeve gastrectomy (SG).

Materials and methods: All participants underwent a 75 g-OGTT before and 2 years after BS. A mixed meal test and a continuous glucose monitoring (CGM) for 7 days by Dexcom G4 PLATINUM were performed after surgery. In fourteen patients (BPG / SG: 8/6, age: 49±7 years, BMI: 32±6 kg / m2, diabetes duration: 3±5 years) with evidence of P-HYPO (blood glucose <70 mg / dl) and in 5 patients (BPG / SG: 1/4, age: 45± 10 years, BMI: 31±6 kg / m2, diabetes duration: 7±3 years) without P-HYPO (NO-HYPO) changes in insulin sensitivity (OGIS), insulin secretion (IGI) and the meal response of the entero-hormones were evaluated.

Results: The two groups were similar for age, duration of DM2 and postoperative BMI. Patients with P-HYPO showed a greater decrease in weight (ΔBMI: 13±6 vs 7±4 kg / m2, p=0.042), a greater improvement in insulin sensitivity (ΔOGIS: 158±54 vs 29±22 ml min -1 ml-2 , p= 0.001), a higher postprandial GLP-1 peak (32±31 vs 7±2 pmol/L, p= 0.031) and a lower GIP meal response (GIP peak: 209±120 vs 371± 27 pg/ml, p=0.009) than NO-HYPO.

Conclusion: In patients with remission of DM2 after BS, P-HYPO is associated with greater weight loss, more marked increase in insulin sensitivity and distinct entero-hormone profile, suggesting that this complication can reflect an "extreme" form of improvement of the metabolic status.

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Impact of early and late dumping syndrome on weight loss after bariatric surgery

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Background and aims: It has been postulated that weight loss after RYGB could be in part attributed to dumping syndrome following sweet ingestion with a consequent aversion for such foods. However, no study has shown a correlation between dumping syndrome and weight loss. On the other hand, other investigators have hypothesised that increased inter-meal hunger, due to reactive hypoglycaemia, could reduce weight loss. We analyzed an integrated database on bariatric subjects, asking the question if there was any correlation between reported early dumping syndrome (ED), or late dumping syndrome (LD) (i.e., reactive hypoglycaemia) and body weight change.

Materials and methods: 136 morbidly obese subjects, who had undergone bariatric surgery and have been followed for at least 30 months were evaluated. After surgery, during regular follow-up visits, a standardized questionnaire (Sigstaad and Arts questionnaires) was administered to each subject in order to diagnose ED/LD. In this dataset, ED/LD was detected 10-20 months after surgery; BMI values were recorded over the ensuing 10 months. A control group (ND) of bariatric subjects not reporting ED/LD was evaluated at the same time intervals. Once ED/LD was detected, patients were advised to change eating habits; in some cases, an OGTT was performed to confirm diagnosis.

Results: In the whole cohort, there was no significant difference in age among the 3 groups (52±10 years for the ND group, 48±11 years for the ED group and 49±11 years for the LD group, p=ns). RYGB was performed in the 72% of ND, 100% ED and 79% LD (p=ns). ED was reported in 19% of the patients and LD in 27%. LD was significantly more frequent in subjects that had undergone RYGB than in those who underwent sleeve gastrectomy (79% vs 21%, p=0.007). The prevalence of type 2 diabetes mellitus was higher in the ND compared to other groups (55, 25, 22%, p=0.01, in ND, ED and LD group, p=0.01 respectively). The ND group had higher BMIs at 10-20 months and 30 months compared to the ED and LD group (p=0.006); however the data show that ND continued to lose weight throughout the 30 months of follow-up, whereas this was not true for the LD and ED groups: once LD or ED disturb came up, subjects on these groups stopped losing weight. In total the ND group lost more weight than the LD and ED group (p=0.009). BMIs and body weights of the 3 groups at 10-20 months and 30 months are reported in table 1.

Conclusion: Patients present ED after RYGB but not sleeve gastrectomy, while LD occurs after both RYGB and sleeve gastrectomy, with a higher prevalence in the former (≈80%). LD/ED occurs more frequently in glucose tolerant than in diabetic subjects. When ED/LD occurs, subjects stop losing weight.

Table 1: BMIs and Body Weights of the 3 groups at 10-20 months and 30 months.

	ND			ED			LD			P value (for group and time interaction)
	N=73			N=26			N=37			
Time interval (months)	10-20	30	P value (for time)	10-20	30	P value (for time)	10-20	30	P value (for time)	
Body Weight (kg)	91±8	89±19	0.002	74±12	77±14	ns	84±16	83±17	ns	0.001
BMI (kg/m²)	33.9±5.9	32.5±5.8	0.02	28.6±5.1	28.7±5.3	ns	29.5±4.9	29.5±5.3	ns	0.001

PS 080 Hypoglycaemia: implications and complications

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Impaired awareness of hypoglycaemia and peripheral autonomic neuropathy in type 1 diabetes

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Background and aims: Impaired awareness of hypoglycaemia (IAH) implies many-fold increased risk of severe hypoglycaemia in type 1 diabetes mellitus (T1DM). Peripheral autonomic neuropathy has been suggested as a mechanism for the development of IAH. The aim of the present study was to evaluate the association between IAH and the presence of peripheral autonomic neuropathy.
Materials and methods: We included 33 subjects with T1DM and IAH (Gold score≥4) and 33 with normal awareness (NAH; Gold score≤2), matched for age, gender and diabetes duration. A thorough clinical and laboratory evaluation including extensive cardiovascular and pupillometric autonomic function testing, nerve conduction studies as well as quantitative sensory testing was performed. Composite abnormality Z-scores were used for group comparisons. Investigators were blinded as to the hypoglycaemia awareness status of the participants.
Results: The IAH and NAH group had similar age, diabetes duration and HbA1c (Table 1). The autonomic composite score was not different between the IAH and NAH groups (mean difference - 0.15 (95% CI -0.46 to 0.16; p=0.33), and neither were the thermal detection or nerve conduction scores (mean difference 0.15(-0.31 to 0.61; p=0.51) and 0.03 (-0.43 to 0.49; p=0.89), respectively.
Conclusion: Sensitive and extensive measures of autonomic neuropathy were similar in closely matched IAH and NAH participants, indicating that peripheral autonomic neuropathy is not a likely mechanism for the development of IAH in subjects with T1DM.

Table 1 Clinical and biochemical characteristics of participants with type 1 diabetes

	All with diabetes	Impaired awareness	Normal awareness
Men/women, n	28/38	14/19	14/19
Age years, median (IQR)	47 (15.0)	48 (14.5)	47 (14.5)
Diabetes duration, years, median (IQR)	31 (13.3)	30 (13.5)	31 (13.5)
Current HbA_{1c} median (IQR)			
%	8.0 (1.8)	7.8 (2.2)	8.1 (1.9)
mmol/mol	64.0 (19.7)	62.0 (24.0)	65.0 (20.8)
Insulin regimen, n (%)			
Long + rapid acting analogues	34 (51.5)	18 (54.5)	16 (48.5)
NPH insulin + rapid acting analogue	12 (18.2)	7 (21.2)	5 (15.2)
Insulin pump with rapid acting analogue	19 (28.8)	8 (24.2)	11 (33.3)
Other	1 (1.5)	0 (0)	1 (3)
Frequency of blood glucose measurement, n (%)			
> 4 times/day	34 (51.5)	17 (51.5)	17 (51.5)
1-4times/day	20 (30.3)	10 (30.3)	10 (30.3)
1-6 times/week	12 (18.2)	6 (18.2)	6 (18.2)
< 1time /week	0	0	0
N° of severe hypoglycemia episodes in the preceding year, n (%)			
None	47 (71.2)	20 (60.6)	27 (81.8)
1-2	15 (22.7)	9 (27.3)	6 (18.2)
≥ 3	4 (6.1)	4 (12.1)	
P-Creatinine, µmol/L, median (IQR)	61.0 (22.0)	60.5 (19.8)	63.0 (25.5)
U-Albumin/Creatinine Ratio			
mg/mmol, median (IQR)	0.8 (1.5)	0.9 (1.5)	0.8 (1.4)
< 3 / ≥ 3 mg mg/mmol, n (%)	51 (77.3) /15 (22.8)	25 (75.8) /8 (24.2)	26 (78.8) / 7 (21.2)

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Sulphonylurea induced hypoglycaemia: How often are we really seeing it?

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Background and aims: Sulphonylureas are oral medications used to reduce blood glucose levels in Type II Diabetes. The current National Institute for Health and Care Excellence (NICE) guidelines recommend their prescription as second line oral hypoglycaemic agents where blood glucose control is suboptimal despite treatment with metformin. While effective at lowering blood glucose levels, sulphonylureas are associated with serious adverse effects especially in the elderly population. These include hypoglycaemia and increased cardiovascular risk. The ultimate outcome can result in falls, fractures, psychological fear and frequent hospital admissions. The authors suspected that the prevalence of sulphonylurea induced hypoglycaemia was higher than anticipated based on in hospital observation and volume of referrals to the diabetic team. The aim of this audit was to ascertain the commonest cause of hypoglycaemia in hospitalised patients. Secondary review points included reviewing the investigations and contributing factors to hypoglycaemia as well as treatment by staff. The data collected would be used to discourage the prescription of sulphonylureas in the elderly population.
Materials and methods: Retrospective data was collected on all patients who sustained an episode of hypoglycaemia while admitted in a district general hospital over a 6 month period. The inclusion criteria required hypoglycaemia to be coded somewhere in the patient's discharge. This included patients admitted with hypoglycaemia and those where hypoglycaemia was a secondary diagnosis during admission. Particular focus was applied to medication prescription and contributing factors such as alcohol excess and prescription errors. The results were collated and statistically analysed for comparative purposes
Results: Analysis revealed the commonest cause of hypoglycaemia in hospitalised patients to be insulin related. In total 14.3% of cases were related to sulphonylureas, much higher than anticipated from the UKPDS study where sulphonylurea associated hypoglycaemia is quoted at approximately 1.8%. Contributing factors played a relatively modest role in this subset of data. Acute kidney injury and liver failure played important roles in two patients. One elderly patient self administered twice the usual dose of insulin. The treatment in all cases was reviewed and deemed appropriate for the level of hypoglycaemia and patients clinical status.
Conclusion: Sulphonylureas are contributing to a higher than expected percentage of hypoglycaemia in hospitalised patients. There are often contributing factors as identified with this audit: A relatively small number of patients were used in the audit and the district general hospital is located near a seaside resort with a predominately retired population. Regardless of this the rate of hypoglycaemia caused by sulphonylureas is significantly higher than expected. It is also likely that we are underestimating the impact and frequency of hypoglycaemia as patients are treated by ambulance services, emergency departments and general practitioners. From this data we recommend avoiding sulphonylurea prescription in elderly patients. We also recommend discontinuing them where patients are at risk of falls, isolation and acute kidney injury. NICE guidelines recommend the use of dipeptidyl peptidase 4 (DPP-4) inhibitors and Thiazolidineiones as alternatives. This data is being used to raise awareness with GP's in the local area.

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Brain lactate concentration is not increased during hypoglycaemia in non-diabetic subjects and patients with type 1 diabetes, as measured with ^{1}H-MRS

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Background and aims: Iatrogenic hypoglycemia is the most frequent acute complication of insulin therapy in patients with type 1 diabetes (T1DM), and limits glycemic management. Recurrent hypoglycemia initiates a process of habituation, characterized by suppression of hypoglycemic symptoms and impaired hypoglycemic awareness. Recent evidence suggests a role for increased brain lactate transport as underlying mechanism. The aim of our study was to investigate the effect of hypoglycemia on both plasma and brain lactate content in non-diabetic subjects and in T1DM patients.
Materials and methods: After an overnight fast, 5 non-diabetic subjects (2 males, mean age: 29±7 yrs, BMI 23.2±1.7 kg/m^2) and 10 T1DM patients (6 males, mean age 27±7 yrs, BMI 24.4±2.5 kg/m^2) underwent a stepped hyperinsulinemic (60 mU m^2 m^{-1}) euglycemic-hypoglycemic clamp. Arterial plasma glucose and lactate levels were determined every 5 minutes. Cerebral ^{1}H-MRS data were acquired with a J-editing semi-LASER sequence (TE 144 ms, TR 3000 ms, 32 averages) at 3.0 T (Siemens, Trio) from a 31.25 cm^3 voxel placed in the supraventricular cortex, and lactate signals were quantified using the AMARES algorithm in jMRUI.
Results: Plasma glucose levels were similar in patients and controls, both during the euglycemic and the hypoglycemic phase (euglycemia: 5.01± 0.18 versus 4.92±0.20 mmol/L, hypoglycemia: 2.84±0.04 versus 2.86± 0.08 mmol/L, *p= NS* for all). In non-diabetic subjects, plasma lactate levels increased from 0.87±0.22 mmol/L during euglycemia to 1.31± 0.33 mmol/L during hypoglycemia (p=0.010). Conversely, plasma lactate levels dropped from 1.16±0.12 mmol/L to 0.92±0.14 mmol/L in T1DM patients (p=0.001). Brain lactate levels did not differ between the eu- and hypoglycemic condition, neither in non-diabetic subjects, nor in T1DM patients (controls: 0.50±0.06 versus 0.45±0.04 μmol/g, and T1DM: 0.48±0.07 versus 0.43±0.06 μmol/g, *p= NS* for all). In addition, brain lactate levels did not differ between patients and controls *(p=* 0.69 for euglycemia, p=0.51 for hypoglycemia).
Conclusion: These data show divergent responses in plasma lactate levels during hypoglycemia between non-diabetic subjects and T1DM patients, which are not accompanied by changes in lactate content in the brain. This could indicate that during hypoglycemia (excess) lactate is either not taken up in the brain or that lactate is immediately utilized as a fuel, potentially to a greater extent in T1DM patients than in controls.
Clinical Trial Registration Number: NCT02146404
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Recovery of cognitive function following brief exposure to hypoglycaemia in adults with type 1 diabetes

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Background and aims: Recovery of cognitive function after one hour of hypoglycaemia is delayed for a protracted period in adults. Whether a shorter period of exposure to hypoglycaemia can cause a similar delay in cognitive recovery is unknown. The aim of this study was to determine whether the duration of hypoglycaemia affects the time of recovery of cognitive function in adults with type 1 diabetes and intact hypoglycaemia awareness.
Materials and methods: A hyperinsulinaemic hypoglycaemic clamp was used to lower blood glucose to 2.5 mmol/L (45 mg/dL) (hypoglycaemia) for 20 minutes or to maintain blood glucose at 4.5 mmol/L (81 mg/dL) (euglycaemia) on two separate occasions at least two weeks apart. The order of exposure to each experimental condition was randomised and counterbalanced. A hypoglycaemia symptom questionnaire (HSQ) and cognitive test battery (Digit Symbol Substitution Test (DSST) and Four Choice Reaction Time (CRT)) were completed before, and during each experimental condition, and repeated at 10 to 15 minute intervals for 90 minutes during the recovery period. Performance on the two experimental conditions was compared using repeated measures ANOVA. A p value of <0.05 is considered significant.
Results: Eighteen adults (5 female) participated in the study (median age 28.5 years (IQR 23.75-33.25), median duration of type 1 diabetes 15.5 years (IQR 12.25-22.00) and median HbA1c 63 mmol/mol (IQR 59-74.25)).
Table 1 shows mean (SD) test scores and scores corrected for baseline performance. HSQ, DSST and CRT deteriorated during hypoglycaemia (p=0.009, <0.001 and 0.001 respectively) with an immediate improvement following restoration of euglycaemia. CRT deteriorated again 20 and 70 minutes into recovery (p=0.016 and 0.032 respectively) while the DSST score also deteriorated 70 minutes (p=0.006) into recovery but both scores had improved by the final time point (85 minutes after euglycaemia was restored).
Conclusion: Following brief hypoglycaemia, although cognitive function recovered immediately once euglycaemia was restored, subsequently a transient deterioration in reaction time was observed in a similar pattern to that observed in previous studies in which the duration of hypoglycaemia was longer. Both DSST and CRT deteriorated 70 minutes into the recovery period but were restored within 15 minutes. This could suggest an effect of boredom or fatigue rather than a direct consequence of hypoglycaemia. However, as the counterbalanced study design should ensure equal levels of ennui during both study conditions, hypoglycaemia may be affecting the ability to sustain attention for mundane, repetitive tasks during the recovery period, even after brief exposure to a low blood glucose. This has practical implications for the time required for recovery of cognitive function following hypoglycaemia in relation to activities such as driving.

TABLE 1: Mean test scores (SD) and scores corrected for baseline performance

	EUGLYCAEMIA		HYPOGLYCAEMIA		EUGLYCAEMIA corrected for baseline		HYPOGLYCAEMIA corrected for baseline		*p* values
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	
Baseline HSQ	21.7	5.7	23.5	7.2					
Experimental HSQ	**22.7**	**6.0**	**31.5**	**10.9**	**0.9**	**2.4**	**8.0**	**10.4**	**0.009**
Recovery 1 HSQ	23.7	6.7	25.0	7.7	2.0	2.9	1.5	7.9	0.72
Recovery 2 HSQ	23.2	7.0	24.5	7.4	1.5	3.5	1.0	6.4	0.614
Recovery 3 HSQ	24.1	7.8	24.4	10.0	2.3	4.3	0.9	8.7	0.41
Recovery 4 HSQ	23.4	6.9	23.7	8.0	1.7	3.3	0.2	7.4	0.187
Recovery 5 HSQ	**22.8**	**6.5**	**22.5**	**7.3**	**1.1**	**3.4**	**-1.0**	**6.3**	**0.041**
Recovery 6 HSQ	21.6	4.3	21.9	6.5	-0.2	3.2	-1.6	5.8	0.124
Recovery Final HSQ	**21.6**	**5.4**	**21.1**	**4.8**	**-0.2**	**3.7**	**-2.4**	**5.7**	**0.011**
Baseline DSST	74.7	13.3	80.3	11.9					
Experimental DSST	**77.4**	**12.5**	**64.8**	**19.8**	**2.8**	**5.9**	**-15.5**	**15.1**	**0.000**
Recovery 1 DSST	77.8	11.5	82.9	15.7	3.2	5.9	2.7	8.1	0.869
Recovery 2 DSST	81.4	15.0	82.4	12.3	6.8	7.5	2.1	9.7	0.084
Recovery 3 DSST	82.4	15.7	86.3	12.6	7.8	10.1	6.1	8.7	0.419
Recovery 4 DSST	84.2	16.2	87.2	14.8	9.6	10.6	6.9	10.4	0.398
Recovery 5 DSST	87.2	15.2	87.8	16.9	12.6	9.3	7.5	11.3	0.074
Recovery 6 DSST	**90.1**	**14.7**	**87.9**	**12.8**	**15.4**	**10.2**	**7.7**	**7.9**	**0.006**
Recovery Final DSST	89.7	15.4	89.5	17.2	15.1	10.8	9.2	11.6	0.064
Baseline CRT	441.4	68.2	423.6	51.9					
Experimental CRT	**438.0**	**71.5**	**467.3**	**70.7**	**-3.3**	**26.9**	**43.7**	**47.7**	**0.001**
Recovery 1 CRT	435.2	66.1	432.6	65.3	-6.2	37.0	8.9	35.1	0.252
Recovery 2 CRT	**432.7**	**65.4**	**434.2**	**53.2**	**-8.6**	**28.0**	**10.6**	**23.0**	**0.016**
Recovery 3 CRT	441.5	70.2	437.4	63.8	0.1	26.4	13.8	32.7	0.222
Recovery 4 CRT	451.3	82.4	436.3	52.1	**9.9**	41.0	**12.8**	28.4	0.979
Recovery 5 CRT	433.9	71.0	430.6	57.8	-7.4	36.8	7.0	34.0	0.186
Recovery 6 CRT	**428.0**	**67.6**	**433.4**	**62.6**	**-13.3**	31.4	**9.8**	**34.8**	**0.032**
Recovery Final CRT	428.9	67.3	429.6	67.1	-12.5	27.0	6.1	41.4	0.122

HSQ=Hypoglycaemia symptom questionnaire
DSST=Digit symbol substitution test (the number of symbols identified in two minutes)
CRT=Choice reaction time (mean reaction time in milliseconds)

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The combined effects of sleep deprivation and insulin-induced hypoglycaemia on cognitive function in adults with type 1 diabetes

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Background and aims: To test the hypothesis that when hypoglycemia is induced during a state of sleep deprivation the severity of cognitive dysfunction would be greater than with hypoglycemia alone, and that recovery following restoration of normoglycemia would be delayed.
Materials and methods: Fourteen adults with type 1 diabetes underwent a hyperinsulinaemic, hypoglycemic clamp on two occasions, separated by at least two weeks. Before one glucose clamp the subjects were sleep deprived overnight. Subjects were randomized and counterbalanced to the experimental condition. Cognitive function tests were performed before and during hypoglycemia, and for 90 minutes following recovery of euglycemia.
Results: Performance in cognitive tests assessed during hypoglycemia did not differ significantly between the sleep-deprived and non-sleep-deprived conditions. Digit symbol substitution scores and choice reaction times were consistently poorer during recovery in the sleep-deprived state, but the difference was only significant at the final time point ($p=0.01$) for digit symbol, and at 20 and 40 minutes into recovery for choice reaction time($p=0.02$ and 0.03 respectively). Hypoglycemia symptom scores were significantly higher in the sleep-deprived state during all stages of recovery ($p=0.002$).
Conclusion: Hypoglycemia per se produced a profound deficit in cognitive function and co-existing sleep deprivation did not have an additive effect. However, during restoration of normoglycemia, sleep-deprived subjects remained more symptomatic and performed more poorly on cognitive testing for the full 90 minute recovery period.

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Hypoglycaemia-induced changes in cerebral metabolic and reward processing: a non-invasive imaging investigation using arterial spin labelling

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Background and aims: Impaired awareness of hypoglycaemia (IAH) affects 30% of people with longstanding type 1 diabetes, increasing risk of severe hypoglycaemia 6-fold. Attenuated neuroendocrine responses to hypoglycaemia are well-described; the central correlates of symptomatic counterregulatory actions are less well understood. To explore the brain responses to hypoglycaemia and the complex interplay between the regions involved we used 3D pseudo-continuous arterial spin labelling (3D pcASL) functional magnetic resonance imaging (fMRI). This approach allowed us to measure regional variation in cerebral perfusion as a surrogate marker of changes in brain activity in non-diabetic volunteers.
Materials and methods: 14 healthy, right-handed volunteers, (mean age ± SD, 40±10 years, 6 male) underwent a two-step hyperinsulinaemic (1.5 mU kg-1 min-1) clamp in a 3 Tesla MR scanner after an overnight fast. Two 3D pcASL scans were obtained at euglycaemia (5 mmol/L) and 2 further scans at established hypoglycaemia (2.6 mmol/L). After each scan subjects quantified autonomic symptoms using a visual analogue scale (VAS). Each pcASL scan (5 min) provided a whole brain cerebral blood flow (CBF) map with a spatial resolution of 2×2×3 mm. Statistical parametric analysis of these images was performed using SPM-8; and a voxel-wise paired t-test was employed to examine the effect of hypoglycaemia. Only those clusters that remained statistically significant ($p<0.05$ from a cluster-forming threshold of $Z>2.31$) after family-wise error correction for multiple comparisons are reported here.
Results: During hypoglycaemia, autonomic symptom scores increased significantly (mean symptom score±SD, euglycaemia 12.39±4.97, hypoglycaemia 21.75±8.44 $p<0.001$). A significant increase in CBF occurred in the left thalamus (a brain region associated with arousal, vigilance and recognition of stressful stimuli), right inferior orbitofrontal cortex and dorsolateral prefrontal cortex (associated with executive function, decision making and complex motivation and reward pathways). Significant decreases were detected in the right hippocampus and superior temporal cortex bilaterally (regions associated with memory and processing sensory input).
Conclusion: The cerebral response to acute hypoglycaemia reflects the activation of stress pathways but also encompasses brain regions involved in reward salience, executive function and memory. Recruitment of these higher cortical areas suggest their involvement in the behavioural response to hypoglycaemia. These networks may also influence the response to the repeat stimulus of future hypoglycaemia. Their investigation in people with type 1 diabetes and different degrees of hypoglycaemia awareness and risk will inform future therapies to reduce severe hypoglycaemia.
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Hypoglycaemia and QTC prolongation in patients with type 2 diabetes

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Background and aims: Hypoglycaemia has been associated with increased risk of death, mainly attributed to lethal arrhythmias and QTc prolongation, both in Type 1 and Type 2 diabetes (T2D) patients. Very few studies have been performed under real-life conditions though. The aim of the present study was to examine the relationship between hypoglycaemic episodes (examined during continuous glucose monitoring subcutaneously [CGMS]) and QTc prolongation (measured by continuous ECG monitoring) in patients with T2D, during routine every-day life.
Materials and methods: A total of 40 (50% males) patients with T2D (mean age [±SD]: 68.3±6.9 years, HbA1c: 7.45±0.91%, diabetes duration 17.7±8.7 years, BMI: 29.9±1.3 kg/m2, treated with insulin with or without oral hypoglycaemic medications) were studied with simultaneous CGMS and 24-hour ECG monitoring. Hypoglycaemia was defined as an episode of blood glucose <70 mg/dl (3.9 mmol/L), lasting for ≥20 minutes. The mean QTc during these episodes was compared with the mean QTc during the period preceding the episode. Hypoglycaemia during day- and night-time was also evaluated separately.
Results: A total of 26 non-severe hypoglycaemic episodes were recorded in 16 patients. Half of these episodes occurred during the night. Mean QTc during hypoglycaemia was significantly longer than during normoglycaemia (443.38±51.38 vs. 424.46±61.49 msec, $p=0.012$). Interestingly, only the night-time hypoglycaemic QTc differed significantly from the preceding period's one (459.62±55.65 vs. 417.62±86.13 msec, $p=0.003$), while, during the day-time, no significant difference was observed (427.15±42.81 vs. 431.31±18.91 msec, $p=0.58$)
Conclusion: Hypoglycaemia in patients with T2D is associated with prolongation of the QTc interval, especially during the night, and may contribute to the increased adverse outcomes seen with intensive treatment of the disease.

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Hypoglycaemia reduces cognitive performance and affects cerebral blood flow in subjects with type 1 diabetes

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Background and aims: This randomised single-blinded, two-period cross-over trial investigated cognitive performance and associated regional cerebral blood flow (rCBF) during hypoglycaemia.
Materials and methods: Subjects (n=26) with type 1 diabetes underwent a hypoglycaemic and euglycaemic clamp (target plasma glucose 2.8±0.2 mmol/L and 5.5±0.55 mmol/L, respectively). Cognitive performance (working memory) was assessed by a modified digit symbol substitution test (DSST), adjusted for sight and finger movements (control DSST [cDSST]), at both visits. During DSST and cDSST, rCBF was measured in 19 pre-specified brain regions using ^{15}O-labelled water positron emission tomography. DSST scores and response time were analysed using a linear mixed-effect model with condition and period as fixed factors and subject as a random factor. All rCBF values were normalised to grey matter and analysed using an ANOVA with condition, period and subject as fixed factors.
Results: The DSST score was significantly lower (estimated treatment difference [ETD]: –0.63 [–1.13; –0.14], p=0.014) and the response time significantly longer (ETD: 2.86 s [0.67; 5.05], p=0.013) during hypoglycaemia vs euglycaemia. During DSST, rCBF was significantly decreased ($p<0.05$) in medial temporal lobe (MTL) and hippocampus, and increased ($p<0.05$) in dorsolateral prefrontal cortex (DPC), middle and inferior frontal gyrus (MFG/IFG), superior parietal lobe (SPL) and thalamus during hypoglycaemia vs euglycaemia. During cDSST, rCBF was significantly decreased ($p<0.05$) in hippocampus, MTL, parahippocampal gyrus and striatum, and increased ($p<0.05$) in DPC, IFG, MFG, orbitofrontal cortex, superior frontal gyrus, ventromedial prefrontal cortex, SPL and thalamus during hypoglycaemia vs euglycaemia. When performing the DSST adjusted for cDSST (DSST-cDSST), rCBF was relatively increased in the striatum during hypoglycaemia vs euglycaemia.
Conclusion: Hypoglycaemia reduced cognitive performance, accompanied by significant changes of rCBF, indicating raised activity in the attentional mode network at the expense of the default mode network.
Clinical Trial Registration Number: NCT01789593
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PS 081 Type 1 diabetes: new clinical insights

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'It makes a difference, coming here': a qualitative exploration of clinic attendance among young adults with type 1 diabetes

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Background and aims: Young adulthood is a time of challenges and risks for people with type 1 diabetes. Thus, appropriate support and education from a diabetes clinic is of particular importance. However, poor outpatient clinic attendance is common among young adults with type 1 diabetes. A significant gap exists in the research related to clinic non-attendance among young adults. The aim of this qualitative study was to develop a theory explaining attendance at a hospital-based diabetes clinic.
Materials and methods: Using a grounded theory methodology, data were collected through semi-structured qualitative interviews. Twenty nine people (21 young adults with type 1 diabetes and 8 service providers) from one hospital-based diabetes clinic were interviewed. Interviews were recorded, transcribed and analysed according to the grounded theory methodology.
Results: Relationships between young adults and service providers is the core category of this theory. Collaborative relationships between young adults and service providers increased the perceived value of attendance and reduced the vulnerability of young adults to the barriers within the existing service, such as meeting unfamiliar service providers. Relationships between young adults and service providers were developed following opportunities for contact (e.g. experiencing a crisis of diabetes or attending a structured education programme), and facilitated engagement with the service and further attendance. Relationships developed outside clinic appointments due to clinic-related barriers such as short, impersonal consultations. Barriers to clinic attendance included young adults' negative perceptions of their self-management and a lack of value associated with attending. Young adults who were engaged with the service were more likely to attend, despite barriers to attendance, due to the knowledge and confidence they had that they would benefit from attending.
Conclusion: The diabetes clinic was described as an important and valued resource by young adults and service providers. Collaborative relationships between young adults and service providers enhanced service provision in this study. Young adults gain the motivation and confidence to engage with diabetes services by experiencing collaboration with service providers. According to the results of this study, clinic attendance may be improved by increasing opportunities for relationship development between service providers and young adults. A focus on relationship development may result in more effective use of limited clinic resources by increasing engagement and reducing non-attendance.
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Dysregulated IGF-system in type 1 diabetes on subcutaneous insulin therapy. Need for a change?

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Background and aims: Under physiological conditions insulin reaches the liver through the portal vein and the liver is exposed to a several fold higher insulin concentration than the rest of the body. In Type 1 diabetes (T1D) treated with subcutaneous insulin high IGFBP-1 and low IGF1 have been reported and this may be due to insufficient insulin delivery to the liver. Our aim was to characterize associations of IGFBP-1 and IGF1 levels to metabolic variables in a large material of patients with T1D of long duration in comparison to matched controls without diabetes.
Materials and methods: Patients diagnosed before 35 years of age, diabetes duration >20 years and not older than 65 years were invited to participate. Controls matched for age were obtained from a national population registry. IGF1 was measured by Immulite® and IGFBP1 by an ELISA. C-peptide was analyzed with a high sensitive ELISA. Blood samples were obtained after fasting overnight
Results: Altogether 773 patients and 708 controls participated. Mean age for the patients was 50.5±9.5 (±SD) and for controls 54.1±9.3. In spite of a long diabetes duration 80 of 578 patients were C-peptide positive and 30 had values>100 pmol/l. IGFBP-1 was 68.1 (36.0 - 159.0) μg/l (median; interquartile range) in T1D and 20.0 (11.0-34.0 in controls) $p<0.0001$ while IGF1 was 112±44 μg/l (mean ± SD) in T1D and 151±60 in controls $p<0.0001$.
IGFBP-1 was strongly associated with fasting P-glucose ($r=0.405$; $p<0.0001$) but not with HbA1c. No associations were found between P-glucose or HbA1c with IGF1. In Spearmans correlation there was a negative association between C-peptide and IGFBP-1 ($p=0.011$) and a positive with IGF1 ($p=0.001$).
Conclusion: There are pronounced changes in the IGF-system in T1D on subcutaneous insulin administration counteracted by persistent endogenous insulin secretion. Although the clinical consequences of the dysregulated IGF-system is still unclear the results raises the question of the possible advantages of intraperitoneal insulin administration.
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Predicting complications and long-term outcomes in type 1 diabetes: the PRIME Diabetes Model

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Background and aims: Health economic models are important for informing healthcare policy that directly affects patients with diabetes. The utility of a model is inherently linked to its accuracy and representativeness. To date, several models of type 1 diabetes mellitus (T1DM) have been published, but often rely on historical data and/or data derived from populations without T1DM. Recent publications describing long-term follow-up from landmark trials and diabetes registries represent an opportunity to revisit T1DM modeling options. Our objective was to develop a new, product-independent model of T1DM, using data from T1DM populations to estimate complication risk, capable of predicting long-term clinical and cost outcomes.
Materials and methods: Following a systematic literature review to identify large-scale clinical trials in populations with T1DM, a model was developed (the PRIME Diabetes Model) to simulate disease progression and the risk of complications. Written in Java and externally audited, the model is structured such that individual, simulated patients are followed over time analogous to a clinical trial. At the start of a simulation, patient characteristics are assigned using a covariance matrix to ensure realistic risk profiles. Each patient is then passed to individual controllers to evaluate the risk of complications (myocardial infarction, stroke, angina, heart failure, nephropathy, retinopathy, macular edema, neuropathy, amputation, hypoglycemia and ketoacidosis), mortality and progression of risk factors such as glycated hemoglobin (HbA1c). Several approaches novel to T1DM modeling are utilized, including covariance of patient characteristics and risk factors, an HbA1c progression model derived from patient-level data, a weighted model averaging approach to estimate cardiovascular risk, and model combination to more accurately assess microvascular complication risk.
Results: Validation analysis comparing outcomes predicted by the model with those of clinical studies has shown that the PRIME Diabetes Model can project long-term patient outcomes that are consistent with those reported for a number of long-term studies in T1DM. Macrovascular endpoint data have been reliably reproduced from the EURODIAB study, the FinnDiane Registry, the Diabetes Control and Complications Trial (DCCT), the Swedish National Diabetes Registry (SNDR), and Wisconsin Epidemiologic Study of Diabetic Retinopathy study (WESDR). Similarly, macrovascular complication risk is accurately reflected in the model based on comparisons with the DCCT and the Epidemiology of Diabetes and its Complications (EDIC) study, the SNDR, EURODIAB and WESDR.
Conclusion: The PRIME Diabetes Model is product-independent, available online for academic use and has been developed in line with good practice guidelines. Validation has indicated that outcomes from long-term T1DM trials can be reliably reproduced. The model offers new approaches to long-standing challenges in T1DM modeling and may be a valuable tool for informing healthcare policy in T1DM.
Supported by: Eli Lilly

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Diet management, lifestyle factors and education needs by target attainment in Italian youth with type 1 diabetes from the Global TEENs study

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Background and aims: TEENs is an international, cross-sectional observational study, conducted in 20 countries in order to assess T1D management and psychosocial parameters in 8-25-year-olds (y/o). Data on diet management, lifestyle factors and education needs by target HbA1c attainment from the Italian cohort are reported.
Materials and methods: Data were collected at 23 centres by participant interview, medical record review and participant/parent survey from 1, 009 Italian youth (46% female) in three age groups: 8-12 y/o (n=330), 13-18 y/o (n=490), and 19-25 y/o (n=189). HbA1c was measured uniformly using A1cNow™ with target HbA1c defined as <7.5% (58 mmol/mol) for ≤18 y/o (ISPAD) and <7% (53 mmol/mol) for >18 y/o (ADA).
Results: Overall, 40% of participants met HbA1c targets. Measuring food intake based on experience was the most common method used by all age groups, followed by carbohydrate counting (Table). Of the participants who used carbohydrate counting, a higher percentage met target HbA1c than did not in all age groups, with a significant effect on target

attainment due to carbohydrate counting compared with other methods observed in 13-18 y/o (p=0.035). Avoiding sugars was the least common method used in all age groups. Across all age groups, participants who did not undertake any exercise were numerically less likely to reach HbA1c target; on the contrary, participants who exercised 1-2 days/week were numerically more likely to reach HbA1c target (Table). Performing exercise had a significant effect on target HbA1c attainment in 8-12 y/o (p= 0.012). The majority of participants were in the underweight/normal body mass index (BMI) category in all age groups, with no clear pattern between BMI class and the proportion of patients reaching HbA1c target. Participants of all ages commonly requested education on diet, carbohydrate counting, how to manage T1D during illness, and how to manage blood glucose levels with exercise.

Conclusion: Carbohydrate counting and exercising at least twice per week help to attain HbA1c target across all age groups. Assessment of lifestyle factors suggests that efforts targeting carbohydrate counting and exercise could promote successful health outcomes and help more patients with T1D to reach the recommended HbA1c target.

Table: Diet management and lifestyle factors by age and HbA_{1c} target attainment

	8–12 y/o		13–18 y/o		19–25 y/o	
	HbA_{1c} target met n=139 (42%)	HbA_{1c} target **not** met n=191 (58%)	HbA_{1c} target met n=203 (41%)	HbA_{1c} target **not** met n=287 (59%)	HbA_{1c} target met n=59 (31%)	HbA_{1c} target **not** met n=130 (69%)
Method used to measure food intake, %*						
Carbohydrate counting	32	24	28	21	19	15
Carbohydrate exchanges	7.2	7.9	11	9.8	3.4	5.4
Weighing/measuring	6.5	9.9	4.9	6.6	5.1	6.2
Based on experience	50	52	48	58	70	70
Avoiding sugars	0.7	1.0	0.0	1.0	0.0	0.0
Number of days per week spent exercising†, %						
0	5.9	12	13	18	21	27
1–2	42	34	34	27	33	28
3–4	44	36	35	34	25	25
5–7	8.1	17	18	20	21	19
Body mass index, %						
Overweight/obese‡	37	34	26	28	41	35

*Columns may not add up to 100% due to missing responses; only one response per participant was allowed

†At least 30 minutes doing any physical activity or exercise

‡BMI-for-age Z-score in classes were used for 8–18 y/o: overweight, >+1 SD to ≤+2 SD; obese, >+2 SD. BMI in classes was used for 19–25 y/o: overweight, >25 kg/m² to ≤20 kg/m²; obese, >30 kg/m²

BMI, body mass index; SD, standard deviation

Supported by: Sanofi

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Freedom for life: 13 years of experience from an accredited patients' education course for type 1 diabetes. Review of long-term outcomes

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Background and aims: The Freedom for Life course is the accredited patients' education course for type 1 diabetes in the catchment area of Bath, UK. It is similar in principle to the DAFNE (Dose Adjustment For Normal Eating) course but with a different structure. It consists of one session a week (3 hours) for five weeks with follow up at 3 months, 6 months and 1 year (2 hours). The aim of our work was to review the long-term outcomes of the course 12 years after its introduction in eleven centres in the Bath catchment area.

Materials and methods: Using the hospital's research diabetes database (DIAMOND), records of 211 adults with Type 1 diabetes (65.5% female) were examined (baseline mean ± SD: age 45.28±12.94 years,duration of diabetes 19.29±13.21 years). Biomedical data for HbA1c and body mass index (BMI) were collected before and then every 12 months after patients completed the education. Additionally, data for proportion of patients progressing to insulin pump were collected.

Results: At one year follow-up HbA1c had greatly decreased from 76± 15.7 to 66±12.3(p<0.001). The results for the years following the Freedom for Life course showed an improved HbA1c compared to the baseline: 67±13.6 at 24 months, 69±13.6 at 36 months, 70±12.1 at 48 months, 71±13.2 at 60 months, 71±10 at 72 months, 72±12.3 at 84 months, 72±11.4 at 96 months and 73±13.4 at 108 months. Further review of the outcomes excluding patients who had insulin pump therapy revealed a significant reduction in HbA1c which remained statistically significant for 6 years following the course. The mean BMI had not significantly changed before (24.35) and after (25.11) the course. Lastly 47 patients (22.27%) progressed to insulin pump with a mean interval of 2.25 years between the course and the initiation of insulin pump treatment.

Conclusion: The impact of a single Freedom for Life course on glycaemic control remains apparent in the long term. The improvement in the HbA1c at 108 months compared to the baseline one remained significant (76±15 to 71±13.4 of 6.57%) even though it had deteriorated from 12 months (66±12.3 or 13.15%). Analysis of the outcomes for patients who didn't have insulin pump revealed that the reduction in HbA1c remains significant for 6 years after the course. 22.3% of the patients who participated in the Freedom for Life course progressed to insulin pump treatment with a mean interval of 2.25 years. The effect of a single Freedom for Life course on glycaemic control seems to be superior to the outcomes of the other educational programmes for people with type 1 DM (both the traditional programmes and the DAFNE 5×1 day trial). This is likely due to the fact that the Freedom for Life course offers follow-up sessions at 3, 6 and 12 months. Repeat training sessions have been shown to have better outcomes in educational trials and other chronic health conditions and also lead to a better relationship between the patients and healthcare professionals.

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Predictors of hospital admission in patients with type 1 diabetes following participation in the structured education programme, Dose Adjustment For Normal Eating (DAFNE)

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Background and aims: Diabetes-related hospital admissions are not only costly to the NHS in the UK but are distressing for patients involved. Structured education programmes such as DAFNE encourage self-management and participation has been shown to reduce hospital admissions post-course. However, there continues to be some patients who are admitted despite training. We aimed to determine the rate of hospital admissions in patients with type 1 diabetes prior to and post DAFNE and examine predictors for admission post course.

Materials and methods: Using clinical case records from the PIC patient database, anonymised data from 99 adults with type 1 diabetes (T1D) who participated in DAFNE between January 2012 and September 2013, was collected and examined. Records were reviewed for evidence of hospital admission for severe hypoglycaemia, hyperglycaemia and diabetic ketoacidosis in the 12 months prior to and 12 months following course participation. Case notes were reviewed for those admitted to hospital and follow-up attendance recorded. HbA1c results for all were recorded 1 year before and after DAFNE training.

Results: In the year before the course, 14 patients were admitted to hospital with a total number of 15 admissions. In the year following DAFNE, 5 patients were admitted with a total of 6 admissions. Of the 94 avoiding admission in the year following DAFNE, 83 attended at least one DAFNE review or diabetes clinic in this time (88% attendance) compared to 2 patients (40%, p<0.05) for those admitted. Evidence of poor blood glucose monitoring was recorded in the notes for 5 of the 5 patients (100%) and HbA1c values increased in 3 of the 5 (60%).

Conclusion: As has been previously demonstrated, we show that hospital admissions for severe hypoglycaemia and diabetic ketoacidosis are

reduced in the year following the DAFNE structured education course. A predictive factor reported for ketoacidosis is a higher HbA1c concentration and our findings are consistent with this. In addition, we also demonstrate that lack of engagement with diabetes services is a further predictor of hospital admission in the year following the course. We suggest that patients such as these could be identified, monitored more closely and offered extra support to improve outcomes following programmes such as DAFNE. Whilst some participants will benefit from group follow-up, these results and indeed, previous studies suggest that there is a need for ongoing individualised support and follow-up. In this way, DAFNE principles are more likely to be reinforced and maintained, and this in turn contributing to reducing hospital admissions, emergency treatment costs, and improving psychosocial outcomes for patients with type 1 diabetes. Structured education remains an important part of the management of type 1 diabetes and is recommended for all adults with the condition. Gaining a more thorough understanding of what factors influence adherence to DAFNE principles will allow for more focused care.

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Partial remission period based on insulin dose-adjusted HbA_{1c} in 3, 661 German and Austrian children and adolescents with type 1 diabetes

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Background and aims: Occurrence of partial remission (PR) period shortly after onset of type 1 diabetes (T1D) might be due to amelioration of insulin sensitivity, but also due to a temporary restoration of pancreatic beta cells affecting insulin dosage. Insulin dose adjusted A1C (IDAA1C) combines required insulin dosage with HbA1c and correlates with stimulated c-peptide. Applying IDAA1c, we studied PR in a large group of children and adolescents with T1D.
Materials and methods: Data of 3,661 patients with T1D (<18 years at diabetes onset, continuous follow-up) from the DPV (Diabetespatienten Verlaufsdokumentation) database were analysed. IDAA1C was calculated as HbA1c (%) + 4 × [insulin dosage (Units per kilogram bodyweight per day)] at 1, 3, 6, 12, 18, 24, 36, 48, 60 and 72 months after T1D onset. We evaluated duration of PR (IDAA1c≤9, based on the first period without and the last period with PR) using Kruskal-Wallis tests and hierarchical linear regression models with random effects accounting for differences between treatment centres.
Results: Median PR period was 9 (0;21) months. After 3 months, 61% of patients had PR. In 4.7% of patients, PR even lasted until 72 months after onset. Median PR duration was lowest in patients aged<5 years at T1D onset (4.5 (0;30) months), whereas it was highest in patients older than 15 years at T1D onset (21 (9;72) months). In those>15 years, 21% still had PR 5 years after T1D onset. Duration of PR showed significant negative correlations with initial HbA1c (R=-0.15, p=<0.001), positivity for islet-autoantibodies at T1D onset (R=-0.06, p=0.003) and year of diagnosis (R=-0.05, p=0.004). Duration of PR was longer in boys than in girls (9 (2;21) vs. 4.5 (0;15) months, p<0.001). This difference remained significant after adjustment for age, HbA1c, ketoacidosis, auto-antibody-positivity at T1D onset and year of diagnosis (p=0.01). Subsequently, linear regression revealed that age at T1D onset>15 years, lower initial HbA1c, and negativity for islet-auto-antibodies were significantly associated with longer duration of PR. Though patients with diabetic ketoacidosis at T1D onset had a significantly shorter PR than patients without, this difference did not remain significant after adjustment.
Conclusion: As IDAA1c does not discriminate between insulin sensitivity and insulin secretion, available data did not allow resolving whether the sex-difference in PR duration reflects an innate higher insulin resistance in girls, or a better beta-cell recovery in boys. Hence, the weighting of HbA1c in the IDAA1c definition could cause underreporting of PR duration in girls. Yet, ketoacidosis at T1D onset, which is strongly associated with initial HbA1c and has been discussed as a main risk factor for a short PR, does not show statistical significance in our regression model with adjustment for age and gender. Further research is needed to clarify the usefulness and performance of IDAA1c in clinical practice.

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Understanding patient and clinician glucose reporting preferences in type 1 diabetes: Ambulatory Glucose Profile (AGP)

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Background and aims: A lack of a standardized glucose report and streamlined data acquisition across glucose monitoring devices has created frustration in clinics and reduced the use of glucose data in clinical encounters. Device specific reports are unique to each glucose monitoring device, are complex and not able to incorporate devices from different manufacturers into a single view. The AGP is simple one page report with 3 components: glucose metrics, graphic summary visualization of a 2 week glucose profile and a calendar view the glucose profiles from each of the days making up the summary profile view. The purpose of this novel research is to evaluate both patient (aim 1) and clinician (aim 3) utility and preference of streamlined standardized glucose reporting using Ambulatory Glucose Profile (AGP) reports. The first two sites participated in Time in Motion (TIM) evaluation as well as work flow mapping to determine an idealized glucose reporting work flow (aim 2).
Materials and methods: Seven geographically and demographically diverse clinics with large patient populations with type 1 diabetes were selected. Twenty patients per clinic and up to four clinicians per clinic utilized a device download station to create a standardized SMBG or CGM AGP report. Both the patients and clinicians reviewed the report then responded to a survey about the AGP reports utility and their preferences when compared with their usual glucose reports.
Results: Patients or their parents have reported a significantly higher preference for the AGP report than logbooks, device specific reports or from device displays (see Table). Patients and parents report significantly higher amounts of information, better information to support health routine changes, better understanding of their glucose patterns, more confidence in managing their glucose levels and seeing new trends from the AGP (see Table). Clinicians reported significantly higher preference of the AGP over other methods for seeing glucose trends and helping to educate patients about trends. TIM portions of the study showed significant savings in staff time (on average 4 to 15 minutes) per patient from this streamlined reporting system.
Conclusion: This novel approach to glucose reporting has the ability to save staff time, improve patient and clinician interactions through shared understanding of the patient's glucose profile and lead to more in depth individualized support for medication adjustment and health related behavior changes. Compared to the use of an HbA1c and inconsistent patient reporting of adverse glucose related events, the standardized use of a one page comprehensive glucose report (AGP) resulted in more personalized clinical decision making and significantly improved clinic workflow.

	Handwritten log book	Normal device report from computer	Looking at your device display/ memory	captūrAGP Report
I prefer to use...				
CGM	1.7%	16.9%	49.2%	64.4%
SMBG	2.0%	18.0%	42.0%	60.0%
I get more information from...				
CGM	**0.0%**	**5.1%**	**18.6%**	**89.8%**
SMBG	**2.1%**	**18.8%**	**16.7%**	**79.2%**
Helps me change my health routines *(eating right, exercise, sleep)*...				
CGM	3.5%	10.5%	29.8%	71.9%
SMBG	4.0%	12.0%	28.0%	68.0%
Helps me understand when to take my insulin and how much to take...				
CGM	**0.0%**	**17.0%**	**50.9%**	**39.6%**
SMBG	**2.0%**	**4.1%**	**59.2%**	**44.9%**
Helps remind me to test more often...				
CGM	1.9%	9.3%	53.7%	40.7%
SMBG	0.0%	6.4%	34.0%	61.7%
Helps me prevent low glucose levels (hypoglycemia)...				
CGM	1.7%	5.1%	54.2%	50.8%
SMBG	0.0%	6.3%	47.9%	56.3%
Helps me understand my glucose trends/ patterns (high, low and in range)...				
CGM	0.0%	6.8%	15.3%	93.2%
SMBG	2.0%	10.0%	24.0%	76.0%

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Where do SGLT2 inhibitors fit in treating my patients with diabetes? Effect of online CME and need for further education

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Background and aims: Successful implementation of new standards of care begins with a thorough understanding of the mechanisms of action of newer agents, and where these agents fit into modern type 2 diabetes (T2D) treatment algorithms. We sought to determine if online continuing medical education (CME) could improve the clinical knowledge and competence of primary care physicians (PCPs) regarding the use of SGLT2 inhibitors in T2D management.

Materials and methods: The effect of two educational interventions on the role of the kidney and SGLT2 in the treatment of T2D was analyzed to determine efficacy of online education presented in the form of a video lecture and interactive case-based text activity. The activities launched online in November or December, 2014 and data were collected through January, 2015. The effects of education were assessed using knowledge- and case-based matched pre-assessment/post-assessments. The effect size was calculated with Cohen's d (>0.8 are large, 0.8-0.4 are medium, and< 0.4 are small).

Results: In total, 1566 PCPs participated. Significant overall improvements were seen for both the video lecture (n=455; large effect d=0.98) and case-based text activity (n=1111; large effect d=1.238). Specific areas of improvements seen include:

•Compared with baseline, 43% more PCPs ($P<.0005$) correctly identified the rationale for glomerular filtration rate (GFR) limits when considering use of SGLT2 inhibitors

•Compared with baseline, 33% more pulmonologists ($P<.05$) correctly identified a strategy for combating hypotension in patients on SGLT2 inhibitor therapy

•In a patient with T2D, more physicians appropriately identified the major pathological contributor to the patient's T2D after the education (47% increase, $P<.05$)

•In a patient with T2D who is starting on an SGLT2 inhibitor, more physicians appropriately educated the patient on genital mycotic infections as the most common side effect associated with this class of antihyperglycemic agents (44% increase, $P<.05$)

Conclusion: This study demonstrates the success of a targeted educational intervention with multiple educational components (serial learning) on improving knowledge and competence of PCPs regarding the use of SGLT2 inhibitors in the treatment of T2D. Additional studies are needed to assess if improved knowledge and competence translates into improved appropriate use of these therapeutic options.

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PS 082 Perceptions, communications and patient outcomes in diabetes care

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Change in HbA_{1c} associated with adherence to newly initiated metformin therapy

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Background and aims: Adherence to newly prescribed anti-hyperglycemic agents is essential for the attainment of glycemic goals but the association between changes in adherence and glycemic control has not been reported. We studied the association between metformin adherence and change in A1C from pre- to post-treatment levels, and the association between change in adherence and change between 2 post-treatment A1C measures.

Materials and methods: First, we analyzed 2,844 patients who newly initiated metformin monotherapy to estimate the effect of adherence on change in A1C that occurred as a result of initiating metformin. A1C change was calculated as the difference between pre-metformin A1C and the first A1C measured 6-12 months following their first metformin dispense. We used a modification of the proportion of days covered (PDC) method to estimate adherence over the 90-day period preceding each A1C to produce a "biologic response based" PDC (BRB-PDC). We categorized BRB-PDC into 4 levels: 0% (no refill of initial dispense), 1-49%, 50-79%, and ≥80%. Change in A1C was modeled as a function of BRB-PDC category. Next, we evaluated change in A1C among 2,416 patients who had at least two post-metformin A1C values. Change in A1C was calculated as the difference between the first and last post-treatment values. We estimated BRB-PDC for each A1C value and modeled change in A1C as a function of change in BRB-PDC category. Analyses were adjusted for age, sex, the first A1C value used in the relevant change calculation, and metformin dose.

Results: There was a graded relationship between adherence and change in A1C following metformin initiation. Relative to the non-adherent group, BRB-PDC 50-79% was associated with a change of -0.45% (95% CI -0.65, -0.26; p<0.001), and BRB-PDC ≥80% was associated with an A1C change of -0.73% (-0.90, -0.55; p<0.001). In addition, change in BRB-PDC was associated with a significant change in A1C. Among patients with at least some initial adherence (1-79%), change to total non-adherence (BRB-PDC=0%) at the 2nd A1C measure was associated with 0.25% (0.07, 0.42; p=0.005) increase in A1C, while change from some to full adherence (BRB-PDC ≥80%) was associated with an A1C decrease of 0.15% (-0.28, -0.02; p=0.027). These results were magnified when limited to the 861 patients whose 1st A1C was ≥7%. Change from some to no adherence was associated with an A1C increase of 0.63% (0.27, 0.99; p<0.001) while change from some to full adherence was associated with an A1C decrease of 0.40% (-0.67, -0.14; p=0.003).

Conclusion: Estimating adherence over a biologically relevant time period produces a stronger association with glycemic control than has been previously reported. Changes in adherence were inversely associated with changes in glycemic control and were especially important among patients not at optimal glycemic goal.

	Change in A1C (95% CI)	P Value
First BRB-PDC (n=2,844)		
0% (n=204)	Reference	--
1% - 49% (n=216)	-0.08 (-0.31, 0.15)	0.498
50% - 79% (n=534)	-0.45 (-0.65, -0.26)	<0.001
≥ 80% (n=1890)	-0.73 (-0.90, -0.55)	<0.001
Change from 1st to Last BRB-PDC (At least 2 measures, n=2,416)		
No Change (n=1,487)	Reference	--
0% → 1-79% (n=47)	0.07 (-0.22, 0.37)	0.646
0% → ≥ 80% (n=40)	0.03 (-0.29, 0.35)	0.872
1-79% → 0% (n=135)	0.25 (0.07, 0.42)	0.005
1-79% → ≥ 80% (n=250)	-0.15 (-0.28, -0.02)	0.027
≥ 80% → 1-79% (n=293)	-0.03 (-0.15, 0.10)	0.683
≥ 80% → 0% (n=164)	-0.01 (-0.17, 0.15)	0.871
Change from 1st to Last BRB-PDC (Not in Glycemic Control, n=861)		
No Change (n=497)	Reference	--
0% → 1-79% (n=32)	-0.18 (-0.67, 0.30)	0.458
0% → ≥ 80% (n=28)	-0.38 (-0.89, 0.13)	0.141
1-79% → 0% (n=51)	0.63 (0.27, 0.99)	<0.001
1-79% → ≥ 80% (n=105)	-0.40 (-0.67, -0.14)	0.003
≥ 80% → 1-79% (n=107)	-0.06 (-0.32, 0.20)	0.642
≥ 80% → 0% (n=41)	0.05 (-0.34, 0.45)	0.795

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Perceptions of diabetes control among physicians and patients with uncontrolled type 2 diabetes using basal insulin

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Background and aims: While clinical definitions of diabetes control are well established, there is limited understanding of how patients perceive control and whether it differs from perceptions of control among physicians. The purpose of the study was to investigate how physicians and patients with uncontrolled type 2 diabetes (T2D) using basal insulin perceive diabetes control.

Materials and methods: A web survey of 1,012 adults with uncontrolled T2D (physician confirmed HbA1c>8% (64 mmol/mol)) and treated with basal insulin and 300 physicians was conducted in Sweden (n=240 patients and 100 physicians), Switzerland (n=152 patients and 100 physicians), and the UK (n=620 patients and 100 physicians).

Results: Analyses revealed significant differences between physicians and patients with uncontrolled T2D in their perceptions of control. In defining control, physicians were significantly more likely than patients to indicate that HbA1c (85% vs. 79%, p<.05), complications from diabetes (89% vs. 75%, p<.001), and frequency/severity of hypoglycaemia (93% vs. 69%, p<.001) were very/extremely important for deciding whether or not diabetes is well-controlled. Patients, on the other hand, were significantly more likely than physicians to report that a wide range of other factors were very/extremely important, including energy levels (75% vs. 33%, p<.001), insulin units/day (78% vs. 29%, p<.001), how

predictable life is (72% vs. 29%, p<.001), and how much one has to think about diabetes (68% vs. 31%, p<.001). The time period for thinking about control also differed; physicians were significantly more likely than patients to think about the last three months (60% vs. 19%, p<.001) and less likely than patients to consider the last week or more recently (7% vs. 51%, p<.001). Patients also saw more obstacles making control very/extremely difficult compared to physicians, including stress (75% vs. 54%, p<.001), medicine side effects (70% vs. 56%, p<.001), other health issues (71% vs. 45%, p<.001), family obligations (61% vs. 33%, p <.001), and lack of patient support groups (56% vs. 11%, p<.001). Patients were significantly more likely than physicians to see uncontrolled diabetes as very/extremely interfering in different aspects of their lives, including general health (70% vs. 51%, p<.001), energy level (71% vs. 36%, p<.001), mood/emotions (63% vs. 33%, p<.001), how much one accomplishes during the day (62% vs. 23%, p<.001), keeping appointments/commitments (63% vs. 17%, p<.001), making plans (62% vs. 16%, p<.001), completing daily chores (60% vs. 21%, p <.001), and family responsibilities (60% vs. 18%, p<.001).

Conclusion: Results revealed a significant disconnect between physicians and patients with uncontrolled T2D in their perceptions of diabetes control, including how they define control, obstacles to control, and the impact of uncontrolled T2D. While physicians generally expressed a more focused and clinical view of diabetes control, patients had a much broader view, considering many different factors in their perceptions of control. The findings suggest that educating physicians about how patients' perceptions of control may differ from theirs could benefit physician/patient communication and improve diabetes management.

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Discussing the need for additional oral drugs in type 2 diabetes: link between perceived physician empathy, patient bargaining and self-reported outcomes in the IntroDia™ study

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Background and aims: IntroDia™ is investigating physician-patient communication during early treatment of type 2 diabetes (T2D); at diagnosis and at the time, often months or years post-diagnosis, when an additional medication is added ("add-on"). Here we examine the issue of patient bargaining (to delay additional medication) and its association with self-reported outcomes.

Materials and methods: As part of IntroDia™, 4235 T2D patients from 26 countries (median age, 46 years; male gender, 52%) were surveyed about their experience with their physician during oral medication add-on conversations (which had occurred, on average, 7.4 months ago). Patients were asked whether, during these conversations, they tried to convince their physician to postpone the additional medication. Perceived physician empathy (PPE) (using elements from CAHPS, TIPS and IPC) was measured as well as current psychosocial status (DDS and WHO-5) and self-care behaviours (SDSCA).

Results: At add-on, 80% of patients did not bargain (DNB), while 20% did bargain (DB). Of note, within the DB group, 60% of patients managed to delay initiating additional medication, while 40% did not. There were no clinically relevant differences between DNBs and DBs on key demographic variables. The mean PPE score (scale 1=poor to 4=high [SD]) was significantly higher (p<0.001) for DNBs vs. DBs (3.25 [0.71] vs. 3.01 [0.68]). DNBs were significantly less distressed by diabetes (DDS emotional score 2.63) than DBs (3.45) (p<0.001). In addition, well-being (WHO-5: 63% vs. 54%) and self-care scores (SDSCA general diet: 4.8 vs. 4.1; SDSCA medication: 5.9 vs. 4.8) were significantly higher for DNBs vs. DBs, respectively (in all cases, p<0.001).

Conclusion: These data show that at least 20% of patients are reluctant to start an additional medication and actively bargain with their physicians to avoid doing so. Those who bargain report poorer PPE, greater diabetes distress, and poorer well-being and self-care than those who do not bargain.

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An informed shared decision making programme on the prevention of myocardial infarction for patients with type 2 diabetes: randomised, controlled trial

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Background and aims: EASD/ADA and the German national guideline committee explicitly recommend the implementation of shared decision making (SDM) in diabetes care. SDM is a complex approach requiring evidence-based patient information and health professionals willing and capable to share decisions with patients. We have developed an informed shared decision making (ISDM) programme on the prevention of myocardial infarction. Diabetes educators teach patients to understand risk information including probabilities of benefits and harms of preventive options, and to set individual treatment goals. We evaluated the efficacy of this programme within a single centre study in the setting of an outpatient diabetes clinic in Germany.

Materials and methods: Patients with type 2 diabetes, 40 to 69 years, without diagnosis of ischemic heart disease or stroke were randomly assigned to the ISDM programme or control intervention. Follow-up was 6 months. The ISDM programme consisted of a patient decision aid, a 90 minutes teaching session provided by diabetes educators, and provider training. The control intervention was structurally identical but addressed lifestyle topics. Primary endpoint was the level of risk knowledge after teaching when patients define their individual treatment goals. Secondary outcomes were sufficient risk knowledge, realistic expectations, prioritisation of treatment goals, knowledge at follow-up, and achievement of treatment goals. Analyses were on intention-to-treat basis. ISDM sessions were video-taped to monitor intervention fidelity.

Results: A total of 154 patients (mean HbA1c 7.0%, blood pressure 145/82 mmHg) were randomised; 72/77 patients in the ISDM group, and 71/77 in the control group attended the teaching sessions and completed the knowledge test (score 0-12). Mean score was 8.25 for ISDM, and 2.62 for the control group (difference 5.63 [95% CI 4.82 to 6.44]; p<0.001). Sufficient risk knowledge (9 correct answers) was achieved by 35/72 patients in ISDM, but no one in the control group. Mean score of realistic expectations (six questions) was 4.51 for ISDM, and 0.85 for the control group (difference 3.67 [95% CI 3.23 to 4.11]; p<0.001). In the ISDM group more patients prioritised blood pressure control (51.4% vs. 25.7%; p=0.002), whereas fewer prioritised glucose control (33.3% vs. 60%; p=0.002). At follow-up the risk knowledge score was 3.68 for ISDM (n=71) and 2.70 for controls (n=70) (difference 0.98 [95% CI 0.15 to 1.80]; p=0.021). More ISDM patients achieved their HbA1c goals (81.7% vs. 65.7%; p=0.036). There was no difference with respect to other treatment

goals. In both groups, most patients achieved their prioritised goals. Analysis of the video-taped ISDM teaching sessions documented that the curriculum was properly implemented.

Conclusion: This study shows that the informed and shared decision making programme is effective under high fidelity conditions. Patients in the ISDM group demonstrated better understanding of risk information, more realistic expectations, and more patients achieved individual HbA1c goals. Involvement of diabetes educators may be essential to implement SDM.

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Physician-patient communication at type 2 diabetes diagnosis and its links to physician empathy and patient outcomes: new results from the global IntroDia™ study

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Background and aims: Effective communication between physicians and type 2 diabetes (T2D) patients may improve patient self-care and outcomes. IntroDia™ is a global study investigating physician-patient communication at the time of T2D diagnosis and during early treatment, and the possible impact of early conversations on patient self-care and self-reported outcomes.

Materials and methods: As part of IntroDia™, 3628 T2D patients from 26 countries (48% male, median age 51 years) were surveyed about conversations with physicians at diagnosis. Parameters examined included the conversation's content (via a 43-item version of PACIC modified for T2D diagnosis), conversation quality (using CAHPS, TIPS and IPC scale items to ascertain perceived physician empathy [PPE]), current psychosocial status (WHO-5, DDS) and self-care behaviour (SDSCA).

Results: Using factor analysis, we identified 4 types of physician action - Collaborative (e.g. "Gave me choices about treatment to think about"), Encouraging (e.g. "Told me that a lot can be done to control my diabetes"), Discouraging (e.g. "Told me that diabetes gets harder…") and Recommending Other Resources (ROR) (e.g. "Referred me to a dietician, health educator, nurse or counselor"). PPE was positively associated with Collaborative (β=0.44, p<0.001) and Encouraging (β=1.69, p<0.001), negatively associated with Discouraging (β=-1.24, p<0.001) and unrelated to ROR (β=0.09, p=0.078). In turn, PPE was also linked to less diabetes distress (DDS: β=-0.37, p<0.001), greater well-being (WHO-5: β=0.39, p<0.001), and greater adherence to self-care behaviour (SDSCA - exercise: β=0.70, p<0.001; diet: β=1.09, p<0.001; medication taking: β=0.91, p<0.001).

Conclusion: Physicians' use of collaborative and encouraging conversation elements at T2D diagnosis may improve physicians' communication with patients, leading to better self-care and enhanced quality of life. Conversations using discouraging elements, however, may be counterproductive.

Supported by: Boehringer Ingelheim/Eli Lilly

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Physician-patient communication at prescription of an additional oral agent for type 2 diabetes: link between conversation elements, physician empathy and patient outcomes

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Background and aims: The global IntroDia™ study is investigating physician-patient communication during the early treatment of type 2 diabetes (T2D) and how such conversations may be linked to critical patient outcomes. Here we investigate patient experiences of the "add-on" moment, i.e. the consultation when, typically months or years after the T2D diagnosis, another oral agent is first prescribed, and how this conversation is associated with patient self-care and quality of life outcomes.

Materials and methods: A total of 4235 patients from 26 countries (52% male, median age 46 years) completed a self-report survey examining the content of the conversation with their physicians at add-on (via a 27-item scale of conversation elements specific for add-on). Perceived physician empathy (PPE) was measured with CAHPS, TIPS and IPC items. Current psychosocial status (WHO-5, DDS) and self-care behaviour (SDSCA) were also evaluated.

Results: Three key elements of the physician-patient conversation were identified by factor analysis - Collaborative (e.g. "Encouraged me to ask him/her questions"), Encouraging (e.g. "Told me that the new medication would improve my quality of life") and Discouraging (e.g. "Told me that my diabetes was getting worse"). PPE was positively associated with Collaborative (β=1.16, p<0.001) and Encouraging (β=1.39, p<0.001) and negatively linked with Discouraging (β=-0.92, p<0.001). Further, PPE was associated with less diabetes distress (DDS: β=-0.39, p<0.001), greater well-being (WHO-5: β=0.54, p<0.001) and better self-care (SDSCA - exercise: β=0.56, p<0.001; diet: β=0.94, p<0.001; medication taking: β=0.76, p<0.001).

Conclusion: Physician use of collaborative/encouraging conversation elements when prescribing an additional oral agent may enhance communication with T2D patients and lead to better self-care and quality of life. Conversely, discouraging elements may have the opposite effect.

Supported by: Boehringer Ingelheim/Eli Lilly

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Hypoglycaemia contributes to insulin discontinuation in US patients newly-initiated on basal insulin: a real-world analysis study

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Background and aims: Hypoglycaemia and fear of hypoglycaemia remain major barriers to persistence with insulin therapy among patients with type 2 diabetes mellitus (T2DM). This study examined if US patients with T2DM who experience hypoglycaemia soon after insulin initiation are at a greater risk of insulin discontinuation.

Materials and methods: This was a retrospective cohort study using claims data from the IMPACT™ medical and pharmacy claims database. Patients (aged≥18 years) with T2DM were identified if they initiated

basal insulin therapy (defined as having no prescription of basal insulin≥ 12 months before initiating insulin glargine, insulin detemir, or NPH insulin) between January 2007 and March 2013. Hypoglycaemic events were identified by health care encounters with an ICD-9-CM diagnosis code of 250.8× during the first 6 months of basal insulin use. Discontinuation was defined as gaps of longer than 45, 60, or 90 days in any insulin prescription coverage. A baseline was established using 12-months patient-level data prior to insulin initiation; follow-up periods of 12 and 24 months were considered. Data were adjusted for confounders using Cox regression analysis.

Results: Among 71,470 patients identified, 3,301 (4.6%) experienced hypoglycaemia within the first 6 months of insulin use. These patients were on average older than those without hypoglycaemia (56 vs 54 years; P<0.0001), and were more likely to discontinue insulin use within 12 months of initiation (45-day gap: 79% vs 74%; P<0.001). Data adjusted for confounders showed that patients with hypoglycaemia had a higher risk of discontinuation over 12 months (hazard ratio 1.16; 95% CI 1.03-1.32). Similar results were seen at 12- and 24-months follow-up, or when using prescription gaps of 60 or 90 days. Furthermore, patients who experienced hypoglycaemia, versus those who did not, were more likely to have a hospitalization (41% vs 23%) or an emergency department visit (56% vs 35%) (both P<0.001) during the first 12 months.

Conclusion: In conclusion, in a US setting, patients with T2DM who experienced hypoglycaemia within the first 6 months after initiating basal insulin use were more likely to discontinue insulin therapy and had greater annual health care use.

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PS 083 Health care delivery in diabetes

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Factors influencing remission phase in type 1 diabetic children

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Background and aims: To evaluate factors influencing the natural course and characteristics of the remission phase while residual beta cell function preservation is important.

Materials and methods: 120 patients with type 1 diabetes under the age of 18 enrolled in the study in 3 years period. Data were collected by reviewing the hospital records of patients from the time of diagnosis through the firs 24 hours after diagnosis. Duration of symptoms,history of prior infection, ketoasidosis at diagnosis,length of hospitalization, initial glucose level, basal C peptide levels at diagnosis and daily insulin requirement per kg/body weight and HbA1C in each visit were recorded.

Results: 68 patients (56.5%)entered partial remission.We observed similar remission rates in those aged 10 years at diagnosis and in boys and girls.History of infection and presentation with ketoasidosis were associated with a lower rate of remission(p<0.001,p<0.0001 respectively) and were commonly observed under the age of 10 years(p<0.001 and p< 0.0001 respectively). The average insulin requirements per kg at diagnosis decreased with increasing age(r:-0.31,p<0.012).The length of time until remission was 1.36+/-1.03 months and positively correlated with insulin requirements at discharge from the hospital(p<0.0001). Mean duration of remission was 11.67+/-5.82 months and was longer in boys than girls(p<0.05). 8 patients,all boys, entered total remission for 3.80+/-3.73 months. HbA1C levels were 7.31+/-1.24% in the first year in remission patients with respect to 8.24+/-1.47 in non remission patients(p< 0.05).

Conclusion: History of infection prior to presentation and diabetic ketoacidosis at diagnosis were associated with young age and were the most important factors negatively influencing the remission rate in newly diagnosed type 1 diabetics.Boys had a longer length of remission, and especially in puberty, had lower insulin requirement than girls. It is important to provide close follow up for patients in remission, since HbA1C levels may become elevated and remain unrecognised for a while in these patients when the remission phase is over.

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Management and mortality in very old patients with type 2 diabetes

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Background and aims: Less stringent treatment goals for intermediate outcome measures such as HbA1c are sometimes proposed for older patients with diabetes according to characteristics and health status of individual patients, but the appropriate ranges for the intermediate outcomes are still unclear. This study aimed to evaluate the management of very old patients with type 2 diabetes, and to explore associations of HbA1c, blood pressure (BP) and total cholesterol (TC) with all-cause mortality.

Materials and methods: A population-based cohort aged 80 years or older, diagnosed with type 2 diabetes, and registered at family practices in the UK Clinical Practice Research Datalink at 1 April 2011 was analysed. The intermediate outcomes were measured between 1 April 2011 and 31 March 2012. Patients were followed up for all-cause mortality from 1 April 2012 to death, the end of their record, or the last data collection date (31 March 2014). Associations of HbA1c, BP and TC with all-cause mortality were estimated in the Cox proportional hazards model, adjusting for age, sex, duration of diabetes, BMI, smoking status, history of coronary heart diseases and stroke, and medications including

antidiabetic, antihypertensive, lipid-lowering and antiplatelet drugs, and clustering by general practice.
Results: Data for 29,909 patients, including 16,739 (56%) of females, were analysed. During the 2-year follow-up period (median 1.8 years, IQR 1.3 to 1.9 years), there were 5,016 deaths (11.1 per 100 person-year). Mortality was higher for HbA1c <6.0% [42 mmol/mol] HR 1.33 (95% CI 1.18 to 1.50); 6.0-6.4% [42-47 mmol/mol] 1.16 (1.03 to 1.30); and ≥8.5% [69 mmol/mol] 1.49 (1.31 to 1.69); BP <130/80 mmHg 1.49 (1.38 to 1.60); and TC <4.0 mmol/L 1.20 (1.11 to 1.29). There was evidence of interaction between HbA1c and prescriptions of antidiabetic drugs (P for interaction <0.05), with low HbA1c only associated with mortality in patients on antidiabetic medications. In patients receiving no antidiabetic drugs, HbA1c 8.0-8.4% (64-68 mmol/mol) was associated with higher mortality (HR 2.07, 95% CI 1.28 to 3.33). However, in patients prescribed antidiabetic drugs, HRs for HbA1c <6.0% (42 mmol/mol), 6.0-6.4% (42-47 mmol/mol) and ≥8.5% (69 mmol/mol) were 1.42 (95% CI 1.23 to 1.64), 1.21 (95% CI 1.06 to 1.38), and 1.52 (95% CI 1.33 to 1.74), respectively. There were no interactions between BP and prescriptions of antihypertensive drugs or TC and prescriptions of lipid-lowering drugs.
Conclusion: In very old people with type 2 diabetes, stringent targets for metabolic and BP control may not be advisable, but control of the intermediate outcomes within appropriate ranges may be required.

	Hazard ratio (95% CI)
HbA1c (%/mmol/mol)	
<6.0 (42)	1.33 (1.18 to 1.50)
6.0–6.4 (42–47)	1.16 (1.03 to 1.30)
6.5–6.9 (48–52)	1.09 (0.98 to 1.22)
7.0–7.4 (53–57)	Ref
7.5–7.9 (58–63)	1.08 (0.93 to 1.25)
8.0–8.4 (64–68)	1.13 (0.96 to 1.33)
≥8.5 (69)	1.49 (1.31 to 1.69)
Blood pressure (mmHg)	
<130/80	1.49 (1.38 to 1.60)
<140/80	1.02 (0.93 to 1.11)
<150/90	Ref
<160/100	1.02 (0.90 to 1.15)
≥160/100	0.95 (0.84 to 1.08)
Total cholesterol (mmol/L)	
<4.0	1.20 (1.11 to 1.29)
4.0–4.9	Ref
5.0–5.9	0.95 (0.85 to 1.05)
≥6.0	0.83 (0.72 to 0.96)

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Clinical inertia affects younger and older adults with type 2 diabetes mellitus equally, with or without CKD

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Background and aims: Clinical inertia (CI) is often defined as a failure to escalate treatment in order to achieve appropriate treatment goals in management of type 2 diabetes mellitus (T2DM). There is no single identifiable fault in CI; rather, it is a multifactorial condition, with contributory factors from the people with diabetes, the physicians and the system in which they operate. The Time2DoMore in Diabetes programme suggested that physicians believe older adults with diabetes and those with chronic kidney disease (CKD) are more susceptible to CI than younger patients with few or no comorbidities, resulting in lower expectations for concordance and adherence to lifestyle changes in the older or multimorbid people with diabetes. It is widely accepted that CI affects less clinical trials, presumably due to the placebo effect. The difference between routine care and this placebo effect in trials represents a correction of non-pharmacological aspects of CI. We used this concept to explore the pre-existing CI by targeting placebo-treated patients from the large vildagliptin clinical trial programme and using CKD as a proxy of diabetes complications in general; comparing the glycaemic outcomes after a placebo intervention in older patients with or without CKD, to those in a younger population, with or without CKD.
Materials and methods: We stratified all placebo-treated subjects, both treatment-naïve or with multiple antidiabetes background medications, from the vildagliptin clinical trial programme, in a factorial design by age (<70 or >70 years) and with or without CKD (impaired renal function defined as eGFR <60 ml/min). Least Square (LS) mean change in HbA1c was assessed at 24 weeks (ANCOVA) and predictors of any differences were explored from the available baseline characteristics.
Results: Our cohort comprised 3081 placebo-treated patients from 25 studies. 80% of patients included in the analysis were <70 years old and 45% of all patients were women. As expected, the duration of T2DM was longer in patients with CKD in both younger and older groups (table), and the renal function was reduced in patients with CKD independent of their age; corresponding stage 3B in both CKD groups. The mean baseline HbA1c was comparable across the groups independent of age or CKD status. The overall mean placebo effect, with or without adjustment for baseline HbA1c value, was similar between all groups (adjusted LS mean HbA1c reductions between -0.23 and -0.32). There was no between-group difference when the HbA1c reductions from baseline were adjusted for CKD status (p=ns, for all comparisons).
Conclusion: In this exploratory analysis we were able to demonstrate a consistent placebo effect across age groups in presence or absence of CKD. This suggests that the non-pharmacological component of CI in patients with diabetes may be independent of age and comorbidities.

	Younger, non-CKD n=2176	Younger with CKD n=304	Older, non-CKD n=338	Older with CKD n=263
Demographics				
Age (years)	54.4±9.22	60.6±6.81	73.5±3.10	74.7±3.93
BMI (kg/m^2)	29.61±5.78	29.94±5.12	28.10±4.35	30.08±4.60
Duration of T2DM (years)	6.46±6.07	12.15±9.28	10.19±8.15	13.66±10.08
eGFR, MDRD (ml/min)	92.21±21.37	42.31±14.48	81.57±16.71	44.44±11.16
Baseline HbA1c (%)	8.13±1.19	7.83±1.09	7.93±1.05	7.80±0.94
Glycaemic outcomes at week 24				
Placebo effect (HbA1c drop, %)	-0.27±1.09	-0.20±1.26	-0.25±0.84	-0.21±0.97
After adjustment for baseline HbA1c				
Placebo effect (LS mean HbA1c drop, %±SEM)	-0.23±0.03	-0.27±0.07	-0.29±0.07	-0.32±0.07

Values are expressed as mean±SD or SEM (for adjusted HbA1c change)
CKD, chronic kidney disease; eGFR, estimated glomerular filtration rate; MDRD, modification of diet in renal disease; T2DM, type 2 diabetes mellitus

Supported by: Novartis

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Impact of socioeconomic status and ethnicity on risk of stroke, hospitalisation for heart failure and death in 371,092 individuals with type 2 diabetes

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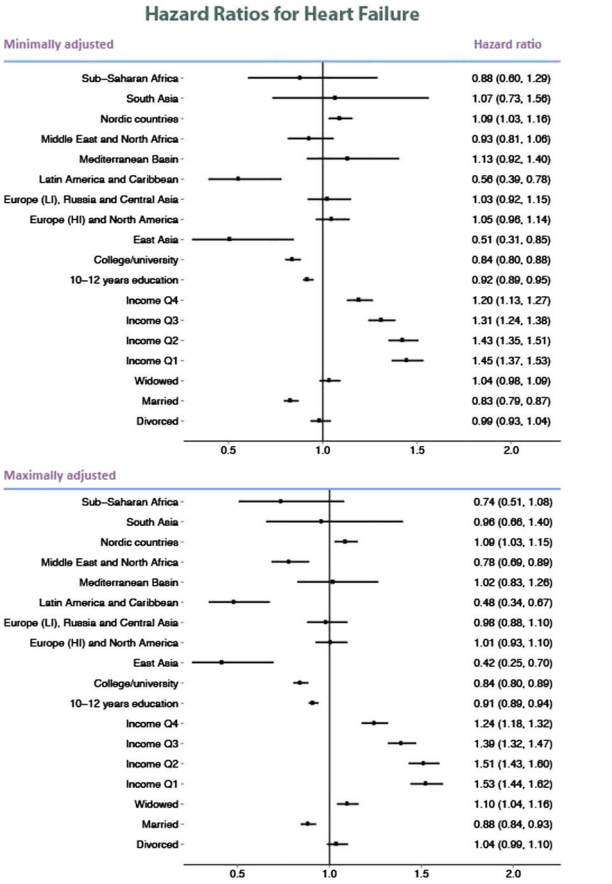
Background and aims: Socioeconomic status (SES) and ethnicity are powerful predictors of coronary heart disease in diabetes, but whether this association extends to heart failure and stroke is unknown. We examined the impact of SES and ethnicity on the risk of stroke, hospitalization for heart failure (HF) and overall mortality in type 2 diabetes.
Materials and methods: We included 371,092 patients (contributing 2, 766,349 appointments) with type 2 diabetes in the Swedish National Diabetes Register (NDR) during 2004-2012. Clinical data from the NDR was linked to national registers, whereby information on income, education, marital status, country of birth, comorbidities and events was obtained. Swedish natives were used as the reference group for ethnicity. Patients were followed until a first incident event (hospitalization for heart failure and fatal/nonfatal stroke), death or end of follow-up. The association between socioeconomic variables and the outcomes was modeled using Cox regression. Two models were computed for each outcome. The first model was adjusted for demographic and diabetes-related covariates. The second model was additionally adjusted for outcome specific covariates.
Results: Mean (SD) follow-up was 4.7 (2.5) years. HF occurred in 26, 448 (7.1%) persons. Fatal/nonfatal stroke occurred in 38,480 (10.1%) persons and 49,829 died (13.4%). Immigrants from Nordic countries had 9% and 14% elevated risk of HF (Figure 1). Immigrants from the Middle East and North Africa had 22% to 50% lower risk of all outcomes. Immigrants from the Mediterranean Basin had 23% lower risk of stroke. Latin Americans had 50% lower risk of HF and 40% lower risk of overall mortality. East Asians had 60% lower risk of HF and 50% lower risk of overall death. As compared with having 9 years or less education, having a college/university degree was associated with 20% lower risk of both HF and overall mortality. Those with 10 to 12 years of education had 10% lower risk of both death and HF, compared with having 9 years or less education. Income was inversely associated with the outcomes. As compared with the highest income quintile, those in the two lowest quintiles had 50% higher risk of all outcomes. As compared with being single, being married was associated with 12% lower risk of HF. Individuals who were widowed had 10% higher risk of HF.
Conclusion: There are marked socioeconomic disparities in the risk of HF, stroke and overall mortality despite equitable access to universal health care. We find indications of a healthy immigrant phenomenon, as non-Western immigrants frequently had lower risk than native Swedes. Socioeconomic status and ethnicity are independent predictors of stroke, HF and death in type 2 diabetes.



MODEL ADJUSTMENTS: Minimally adjusted: age, sex, duration of diabetes, marital status, income, education, ethnicity. **Maximally adjusted**: as the minimally adjusted model and additionally smoking, systolic blood pressure, HbA1c, body mass index, previous myocardial infarction, previous stroke, diabetes treatment, blood pressure lowering medications, atrial fibrillation.

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Exploring the distribution of cardiovascular disease and risk factors amongst patients with diabetes on sulfonylurea in a Canadian primary care dataset

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Background and aims: A growing body of evidence generated from observational studies and meta-analyses has begun to illustrate the potential adverse cardiovascular (CV) risk profile associated with sulfonylurea (SU) use. Studies reported an increased risk of death with SU drugs. The use of SU was associated with increased mortality and a higher risk of stroke. Also, more CV events with SU use have been reported in subgroups of patients with history of CV disease, elderly and higher body mass index. The objective of this study was to explore the distribution of CV disease and CV risk factors amongst patients with diabetes on SU in a Canadian primary care dataset for 2013 calendar year.
Materials and methods: The Canadian Primary Care Sentinel Surveillance Network (CPCSSN), which is a multi-disease surveillance system based on primary care electronic medical record data, was utilized for this research study. Patients with a diagnosis of type 2 diabetes and exposure to SU were identified. Distribution/prevalence of CV risk profile amongst this sub-cohort was explored.
Results: Through database for 2013 calendar year there were 6146 patients who had diabetes, had at least one visit with their family doctor, and were on SU. For this sub-cohort demographic data included: age [mean

(SD)] 65.4(12.8) years-old; 56.4% male and mean BMI 31.3(10.0). Established CV disease was found in 16% of the patients with the following distribution: 13% had ischemic heart disease/myocardial infarction or coronary artery disease; 2% had stroke; and 2% had peripheral vascular disease. Regarding aggregation of CV risk factors a very significant proportion (65%) of patients without established atherosclerotic CV disease had 2 or more CV risk factors including: hypertension (62%), dyslipidemia (33%), active smoking (13%), and obesity (43%). Almost half of the cohort (45%) were males older than 55 years of age or females more than 60 years of age with at least one of the following risk factors: dyslipidemia, hypertension or current smoking, but without established cardiovascular disease. A large proportion of patients (19.5%) had a diagnosis of cardiac specific issues including ischemic heart disease, heart failure or arrhythmia. Almost 82% of patients had established atherosclerotic CV disease or 2 or more CV risk factors without established atherosclerotic CV disease.

Conclusion: This study illustrated that in this dataset a significant proportion of patients with diabetes on SU in 2013 had established CV disease and/or aggregation of multiple CV risk factors. In light of recent data on association between SU utilization and CV events and increased mortality, pharmacovigilance programs should actively monitor SU utilization in patients with diabetes and high risk CV profile in real world clinical settings.

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LEADER-8: Type 2 diabetes patients at high cardiovascular risk: a comparison of Eastern and Western European participants in the trial

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Background and aims: The prevalence of type 2 diabetes (T2D) may reach 600 million patients worldwide in 2035. There are many regional and national differences in incidence and prevalence for the disease itself as well as its complications. The 45 years of separation between Western and Eastern Europe may have resulted in some of these differences, but also in distinct differences in the cardiovascular disease (CVD) epidemiology. It is unknown how the baseline demographics and anthropometric differences for patients with T2D and high risk of CVD vary between these two regions.

Materials and methods: This study compares metabolic control, diabetic risk factors and treatment at baseline between regions (Western EU (WEU), Eastern EU (EEU) and Russia/Serbia (Ru/Se)) in a subpopulation of subjects with T2D and high cardiovascular risk (n=2845) enrolled in the Liraglutide Effect and Action in Diabetes: Evaluation of cardiovascular outcome Results (LEADER) trial. We performed descriptive statistics to describe the differences in baseline characteristics among the regions.

Results: HbA1c levels were similar between WEU and EEU, 8.3±1.3% and 8.3±1.3%, respectively. Western patients were older (65.9 years vs. 62.1, $p<0.0001$) and had longer diabetes duration (12.4±7.7 years vs 9.5 ±6.5, $p<0.0001$). Mean systolic blood pressure (BP) was lowest for participants from Ru/Se (137±14 mmHg), whereas diastolic BP was lowest in WEU (81.3±9.5 mmHg). Control of dyslipidaemia was best in WEU, and worst in Ru/Se. The percentage of patients meeting all three guideline targets for Hba1c (<7%), BP (140/80 mmHg), and LDL cholesterol (70 mg/dL) was 9.7%, 6.4% and 3.2% ($p<0.05$) in WEU, EEU and Ru/Se, respectively. For antidiabetic treatment, metformin and sulphonylurea treatment was more frequent in the EEU than in WEU. Insulin was used less frequently in EEU than in WEU and Ru/Se. Statins were used most frequently in the WEU countries and least frequently in Ru/Se.

Conclusion: In spite of longer diabetes duration and higher age, patients with T2D and high CV risk from WEU had lower BMI, better blood pressure and lipid control than those in the EEU. The differences, however, were small and the percentage of subjects meeting all the three targets of treatment was low in all regions of Europe, suggesting that despite socioeconomic differences patients recruited in Europe were overall comparable at baseline.

Table: Baseline differences between European regions.*

Parameter	Western EU (1905)	Eastern EU (564)	Russia and Serbia (R/S) (n=376)	P value, Eastern EU vs. R/S	P value, Eastern EU vs. Western EU
Age [y]	65.9±7.5	62.1±6.9	60.8±6.4	<0.01	<0.0001
BMI [kg/m^2]	32.6±5.7	33.7±5.9	34.1±5.8	NS	<0.0001
Diabetes duration [y]	12.4±7.7	9.5±6.7	9.0±6.0	NS	<0.0001
Current smokers [n(%)]	252 (13.2)	67 (11.9)	44 (11.7)	NS	NS
Previous smokers [n(%)]	1087 (57.1)	244 (43.3)	106 (28.2)	<0.0001	<0.0001
Never smoked [n(%)]	566 (29.7)	253 (44.9)	226 (60.1)	<0.0001	<0.0001
HbA1c [%]	8.3±1.3	8.3±1.3	8.5±1.3	<0.005	NS
Systolic BP [mmHg]	140.8±19.0	140.1±15.8	137.3±14.0	<0.05	NS
Diastolic BP [mmHg]	77.4±10.5	81.3±9.5	82.3±8.1	NS (0.06)	<0.0001
eGFR (MDRD) [ml/min/m^2]	77.7±27.1	88.2±24.5	89.3±20.2	NS	<0.0001
Albumin/creatinine ratio [mg/mmol]	18.2±62.0	8.8±29.7	6.2±16.0	NS	=0.0001

*P-values are for difference between regions on covariates (t-test) and factors (Chi-square).
BMI: Body mass index, BP: Blood pressure, eGFR: estimated Glomerular Filtration Rate, MDRD: Modified Diet in Renal Disease, LDL: Low Density Lipoprotein, HDL: High Density Lipoprotein

Clinical Trial Registration Number: NCT01179048
Supported by: Novo Nordisk

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LEADER-7: US and European participants in the trial are different: implications for the interpretation of the trial results

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Background and aims: The usefulness of trial results for a particular clinician depends on how well the trial population mirrors the population of patients in that clinician's practice. When participants differ with respect to attributes that modify treatment, the results will be less applicable. We examined whether there are important differences in cardiovascular risk factors among LEADER participants enrolled in the US and in Europe.

Materials and methods: The Liraglutide Effect and Action in Diabetes: Evaluation in cardiovascular outcome Results (LEADER) trial in 9340 patients with type 2 diabetes mellitus (T2DM) will provide data on the cardiovascular safety of liraglutide compared to placebo. Baseline data from US (n=2488) and European (n=3521) participants were compared, and stratified for prior cardiovascular disease (CVD). Multivariable logistic regression analyses were used to determine whether region was an independent determinant of achieved targets for HbA1c, blood pressure and LDL-cholesterol.

Results: Irrespective of CVD history, US participants were more often of non-white origin, had a longer history of T2DM and a higher body weight (Table). They had a 0.5% higher baseline HbA1c and a substantially lower blood pressure, but were less often at target for their 'region-specific' blood pressure target (OR 0.60; 95% CI 0.42-0.87). They had a marginally lower LDL-cholesterol level. In the group of patients with prior CVD less US participants had a prior myocardial infarction (15.5% vs 19.4%). 'Region' was independently associated with participants being at the HbA1c, BP and LDL-targets, likely partially due to different diabetes guidelines in Europe and the US.

Conclusion: The differences between US and EU participants and the role of the region might cause clinically important heterogeneity in treatment effects across regions and subgroups. These differences should be taken into account in the analysis and reporting of the results.

Table: Baseline differences between Europe and US patients stratified by prior CVD.*

	No Prior CVD group (n=1013)			Prior CVD group (n=4996)		
	Europe (n=599)	United States (n=414)	p-value	Europe (n=2922)	United States (n=2074)	p-value
Gender (N,% F)	223 (37.2)	177 (42.8)	0.077	857 (29.3)	710 (34.2)	**0.001**
Age, years (mean, sd)	66.0 (15.0)	66.1 (15.6)	0.783	64.6 (11.3)	64.3 (11.7)	0.166
50-59 yrs (N,%)	10 (1.7)	8 (1.9)		789 (27.3)	589 (28.4)	
60-69 yrs (N,%)	454 (75.8)	310 (74.9)		1364 (46.7)	971 (46.8)	
70-79 yrs (N,%)	127 (21.2)	84 (20.3)	0.355	694 (23.8)	451 (21.7)	0.240
80-89 yrs (N,%)	8 (1.3)	12 (2.9)		65 (2.2)	62 (3.0)	
90-99 yrs (N,%)	0 (0)	0 (0)		1 (0.0)	1 (0.0)	
Diabetes duration, years (mean, sd)	11.2 (6.7)	13.2 (7.9)	**<0.001**	11.7 (7.6)	13.5 (8.8)	**<0.001**
Race (N,%)						
Asian	3 (0.5)	9 (2.2)		25 (0.9)	37 (1.8)	
Black	2 (0.3)	101 (24.4)	**<0.001**	14 (0.5)	366 (17.6)	**<0.001**
White	592 (98.8)	295 (71.3)		2871 (98.3)	1622 (78.2)	
Other	2 (0.3)	9 (2.2)		12 (0.4)	46 (2.4)	
Body weight (kg)	84.0 (16.2)	98.9 (23.4)	**<0.001**	83.6 (18.6)	101.1 (21.8)	**<0.001**
BMI, kg/m² (mean, sd)	33.0 (5.6)	34.4 (6.9)	**0.001**	32.6 (5.3)	34.6 (6.8)	**<0.001**
18-25 kg/m² (N,%)	25 (4.2)	24 (5.8)		180 (6.2)	96 (4.6)	
25-30 kg/m² (N,%)	176 (29.4)	91 (22.0)		843 (28.9)	438 (21.1)	
30-35 kg/m² (N,%)	216 (36.1)	125 (30.2)	**<0.001**	1024 (35.0)	686 (33.1)	**<0.001**
35-40 kg/m² (N,%)	113 (18.9)	91 (22.0)		554 (19.0)	478 (23.0)	
> 40 kg/m² (N,%)	69 (11.5)	83 (20.0)		316 (10.8)	375 (18.1)	
Waist circumference, cm (mean, sd)	112.2 (15.5)	114.1 (18.4)	0.072	111.2 (15.3)	114.5 (17.0)	**<0.001**
HbA1c, % (mean, sd)	8.4 (1.3)	8.9 (1.6)	**<0.001**	8.3 (1.3)	8.8 (1.5)	**<0.001**
HbA1c on target (N, %) (≤ 8%)	307 (51.3)	146 (35.3)	**<0.001**	1494 (51.1)	837 (40.4)	**<0.001**
SBP, mmHg (mean, sd)	144.1 (17.5)	135.3 (17.1)	**<0.001**	140.0 (18.4)	133.3 (18.3)	**<0.001**
DBP, mmHg (mean, sd)	81.0 (9.7)	76.0 (9.3)	**<0.001**	78.3 (10.2)	74.7 (10.3)	**<0.001**
BP on target (N,%) (EU' <140/80 &US ≤130/80 mmHg)	482 (79.3)	280 (67.6)	**0.005**	1944 (66.5)	1274 (61.4)	**<0.001**
LDL, mmol/L (mean, sd)	2.5 (0.9)	2.3 (0.9)	**<0.001**	2.3 (0.9)	2.2 (0.9)	0.113
On target (N,%)	319 (53.3)	279 (65.9)	**<0.001**	1934 (66.2)	757 (36.5)	**<0.001**
HDL, mmol/L (mean, sd)	1.3 (0.3)	1.2 (0.3)	0.104	1.2 (0.3)	1.2 (0.3)	**0.008**
Triglycerids, mmol/L (mean, sd)	2.0 (1.3)	2.0 (2.0)	0.740	2.1 (1.3)	2.0 (1.4)	0.038
Creatinine, umol/L (mean, sd)	71.6 (14.6)	73.1 (12.6)	0.106	83.4 (31.2)	94.7 (38.2)	**<0.001**
ACR, mg/mmol (mean, sd)	10.6 (30.5)	14.8 (50.5)	0.135	16.4 (37.3)	26.5 (82.0)	**<0.001**
eGFR (mean,sd)	90.0 (24.0)	90.3 (20.5)	0.847	81.3 (26.8)	74.8 (27.1)	**<0.001**

*P-values are for difference between region (Europe versus US) on covariates (t-test) and factors (Chi-square). BMI: Body mass index, SBP: Systolic blood pressure, DBP: Diastolic blood pressure, LDL: Low Density Lipoprotein, HDL: High Density Lipoprotein, ACR: Albumin creatinine ratio, eGFR: estimated Glomerular Filtration Rate (calculated using the MDRD (Modified Diet in Renal Disease) equation)

Clinical Trial Registration Number: NCT01179048
Supported by: Novo Nordisk

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Effectiveness and feasibility of a computerised prompt for primary and secondary care physicians to refer or refer back type 2 diabetes patients

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Background and aims: Patients with type 2 diabetes mellitus (T2DM) are treated according to clear guidelines. However, guidelines on treatment setting (primary or outpatient clinic based care) are mostly lacking. In the Netherlands, ~85% of T2DM patients are treated by general practitioners in primary care and clear guidelines exist when to refer a patient. However, many patients are not treated in the correct setting. We introduced an automated signal in the Electronic Medical Record (EMR) of general practitioners and internists that warns if a patient should be treated in another setting, i.e. be referred or referred back. This signal gives a recommendation according to guidelines. We hypothesize that this can create a shift from primary to secondary care and vice versa in such a way that only individuals who need intensive diabetes treatment will be offered hospital based diabetes care.
Materials and methods: In a cluster randomized trial general practices and practices of hospital based specialists who all share a common EMR were randomized. In the intervention group an automated message in the EMR popped up to physicians during a consultation if their patients were not treated in the correct setting; it also recommended the preferred allocation of care. The signal was based on HbA1c, blood pressure, lipids and end organ damage. In the general practices 3 types of recommendations were provided: 1. e-consultation with a specialist; 2. referral to specialist; 3. decrease consultation frequency combined with self-monitoring by the patient. A single patient could be treated according to both recommendations 1. and 2, depending on treatment values. In the outpatient clinic specialists could receive only one signal to refer a patient back to the general practitioner. At the same time, we asked physicians if they would follow the recommendation and if not, why. The control group provided care as usual. Follow-up: 12 months. The primary outcome is the change in proportion of patients who are treated in the right care setting.
Results: We included 38 primary care and 10 specialist practices. Together they included 2779 patients (1418 intervention, 1361 control group). At the start, in the intervention group there were 818 patients (57.7%) with one or more recommendations to change treatment setting (559 for e-consult; 451 for referral to specialist care; 53 for self-monitoring and 30 for referral back). In the control group there were 764 patients (56.1%) who qualified for another treatment setting (501 for e-consult; 417 for change to specialist care; 49 for self-monitoring and 39 for change to primary care). After one year, in the intervention group 542 of these individuals (66.3%) and in the control group 552 (72.3%) still were not treated in the correct setting (p=0.29). The main reasons for not adhering to the guideline-based recommendation were: 1) at patient's request and 2) the current leading physician would like to adjust treatment first.
Conclusion: Over half of T2DM patients are not treated in the correct treatment setting as stipulated in the Dutch National Guidelines. After one year this situation was unchanged in over two-thirds of these patients. The signal to the treating physician in the EMR had no added value to improve triage and change treatment setting in this population.
Clinical Trial Registration Number: NCT02229110
Supported by: Stichting Diamuraal and UMC Utrecht

904

Blood glucose and blood pressure related treatment target achievement: results of the prospective DIALOGUE registry

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Background and aims: Individualized treatment target achievement is difficult to predict, based on patient characteristics at baseline. Therefore, we analyzed the baseline characteristics of 6,092 patients included into the prospective, observational, multi-center DIALOGUE registry in Germany which had a 12 months follow-up to identify patients with good treatment outcomes.
Materials and methods: A total of 8,594 patients with type-2 diabetes and hypertension were included of which 6,092 had a 12 months follow-up and complete data to assess treatment target achievement. Treatment targets were individually assigned by the physicians to strict, moderate or loose blood glucose (BG) targets (HbA1c <6.5%; 6.5-<7.0%; 7.0-<7.5%) and blood pressure (BP) targets (sBP <130 mmHg; 130-<135 mmHg; 135-140 mmHg).
Results: Out of 6,092 patients 32.3% (n=1,967) had both their blood pressure (BP) and glucose (BG) controlled at 12 months according to their prespecified individualized goals. 24.6% (n=1,500) missed their BG goal, 20.8% (n=1,267) missed their BP goal and 22.3% (n=1,358) missed both treatment targets. Patients who achieved their individual BP and BG targets (BP+/BG+) were older, had a shorter diabetes duration and a lower BMI compared to the other patient groups (p<0.0001). With a mean admission HbA1c of 7.5±1.3% in this group, 30.4% had a strict treatment target of HbA1c ≤6.5% assigned which were fewer patients compared to the other groups (p<0.0001). For this purpose less

antidiabetic drugs were needed in this group after baseline. The same pattern was seen for blood pressure targets. With a mean admission BP of 137.5±14.0 which was less than in the other groups, less stringent treatment targets were pursued ($p<0.0001$). On the other hand there was no difference in the number of antihypertensive drugs used.

Conclusion: In this observational study, blood pressure and blood glucose treatment targets were achieved in a substantial portion of patients associated with being older, having a shorter diabetes duration and lower weight.

	BP+/BG+	BP+/BG-	BP-/BG+	BP-/BG-	p-value
Patient number	1967 (32.3%)	1500 (24.6%)	1267 (20.8%)	1358 (22.3%)	
Age in years	66.1±11.1	63.8±11.3	65.9±10.7	63.6±11.1	<0.0001
Female gender	47.3	45.5	47.8	44.3	0.20
Diabetes Duration	6.3±5.8	7.5±5.5	6.9±5.8	7.5±5.5	<0.0001
BMI in kg/m^2	30.5±5.4	31.3±5.9	31.7±6.2	32.1±5.8	<0.0001
HbA1c goal ≤6.5 %	30.4	39.3	39.2	50.4	<0.0001
Admission HbA1c %	7.5±1.3	8.2±1.3	7.4±1.3	8.1±1.4	<0.0001
SBP goal <130 mmHg	31.5	37.1	39.7	48.7	<0.0001
Admission SBP mmHg	137.5±14.0	136.7±13.9	144.9±16.5	144.5±16.0	<0.0001
Antidiabetic drugs at BL	1.7±0.7	1.8±0.7	1.7±0.7	1.9±0.7	<0.0001
Antihypertensive drugs at BL	2.1±1.1	2.1±1.1	2.2±1.2	2.1±1.1	0.40

BP+: individual blood pressure treatment target was achieved after 12 months; (BP-: target missed.)

BG+: individual HbA1c treatment target was achieved after 12 months. (BP-: target missed.)

Supported by: Novartis Pharma GmbH

PS 084 Diabetes devices

905

GLP-1 receptor agonist device- and regimen-related features important to injection-experienced and injection-naïve patients with type 2 diabetes mellitus: a multinational interview study

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Background and aims: GLP-1 receptor agonist injectable treatments for type 2 diabetes mellitus (T2DM) vary with respect to device and regimen-related features. The objective of the study was to examine importance and acceptability of GLP-1 receptor agonist treatment features among injection-experienced and injection-naïve patients.

Materials and methods: One-on-one interviews were performed with injection-experienced and injection-naïve patients with T2DM in 5 countries: Brazil, China, Germany, Japan, and the UK. Interviews were conducted in the patients' native language by trained interviewers using a semi-structured interview guide. All patients ranked the importance of 5 device- and regimen-related attributes: dosing frequency, preparation method, needle size, device size, and need for refrigeration. Injection-naïve patients also were asked both open-ended and prompted questions about the acceptability of an injectable medication and the most important aspects of injection treatment. Interviews were audio-recorded and transcribed into English. A thematic and content analysis was performed using MaxQDA 11 software.

Results: Fifty patients, 10 in each target country, were interviewed and included injection-experienced patients (n=28, 56%; n=9, 18% exenatide QW users and n=19, 38% liraglutide users) and injection-naïve patients on oral anti-diabetic medications (n=22, 44%). Mean age was 52.8±12.5 years (range 21 to 79), and 54% (n=27) were male. The 3 most important attributes were the same for both the injection-experienced and injection-naïve patients; dosing frequency (experienced: n=23, 82%; naïve: n=18, 82%), needle size (experienced: n=19, 68%, naïve: n=15, 68%), and injection preparation (experienced: n=16, 57%; naïve: n=11, 50%). For the remaining two attributes, need for refrigeration was ranked as more important than device size for the injection-naïve patients. Almost two-thirds of the injection-naïve patients (n=14; 64%) reported that they would be hesitant to use an injectable treatment. Top concerns regarding injectable treatment were related to self-injection, such as knowing how and where to inject, (n=7, 32%), side effects (n=6, 27%), dosing frequency (n=4, 18%) and preparation involved, including amount and ease of preparation (n=4, 18%). Injection-naïve patients identified important aspects of injectable treatment as needle size (n=13, 59%), the frequency of dosing (n=11, 50%), injection preparation (n=9, 41%), efficacy (n=5, 23%) and device size (n=5, 23%).

Conclusion: Injection-experienced and injectable-naïve patients both identified dosing frequency, needle size and preparation as the most important device- and regimen-related attributes. Injection-naïve T2DM patients have concerns about injectable treatments and are hesitant to begin this type of therapy. Comprehensive education and support by healthcare providers on the preparation and proper use of injectable diabetes treatments would be important in relieving patients' fears and concerns about self-preparation and administration.

Supported by: AstraZeneca

906

Sustained effects of duodenal-jejunal bypass liner on weight loss and glycaemic control

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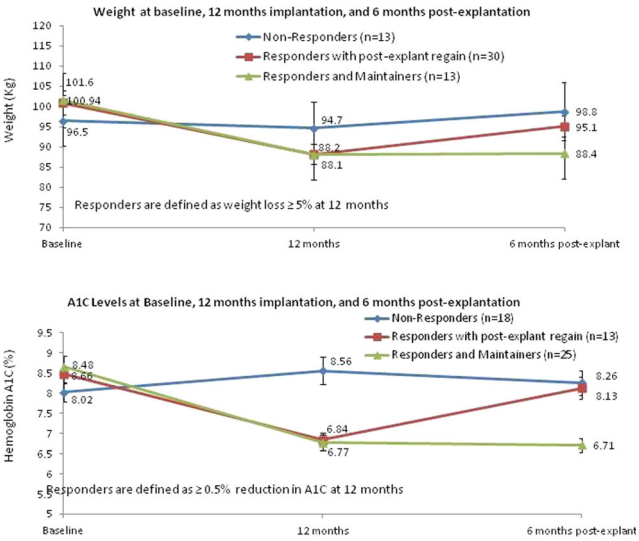
Background and aims: Behavioral and pharmacologic interventions in patients with obesity and type 2 diabetes (T2DM) have a poor record of sustainable efficacy in a real world setting. This has been thought to result from advancing disease, waning therapeutic potency and progressive non-adherence to therapy. The safety and efficacy of the duodenal-jejunal bypass liner (DJBL) for the treatment of patients with obesity and T2DM has been established during the intended 12-month implantation period. Possible longer-term effects of DJBL on weight and glycemic control, after device removal, are not well characterized. We examined the effects of DJBL on weight and hemoglobin A1c at 12 months of implantation and 6 months after removal.
Materials and methods: Data were extracted from one controlled and five single-arm clinical studies, involving subjects who completed a 12-month DJBL implantation period and a 6-month post-explantation follow-up. Data are presented as mean±standard error of the mean.
Results: 56 subjects (aged 51.4±1.0 years; 54% male) with obesity (100 ±2.6 kg, BMI 36.1±0.8 kg/m2) and T2DM (A1c 8.4±0.15%) were included. The mean implant duration was 368±0.6 days. The mean change in weight from baseline to 12 months was -10.4±0.96 kg and from 12 months to 6 months post-explantation was 4.75±0.55 kg. The overall weight change from baseline to 6 months post-explantation was -5.6± 1.01 kg. The percent weight change was -10.3±0.89% at 12 months and -5.5±0.95% at 6 months after explantation. The mean change in A1c was -1.05±0.2% from baseline to 12 months and 0.19±0.15% from 12 months to 6 months after explantation. The overall A1c change from baseline to 6 months post-explantation was -0.86±0.18%. At 12 months, 43/56 (76.8%) achieved≥5% weight loss. Of these "responders," 13/43 (30%) exhibited≤25% weight regain 6 months after explantation. Among the 13 subjects who did not achieve≥5% weight loss at 12 months, 8 (62%) nonetheless exhibited≥0.5% reduction in A1c at 12 months. Similarly, 38/56 (67%) achieved≥0.5% reduction in A1c at 12 months, and of these responders, 25/38 (66%) maintained at least 75% of this reduction 6 months after explantation (Figure 1).
Conclusion: DJBL was associated with a significant decline in both weight and A1c during the 12-month implantation period. At 6 months after device removal, there was a surprisingly modest increase in weight and A1c overall. Following a 12-month implantation with DJBL, 30% of subjects maintained their weight loss and 66% maintained their A1c 6 months after device removal. These observations, if confirmed by longer post-explantation follow-up, suggest that device may influence the underlying pathogenesis and progression of obesity and diabetes.



Clinical Trial Registration Number: 00985491, 00985114, 00986349, 01114438, 00985491, 01372501
Supported by: GI Dynamics

907

The EndoBarrier® gastrointestinal liner reduces food intake in obese subjects and improves postprandial glucose metabolism acutely in obese patients with type 2 diabetes

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Background and aims: The EndoBarrier® gastrointestinal liner is an endoscopic deployable (and removable) duodenal-jejunal bypass sleeve (DJBS) developed to treat obesity and type 2 diabetes (T2D). To evaluate the acute effect of the EndoBarrier® gastrointestinal liner on appetite, food intake, gallbladder emptying and postprandial glucose excursions we investigated obese subjects with normal glucose tolerance (NGT) and obese patients with T2D before and one week after DJBS implantation.
Materials and methods: Eleven obese metformin-treated T2D patients (diabetes duration: 40±18 months; age: 51±12 years; body weight 108± 18 kg; BMI: 36.6±3.3 kg/m^2; fasting plasma glucose (FPG): 9.3± 2.8 mmol/l; HbA_{1c}: 49±11 mmol/mol) and 12 gender, age and BMI-matched NGT subjects (age: 45±12 years; body weight 102±10 kg; BMI: 34.3±2.1 kg/m^2; FPG: 5.5±0.5 mmol/l; HbA_{1c}: 32±5 mmol/mol) underwent a standardised 4-hour liquid mixed meal test during which blood samples were collected, gallbladder emptying measured via ultrasound and appetite evaluated using visual analogue scales, and a subsequent ad libitum meal before and one week after DJBS implantation.
Results: Postprandial glucose excursions declined significantly after one week of implantation in obese patients with T2D (AUC: 638±115 vs. 431±83 min×mmol/l, p=0.02), but did not change in the NGT subjects. NGT subjects experienced significantly greater fullness and satiety and less hunger and thirst and T2D patients reported less thirst after DJBS implantation. Food intake during the ad libitum meal decreased significantly and similarly in both groups after implantation (NGT: 393±42 vs. 261±34 g, p=0.002; T2D: 463±64 vs. 269±33 g, p=0.02). Gallbladder emptying did not change in any of the groups.
Conclusion: After only one week of implantation, DJBS improves postprandial glucose tolerance in obese patients with T2D and alters satiety sensation and reduces food intake in obese patients with or without T2D.

Gallbladder emptying was left intact despite lining of the duodenal mucosa by the device.
Clinical Trial Registration Number: NCT02360878

908

Ten months of treatment with endoscopic duodeno-jejunal bypass liner reduce glycaemic variability and partially restore the incretin effect in obese type 2 diabetic subjects

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Background and aims: Duodenal-jejunal by-pass liner (DJBL) is an endoscopically implantable device designed to non-invasively mimic the effects of surgical gastrointestinal by-pass operations. The aim of our study was to comprehensively assess the effects of DJBL on body composition, glucose control and metabolic and hormonal profile of subjects with obesity and type 2 diabetes mellitus (T2DM).
Materials and methods: Thirty obese patients with T2DM (22 males, age 51.8±1.8 years) underwent the implantation of the EndoBarrier DJBL (GI Dynamics, USA). Anthropometric, biochemical and hormonal parameters were measured before and 1, 6 and 10 months after the implantation of DJBL. Glycemic variability was assessed using continuous glucose monitoring (CGM). Postprandial effects of DJBL were evaluated during a 2-hour liquid meal test (LMT).
Results: Over 10 months the implantation of EndoBarrier lead to a sustained decrease in body weight (BMI 42.7±1.2 vs. 38.4±1.2, $p<0.05$) and body fat (40.2±1.2 vs. 30.5±2.6%, $p<0.05$) and improvement in glucose control (fasting blood glucose 12.3±0.7 vs. 9.1±0.7 mmol/l, $p<0.05$; HbA1C 75.0±3.4 vs. 55.4±3.8 mmol/mol, $p<0.05$) accompanied by reduction of glycemic variability as calculated from a 7-day CGM (SD 2.5±0.2 vs. 1.8±0.2, $p<0.05$). Conversely, the levels of fibroblast growth factor 19 (FGF-19), a potent regulator of bile acid synthesis, markedly increased after implantation (80.3±9.8 vs. 154.4±26.1 pg/ml, $p<0.05$). Moreover, EndoBarrier also partially restored the incretin effect 1 month after insertion (serum GLP-1 at 15 min of LMT 129.7±17.0 vs. 105.8±12.0 pg/ml, $p<0.05$).
Conclusion: The implantation of EndoBarrier DJBL leads to a lasting reduction of body weight and improvement of all measures of glycemic control. Changes in the incretin system and the increase of FGF19 might be at least partially responsible for these effects.
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909

Advanced correction of marked hyperglycaemia with insulin administered by needle-free jet injection in patients with insulin-treated diabetes

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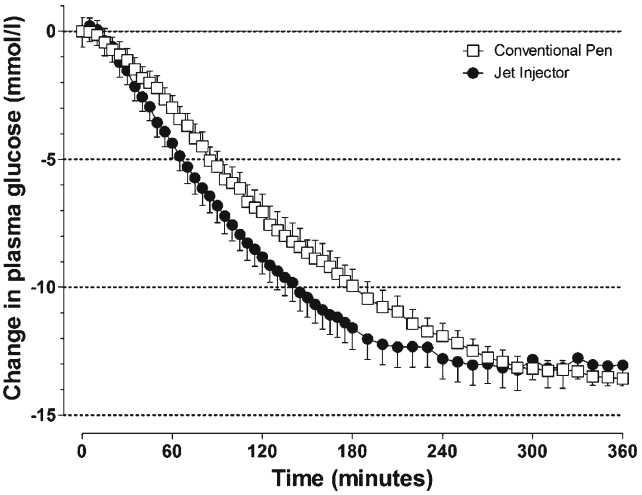
Background and aims: Rapid correction of marked hyperglycaemia in patients with insulin-treated diabetes is important to reduce symptoms and revert the risk of progression to ketoacidosis or hyperosmolar hyperglycaemic states. Insulin administered by jet injection may more rapidly correct marked hyperglycaemia than injection with a conventional pen, because insulin is dispensed over a larger subcutaneous area, facilitating faster absorption, especially in patients with body weights above normal. We compared the pharmacologic properties of the jet injector and conventional pen for the correction of marked hyperglycaemia by rapid-acting insulin.
Materials and methods: Adult, overweight or obese (BMI ≥25 and ≤40 kg/m^2) patients with type 1 diabetes (n=10) or insulin-treated type 2 diabetes (n=10) were enrolled in a randomized, controlled, cross-over study. On two separate occasions, after reaching hyperglycaemia (18-23 mmol/l) by decreasing prior insulin use, they were injected with insulin aspart by jet injection or by conventional pen, in a dose based on an estimate of their individual insulin sensitivity. Pharmacologic profiles were derived from plasma glucose and insulin levels, measured for six hours after injection.
Results: Insulin administered by jet injection resulted in faster correction of hyperglycaemia (fig 1). Plasma glucose values dropped with ≥10 mmol/l after 147.9±14.4 minutes with jet injection versus 192.5±13.6 minutes after conventional administration (difference 44.6 [95% CI 4.3- 84.8]; $P=0.03$). After one hour, plasma glucose had dropped with 4.4±0.3 mmol/l versus 3.0±0.2 mmol/l ($P=0.001$). The hyperglycaemic burden was significantly reduced during the first two hours (2041±37.2 versus 2167±26.1 mmol·min^{-1}·l^{-1}; $P=<0.012$). The risk for late hypoglycaemia was not increased after insulin administration by jet injection. The jet injector performed better in patients with a higher BMI.
Conclusion: Administration of insulin aspart by jet injection resulted in faster correction of marked hyperglycaemia compared to administration by conventional pen. The jet injector provided the most benefit in obese patients who needed high insulin doses.



Clinical Trial Registration Number: NCT01947556
Supported by: European Pharma Group

910

Lipohypertrophy: prevalence, risk factors, clinical characteristics, and economic burden of insulin-requiring patients in China

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Background and aims: Lipohypertrophy (LH) is a poorly studied complication of insulin injection/infusion therapy that impairs insulin absorption/glycemic control. This study aims to understand the prevalence, risk factors, clinical characteristics and economic burden associated

with LH to provide optimal patient care as the insulin-requiring population in China grows.

Materials and methods: Prevalence, risk factors and clinical features of LH patients were evaluated in 4 Chinese cities. Adult patients injecting insulin by pen ≥1 yr provided detailed information on diabetes/injection history, injection technique/training, pen needle (PN) reimbursement, and insulin doses, followed by physical exam and HbA1c testing. Differences from those without LH were evaluated by Student's t-test or Wilcoxon rank sum test. P-values<0.05 were considered significant.

Results: The 401 patients were mean(SD) 59.6(11.5) yrs; BMI 25.4(3.2) kg/m2; 50% male; 93.5% T2DM. HbA1c=8.0(1.7)%; total daily insulin dose=33.0(18.4) U with 2.1(1.0) injections daily. Durations of diabetes and of insulin therapy=11.9(7.6) and 5.6(4.6) yrs, respectively; 95% of patients reused PNs median 10 (max 360) times; 35.6% had PN reimbursement. LH prevalence was 53% overall (range 38-76%), mostly abdominal. Patients with LH had higher BMI and HbA1c, took 11 U (0.13U/kg or 31.7%) more insulin daily, took more injections, reused PNs more times and had less PN reimbursement (all p≤0.003) versus those without LH. LH patients rotated injection sites less and had slightly more injection training, but these were marginally significant (see Table). BMI and # daily injections remained significantly associated with LH prevalence by logistic regression (p≤0.01). Based on an estimated 8.4 million insulin injectors, the cost of excess insulin use associated with LH is estimated at RMB 3,892,593,600 ($630 million) per year.

Conclusion: LH was present in over half of insulin-requiring patients in China. LH is associated with worse glycemic control despite higher insulin consumption. Needle reuse frequency, # daily injections, BMI and lack of PN reimbursement are risk factors, suggesting local tissue trauma and/or insulin exposure as important contributors to LH development. Additionally, patients with LH contribute to a significant and avoidable economic burden in China of up to RMB 3.9 billion due to excess insulin consumption.

Characteristic	With LH (n=213)	Without LH (n=188)	*P*
HbA1c	8.2 (1.8)%	7.7 (1.5)%	0.003
Daily insulin dose (Units)	38.1 (20.1)	27.1 (14.3)	<0.001
Daily insulin dose (U/kg)	0.54 (0.28)	0.41 (0.21)	<0.001
BMI (kg/m²)	26.0 (3.3)	24.8 (3.0)	<0.001
Needle reuse, median # times	13.0	7.5	0.003
Daily injections, #	2.3 (1.0)	1.9 (0.9)	<0.001
Lack of site rotation, patients	4.7%	1.6%	0.080
Needle reimbursement, patients	27.8%	44.1%	<0.001
Injection training, patients	92.5%	87.2%	0.113
Average daily insulin costs, RMB	8.2	5.8	<0.001

911

Insulin injection into regions with lipohypertrophy worsens postprandial blood glucose versus injections into normal adipose tissue

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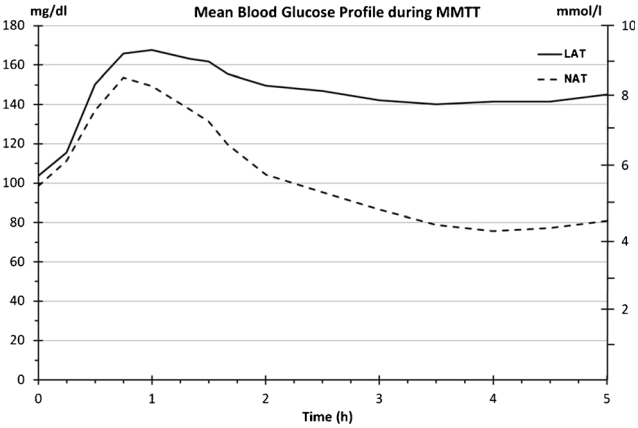
Background and aims: Lipohypertrophy (LHT) is a common side effect of long-standing insulin therapy, however, the impact of LHT on insulin absorption and blood glucose variability has not been investigated systematically yet. Therefore we compared the effects of subcutaneous insulin (INS) injection into LHT and regions with normal adipose tissue (NAT) on postprandial blood glucose (BG) control and INS absorption in 13 patients with type 1 diabetes (mean ± SD age: 50.1±10.1 years, HbA1c: 7.55±1.15%) during a mixed meal tolerance test.

Materials and methods: Patients received two identical standardised mixed meals (75 g CHO) separated by at least 6 h, each covered with a single dose of 0.15 U/kg insulin lispro injected at meal start into LHT or NAT, in randomised order. LHT was assessed and confirmed by physical examination and ultrasound. Pre-meal BG was adjusted to 100±20 mg/dL. BG and INS (lispro-specific radioimmunoassay) were measured over 5 hours after each mixed meal test.

Results: Injection into LHT led to reduced INS exposure ($AUC_{INS\ 0\text{-}5h}$ 46% lower, p=0.053), which was in line with a less pronounced maximum exposure versus injection into NAT ($C_{max\text{-}Ins.}$ 42% lower, p=0.72). Mean postprandial BG concentrations were significantly increased (17% higher in the first 2 hours, 58% higher from 2 to 5 h, 39% higher over 5 h, all p<0.05) after injection into LHT resulting in a 25% increase in maximum BG (193 vs. 154 mg/dL, p=0.043) (Figure). In addition maximum BG concentrations were reached 15 min later after injection into LHT ($T_{BG\text{-}max}$: 60 min vs. 45 min, p<0.05). Postprandial hypoglycemia (BG≤ 50 mg/dL) occurred slightly less frequently with LHT injection (2 vs. 6 patients, p=0.20), whereas postprandial hyperglycaemia (BG≥300 mg/dL) occurred only with LHT injection (2 patients).

Conclusion: Insulin injection into LHT regions leads to a pronounced impairment in insulin absorption leading to a deterioration in postprandial blood glucose control compared with injection into NAT. These results reinforce the importance of good injection technique, particularly site rotation, and provide the rationale for patients to avoid injecting insulin into areas with LHT.



Clinical Trial Registration Number: NCT02221323
Supported by: Becton Dickinson Diabetes Care

912

Lipohypertrophy leads to blunted, more variable insulin absorption and action in patients with type 1 diabetes

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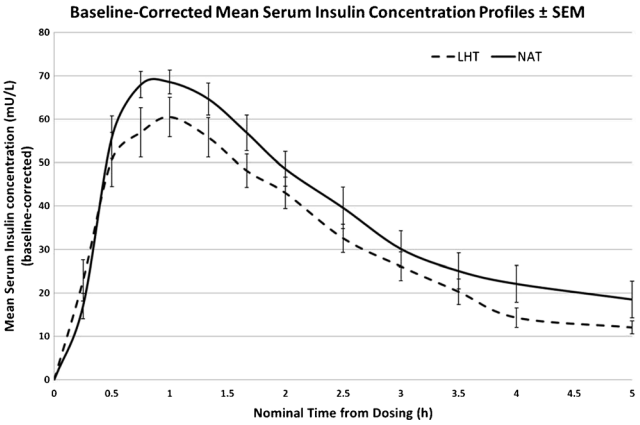
Background and aims: Lipohypertrophy (LHT) is a common side effect of long-standing insulin therapy; however, the impact of LHT on insulin (INS) absorption and efficacy has not yet been investigated using a sophisticated method such as the euglycaemic clamp technology. Therefore, we compared the pharmacokinetics (PK) and pharmacodynamics (PD) of subcutaneous injection of insulin lispro into abdominal areas with LHT and normal adipose tissue (NAT) in 13 patients with type 1 diabetes (T1D) under automated glucose clamp conditions.

Materials and methods: Patients (mean ± SD age: 50.1±10.5 years, BMI: 26.7±2.2 kg/m^2, HbA1c: 7.6±1.2%) received 4 single doses of 0.15 U/kg insulin lispro approximately every 6 hours, twice into a region with LHT and twice into NAT in random order. LHT was assessed and confirmed by physical examination and ultrasound. The clamp target level after dosing was 100 mg/dL and the glucose infusion rate (GIR) and serum insulin concentration were measured for 5 hours post-dosing.

Results: Mean INS profiles are shown in the figure. Comparing LHT with NAT injection, LS-mean INS concentrations were comparable during the first 30 min ($AUC_{INS0\text{-}0.5h}$ 8.8 vs. 9.4 h*mU/L), but significantly

lower thereafter ($AUC_{INS0-1h}$ 29.3 vs. 41.5 h*mU/L, $AUC_{INS.0-4h}$ 97 vs. 154 h*mU/L, all p<0.02). Maximum INS exposure was reduced by 34% (C_{INSmax} 49.7 vs. 75.4 mU/L, p<0.002). These differences were reflected in the GIR profiles: The PD effect in the first 30 min was comparable between LHT and NAT ($AUC_{GIR0-0.5h}$ 25 vs. 23 mg//kg, p=0.273) but was significantly and nearly 30% lower after 4 to 5 hours with LHT injection ($AUC_{GIR0-4h}$ 529 vs. 720 mg/kg, $AUC_{GIR0-5h}$ 576 vs. 803 mg/kg, all p<0.05). Maximum GIR was also reduced with LHT injection but this did not reach statistical significance (GIR_{max} 5.5 vs. 6.0 mg/kg/min, p=0.378). Intra-subject variability was substantially higher after dosing into LHT compared to NAT (coefficients of variation 52 vs. 11% [$AUC_{INS0-4h}$], 55 vs. 15% [C_{max}], and 57 vs. 23% [$AUC_{GIR0-4h}$], all p<0.01).

Conclusion: This first glucose clamp study in patients with LHT explains clinical experience that both insulin absorption and action are substantially blunted and considerably more variable when insulin is injected into areas with LHT. These findings likely also apply to subcutaneous insulin infusion and warrant further study.



Clinical Trial Registration Number: NCT02221323
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PS 085 Insulin pump therapy

913

User interaction are associated with glycaemic control in regular-use of real-time CGM

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Background and aims: The clinical benefit from real-time CGM (RT-CGM) in the management of diabetes has been demonstrated in many studies. However, there is limited information reported regarding the everyday use of RT-CGM. This study was to evaluate relationships between user interaction, demographics,and glycemic control in real-life patient daily use.

Materials and methods: The RT-CGM data was voluntarily uploaded by users seeking diagnostic technical support. The Dexcom G4 Platinum CGM system records the interaction users have with their alert settings and logs screen views. The alert settings are a customizable threshold for when to sound a hypoglycemia or hyperglycemia alarm, while screen views are button pushes that result in the display of glucose and/or glucose trend information. RT-CGM users' most common alert settings, the frequency of their average daily screen views (repeated screen views within a minute were counted as one view), and their overall glucose average and standard deviation were compared with age by grouping into four categories:Seniors (50 YO+), Adults (18 to 49 YO), Adolescents (12-17 YO) and Children (2-11 YO).

Results: A total of 229 (~50% male) users provided their CGM data and their demographics. There were ~4.6 M records of CGM data and the average time of CGM use was 77±68 (mean±s.d.) days. As age increased, average CGM glucose decreased; the highest average glucose was 9.6±1.9 mmol/l in Children and the lowest of that was 8.1±1.5 mmol/l in Seniors. The average standard deviations of CGM glucose were 4.2 mmol/l in Children, 4.1 mmol/l in Adolescents, 3.6 mmol/l in Adults and 3.3 mmol/l in Seniors. There was a consistent decrease in both average standard deviation of glucose from one age group to the next. The majority used the manufacturer default glucose alert settings of 4.4 mmol/l (for low) and 11.1 mmol/l (for high), 70% and 75% of users, respectively. However, as users increased their low glucose alert settings the average CGM glucose did as well. There was a positive correlation between the low glucose alert setting and the average glucose (r=0.313, p<.0001, displayed in Figure 1). The same relationship was noted between the high glucose alert setting and the average glucose (r=0.299, p<.0001). Furthermore, an examination of daily average screen views by subject within age group revealed that Children interacted with their CGM receiver the most with an average of 59±22 views per day. The next closest group was Adolescents with an average of 54±48 views per day. On average, Adults and Seniors viewed their receivers 36 times per day.

Conclusion: This study found an inverse relationship between age and average glucose, and also found that the older age groups had tighter glycemic control. Furthermore, CGM alert settings are associated with glucose levels and variability. Although younger populations interact with their RT-CGM more frequently, this study suggests there may be other factors preventing them from tighter glycemic control. The impacts of user interaction in glycemic control should be evaluated further within patient age population in a larger scale study.

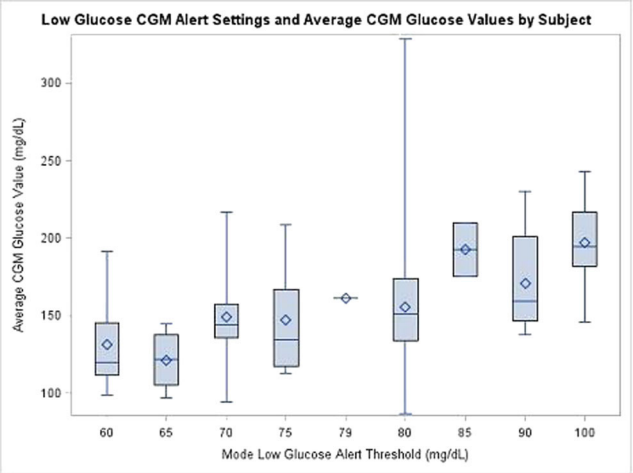

Figure 1: Box-Whisker plot of Average CGM Glucose Value across the Mode of Low Glucose Alert Threshold Settings

914

Resolution of the main indication and sustainability of long-term glycaemic control in type 1 diabetes with continuous subcutaneous insulin infusion

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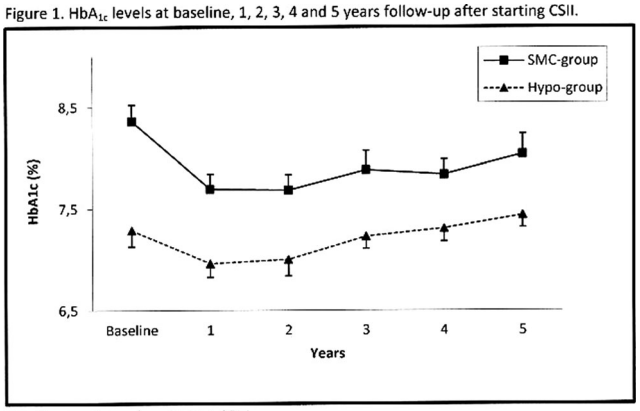
Background and aims: The effectiveness of continuous subcutaneous insulin infusion (CSII) in Type 1 diabetes (T1D) has been established in the short-term. However, less information is available regarding the impact on solving main indications for initiating CSII and whether good control can be maintained over several years. Our objective was to assess the resolution of the main indication and sustainability of long-term glycaemic control in T1D using CSII.

Materials and methods: Retrospective observational study including 178 T1D patients who started CSII treatment in our Center (2003-2008). All patients were followed in our CSII program for outpatients for at least 5 years. Data on annually HbA1c was collected and the resolution of the main indication for starting CSII was analysed.

Results: 27 out of 178 patients were excluded because of loss of follow-up or withdrawal from CSII. 151 patients (37.4±10.5 years, 64% women) were analysed. The main indications to start CSII were suboptimal metabolic control (SMC-group, 60.9%), severe hypoglycaemia/hypoglycaemia unawareness (H-group, 25.5%) and other (13.6%). HbA1c at the start of CSII was 8.0±1.2 and 7.8±1.2 after 5 years in the total cohort ($p<0.05$). In the SMC-group HbA1c dropped from 8.37±1.09% to 8.04±1.33% ($p=0.016$) and 37.4% of patients in this group had an HbA1c≤7.5% after 5 years. The resolution of the main indication was obtained in 64% of the SMC-group and 93% in H-group.

Conclusion: CSII therapy maintains long term glycaemic benefits after 5 years of follow up. In addition, the main indication for this treatment can be resolved in two thirds of T1D patients.

Figure 1. HbA_{1c} levels at baseline, 1, 2, 3, 4 and 5 years follow-up after starting CSII.



Results are showed as mean ± SEM.

915

Optimal glucose control during insulin pump therapy requires self-monitoring of blood glucose at least six times daily

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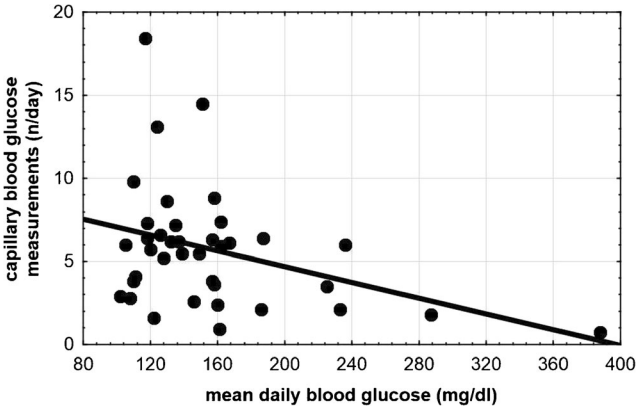
Background and aims: Due to economical reasons sensor-augmented pumps are not in common use among type 1 diabetes patients in many European countries. Subsequently, the subjects treated with continuous subcutaneous insulin infusion (CSII) are strongly advised to measure blood glucose (self-monitoring of blood glucose, SMBG) at least four times a day. However, the minimal frequency of SMBG required for having optimal glucose control remains unknown. Optimally, individuals with type 1 diabetes should maintain their HbA1c<6.5%, which is equivalent to the average blood glucose (BG) <140 mg/dl [7.8 mmol/l]. We conducted a study aiming at identifying minimal frequency of SMBG which would allow type 1 diabetes patients achieving good metabolic control.

Materials and methods: The group comprised 40 type 1 diabetes patients (mean age 27±8 years, diabetes duration 9±8 years, CSII duration 3.1±2.5 years, body weight 70±12 kg, BMI 24.2±3.7 kg/m2, HbA1c 7.7±1.9%, average BG 155±56 mg/dl, number of SMBG 5.7±3.7/day). Data from 2 last weeks before the study visit were analysed. All subjects were treated with CSII for at least for 6 months, thus they had obtained sufficient knowledge about this therapy mode. The pump settings (basal rate, bolus wizard) were assessed with standard tests.

Results: A negative correlation between the frequency of SMBG and the average number of capillary BG measurements (figure) was revealed ($r=-0.37$; $p<0.05$); the recommended target of metabolic control was obtained in subjects who had performed SMBG at least six times a day.

Conclusion: In conclusion, CSII therapy may lead to good metabolic control, but the acceptance and willingness of a patient to measure blood glucose six or more times a day is crucial.



916

Continuous subcutaneous insulin infusion in patients with type 1 diabetes: results of a prospective study with three years of follow up

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Background and aims: Several studies have found improved glycemic control with continuous subcutaneous insulin infusion compared with multiple daily insulin injections for patients with type 1 diabetes albeit for a relatively short-period of follow-up. This prospective study presents for the first time the optimization of glycemic control with insulin pumps in a cohort of Greek patients with type 1 diabetes during a 3-year follow-up period.
Materials and methods: Seventy-nine patients (30 males), previously on intensified basal-bolus insulin therapy with poor glycemic control, were recruited. HbA1c, hypoglycemic and diabetic ketoacidosis episodes, pump related side effects, lipidemic profile, 24-hour urine albumin excretion, body mass index, blood pressure, total daily insulin requirements (bolus and basal) were recorded during the 3-year follow-up. Statistical analysis was initially conducted for the entire study population. Statistical analysis was performed using STATA 9.0 software.
Results: A statistically significant decrease during the 3 year period of study was noted for the following parametres: HbA1c ($p<0.001$), basal rate ($p<0.001$), basal/bolus ratio ($p<0.001$), hypoglycemic episodes ($p=0.007$) and total/HDL-cholesterol ratio ($p=0.01$). On multivariate analysis, an independent and significant association was observed for hypoglycemic episodes (95%CI: -0.014 - -0.0038, $p=0.001$), basal rate (95%CI: -0.0322 - -0.0148, $p<0.001$) and total/HDL-cholesterol ratio (95%CI: -0.1222 - 0.0078, $p=0.026$). Insulin pump treatment was not accompanied with weight changes across all body mass index strata.
Conclusion: Continuous subcutaneous insulin infusion achieved almost optimal glycemic control, reduced hypoglycemic episodes without weight gain and was well tolerated for the whole study period. Finally, this therapeutic approach was accompanied with lower daily insulin requirements.

917

Long-term glycaemic remission of type 2 diabetes mellitus with insulin pump therapy regardless of disease duration

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Background and aims: Intensive insulin therapy (IIT) is known to improve β-cell function and induce glycemic remission in newly diagnosed type 2 diabetes mellitus (T2DM) patients. However, it is not known whether IIT can induce long-term remission in patients with long T2DM duration. We aimed to analyze T2DM remission cases achieved by insulin pumps.
Materials and methods: The patients who experienced T2DM remission were included between March and July in 2014. We collected the medical records retrospectively and prospectively. Remission is defined as maintaining normal fasting and postprandial glucose levels for 6 months after discontinuation of all anti-diabetic medications. Blood samplings were performed at fasting and 120 minutes after ingestion of a mixed meal (500 kcal), at baseline and 6 month intervals during the treatment and follow-up periods. Vales are presented as median (minimum-maximum) or mean±standard deviation.
Results: Nineteen patients (9 males, 10 females) were enrolled during the 5 months [age at diagnosis, 49 (32-57) years; duration of T2DM, 1.0 (0.0 - 23.0) years]. Two patients (11%) had been newly diagnosed and 17 patients (89%) were on oral anti-diabetic medications before insulin pump therapy. Baseline HbA1c level was 7.4±2.0%, and total daily insulin dose at the initiation of insulin pump therapy was 50 (22 - 344) IU. The time to remission was 23 (5 - 108) months and the duration of remission was 38 (12 - 108) months. During the follow-up of 5.0 (2.3 - 10.4) years, 5 subjects had T2DM relapse. After restart of insulin pump therapy, 3 subjects had remission again by 4 (3 - 8) months. In 17 patients with sustained remission, the latest HbA1c level was significantly lower than the initial level (6.1±0.4 vs. 7.4±2.1%, $p=0.017$) and their latest body mass index was not significantly changed (24.7±3.6 vs. 25.7±4.0 kg/m2, $p=0.053$). Their disposition index increased from 0.14±0.09 (initial) to 0.34±0.15 (maximum) ($p=0.001$).
Conclusion: In conclusion, insulin pump therapy improved β-cell function and induced long-term glycemic remission regardless of T2DM duration.

918

Glycaemic control in a large cohort of patients with type 1 diabetes treated with continuous subcutaneous insulin infusion

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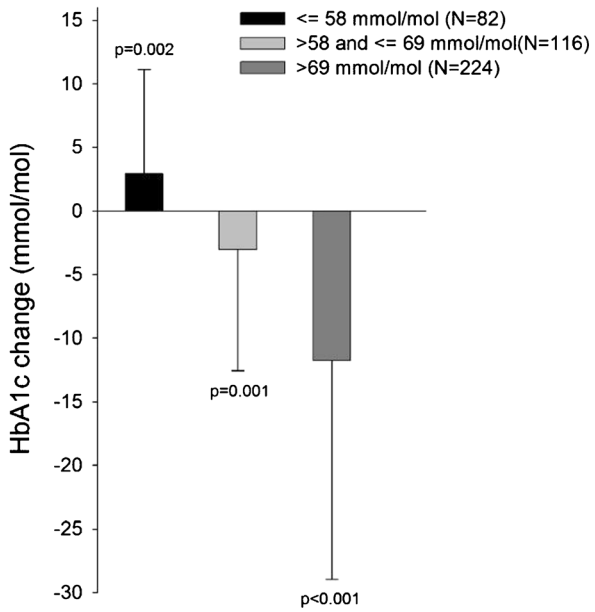
[1]Manchester Diabetes Centre, [2]University of Manchester & Manchester Academic Health Science Centre, UK.

Background and aims: There is limited data describing glycemic control in patients with type 1 diabetes (T1DM) treated with continuous subcutaneous insulin infusion (CSII) during routine care. In a tertiary referral center we undertook one of the largest studies of the effectiveness of CSII in adults with T1DM.
Materials and methods: In T1DM patients with at least 12 months of CSII therapy we obtained demographic and HbA1c data from hospital records and central laboratory. For each patient the average post-CSII HbA1c was computed from results obtained over 30 months since June 2012. We also compared pre- and average post-CSII HbA1c in the subgroup with paired data.
Results: We analysed 2079 HbA1c results from 484 patients (% females 57; mean (SD) ; age: 45 (13) years, CSII duration: 4.4 (2.5) years; HbA1c tests per patient: 4.3 (2.5); time between pump start and average HbA1c 40.4 (27.5) months). Insulin pumps used were: Medtronic: 43%; Omnipod: 29%; Animas: 14%; Accu-Chek: 13%. There were no differences in HbA1c comparing different pump users ($p=0.65$, ANOVA) or those using catheter pump vs. Omnipod (66 (15) vs. 68 (16) mmol/mol, $p=0.34$). CSII therapy was associated with a significant improvement in glycemic control (N=422 with paired data; mean (SD), pre vs. average post-CSII HbA1c: 73 (19) vs. 66 (15) mmol/mol, $p<0.001$, mean (SD) change -7 (15) mmol/mol). For those with a pre-CSII HbA1c more than 69 mmol/mol, improvements in glycemic control were even larger (N=224, pre- vs. average post-CSII HbA1c, 86 (16) vs.74 (15), $p<0.001$, mean (SD) change -12 (17) mmol/mol) (Figure). Despite improvements

only 30% of patients achieved good glycaemic control (HbA1c<or= 58 mmol/mol) and 39% of patients had a HbA1c more than 69 mmol/mol. We found a negative correlation between post-CSII HbA1c and age (r=-0.17, p=0.001) and diabetes duration (r=-0.23, p=0.001) but no association with CSII duration (p=0.30). In contrast no demographic factors were associated with improvements in HbA1c with CSII therapy.
Conclusion: We found significant improvements in glycaemic control with CSII particularly in those with a higher pre-CSII HbA1c under routine care conditions. Despite improved control, a large proportion of T1DM patients treated with CSII have poor glycaemic control, highlighting the challenges of managing T1DM under real-life conditions. Further work is required to understand the factors associated with poor control.

Mean(SD) change in HbA1c associated with CSII therapy by pre-CSII HbA1c level



919

Diabetes distress and fear of hypoglycaemia: What are the psychological benefits of insulin pump therapy?

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Background and aims: Living with Type 1 diabetes imposes a considerable burden on the individual to continuously self-manage their condition. As part of on-going audit the psychological outcomes of insulin pump therapy were evaluated over a 3 year period.
Materials and methods: Adult patients completed self-report questionnaires at initiation and after 12 months of pump therapy. Diabetes emotional distress was assessed using the Problem Areas in Diabetes Scale (PAID) and cognitive and behavioural aspects of fear of hypoglycaemia using the Hypoglycaemia Fear Survey (HFS-W, HFS-B). HbA1c and demographic data were obtained from the clinical record. Data were analysed using Predictive Analytic Software (PASW) Version 19.
Results: Data were collected from 142 adults, mean age 40 years (range 15-72), mean duration of diabetes 22 years (range 3-57). Outcomes improved significantly from baseline to 12 months for all variables (mean (sd)): HbA1c mmol/mol 73.7 (15.6), 63.2 (12.6) p<0.001; PAID 24.6 (15.7), 16.7 (13.22) p<0.001; HFS-W 24.9 (15.9), 16.4 (13.0) p< 0.001; HFS-B 19.1 (10.0), 13.8 (8.9) p<0.001. Paired data analysis did not indicate an association between HbA1c reduction and reduced PAID and HFS scores. There was no between group difference in baseline psychological variables above and below HbA1c <70 mmol/mol and >-70 mmol/mol.
Conclusion: Pump therapy is associated with improvements in medical and psychological variables. Whilst a reduction in both diabetes emotional distress, and worries and behaviours related to fear of hypoglycaemia appear to be unrelated to improvements a reduction in HbA1c, the overall burden of diabetes indicated by diabetes distress and fear of hypoglycaemia is reduced significantly 12 months after initiation of insulin pump therapy.

920

Is the pump bolus calculator a useful tool or the man behind the curtain?

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[1]Keck School of Medicine of the Unversity of Southern California, Los Angeles, [2]Jaeb Center for Health Research, Tampa, [3]Barbara Davis Center for Childhood Diabetes, Aurora, [4]University of California San Francisco, , USA.

Background and aims: Currently, little data exist on the utility of bolus calculators included in insulin pumps. It is unclear if this feature is a crucial part of pump management, a reflection of user ability, or simply a device of the program. Their utility depends on multiple factors_how well they are set up and adjusted by the provider and the patient, whether or not carb counting is done accurately and if a blood glucose level is entered into the pump so dose adjustments can be made. The aim of this analysis is to assess the frequency of bolus calculator use and determine if there are associations with demographic and clinical characteristics/outcomes among T1D Exchange clinic registry participants.
Materials and methods: Analysis included data from 1,944 T1D Exchange registry participants using an insulin pump who were age≥ 13 years with type 1 diabetes (T1D) duration≥1 year (mean age 33.1 years [range 13.0-83.7], duration 18.8 years [2.2-71.7], 60% female, 93% non-Hispanic white). Participants who self-reported using a bolus calculator most of the time or always were counted as frequent users. The most recent HbA1c from the medical record was used for analysis and frequencies of≥1 diabetic ketoacidosis (DKA) and severe hypoglycemic (SH), defined as seizure/loss of consciousness, events in the past 3 months were self-reported. Chi-square tests and t-tests were performed and adjusted models were conducted using logistic and linear regression.
Results: Among the 1,944 participants, 1532 (79%) reported frequently using a pump bolus wizard/calculator and 412 (21%) did not. Bolus calculator use was more common among adolescent participants, females, and participants with shorter duration of T1D and a lower education level (Table). Use of a bolus calculator was reported by 82% of participants who frequently check their blood glucose prior to giving a bolus for a meal vs. 66% among those who infrequently check (P<0.001). Bolus calculator use was similar among participants who reported giving a bolus prior to a meal vs. during or after a meal (80% vs. 76%, P=0.06). There were no significant differences between frequent vs. infrequent use of a bolus calculator in # of boluses per day (5.4 vs. 5.3, P=0.23), HbA1c (adjusted HbA1c 8.0% vs. 8.1%, P=0.04), occurrence of DKA in the prior 3 months (2% vs. 1%, P=0.18), and occurrence of SH in the prior 3 months (6% vs. 7%, P=0.56).
Conclusion: The majority of T1D patients using an insulin pump report frequently using a bolus wizard/calculator. Yet, in this study frequent use of the wizard/calculator was not a significant factor in the safe and effective use of insulin pumps. Common use of the bolus calculator should drive the development of further technology, as well as support the need for automatic entry of blood glucose values into the pump.

N=1944	Frequent Use of Bolus Wizard/Calculator	P Value
Overall	1532 (79%)	
Age Group – N(%)		0.003
13-<18 years old	335 (86%)	
18-<26 years old	361 (76%)	
26-<50 years old	548 (77%)	
≥50 years old	288 (78%)	
Diabetes Duration		0.005
1-< 10 years	494 (83%)	
10-< 20 years	488 (75%)	
≥ 20 years	550 (78%)	
Gender		0.005
Female	950 (81%)	
Male	582 (76%)	
Race/Ethnicity		0.80
White Non-Hispanic	1424 (79%)	
Black Non-Hispanic	20 (74%)	
Hispanic or Latino	53 (83%)	
Other Race/Ethnicity	35 (80%)	
Annual Household Income		0.25
Less than $35,000	143 (76%)	
$35,000 - < $75,000	363 (82%)	
$75,000 or more	711 (78%)	
Education Level		0.01
High School Diploma or Less	679 (82%)	
Associate or Bachelor degree	515 (77%)	
Masters, doctorate or profess	318 (75%)	

Supported by: Leona M. and Harry B. Helmsley Charitable Trust

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Elastargene 3C helps to improve glycated haemoglobin in children and adolescents with type 1 diabetes using insulin pump therapy

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Background and aims: Elastargene 3C is a cream specifically designed to improve lipoatrophy in patients with diabetes. It is made by many ingredients; the most important ones are elastin, arnica, collagen, caffeine, and L-carnitine. We started a 6-month, double-blind, randomized trial to test the efficacy of elastargene 3C in children with type 1 diabetes using insulin pump, in whom infusion set usually left little withe scars.
Materials and methods: Forty children and adolescents using insulin pump therapy, were randomized into 2 arms: a) n=20 elastargene 3C once a day on the skin of abdomen or other sites where infusion sets have been placed, administered before sleeping; b) n=20 placebo once a day on the skin of abdomen or other sites where infusion sets have been placed, administered before sleeping. BMI, HbA1c, insulin requirement, were determined in each child before staring the study and after 6 months.
Results: At the end of the study, 5 patients dropped using the elastargene cream or placebo and were excluded from the analysis. In elastargene group, 18 patients with type 1 diabetes were evaluated: age 15.2±4.8 yrs, diabetes duration 8.0±5.3, time using a pump 4.1±3.0; in the placebo group, 17 patients with type 1 diabetes were evaluated: age 15.1±5.7 yrs, diabetes duration 8.3±5.8, time using a pump 4.7±3.0. No significant difference has been observed for age, disease duration and time since insulin pump started. HbA1c significantly improved in patients using elastargene 3C (baseline 8.08±0.80%, after 6 month 7.51±0.53%, p=0.005, delta -0.53%), but not in placebo group (baseline 7.98±0.74%, after 6 month 7.76±0.79%, p=0.19, delta -0.22%). No difference has been observed regarding BMI or insulin requirement. In the elastargene 3C group, withe scars completely disappeared in 8 patients and improved in 10; in the placebo group withe scars did not change in any of the patients.
Conclusion: To our knowledge this is the first time that a direct effect of elastargene 3C have been shown in improving little withe scars that appear on the skin after infusion set removal in children with type 1 diabetes using insulin pump therapy. Moreover, HbA1c significantly improved only in the elastargene 3C group, probably because improved insulin absorption.

PS 086 Fine-tuning insulin therapy

922

Effects of advanced carbohydrate counting guided by an automated bolus calculator in type 1 diabetes (ABC): a 12-month, randomised clinical trial

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Background and aims: Advanced carbohydrate counting (ACC) improves metabolic control in patients with type 1 diabetes (T1D). We studied whether concomitant use of an automated bolus calculator (ABC) results in further improvement in HbA_{1c}.
Materials and methods: The study was a 12-month randomised, parallel group, open-label, single-centre, investigator-initiated clinical trial. ACC-naïve and poorly controlled T1D adults on multiple daily insulin injections therapy and HbA_{1c} 64-100 mmol/mol were recruited from a specialized diabetes centre. Between August 2012 and September 2013, 168 participants (96 men, 72 women) were enrolled and randomly assigned to receive training in either ACC using mental calculations (MC-group, n=84) or ACC using an ABC (ABC-group, n=84). All patients received a 3.5-hour group training course in general T1D management guidelines as well as theoretical and hands-on training in ACC. The patients were subsequently followed in routine clinical practice for 12 months. The primary outcome was change in HbA_{1c} from baseline to 12 months.
Results: Baseline HbA_{1c} was 75.0±8.9 mmol/mol in the MC-group and 73.7±8.1 mmol/mol in the ABC-group. Drop-out rates were similar, 23.8% and 21.4%, respectively (p=0.712); 130 patients completed the study. At 12 months, change in HbA_{1c} was significant within both groups; MC-group -2.1 mmol/mol/year (95% CI -3.8 to -0.5, p=0.017) and ABC-group -4.6 mmol/mol/year (95% CI -6.2 to -3.0, p<0.0001). The decrease in HbA_{1c} was significantly greater in the ABC-group (p=0.033). No participants experienced a severe hypoglycaemic episode during the study. Patients wore a continuous glucose monitoring (CGM) device for 6 days before the intervention and for 6 days after the intervention. Glucose levels were categorized as below, within and above target, using a target range 3.9-10.0 mmol/l. We compared the distribution of CGM measurements between the two study groups. At baseline, there was no difference in time spent in the different target ranges between patients in the MC-group and the ABC-group. At 12 months, ABC-patients spent a larger amount of time within target compared with MC-patients (50% vs. 41%, p<0.001) and a smaller amount of time above target (45% vs. 55%, p=0.002). There was no difference in time spent below target (p=0.902). Overall, weight increased significantly (p=0.045) but with no indication of a difference in weight development between the ABC-group and the MC-group (0.67 vs. 0.24 kg/year, p=0.353).
Conclusion: Twelve months after training poorly controlled adult T1D patients in advanced carbohydrate counting, patients using an automated bolus calculator achieved significantly greater improvements in HbA_{1c} and spent more time in the glycaemic target range.
Clinical Trial Registration Number: NCT02084498
Supported by: Roche; Steno Diabetes center

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Efficacy and safety of standardised glycaemic control in adult and geriatric hospitalised patients with type 2 diabetes mellitus

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Background and aims: Insulin therapy as the preferred method is recommended to achieve glycaemic control in non-critically ill hospitalized patients with hyperglycaemia. The aim of the study was to investigate the efficacy and safety of a standardized insulin therapy supported by a computerized algorithm (GlucoTab) in adult (<70 years) and geriatric (≥70 years) hospitalized patients with diabetes mellitus type 2 (T2DM).
Materials and methods: The GlucoTab system provided automated dosing for basal-bolus insulin therapy for health care professionals in 191 non-critically ill hospitalized patients with T2DM for 8.3 days. Insulin therapy was started with a total daily dose (TDD) of 0.5 units per kg bodyweight in adult patients (<70 years) and 0.3 units per kg bodyweight in geriatric patients (≥70 years) or in patients with a creatinine value ≥2 mg/dl.
Results: 97 adult patients (30 female, age 60±7 years, BMI 31±7 kg/m2, creatinine 1.7±1.4 mg/dl, HbA1c 72±26 mmol/mol; mean ± SD) and 94 geriatric patients (48 female, age 77±5 years, BMI 29±6 kg/m2, creatinine 1.5±0.9 mg/dl, HbA1c 64±19 mmol/mol) were treated with the standardized insulin therapy using the GlucoTab system. First provided TDD was 0.49±0.16 vs. 0.37±0.24 units per kg bodyweight in adult and geriatric patients, respectively. The overall mean daily blood glucose value (BG) was 155±33 mg/dl (adult patients) and 157±31 mg/dl (geriatric patients). 69.6% vs. 71.8% of BG values were in the range 70-<180 mg/dl for adult and geriatric patients, respectively. In the range <70 mg/dl 2.5% vs. 1.3% of BG values occurred in adult and geriatric patients. In both groups no BG value <40 mg/dl was detected. 25.1% vs. 25.2% and 2.8% vs. 1.7% of BG values were in the hyperglycaemic ranges of 180-<300 mg/dl and ≥300 mg/dl in adult and geriatric patients, respectively.
Conclusion: The standardized insulin starting dose suggested by the GlucoTab with 0.5 and 0.3 units per kg bodyweight supported an efficacious and safe glycaemic control for adult and geriatric hospitalized patients.
Clinical Trial Registration Number: NTC01932775, NCT01766752, NCT02053077
Supported by: FB7 248590, FFG 844737

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An individualised calculation of insulin dose to correct marked hyperglycaemia in adult patients with insulin-treated diabetes

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Background and aims: Marked hyperglycaemia frequently occurs in patients with diabetes, the correction of which may be difficult because of glucose toxicity induced insulin resistance and fear that too aggressive correction may lead to hypoglycaemia. Usually, standard sliding scale insulin regimens, barely adjusted to and often underestimating individual needs, are applied both in outpatient and in-patient settings. We tested a simple formula based on the insulin sensitivity factor (ISF, calculated by dividing 100 by the total daily insulin dose [TDID]) for the estimation of the individual insulin dose needed to correct substantial hyperglycaemia in patients with insulin-treated diabetes.
Materials and methods: This analysis was part of a randomised controlled cross-over trial, comparing the efficacy of two insulin pens to

correct hyperglycaemia with the rapid-acting insulin analogue aspart. Adult patients with type 1 or type 2 diabetes on basal-bolus injection or pump therapy were instructed to decrease insulin doses in order to reach morning hyperglycaemia (18-23 mmol/l). Subsequently, aspart insulin was administered subcutaneously in a dose calculated as follows: 1.5 times the difference between the initial and target glucose value (set at 6 mmol/l) divided by the ISF. Plasma glucose levels were determined during 6 hours thereafter. The primary endpoint was the time in minutes until plasma glucose concentration had dropped by at least 10 mmol/l.

Results: 20 patients (10 type 1 diabetes, 10 type 2 diabetes, mean age 53.4 years, HbA1c 70 mmol/mol, TDID 96.1 U/day) underwent 40 experiments. Glucose values dropped from 21.3±0.4 to 7.3±0.4 mmol/l after injection of 21.7±1.9 (range, 8-40) insulin units (figure 1). After 196.2±11.7 minutes, plasma glucose levels dropped below 10 mmol/l. the fall in plasma glucose was about similar in patients with type 1 compared to those with type 2 diabetes. On 6 (15%) occasions the formula under-predicted insulin needs, and glucose values dropped to 12.3± 0.5 mmol/l, whereas in 7 (17.5%) occasions over-prediction required some exogenous glucose administration to prevent hypoglycaemia.

Conclusion: This easy-to-use formula was fairly accurate in calculating individualized insulin doses to correct marked hyperglycaemia in patients with insulin-treated diabetes. In the majority of patients, much larger insulin doses are required than usually recommended.

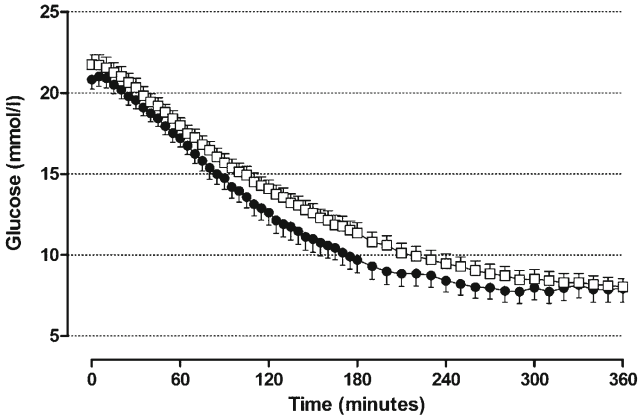

Fig 1. Changes in plasma glucose levels

Data are shown as mean ± SE, from baseline (start of insulin administration) to six hours after insulin administration by patients with T1DM (black circles) or T2DM (open squares).

Clinical Trial Registration Number: NCT01947556
Supported by: europharma group

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Evaluation of accuracy of continuous glucose monitoring when using capillary compared to venous samples as reference

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Background and aims: Accuracy of continuous glucose monitoring (CGM) is generally evaluated using venous reference samples even though CGM-systems are calibrated with capillary glucose samples. Venous glucose might be assessed with higher accuracy and reliability than capillary samples and can be taken more frequently, and have therefor become a prerequisite from regulatory perspective. However, capillary and venous glucose levels differ, and using venous reference could thus affect measured sensor accuracy. The aim was to evaluate the impact of using either capillary or venous samples as reference when evaluating accuracy of CGM calibrated with capillary values.

Materials and methods: We evaluated 41 patients with type 1 diabetes examined in Sweden. All patients used the Dexcom G4 during 6 days and calibrated their CGM by capillary values (HemoCue meter). At one occasion during days 1-3 and 1 occasion during days 4-6 the patients visited the research unit for measuring both capillary and venous samples at the same time using the HemoCue meter. The Mean Absolute Relative Difference (MARD) and correlation coefficient were used to estimate accuracy of CGM in relation to capillary and venous samples. The Wilcoxon Signed Rank test was used in statistical comparisons.

Results: Mean age was 51 years, 14 (34%) were women, and 31 (76%) were treated with multiple daily insulin injections (MDI). Mean HbA1c was 59.3 mmol/mol, diabetes duration 23 years and BMI 24.9 kg/m2. The venous samples were overall 0.83 mmol/l lower than capillary values (8.15 vs. 8.98), $p<0.0001$. In values below 4 mmol/l (22 values from 14 individuals) the difference was 1.25 mmol/l, $p=0.0001$, in the range 4-10 mmol/l (153 values from 38 individuals) 0.67 mmol/l, $p<0.0001$ and above 10 mmol/l (60 values from 24 individuals) 0.95 mmol/l difference, $p<0.0001$. The MARD using capillary values as reference was 11.7% compared to 13.7% for venous samples as reference, $p=0.037$. Below 4 mmol/l the MARD was 16.6% and 31.8% respectively, $p=0.048$, at 4-10 mmol/ 12.1% and 12.6%, $p=0.32$, and above 10 mmol/l 8.7% and 9.2% respectively, $p=0.82$. The correlation coefficient did not differ using capillary and venous samples, 0.84 and 0.85 respectively, $p=0.75$.

Conclusion: Measured CGM accuracy is significantly affected by the used reference method. MARD in the hypoglycaemic range is significantly lower with capillary values as reference as compared to venous samples as reference. We recommend taking capillary reference values when evaluating CGM accuracy in the hypoglycaemic range.

Clinical Trial Registration Number: NCT02159638
Supported by: The Region of Västra Götaland, Swedish state, Swedish Society for Physicians

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Point-of-care assessment of salivary protein glycosylation as a short-term glycaemic index

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Background and aims: Assessment of short-term (2 to 3-week) glycemia can complement daily self-monitoring of blood glucose and long-term (3-month) average blood glucose estimation based on HbA1c levels. Short-term glycemic monitoring can be particularly useful in individuals with type-2 diabetes, where occasional HbA1c results may not adequately reveal significant glycemic fluctuations and the potential for adverse effects of glycemic variability. A short-term index is also useful in determining response therapy, where a more rapid determination of efficacy can enable more efficient treatment regimes. Ideally, a short-term glycemia test would be simple, non-invasive, and feasible in a point-of-care (POC) format. We have recently described salivary protein glycosylation (SPG) as a non-invasive biomarker for assessment of glycemia over the previous 2-3 weeks with performance superior to that of fructosamine and 1,5-anhydroglucitol in comparison to continuous glucose monitoring reference values. We have now expanded the validation of SPG as a glycemia biomarker in a larger population and have developed a prototype POC device (Salivary GlyChek).

Materials and methods: One hundred and thirty-two subjects (47 controls with HbA1c less than 5.7 and 85 type 2 diabetes (T2D) subjects with

HbA1c greater than 6.5) participated in a 4-week study with daily 6-point capillary blood sugar monitoring (pre and post meal). Fasting and post-breakfast saliva and serum samples were collected weekly. HbA1c levels were determined at baseline and at the end of the study. Salivary protein glycosylation (SPG) was measured using a microplate assay involving sample oxidation, neutralization, and color development with a chemical reagent under conditions optimized for detection of fucose and sialic acid residues. Serum fructosamine was measured using an automated chemistry analyzer. The SPG plate assay chemistry was also adapted to a POC format comprised of a lateral-flow test strip containing multiple zones for glycoprotein oxidation, neutralization, and color development. A proprietary hand-held reader was also developed that utilizes colorimetric diffuse reflectance for quantitative test results.
Results: Classification performance analyses demonstrated mean SPG levels of 6.3 ug/ml and 9.5 ug/ml in controls and T2D, respectively with $p<0.0001$. Mean fructosamine values were 284 umol/L and 480 umol/l in controls and T2D, respectively with $p<0.0001$. Baseline fasting blood glucose correlated better with SPG than with with fructosamine ($r=0.61$, $p<0.0001$ vs $r=0.51$, $p<0.0001$). There were no significant differences between fasting and post-prandial sampling. 7 and 14-day mean BG measures were strongly correlated with SPG ($r=0.82$ and 0.88, respectively, $p<0.0001$). The microplate and POC tests for SPG demonstrated similar performance ($r=0.89$), and the prototype POC device exhibited a cv of 8% across the dynamic range of the assay.
Conclusion: The non-invasive GlyChek POC test, in conjunction with the hand-held reader, can greatly facilitate self-monitoring of T2D by patients and their clinical management by healthcare providers.
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Which principles patients with diabetes type 2 and flexible insulin therapy use for self-adjustment of insulin dose?
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Background and aims: Multiple insulin injection therapy is increasingly used in the treatment of people with diabetes type 2 (DM2) to improve metabolic control and reduce hypoglycaemia. Structured treatment and education programmes for people with flexible preprandial insulin therapy provide rules for self-adjustment of insulin dose according to carbohydrate amount of the meal, current blood glucose level, physical activity, stress or illness, which are extensively trained. The aim of this study was to analyze current principles and frequency of self-adjustment of insulin dose and the association with metabolic control in people with DM2.
Materials and methods: In a large university outpatient department for endocrinology and metabolic diseases over a period of three months 149 people with DM2 with flexible or intensive insulin therapy (mean HbA1c 7.1%, age 65y, diabetes duration 19.0y, female 38.9%, BMI 33.8 kg/m^2) were interviewed. 147 (98.7%) of 149 participants had participated in a therapy specific structured education programme. Self-adjustment of insulin dose was defined as each change of insulin dosage compared with the same time the previous day. Additional and skipped injections were counted as dose adjustments. The type of insulin adjustment, insulin-to-carbohydrate ratio (insulin units per 10 g of carbohydrate) and the factor for correction (e.g. 1 insulin unit lowers plasma glucose by 1.5 mmol/l) were assessed by a structured interview. Two principles for insulin dose adjustment were set: firstly the use of adjustment patterns/rules, e.g. insulin-to-carbohydrate ratio and secondly use of personal experience without a nameable rule. The frequency of insulin dose adjustments was drawn from the last 28 days of the patients' diary.
Results: Insulin dose adjustment by pattern/rule was used in 33 people (22.1%) and by personal experience in 111 of 149 participants (74.5%). People adjusting by rule/pattern were younger (60.9±9.8 vs. 65.7±9.2, $p=0.011$) and performed more insulin dose adjustments per 28 days (50.0 ±31.0 vs. 33.4±23.5, $p=0.016$). HbA1c and incidence of hypoglycaemia were comparable at time of data collection. People adjusting by pattern/rule had on average lower HbA1c three years prior to the survey than people adjusting by personal experience (6.8±0.9 vs. 7.2±0.9%, $p=0.026$). Moreover, no differences regarding satisfaction of treatment, quality of life as well as current well-being was found between the two groups. In case of high premeal blood glucose levels 77 of 149 participants (51.6%) reported to use a higher insulin dosage, 30 (20.2%) decrease their carbohydrate intake and 42 (28.2%) correct with increased physical activity.
Conclusion: Patients with DM2 and flexible preprandial insulin therapy in Germany are specifically trained to adjust their insulin dose using an insulin-to-carbohydrate factor respectively a factor for correction. Only 22% use this trained concept in daily life. The majority of the patients adjust their insulin dose by personal experience or feeling. Although people using insulin-to-carbohydrate ratio adjust their insulin dose more frequently, HbA1c and incidence of hypoglycaemia is similar compared to those using personal experience. The importance and effectiveness of self adjustment of insulin dose by rules has to be examined in a RCT.

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Two-year clinical study of a mHealth remote diabetes care system: interim results
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Background and aims: There is a lack of long-term clinical data from mHealth interventions in diabetes management. The objective of this study is to evaluate the clinical and economical effects of Triabetes, a smartphone and online based decision support tool for coaching of patients with type 2 diabetes. The system coaches patients on a daily basis and remotely monitors their progress.
Materials and methods: In a two-year, open-label, parallel-group, multicenter study, nine private care clinics were randomized in clusters with healthcare professionals and adult patients (n=225) with type 2 diabetes (≥6 months) and HbA1c≥53 mmol/mol to usual quarterly follow-up or use of the smartphone based system with quarterly follow-ups. The primary endpoint was changes in HbA1c after 6, 12 and 24 months. The study further measures other clinical biomarkers and patient-reported health, prescription use and disease progression.
Results: Population characteristics are shown in Table 1. The six-month median HbA1c change was significantly larger in the intervention group than in the control group, with a 5.3 mmol/mol reduction (95% CI: -8.5 - (-2.7); p-value<0.001). A total of 197 patients were included in the analysis. There were no significant differences in the secondary endpoints. The study is currently awaiting 12 months results.
Conclusion: Using the Triabetes software in type 2 diabetes treatment helps reduce long-term blood glucose levels with clinically relevant effects.

	Intervention n=109	Control n=108
	Mean ± SD	**Mean ± SD**
Age (years)*	53,0 ± 10,3	57,6 ± 11,2
Gender (male)*	77,8%	59,1%
Diabetes duration (years)	7,0 ± 4,8	8,0 ± 5,2
HbA1c (mmol/mol)	66,1 ± 13,2	63,3 ± 13,0
BMI (kg/m^2)	31,7 ± 4,9	31,6 ± 4,2
Waist circumference (cm)	107,5 ± 13,0	107,4 ± 12,3
Blood pressure (mmHg)	133/83 ± 15/8	136/82 ± 15/10

*Significant difference

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Which mean absolute relative difference is acceptable with respect to ISO 15197 system accuracy limits?

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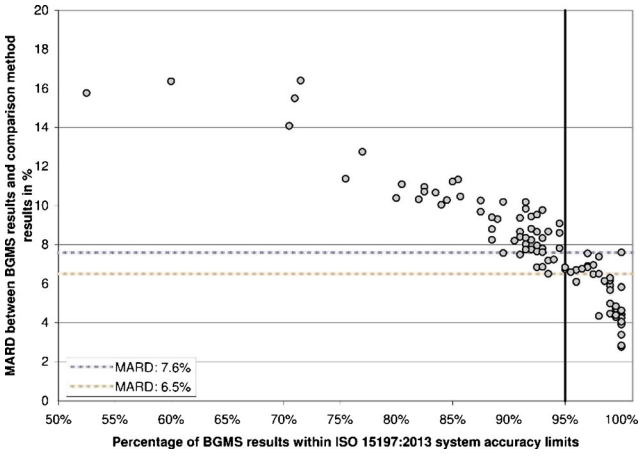
Background and aims: In the literature, the analytical quality of a blood glucose (BG) monitoring system (BGMS) is often described with respect to the system accuracy criteria of the international standard ISO 15197. Sometimes, the mean absolute relative difference (MARD) between the results of the BGMS and a comparison method is used as well; however, MARD is more commonly used to describe the analytical quality of continuous glucose monitoring systems. With regard to BGMS the question arises: which MARD corresponds to the system accuracy limits of ISO 15197?

Materials and methods: In this retrospective analysis, data from system accuracy evaluations of 69 different BGMS types (96 different test strip lots) were used to calculate the MARD between the BGMS results and the corresponding results obtained with the manufacturer's comparison method. Additionally, the same data were used to calculate the percentage of BGMS results within the system accuracy limits of ISO 15197:2003 (±15 mg/dL for BG concentrations below 75 mg/dL, ±20% for BG concentrations equal to or above 75 mg/dL) and ISO 15197:2013 (±15 mg/dL for BG concentrations below 100 mg/dL, ±15% for BG concentrations equal to or above 100 mg/dL), respectively. The system accuracy data were then used to establish which MARD result will likely lead to having at least 95% of results within ISO 15197:2003/2013 accuracy limits.

Results: The MARD results for these 96 test strip lots ranged from 2.8% to 16.4%. The lowest MARD of a test strip lot that showed <95% of results within ISO 15197:2003 accuracy limits was 7.5%, whereas the highest MARD of a test strip lot that showed ≥95% of results within ISO 15197:2003 accuracy limits was 10.2%. For the ISO 15197:2013 accuracy limits, the corresponding MARD values were 6.5% and 7.6%, respectively (see Figure).

Conclusion: Based on our analysis we conclude that a BGMS likely has acceptable analytical quality with respect to ISO 15197:2003, if its MARD result is below 7.5%. If the BGMS' MARD result is even below 6.5%, the BGMS is likely to have ≥95% of results within ISO 15197:2013 system accuracy limits, thus reaching an acceptable analytical quality with respect to ISO 15197:2013.



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Long-term accuracy surveillance of a system for self-monitoring of blood glucose

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Background and aims: Systems for self-monitoring of blood glucose (SMBG) require a CE mark to be distributed in the EU. A one-time conformity assessment, e. g. according to ISO 15197, is sufficient to obtain such a CE mark. While ISO 15197:2013 was released in 2013, ISO 15197:2003 is still valid throughout a transition period of 3 years that ends in 2016. In this study, a CE-marked SMBG system was monitored over a period of 1.5 years by repeatedly evaluating system accuracy of different test strip lots.

Materials and methods: A total of 11 test strip lots were evaluated in 4 individual studies between August 2013 and February 2015. System accuracy assessments were performed following procedures described in ISO 15197 and values obtained with the SMBG system were compared to a laboratory glucose oxidase method, which is also the manufacturer's comparison method. All materials were purchased from pharmacies; storage conditions were controlled and evaluations were performed prior to the labelled expiration dates. Results were analyzed by applying accuracy criteria of ISO 15197:2003 and criterion A of ISO 15197:2013 for every test strip lot individually. ISO 15197:2003 states that for acceptable system accuracy at least 95% of results have to fall within ±15 mg/dL at BG concentrations below 75 mg/dL and within ±20% at BG concentrations equal to or above 75 mg/dL. Criterion A of ISO 15197:2013 has more stringent criteria: at least 95% of results have to fall within ±15 mg/dL and ±15% for BG concentrations below and equal to or above 100 mg/dL, respectively.

Results: Out of 11 test strip lots, 9 lots fulfilled the criteria of ISO 15197:2003 with ≥95% of the results within the stipulated limits (see table). The more stringent criteria of ISO 15197:2013 were fulfilled by 5 test strip lots. The relative bias was between 0.2% and 10.7%. No clear trend in accuracy could be observed, but lot-to-lot variations between lots evaluated at the same time seemed to have decreased over time.

Conclusion: This long-term surveillance indicates variations in system accuracy of a CE-marked SMBG system after market-release. Getting a CE mark for an SMBG system is a one-time process with long-term surveillance of system accuracy by manufacturers not being required. However, consistent acceptable system accuracy of SMBG systems is an important prerequisite of adequate insulin-based diabetes therapy. Therefore, regular post-marketing evaluations are important to identify outliers (such as lot 4) and to ensure continuing compliance with the respective standards, thus providing people with diabetes the opportunity of properly managing their diabetes.

	Lot	Within ISO 15197:2003 limits	Within ISO 15197:2013 limits	Bias [%]
1	08-2013-1	**194 / 200 (97%)**	186 / 200 (93%)	5.1
2	08-2013-2	**198 / 200 (99%)**	**194 / 200 (97%)**	0.6
3	08-2013-3	185 / 200 (92.5%)	170 / 200 (85%)	9.2
4	11-2013-1	171 / 200 (85.5%)	154 / 200 (77%)	10.7
5	11-2013-2	**198 / 200 (99%)**	**196 / 200 (98%)**	0.2
6	05-2014-1	**196 / 200 (98%)**	**190 / 200 (95%)**	0.8
7	05-2014-2	**197 / 200 (98.5%)**	**195 / 200 (97.5%)**	2.6
8	05-2014-3	**197 / 200 (98.5%)**	186 / 200 (93%)	5.0
9	02-2015-1	**192 / 200 (96%)**	185 / 200 (92.5%)	4.8
10	02-2015-2	**190 / 200 (95%)**	181 / 200 (90.5%)	5.4
11	02-2015-3	**195 / 200 (97.5%)**	**193 / 200 (96.5%)**	1.8

PS 087 New and emerging insulin therapies

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Ultra-rapid BioChaperone insulin Lispro (BC-LIS): linear dose-response and faster absorption than insulin Lispro (LIS)

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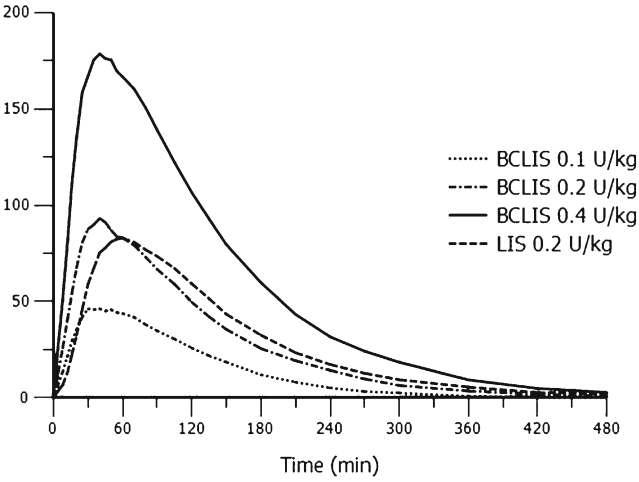
Background and aims: We investigated dose-response and dose-exposure relationship of BC-LIS, a novel, ultra-rapid formulation of the insulin analogue lispro using the BioChaperone technology, and LIS.
Materials and methods: Thirty-eight patients with type 1 diabetes mellitus (age 47.0±11.7 years, BMI 24.7±1.9 kg/m^2, HbA1c 7.5±0.8% [mean ± SD]) were included in this double-blind, randomised, four period crossover phase IIa study. They received single doses of 0.1, 0.2 or 0.4 U/kg of BC-LIS or 0.2 of LIS under automated euglycaemic clamp conditions (target blood glucose 100 mg/dL, clamp duration 12 h post-dosing).
Results: Mean baseline adjusted Insulin profiles are given in the figure. Ultra-rapid properties of 0.2 U/kg BC-LIS were demonstrated with significantly earlier time to maximum (t_{max}) and time to half-maximum insulin levels ($t_{50\%\ Cmax\ early}$), (median tmax 40 vs. 60 min, $t_{50\%\ Cmax\ early}$ 14 vs. 27 min, both comparisons $p<0.0001$), greater early exposure up to 2 h ($AUC_{Ins(0\text{-}30min)}$) 23.2 vs. 9.2 h*mU/L, $p<0.0001$; AUC_{Ins}(0-2 h) 132.3 vs. 122.6 h*mU/L, $p=0.0057$) and lower late exposure ($AUC_{Ins(2\text{-}8h)}$ 69.0 vs. 87.6 h*mU/L, $p<0.0001$) than 0.2 U/kg LIS. BC-LIS had significantly earlier time to half-maximum GIR and time to maximal GIR ($t_{0.5(GIRmax)}$ 32 vs. 42 min, $p<0.0001$; t_{GIRmax} 86 vs. 117 min, $p=0.0005$), stronger early metabolic effect up to 3 h post-dosing ($AUC_{GIR(0\text{-}3h)}$ 952.8 vs. 812.9 mg/kg, $p=0.0090$) and lower late metabolic effect ($AUC_{GIR(3\text{-}8h)}$ 357 vs. 446 mg/kg, $p=0.0115$). Total exposure and total metabolic effect were similar with either BC-LIS or LIS. Dose-proportionality was established for early and total exposure of BC-LIS ($AUC_{Ins(0\text{-}12h)}$ 102 vs 205 vs 444 h*mU/kg, 0.1 vs 0.2 vs 0.4 U/kg) and dose-linearity for early and total metabolic effect ($AUC_{GIR(0\text{-}12h)}$ 726, 1357 and 2422 mg/kg for 0.1, 0.2 and 0.4 U/kg). Ultra-rapid properties were preserved between doses as shown by unchanged t_{max} and $t_{50\%\ Cmax\ early}$. Both insulin formulations were well tolerated and no injection site reactions were observed with BC-LIS.
Conclusion: BC-LIS presents ultra-rapid, dose-proportional absorption, resulting in significantly improved glucose-lowering effect and dose-linear gluco-dynamic activity in subjects with type 1 diabetes. In addition, ultra-rapid properties are preserved at clinically relevant doses of up to 0.4 U/kg. Therefore, BC-LIS has the potential to improve postprandial glucose control versus currently available fast-acting insulin analogues such as LIS.Dose-proportional early and total exposures of clinically relevant doses (0.1, 0.2, and 0.4 units/kg) was established for BC-LIS

Figure: Mean baseline-adjusted serum insulin concentrations (mU/L)



Clinical Trial Registration Number: NCT02146651
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932

Reduced hypoglycaemia is observed with inhaled insulin versus subcutaneous insulin aspart in patients with type 1 diabetes mellitus

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Background and aims: Technosphere Insulin Inhalation Powder (TI) is a dry powder formulation of regular human insulin adsorbed onto Technosphere microparticles for oral inhalation in patients with diabetes. We conducted a post hoc analysis of hypoglycaemia rates from a 24-week, phase 3 randomized study of prandial TI (N=172) versus insulin aspart (IA; N=167) added to basal insulin in patients with type 1 diabetes mellitus (T1DM).
Materials and methods: Annualised hypoglycaemia event rates were determined in the overall study population, patients taking≥1 postmeal supplemental dose (TI, n=111; IA, n=91), and patients not taking a supplemental dose (TI, n=61; IA, n=76). Hypoglycaemia rates during study weeks 0-12 (dose adjustment) and 12-24 (stable dosing) were assessed. Hypoglycaemia was defined as total (all events), confirmed (blood glucose<49 mg/dL), nocturnal (0:00−6:00 AM), and severe (assistance required). Data were adjusted for baseline HbA_{1c} level.
Results: There was no significant difference in the mean number of supplemental doses taken between the TI and IA arms (34.1 vs 26.6, respectively; $P=0.1907$). Significantly higher hypoglycaemia rates (events per patient/year) were seen for IA versus TI in the overall study population (total 81.1 vs 55.2; confirmed 15.0 vs 9.0; nocturnal 8.8 vs 5.9; and severe 0.9 vs 0.5; all $P<0.05$) and patients taking≥1 supplemental dose (total 96.3 vs 60.9; confirmed 18.6 vs 9.8; nocturnal 11.5 vs 6.5; and severe 1.1 vs 0.6; all $P<0.05$). In patients not taking a supplemental dose, a significantly higher total hypoglycaemia rate was seen with IA versus TI (64.9 vs 46.0 events per patient/year; $P=0.0160$). Within each arm there was a trend for higher total hypoglycaemia rates≥1 hour after supplemental dosing (events per patient/year) in patients with a higher supplemental dose frequency (TI vs IA: 0.4 vs 0.4; 1.7 vs 2.2; and 4.9 vs 6.6 for patients receiving 1-5, 6-20, and 21-60 supplemental doses during the study); there was no significant difference between the TI and IA arms. In the overall study population, within both treatments arms there was a trend for a higher rate of all types of hypoglycaemia during weeks 0-12 (titration) versus 12-24 (stable dosing). In patients taking a supplemental dose, a similar trend was seen in the IA arm only. In patients not

taking a supplemental dose, there was a trend for higher total, confirmed, and nocturnal hypoglycaemia rates during weeks 0-12 versus 12-24 for IA; and total, confirmed, and severe hypoglycaemia for TI.
Conclusion: These data show that the more rapid onset and offset of action with TI versus IA was not associated with greater supplemental dosing. There was a consistently lower hypoglycaemia rate in patients with T1DM treated with TI versus IA, including those taking supplemental doses, and during dose adjustment and stable dosing. These data show that supplemental TI dosing does not result in an increased hypoglycaemia risk.
Clinical Trial Registration Number: NCT01445951
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933

Higher early insulin exposure and greater early glucose-lowering effect with faster-acting insulin aspart vs insulin aspart in elderly and younger adults with type 1 diabetes

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Background and aims: Faster-acting insulin aspart (faster aspart) is a new formulation of insulin aspart (IAsp), with initial faster absorption following subcutaneous (s.c.) injection. This single-dose, double-blind, two period, cross-over trial compared the pharmacokinetic (PK) and pharmacodynamic (PD) profiles of faster aspart vs IAsp in elderly and younger adults with type 1 diabetes (T1D).
Materials and methods: Sixty-seven patients (n=37 aged 18-35 years; n=30 aged ≥65 years) received a single dose (0.2 U/kg body weight s.c.) of faster aspart or IAsp in a euglycaemic glucose clamp setting (blood glucose target 5.5 mmol/L (100 mg/dL;duration 12 h post-dose).
Results: For both age groups, onset of appearance with faster aspart occurred twice as fast (relative difference: 55%, elderly, 47%, younger adults; Table) than with IAsp (mean difference [95% CI]: elderly, -2.88 min [-3.83; -1.94]; younger adults, -2.78 min [-4.21; -1.35]), with a greater early insulin exposure during the first 1 h; total exposure was similar in both groups (Table). Earlier onset of action with faster aspart vs IAsp (relative difference: 35%, elderly, 33% younger adults; Table) occurred in both age groups (elderly, -10.17 min [-15.29; -5.06]; younger adults, -8.70 min [-15.07; -2.34]) and was supported by shorter $t50\%GIR_{max}$ vs IAsp (elderly, -5.58 min [-8.99; -2.17]; younger adults, -10.28 min [-15.41; -5.15]). Faster aspart also had a greater early glucose-lowering effect within 2 h post-dose vs IAsp (greatest [i.e., 2-fold] difference within the first 30 min; LSmean, mg/kg: elderly, 44.72 vs 21.41; younger adults, 42.83 vs 20.52); total glucose-lowering effect was similar [Table]). Treatment effects did not differ significantly between age groups. Both treatments were well tolerated and no safety issues were identified, including no injection-site reactions.
Conclusion: Earlier onset and greater early insulin exposure with faster aspart led to a greater early glucose-lowering effect vs IAsp, which was retained in both elderly and younger adults with T1D.

Table: PK and PD results for faster aspart vs IAsp

	Treatment ratio faster aspart/IAsp [95% CI]	
	Elderly	Younger Adults
PK endpoints (insulin exposure*)		
Onset		
Onset of appearance	0.45 [0.30; 0.60]	0.53 [0.34; 0.74]
$t_{50\%C_{max}}$	0.65 [0.56; 0.74]	0.71 [0.62; 0.81]
Early		
$AUC_{0-15min}$	3.17 [2.43; 4.13]	2.51 [1.78; 3.54]
$AUC_{0-30min}$	1.86 [1.53; 2.27]	1.67 [1.35; 2.07]
AUC_{0-1h}	1.33 [1.17; 1.52]	1.26 [1.07; 1.48]
AUC_{0-2h}	1.15 [1.04; 1.27]	1.11 [0.95; 1.29]
Total		
AUC_{0-12h}	1.05 [0.98; 1.14]	1.07 [0.92; 1.25]
C_{max}	1.11 [1.00; 1.23]	1.04 [0.91; 1.18]
PD endpoints (glucose-lowering effect)		
Onset		
Onset of action	0.65 [0.51; 0.81]	0.67 [0.49; 0.89]
$t_{50\%GIR_{max}}$	0.85 [0.77; 0.94]	0.75 [0.65; 0.86]
Early		
$AUC_{GIR,0-30min}$	2.09 [1.37; 4.66]	2.09 [1.31; 4.30]
$AUC_{GIR,0-1h}$	1.42 [1.14; 1.75]	1.55 [1.16; 2.07]
$AUC_{GIR,0-2h}$	1.20 [1.00; 1.43]	1.19 [0.97; 1.46]
Total		
$AUC_{GIR,0-12h}$ †	1.12 [0.94; 1.35]	1.03 [0.90; 1.17]
GIR_{max}	1.13 [0.96; 1.33]	1.04 [0.88; 1.22]

*Based on free serum insulin aspart; †Primary endpoint; AUC=area under the curve; C_{max}=maximum observed concentration; GIR_{max}=maximum glucose infusion rate; onset of appearance=time from dosing until the first time serum IAsp concentration ≥lower limit of quantification; $t50\%_{Cmax}$=time to 50% of maximum serum insulin aspart concentration; $t50\%GIR_{max}$=time to 50% of maximum glucose infusion rate.

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Supported by: Novo Nordisk

934

Insulin degludec/insulin aspart lowers fasting plasma glucose and rates of confirmed and nocturnal hypoglycaemia independent of baseline HbA_{1c} levels

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Background and aims: Individual patient characteristics can influence the selection of appropriate glycaemic targets by clinicians. The availability of individual patient data, including baseline HbA_{1c} levels may improve the achievement of individualised glycaemic targets. IDegAsp, a novel co-formulation of 70% insulin degludec (IDeg) and 30% insulin aspart (IAsp) has been previously shown to provide effective glycaemic control in individuals with type 2 diabetes mellitus (T2DM). This *post-hoc*, pooled analysis evaluated the efficacy of IDegAsp vs BIAsp 30 and IDeg OD + IAsp in adequately controlling glycaemic parameters and lowering hypoglycaemia in subjects with T2DM stratified according to baseline HbA_{1c}.
Materials and methods: Data were pooled from five 26-week treat-to-target phase 3a/b clinical trials in subjects with T2DM in the IDegAsp clinical development programme. Comparators from the individual trials were pooled and included biphasic insulin aspart 30 (BIAsp 30) twice-daily (BID) or IDeg OD + IAsp (2-4 injections). End of trial (EOT) HbA_{1c}, confirmed and nocturnal confirmed hypoglycaemia, and insulin dose were stratified according to four baseline HbA_{1c} categories: <7.5%

(n=263), ≥7.5-<8.5% (n=760), ≥8.5-<9.0% (n=309), ≥9.0% (n=476). EOT fasting plasma glucose (FPG) was stratified according to baseline FPG: 5.5-7.0-10.0 (n=523). For each separate group, continuous variables were analysed using ANOVA and event rates were analysed using negative binomial regression.

Results: There was no difference in HbA_{1c} EOT across HbA_{1c} categories between IDegAsp (n=1111) and the comparator group (n=697). FPG EOT was significantly lower in subjects in three baseline FPG categories (>5.5-7.0-10.0, n=523) with IDegAsp vs comparator (Table 1). Episodes of confirmed hypoglycaemia were significantly lower with IDegAsp ($p<0.05$) (Table 1) as were episodes of nocturnal confirmed hypoglycaemia in all four HbA_{1c} categories (Table 1). IDegAsp BID vs BIAsp 30 BID achieved a significant reduction in total daily insulin dose at EOT in subjects with baseline HbA_{1c} ≥7.5-<8.5% and ≥9.0 ($p<0.0001$). Similarly, when compared with IDeg OD + IAsp, IDegAsp was associated with a significant reduction in EOT daily insulin dose in subjects with baseline HbA_{1c} ≥7.5-<8.5% ($p<0.05$).

Conclusion: No differences in EOT HbA_{1c} levels were observed between IDegAsp and comparator in any of the baseline HbA_{1c} categories. IDegAsp significantly lowered FPG, particularly when baseline FPG exceeded 5.5 mmol/L. IDegAsp was associated with significantly lower rates of confirmed hypoglycaemia and nocturnal confirmed hypoglycaemia compared with BIAsp 30 and IDeg OD + IAsp, independent of baseline HbA_{1c}.

Table 1. Trial endpoints stratified by baseline HbA_{1c}

Endpoint	IDegAsp	Comparator (BIAsp 30 BID and IDeg OD+ IAsp)	Treatment contrast, p value
HbA_{1c} (%)*			
7.0–7.5	6.5	6.5	0.03, p=ns
≥7.5–<8.5	6.8	6.7	0.04, p=ns
≥8.5–<9.0	7.1	7.0	0.11, p=ns
≥9.0	7.3	7.3	0.00, p=ns
FPG (mmol/L)*			
<5.5	5.5	6.1	–0.56, p=ns
>5.5–<7.0	5.4	6.3	–0.88, p=0.0002
>7.0–<10.0	5.7	6.7	–1.01, p<0.0001
>10.0	6.7	7.7	–0.95, p=0.0002
Confirmed hypoglycaemia (Events/100 PYE)*			Rate ratio
HbA_{1c} (%)			
<7.5	798	1201	0.66, p=0.0299
≥7.5–<8.5	792	1093	0.72, p=0.0049
≥8.5–<9.0	676	998	0.68, p=0.0172
≥9.0	870	1175	0.74 p=0.0474
Nocturnal confirmed hypoglycaemia (Events/100 PYE)*			Rate ratio
HbA_{1c} (%)			
<7.5	73	137	0.54, p=0.0132
≥7.5–<8.5	86	198	0.43, p<0.0001
≥8.5–<9.0	68	166	0.41, p=0.0017
≥9.0	82	226	0.36, p<0.0001
Total daily insulin dose (U)*		BIAsp 30 BID	
HbA_{1c} (%)			
<7.5	52.95	72.03	–19.07, p=0.0008
≥7.5–<8.5	63.20	69.31	–6.11, p=ns
≥8.5–<9.0	71.04	81.82	–10.78, p=0.0163
≥9.0	81.41	86.93	–5.51, p=ns
Total daily insulin dose (U)*		IDeg OD + IAsp	
HbA_{1c} (%)			
<7.5	50.86	85.61	–34.75, p=ns
≥7.5–<8.5	85.34	112.09	–26.74, p=0.0346
≥8.5–<9.0	117.34	143.60	–26.26, p=ns
≥9.0	104.36	121.15	–16.79, p=ns

Data are from full analysis set, last observation carried forward
*Estimated
BIAsp 30, biphasic insulin aspart 30; BID, twice-daily; OD, once-daily; PYE, patient years of exposure
Data are pooled from BOOST: INTENSIFY PREMIX I, BOOST: INTENSIFY ALL, BOOST: Start Twice Daily, BOOST: SIMPLE vs STEP-WISE and BOOST: TWICE DAILY vs BB, unless otherwise specified
n numbers for the total daily insulin dose groups are different from the n numbers for the number of patients in each HbA_{1c} category, (as listed in the Materials and Methods) as the comparator groups have been divided differently for the total daily insulin dose groups (IDegAsp BID vs BIAsp 30 BID and IDegAsp vs IDeg OD + IAsp).

Supported by: Novo Nordisk A/S

935

A novel once weekly combination of a long-acting GLP-1R agonist and an insulin analogue (^{LAPS}Insulin Combo) offers favourable PK/PD and mitogenic properties

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Background and aims: Developments of a basal insulin and a GLP-1 agonist combination have received great interest due to greater efficacy and fewer side effects. However, recently developed combination products still require a once-daily administration, which may negatively affect treatment adherence. We have developed a long-acting basal insulin and an exendin-4 analog, ^{LAPS}Insulin 115 (HM12470) and ^{LAPS}*CA*-Exendin-4 (efpeglenatide) with PK indicating that once-weekly administration would achieve continuous therapeutic plasma levels. This study investigated the PK/PD and mitogenic properties of ^{LAPS}Insulin Combo (HM14220: ^{LAPS}Insulin 115 and efpeglenatide combination) *in vitro* and *in vivo.*

Materials and methods: Serum concentration of ^{LAPS}Insulin 115 and efpeglenatide were quantified after single administration of ^{LAPS}Insulin 115 and/or efpeglenatide in various species. The Human PK profile of ^{LAPS}Insulin 115 was predicted using WajimaC_{ss}-MRT method. A thymidine incorporation assay was performed to evaluate the mitogenic effect of ^{LAPS}Insulin Combo on SaOS2, MCF7, and COLO205 cells. DIO/STZ rats were chronically treated with ^{LAPS}Insulin 115 and/or efpeglenatide. Blood glucose and weight changes were monitored throughout the study. At the end of treatment, HbA1c levels were determined. Various dosing regimens were investigated,including a forced flexible regimen (10 to 50 hr delayed administration at every other dosing).The effect on β-cell preservation was evaluated by insulin staining of pancreas of *db/db* mice after 12 weeks treatment of ^{LAPS}Insulin 115 and/or efpeglenatide. A switching study, with initial administration of either insulin glargine or liraglutide for 2 weeks, followed by administration of ^{LAPS}Insulin Combo for another 4 weeks in *db/db* mice was conducted.

Results: After single administration, ^{LAPS}Insulin 115 and efpeglenatide showed harmonized PK without drug to drug interaction. The half-life of ^{LAPS}Insulin 115 and efpeglenatide was determined as 32.2~106.8 h and 38.4~79.6 h, respectively, in rodents and cynomolgus monkeys. Based on these results, ^{LAPS}Insulin 115 half-life in human was predicted as 132 h which was similar to that of efpeglenatide (~140 h).The addition of efpeglenatide did not alter the low mitogenicity of ^{LAPS}Insulin 115 (~5% *vs.*rh-Insulin) in all cell systems tested. Combined administration (Q3D) in DIO/STZ rats mimicked human once-weekly administration of ^{LAPS}Insulin 115 and efpeglematide achieved significantly greater HbA1c reductions (endpoint HbA1c: 4.8±0.25%) relative to the groups treated with the individual compounds (6.3±0.28% for ^{LAPS}Insulin 115; 5.4± 1.3% for efpeglenatide). The improved efficacy was maintained even after forced-flexible dosing without fluctuation in blood glucose profile. Moreover, switching from either insulin glargine or liraglutide to ^{LAPS}Insulin Combo led to additional HbA1c reduction. Finally, insulin staining indicated that ^{LAPS}Insulin Combo showed an increased β-cell mass compared the groups treated with the individual compounds.

Conclusion: ^{LAPS}Insulin Combo may offer the convenience of once-weekly regimen with a potential for flexible dosing together with improved effects on blood glucose for the management of diabetic patients.

936

Faster-acting insulin aspart by continuous subcutaneous insulin infusion: earlier exposure and greater early pharmacokinetic / pharmacodynamic effects vs insulin aspart

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Background and aims: Faster-acting insulin aspart (faster aspart) is insulin aspart (IAsp) in a new formulation with a faster initial absorption after subcutaneous injection. The aims of this study were to evaluate the pharmacokinetic (PK) and pharmacodynamic (PD) properties of faster aspart during continuous subcutaneous insulin infusion (CSII).
Materials and methods: PK/PD properties were investigated in a randomised, double-blind, crossover trial in 48 patients with type 1 diabetes mellitus (mean ± SD age: 46.3±8.6 years; HbA_{1c}: 7.4±0.6% [57± 6 mmol/mol]). Patients received faster aspart or IAsp as a CSII bolus dose (0.15 U/kg) on top of basal CSII (0.02 U/kg/h) under glucose clamp conditions (ClampArt; target 5.5 mmol/l [100 mg/dl]; duration 27 h, 13 h run-in, 14 h post bolus dosing).
Results: After the bolus dose, $t_{50\%\ Cmax}$ and t_{max} (Table) occurred 36% (12 min [95% CI 9;14]) and 31% (26 min [17;34]) earlier, respectively, with faster aspart compared to IAsp. Moreover, early insulin exposure in the first 2 h was greater with faster aspart than IAsp; for example, exposure was threefold greater in the first 30 min for faster aspart ($AUC_{IAsp,\ 0\text{-}30\ min}$, Table), whereas total exposure was similar ($AUC_{IAsp,\ Total}$, Table). Faster aspart had earlier $t_{50\%\ GIRmax}$ (21%; 11 min [7;15]) and t_{GIRmax} (14%; 19 min [3;34]) and a greater glucose-lowering effect during the first 2 h after bolus dosing vs IAsp; whereas total glucose-lowering effect was similar ($AUC_{GIR,\ Total}$, Table). Both treatments were well tolerated.
Conclusion: In summary, faster aspart showed enhanced early exposure and action compared with IAsp in CSII. Improvements in onset of exposure and action were more pronounced than those previously reported for subcutaneous injection of faster aspart.

Table: Pharmacokinetic and pharmacodynamic results for faster-acting insulin aspart vs insulin aspart

PK endpoints	Ratio [95% CI]	PD endpoints	Ratio [95% CI]
Early insulin exposure		**Early glucose-lowering effect**	
$AUC_{IAsp,\ 0\text{–}15\ min}$	7.05 [3.73;136.57]†	$AUC_{GIR,\ 0\text{–}15\ min}$	NA
$AUC_{IAsp,\ 0\text{–}30\ min}$	2.95 [2.32;3.73]	$AUC_{GIR,\ 0\text{–}30\ min}$	2.18 [1.33;5.04]†
$AUC_{IAsp,\ 0\text{–}1\ h}$	1.52 [1.37;1.69]	$AUC_{GIR,\ 0\text{–}1\ h}$	1.52 [1.29;1.83]†
$AUC_{IAsp,\ 0\text{–}2\ h}$	1.18 [1.10;1.26]	$AUC_{GIR,\ 0\text{–}2\ h}$	1.21 [1.07;1.36]
Onset of exposure		**Onset of action**	
$t_{50\%\ Cmax}$	0.64 [0.57;0.71]†	$t_{50\%\ GIRmax}$	0.79 [0.72;0.86]†
t_{max}	0.69 [0.60;0.78]†	t_{GIRmax}	0.86 [0.75;0.97]†
Total exposure		**Total glucose-lowering effect**	
$AUC_{IAsp,\ Total}$	0.97 [0.90;1.05]	$AUC_{GIR,\ Total}$	1.04 [0.95;1.13]
C_{max}	1.11 [1.03;1.19]	GIR_{max}	1.04 [0.94;1.14]

†Ratio calculated using Fieller's method. Endpoints were corrected for basal CSII. Ratio = faster aspart/IAsp. CSII, continuous subcutaneous insulin infusion; faster aspart, faster-acting insulin aspart; GIR, glucose infusion rate; IAsp, insulin aspart; NA, not available; PD, pharmacodynamic; PK, pharmacokinetic.

Clinical Trial Registration Number: NCT01992588
Supported by: Novo Nordisk

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Cardiovascular events and all-cause mortality: effects of dual therapy intensification with insulin vs glucagon-like peptide-1 analogue

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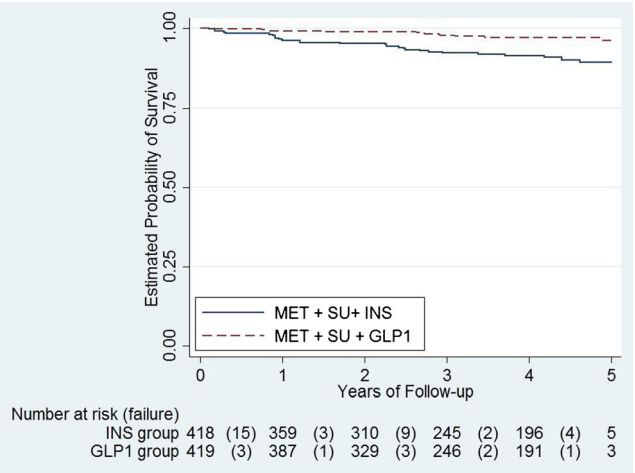
Background and aims: Recent observational studies have raised concerns about the CV safety of insulin therapy in type 2 diabetes (T2DM) after monotherapy with metformin (MET) has failed to maintain glycaemic control. We therefore analysed the time to non-fatal acute myocardial infarction, non-fatal stroke or all-cause mortality in patients with T2DM who had their treatment intensified with insulin or a Glucagon like peptide-1 (GLP-1) analogue following dual therapy failure with MET and SU.
Materials and methods: A retrospective cohort study was conducted in 2003 patients who were newly treated with a GLP-1 or insulin following dual therapy (MET + SU) failure between 2006 and-2014. Data was sourced from UK General Practices via The Health Initiative Network (THIN) database. The risk of the composite outcome (CV events) was compared between 2 treatment groups: MET+SU+Insulin (N=1584) and MET+SU+GLP-1 (N=419). Follow-up was 5 yrs (total of 6614 person-yrs), and propensity score matching analysis and Cox proportional hazard models were employed.
Results: Mean age was 52.8±14.1 years. Overall, the number of CV events was 231 vs 11 for patients who added insulin vs GLP-1 respectively, (44.5 vs 7.7 per 1000 person-years adjusted Hazard Ratio (aHR): 0.27; 95%CI: 0.14-0.53; p<0.0001). Insulin was associated with significant increase in weight compared with GLP-1; (1.78 vs -3.93 kg; p <0.0001) but HbA1c reduction was similar between both treatment groups; (-1.29 vs -0.98; p=0.156). In a subgroup analysis of obese patients, (BMI>30 kg/m^2) there were 84 vs 11 composite outcomes (38.6 vs 8.1 per 1000 person-years; aHR: 0.31; 95%CI: 0.16-0.61; p=0.001) in the Insulin and GLP-1 groups respectively.
Conclusion: Therefore, in this large cohort study tracking outcomes in routine clinical practice, intensification of dual oral therapy by adding insulin is associated with a higher risk of CV events, irrespective of weight compared with adding a GLP-1 therapy as the third oral agent.



938

Rapid-acting insulin lispro products SAR342434 and US-and EU-approved insulin lispro solution show similar pharmacokinetics and pharmacodynamics in subjects with type 1 diabetes mellitus

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Background and aims: SAR342434 solution and US- and EU-approved insulin lispro solution are rapid-acting insulin lispro products. Despite identical amino sequences, protein-based therapeutics must be shown to be pharmacologically similar to be approved as biosimilars. We compared the pharmacokinetics (PK) and pharmacodynamics (PD) of SAR342434 (T) and US-approved (R1) and EU-approved (R2) insulin lispro in a single-centre, randomized, double-blind, 3-treatment, 3-period, 6-sequence, crossover, euglycaemic clamp study.
Materials and methods: 30 male subjects with T1DM (20-59 years) were randomized to receive 0.3 $U.kg^{-1}$ T, R1 and R2 in separate periods;

28 subjects completed all 3 treatment periods. PK and PD (Glucose Infusion Rate, GIR) by euglycaemic clamp were assessed up to 12 hours.
Results: Mean concentration and GIR vs time profiles for T, R1 and R2 were similar. Exposure to and activity of T, R1 and R2 were similar between treatments, with the 90% CI for the ratios of geometric least squares means contained in the pre-specified bioequivalence limit of 0.80 to 1.25, and non-significant differences in time related parameters (Table). The within-subject variability of exposure and activity across the 3 clamps was low: 6.8% (90% CI 5.9 to 8.1) for INS-AUC; 15.6% (13.5 to 18.6) for INS-C_{max}, 16.7% (14.4 to 19.9) for GIR-$AUC_{0\text{-}12}$; and 15.2% (13.1 to 18.1) for GIR_{max}, indicating high day-to-day reproducibility in clamp performance, regardless of the product. Adverse events were similar for T, R1 and R2. No safety concerns were noted in laboratory, vital signs, or ECG data.
Conclusion: This study demonstrated a similar insulin lispro exposure profile, PK and PD activity for SAR342434 solution compared to both US- and EU-approved insulin lispro solution, and between both US- and EU-approved insulin lispro, rendering SAR342434 a biosimilar candidate.

Table. Analysis of the primary PK and PD parameters of rapid-acting insulin lispro products, SAR342434 solution (T), and US-approved (R1) and EU-approved (R2) insulin lispro.

Parameter	Treatment ratio	Point estimate (90% CI)	Parameter	Treatment difference	Hodges-Lehmann shift
INS-C_{max}	T vs R1	0.97 (0.89 to 1.05)	INS-t_{max}, hours	T - R1	-0.17 (-0.25 to -0.08)
	T vs R2	0.96 (0.89 to 1.04)		T - R2	-0.17 (-0.25 to 0.09)
	R1 vs R2	0.99 (0.94 to 1.03)		R1 - R2	0.08 (-0.00 to 0.17)
INS-AUC	T vs R1	0.95 (0.92 to 0.99)	150%-INS-$AUC_{t=12h}$, hours	T - R1	-0.13 (-0.20 to -0.08)
	T vs R2	0.97 (0.94 to 1.00)		T - R2	-0.10 (-0.17 to -0.01)
	R1 vs R2	1.02 (1.00 to 1.05)		R1 - R2	0.05 (-0.01 to 0.08)
GIR-$AUC_{0\text{-}12}$	T vs R1	1.00 (0.94 to 1.07)	GIR-t_{max}, hours	T - R1	-0.30 (-0.56 to -0.03)
	T vs R2	1.06 (0.97 to 1.15)		T - R2	-0.26 (-0.61 to 0.00)
	R1 vs R2	1.05 (0.98 to 1.14)		R1 - R2	-0.07 (-0.42 to 0.28)
GIR-$_{max}$	T vs R1	1.04 (0.98 to 1.10)	T50% GIR-$AUC_{t\text{-}t_{1/2}}$, hours	T - R1	-0.18 (-0.28 to -0.07)
	T vs R2	1.07 (0.99 to 1.14)		T - R2	-0.12 (-0.23 to -0.00)
	R1 vs R2	1.03 (0.95 to 1.10)		R1 - R2	0.04 (-0.07 to 0.12)

GIR, body weight standardised glucose infusion rate; INS, plasma insulin lispro concentration.

Point estimate method: Hodges-Lehmann, CI method: Moses (exact), GIR_{max} and GIR-t_{max} are based on smoothed GIR profiles (tension 0.06).

Clinical Trial Registration Number: NCT02273258
Supported by: Sanofi

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Exploratory study of a dose-response curve for basal insulin
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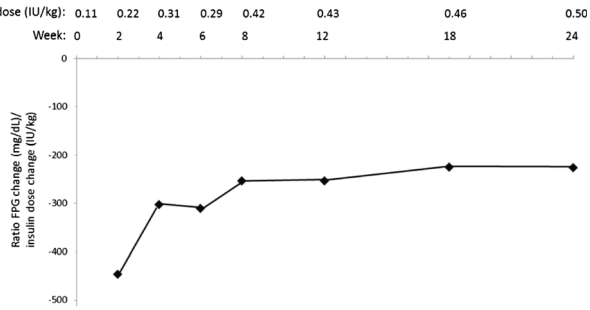
Background and aims: Many primary care physicians (PCPs) are challenged by decisions on transitioning their patients with type 2 diabetes mellitus (T2DM) from basal insulin alone to basal insulin plus prandial therapy. PCPs often continue to titrate basal insulin beyond a point of optimal efficacy and, for many patients, this results in inadequate HbA1c control and increased risk of hypoglycaemia and weight gain. With the aim of guiding PCPs in utilizing basal insulin properly, we assessed if there is a diminishing response to increasing basal insulin by performing a post hoc analysis of 3 insulin glargine titration studies of≥24 weeks among patients with T2DM.
Materials and methods: A total of 458 patients were included; mean age 56 years, half were male, mean BMI 33 kg/m^2, mean HbA1c 8.7%, and mean fasting plasma glucose (FPG) 199.6 mg/dL. Ratios for FPG change:insulin dose change were calculated and plotted at various time points.
Results: We observed that with increasing dose of basal insulin, FPG reduction becomes proportionally smaller, levelling at around 0.5 IU/kg (Figure).
Conclusion: Basal insulin shows a dose-response curve; as the basal insulin dose approaches 0.5 IU/kg, there is little incremental benefit of further increasing the basal insulin dose. Therefore, at basal insulin doses>0.5 IU/kg it may be advisable for PCPs to consider initiating prandial therapy. In patients requiring high basal insulin doses, decreasing glucose-lowering responses may be indicative of either a dose-response curve or a greater degree of insulin resistance. However, despite a dose-response curve seen at a population level, it is important to stress that appropriate treatment decisions for patients with T2DM should be based on individualization of therapy and response.

Figure. FPG change: insulin dose change ratios; changes are from baseline to indicated time point.



Supported by: Sanofi US, Inc.

PS 088 Psychological aspects of diabetes

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Excessive daytime sleepiness, daytime napping, and risk of type 2 diabetes: a meta-analysis

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Background and aims: Sleep is an important component of a healthy life, along with a good diet and appropriate physical activity. Excessive daytime sleepiness is widely prevalent around the world and so is the habit of napping. Daytime naps are usually brief, but can range from a few minutes to a few hours. The frequency varies from taking an occasional nap to planned rest periods several times daily for habitual nappers. Some individuals take a nap because they are excessively sleepy during the daytime as a result of a sleep disorder. We performed a meta-analysis to investigate the association between daytime sleepiness or napping and the risk of type 2 diabetes.
Materials and methods: We searched Medline, the Cochrane Library, and Web of Science for articles published up to November 2014 using the keywords daytime sleepiness, nap, and diabetes. To ascertain the validity of the eligible studies, the quality of each report was appraised with reference to the STROBE statement. In addition, the Newcastle-Ottawa Scale for assessing the quality of observational studies in meta-analyses was used to quantify the validity of each study. The adjusted relative risk and 95% confidence interval were calculated with the random effect model. Dose-response relations were also evaluated. Two observers independently assessed eligibility, extracted data, and assessed possible bias. Sex was employed as an independent category for comparison. Publication bias was evaluated by the funnel plot, Begg's test, and Egger's test.
Results: Among 683 studies initially identified, 261,365 Asian and Western subjects stratified into 13 categories (reports) were selected. In each study, analyses were well adjusted for several confounders for diabetes. Pooled analysis revealed that excessive daytime sleepiness and a longer nap (≥60 min/day) each significantly increased the risk of type 2 diabetes by about 50% comparted with the absence of these factors. The relative risk was 1.56 (95% CI 1.13-2.14, p=0.006) for excessive daytime sleepiness, while the relative risk was 1.46 (1.23-1.74, p<0.001) for a longer nap. In contrast, a shorter nap (<60 min/day) did not increase the risk of diabetes (0.95 (0.75-1.21, p=0.68)). A dose-response meta-analysis using the cubic spline model showed a J-shaped relation between nap time and the risk of diabetes (p for non-linearity=0.003), with no effect of napping up to about 40 minutes/day followed by a sharp increase in the risk of diabetes at longer times. Analyses stratified by study location, study quality, and study type also yielded similar results. Moreover, the funnel plot, Begg's test, and Egger's test did not suggest any evidence of publication bias.
Conclusion: Excessive daytime sleepiness and taking longer naps were associated with increased risk of type 2 diabetes, with a short nap not increasing this risk.

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Curvilinear associations of irregular sleep patterns during weekends with glycaemic control in type 2 diabetes: the Hong Kong Diabetes Registry

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Background and aims: Both long and short duration of sleep are associated with hyperglycemia. Nonetheless, the associations between glycemia and sleep duration during weekdays and weekends are less well studied. We examined the associations of glycemic control with sleep patterns during weekdays and weekends in patients with type 2 diabetes.
Materials and methods: A consecutive cohort of 2,531 type 2 diabetic patients was studied between July 2010 and February 2013. We measured indices of glycemic control including glycated hemoglobin (HbA_{1c}) and fasting plasma glucose (FPG), and sleep parameters including sleep duration during weekdays and weekends, chronotype (a construct capturing an individual's preference for being a "morning" or "evening" person) and social jetlag (a measure of misalignment between biological and social clocks). Restricted cubic spline (RCS) regressions were used to examine the relationships between the parameters of glycemic control and sleep pattern in the present study.
Results: In this cohort, mean age was 54.6 (standard deviation, SD 8.6) years and mean disease duration of diabetes was 7.9 (SD 7.0) years. 59.5% were men with 60.7% reported to have full or part-time employment. 4.5% were regular shift-workers. The mean sleep duration was 7.8 ±1.4 and 8.4±1.5 hours during weekdays and weekends respectively. After adjustment for potential confounders including employment and shift work, we found that chronotype and social jetlag had a significant U-shaped and J-shaped like relationship with HbA_{1c} and FPG respectively (p for non-linear association=0.002 and<0.001 respectively).
Conclusion: In type 2 diabetes, irregular sleep patterns with excessive and insufficient sleep compensation during weekends are associated with poor glycemic control. These novel data underpinned the importance of regularity of sleep habits and circadian influences in glucose metabolism.
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Serum insulin degrading enzyme level and other factors in type 2 diabetic patients with mild cognitive impairment

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Background and aims: Insulin degrading enzyme (IDE) contributes to the degradation process of insulin and Aβ. We aimed to investigate the role of IDE in patients with type 2 diabetes patients with mild cognitive impairment (MCI).
Materials and methods: A total of 146 individuals with type 2 diabetes were enrolled, including 75 patients with MCI and 71 patients without MCI. Demographic characteristics, cognitive function and serum IDE level were examined.
Results: Among patients with MCI, the Montreal Cognitive Assessment (MoCA) score was positively correlated with serum IDE (r=0.906; p< 0.001) but negatively correlated with fasting blood glucose (FBG) (r= −0.314; p=0.026), glycosylated hemoglobin (HbAlc) (r=−0.349; p= 0.003), homeostasis model assessment of insulin resistance (HOMA-IR) (r=−0.619; p<0.001) and glucose fluctuation (r=−0.508; p<0.001). Spearman rank correlation analysis demonstrated that IDE was positively correlated with MoCA score (r=0.847; p<0.001) but negatively

correlated with Trail Making Test B (r=−0.413; p<0.001), FBG (r=−0.372; p<0.001), HbAlc (r=−0.214; p=0.015), HOMA-IR (r=−0.582; p<0.001) and glucose fluctuation (r=−0.551; p<0.001) in all subjects. In logistic regression analysis for MCI, only fasting C-peptide (FCP) (p=0.003), HbAlc (p=0.056), and IDE (p=0.010) were independent variables.
Conclusion: This study demonstrated greater likelihood of MCI with decreasing serum IDE in patients with type 2 diabetes.
Clinical Trial Registration Number: ChiCTR-OCC-15006060

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Mental disorders, suicidal ideation and diabetes mellitus: data from primary care
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Background and aims: Data on mental disorders and suicidal ideation in relation to diabetes mellitus in primary care (PC) are scarce. The aim of the study was to evaluate the rate and association of depressive, anxiety disorders, and suicidal ideation in relation to diabetes mellitus (DM) in PC.
Materials and methods: A cross-sectional study was performed. 1170 adult consecutive patients approached; the final study group consisted of 998 patients. Mental status was evaluated by standard method, by using MINI International Neuropsychiatric Interview (MINI), performed by trained investigator (JP). Information on the clinical diagnoses was obtained from patients' medical records.
Results: Most commonly detected mental disorders were: major depressive episode (MDE) - 15.2%, anxiety disorders - 26.3% (generalized anxiety disorder (GAD) - 18.1%), alcohol misuse/probable alcohol abuse - 34.8%. 6.1% of the sample had suicial ideation, according to the MINI. DM was not the main reason of the visit of the primary care patients to contact their general practitioner (1.9%). When the probability of having mood or anxiety disorders among males and females according to somatic diagnosis was calculated, GAD but not MDE had significant association with both DM and suicidality in all patients (odds ratio (OR) (95% confidence interval (CI)=3.49 (1.35-9.19) and OR (95% CI) =3.26 (1.89-5.60)), and both were found higher in men, respectively (OR (95% CI)=6.88 (1.98-23.8) and OR (95% CI)=3.7 (1.2-11.4)). Neurotic illness had significant association with DM (OR (95% CI)=2.7 (1.09-6.96)) and suicidality (OR (95% CI)=3.01 (1.76-5.12)) in all patients, and was found significantly higher in men with diabetes, and in men with suicidality (OR (95% CI)=5.54 (1.60-19.18) and OR (95% CI)=2.96 (1.60-5.47)) in PC. The differences of clinic variables between suicidal and non-suicidal patients were evaluated. Subjects with type 2 DM were at the greatest risk for suicidality (OR (95% (CI)=4.3 (1.38-13.4), p=0.012), and the risk was greater than this of both GAD and MDE diagnoses taken together (OR (95% CI)=3.32 (1.97-5.6), p<0.001).
Conclusion: The rate of depressive, anxiety disorders and suicidal ideation is prevalent in PC. Psychiatric disorders are associated with DM, especially in men. Suicidal ideation is associated with male gender, mood, anxiety disorders and their coexistence, as well DM in PC. Primary care patients with type 2 DM have very high risk for suicidal ideation. General practitioners should be recommended to screen patients with diabetes for mental disorders and suicidal ideation in their routine practice.
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Screening for sexual problems among type 2 diabetes patients in primary care
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Background and aims: Although sexual problems are prevalent among men and women with type 2 diabetes, these problems receive little attention in diabetes care. To identify patients who are dissatisfied about their sexual functioning, routine screening was performed in primary care as part of a randomized clinical trial. The aim of this study was to determine the prevalence of sexual dissatisfaction, sexual problems and need for help among men and women with type 2 diabetes in primary care.
Materials and methods: Data was collected from January - March 2015 by practice nurses from 40 general practices in the Netherlands. The Brief Sexual Symptom Checklist for men (BSSC-M) and women (BSSC-W) was used to screen among 40-75 year old men and women with type 2 diabetes. The BSSC identifies men and women with self-reported dissatisfaction about sexual functioning, among who the self-reported type of sexual problem and need for help is further screened.
Results: In total, 251 patients with type 2 diabetes (61.4% men) were screened. Mean age did not differ between men and women: 63.2 versus 61.4 years. The prevalence of sexual dissatisfaction was 33.1%, and was significantly higher among men (39.0%) than among women (23.7%). Moreover, dissatisfied men were significantly older than satisfied men (65.1 vs. 61.9 years), yet no significant age difference was observed among women. Dissatisfied men most often reported erectile dysfunction (76.7%), low sexual desire (31.7%) and ejaculatory problems (31.7%). Among dissatisfied women, lubrication problems (52.2%), low sexual desire (39.1%), and dyspareunia (34.8%) were common. Furthermore, significantly more men (70.0%) than women (39.1%) wanted to talk about their sexual problems with their general practitioner.
Conclusion: Preliminary findings suggest that the majority of sexually dissatisfied men with type 2 diabetes would like help for their sexual problems in primary care, as well as over a third of dissatisfied women. Research should further look into the gender differences and their possible implications for care.
Clinical Trial Registration Number: NTR4807
Supported by: Dutch Diabetes Research Foundation (Grant: 2012.00.1554)

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Living without a spouse/partner and psychosocial health: results of the second Diabetes Attitudes, Wishes and Needs (DAWN2) study
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Background and aims: Research has shown that people living without a partner have lower quality of life compared to people living with a partner. Few studies have explored the association between living without a partner and poor psychological health in people with diabetes (PWD). The aim of the current study was to compare PWD living without a partner to those living with a partner regarding psychosocial health across countries.
Materials and methods: The present study is part of the DAWN2 study, a multinational, interdisciplinary and multi-stakeholder study conducted in 17 countries across four continents. The population comprised 8596 PWD, approximately 500 PWD from each country. Measures included *cohabitation status* (living with a partner or not), *patient characteristics*

(age, gender, complications, education and diabetes type/treatment and duration), *social support provision* (who and how many supports), *social support level* (what is the level of the support), and *psychological health* (wellbeing, diabetes distress, diabetes impact, worries of hypoglycaemia and diabetes empowerment). Analyses used multiple regression (linear and binary) to control for potential confounding with patient characteristics. The analyses examined whether associations between cohabitation status and psychological outcomes were mediated by social support. Multilevel regression models were applied to adjust for clustering within countries. Data were weighted in comparison with the general population in each country by age, gender, region and level of education.

Results: About one third of PWD lived without a partner, and there were significant country differences in the proportion of PWD living without a partner. Compared to PWD living with a partner, PWD living without a partner more often had type 1 diabetes than type 2 diabetes (p=0.004), more frequently were women (p<0.0001), had higher rates of complications (p<0.0001), were younger (p=0.002) but had longer diabetes duration (p=0.014). There was no significant association between cohabitation status and education. PWD living without a partner more frequently experienced lack of social support (p<0.01), were less empowered (p=0.001) and the proportion with high depressive symptom scores was larger (18% vs. 12%, p<0.0001) compared to PWD living with a partner. Significant differences with regards to diabetes distress and worrying about night time hypoglycaemia were found, however, these differences were small and probably clinically insignificant. There was no significant difference between PWD living with or without a partner on overall diabetes impact. Overall, the associations between cohabitation status and different psychological outcomes were not fully mediated by social support. The associations between cohabitation status and poor psychological health varied by (interacted with) age, diabetes type and country.

Conclusion: The study indicates social and psychological vulnerability of PWD living without a partner. Further research is needed into the mechanisms underlying this association. In order to enhance psychological health in PWD living without a partner appropriate support interventions have to be developed and tested.

Supported by: Novo Nordisk A/S

946

Development and evaluation of a psychometric instrument to assess problems related to illness acceptance in diabetes: the Denial versus Integration of Diabetes Scale (DIDS)

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Background and aims: Insufficient diabetes acceptance has been associated with reduced self-care and glycaemic control. However, satisfactory tools to measure diabetes acceptance are lacking. Therefore, the *Denial versus Integration of Diabetes Scale (DIDS)* was developed. This report presents its development and preliminary evaluation.

Materials and methods: 56 items were generated and revised based on patient feedback and expert reviews (27 directed towards acceptance/integration, e. g. 'I accept diabetes as a part of my life', 29 towards denial/non-acceptance/avoidance, e. g. 'I often push diabetes to the back of my mind'). A four-point Likert scale (3 - 'applies to me very much' to 0 - 'does not apply to me') was used for responses. Negatively keyed items were reverse-scored; hence, higher scores indicate higher acceptance. The items were tested in a pilot study with 222 patients (age 49±16 y.; 49% female; BMI 30±7; 64% type 1 DM; duration 17±11 y.; HbA_{1c} 8.5±1.7%) to exclude unsatisfactory items and define the scale. A subsequent validation study is recruiting; at the time of this report, 66 patients (age 48±13 y.; 46% female; BMI 28±5; 62% type 1 DM; duration 14±10 y.; HbA_{1c} 8.0±1.0%) had been included, providing data on diabetes non-acceptance (AADQ), self-care (DSMQ), treatment satisfaction (DTSQ), diabetes distress (PAID), depression (PHQ-9) and HbA_{1c} (central lab). Analyses comprised item and scale properties, exploratory factor analyses (EFA), correlations and *t*-Test.

Results: *Item selection:* Initially, 5 items were excluded for psychometric problems. Through EFA, 8 items were excluded for loadings on non-interpretable factors, finally yielding an interpretable four-factor structure. Based on this, 11 items were excluded for poor statistical or semantic fit and 4 for redundancy, leading to the final 28-item scale. *Scales/reliability:* EFA of the selected items yielded four factors (71% explained variance), interpreted as 'acceptance/integration' (7 items, Cronbach's α=0.93), 'treatment motivation' (7 items, α=0.93), 'denial/defence' (7 items, α=0.91) and 'emotional suffering' (7 items, α=0.92). The derived subscales were highly correlated, providing summing to a reliable total score (α=0.97). Reliability was again tested on the validation sample, yielding the following α coefficients (scales in above order): 0.91, 0.91, 0.90, 0.87 and 0.96. *Validity:* A correlation of -0.76 (P<0.01) was found with the AADQ, a measure of diabetes non-acceptance. The correlation with HbA_{1c} was -0.45 (P<0.01). Patients with higher DIDS scores (suggesting higher acceptance; n=34) compared to those with lower ones (n=32; median split) reported better self-care, particularly regarding diet (6.4±1.9 vs. 4.0±2.0, P<0.01), glycaemic self-management (8.7±1.9 vs. 7.1±2.3, P<0.01) and physician visiting (9.2±1.3 vs. 7.9±3.3, P<0.01), and showed better glycaemic control (HbA_{1c}: 7.6±1.1 vs. 8.3±1.1%, P<0.05). They also reported higher treatment satisfaction (30±4 vs. 24±6), less diabetes distress (21±15 vs. 37±16) and less depressive symptoms (6±4 vs. 9±6); all P<0.01.

Conclusion: The DIDS appears reliable and valid in assessing problems related to illness acceptance in both major types of diabetes. It may help explain inadequate self-care and suboptimal glycaemic control. Further data to expand these initial findings are being collected.

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Sleep and health-related quality of life in adults with type 2 diabetes

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Background and aims: Sleep is becoming recognized as an important behavior for metabolic control and mental health. The aim of this study was to examine the relationship between objective estimates of sleep quantity and quality and health-related quality of life (HRQL) in adults with type 2-diabetes (T2DM).

Materials and methods: Adults with T2DM were recruited as a subsample of a large ongoing cohort study in Alberta, Canada. Participants completed an extensive survey of health status and health-related behaviours. HRQL was measured with the SF-12 version 2, which provides Physical and Mental Composite Summary (PCS and MCS) scores, and the EQ-5D (5-Level) index score. Participants also wore an accelerometer (Actigraph GT3X+; 60-epochs @ 30Hz) on their wrist during sleep periods over 7 consecutive days, to derive nightly estimates of total sleep time (TST), sleep latency (SLAT) and sleep efficiency (SEFF) calculated using total number of sleep minutes divided by self-reported number of minutes in bed. Multivariate linear regression models, adjusting for age, sex, diabetes duration, BMI, depressive symptoms, medical comorbidities and household income, were used to examine the association between accelerometer-derived TST, SLAT and SEFF and the PCS, MCS or EQ-5D index scores.

Results: Average age of participants (N=168) was 65 years (SD 10), BMI 31(6.5) kg/m2, 46% female, with a diabetes duration 13(9) years.

Average TST and SLAT were 451.1 (59.1) and 9.9(7.6) minutes, respectively and SEFF was 82.7 (6.1)%. Multivariate regression models showed an inverse association between TST and PCS where every additional 60 min of sleep was associated with a 1.31 unit lower PCS (p=0.036); there was no relationship between TST and MCS (p=0.86). SEFF was positively associated with both PCS and MCS, where a 10% greater SEFF was associated with 2.73 units higher PCS (p=0.005) and 1.77 unit higher MCS (p=0.056). SLAT was not associated with either PCS or MCS, and there were no significant relationships with any sleep variable and the EQ-5D Index.

Conclusion: Among adults with T2DM, both sleep quantity and quality are important contributors to HRQL. An inverse relationship was observed between the quantity of sleep and physical health in this sample, but the magnitude of this difference was quite small. Regardless of the quantity of sleep, better sleep quality (efficiency) was associated with better physical and mental health.

Supported by: Alberta Health

948

Coping skills and quality of life of adolescents with type 1 diabetes

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Background and aims: Biological-psychological changes of puberty are a major challenge in the treatment and care of diabetic patients. In these ages, the adolescents develop various coping strategies that determine stress tolerance, quality of life (QoL) in their later life. The ultimate goal of diabetes management is to achieve good QoL perception. In our research, we compared the coping skills (resilience level) (RL), QoL and glycemic control (GC) in patients with type 1 diabetic adolescents; and we also aimed to examine the effect of regular exercise and use of insulin pump.

Materials and methods: Two hundred and twenty-nine patients with type 1 diabetes (118 males and 111 females) participated in the study. Their mean age was 15.38±2.37 years. Diabetic young people were recruited from 3 Diabetes Outpatient Clinics of the country. QoL was assessed with the Pediatric Quality of Life Inventory 3.0 Diabetes Module. Coping skills were evaluated with the Resilience Scale-15 that was completed with an additional question regarding the regular exercise. GC was indicated with the HbA1c measured during the assessment. Data were analyzed with F-probe and regression analysis using the SPSS 22.0 statistical software.

Results: Regular exercise had a positive impact both on RL ($F(2,225)=8.627$, $p<.001$) and QoL ($F(2,223)=6.476$, $p=.002$), whereas the use of insulin pump positively affected QoL ($F(1,225)=14.502$, $p<.001$) and tended to affect the GC ($F(1,222)=2.948$, $p=.087$). In our regression model, RS was predicted by regular exercise ($\beta=-.207$, $t=-3.395$, $p=.001$) and QoL ($\beta=.374$, $t=6.153$, $p<.001$) ($R=.476$, $R2=.226$). QoL was predicted by gender ($\beta=-.148$, $t=-2.482$, $p=.014$), RL ($\beta=.383$, $t=6.381$, $p<.001$) and intensive insulin therapy modality ($\beta=-.209$, $t=-3.389$, $p=.001$) ($R=.472$, $R2=.223$). GC was significantly predicted by QoL ($\beta=-.138$, $t=-2.102$, $p=.037$) and family background ($\beta=-.187$, $t=-2.852$, $p=.005$) ($R=.239$, $R2=.057$). Independent predictors were age, gender, diabetes duration, HbA1c, intensive insulin therapy modality (1=CSII, 2=MDI), RS, QoL, family background (1=1 parent, 2=2 parents, 3=other), and financial background (1=below average, 2=average, 3=above average)

Conclusion: In addition to the healthy physical development, regular exercise can positively affect the diabetic adolescents' coping skills and QoL. Treatment with insulin pump therapy, male gender and higher RL seemed to be determining factors of better QoL. Favorable GC showed relationship with better QoL and living in intact family. The main goals in diabetes treatment and care are to achieve as good GC and QoL as possible. Some factors cannot be controlled as gender, family and financial background, but improving and developing coping skills, treating with insulin pump therapy and encourage patients to exercise regularly seems effective way to achieve the aims.

PS 089 Hypoglycaemia: that sinking feeling

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Incidence of hypoglycaemia in patients with type 2 diabetes mellitus recently initiating basal insulin in a real-world US setting

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Background and aims: Hypoglycaemia and fear of hypoglycaemia remain major barriers to persistence with insulin therapy. This retrospective cohort study of electronic medical record (EMR) data examined the incidence of hypoglycaemia soon after insulin initiation in US patients with type 2 diabetes mellitus (T2DM) and if they are at a greater risk of insulin discontinuation.

Materials and methods: The Predictive Health Intelligence diabetes dataset integrates anonymous de-identified EMR patient-level data from 20 Integrated Delivery Networks in the USA, with>325 hospitals and 300,000 providers (including some of the largest integrated delivery networks in the US). This was used to identify adult patients with T2DM who initiated basal insulin therapy (defined as having no prescription of basal insulin≥12 months before initiating insulin glargine, insulin detemir, or NPH insulin) between January 2008 and March 2013. Hypoglycaemic events were identified by health care encounters with ICD-9-CM diagnosis codes for hypoglycaemia, or blood glucose values≤70 mg/dL (≤3.89 mmol/L) during the first 6 months of basal insulin use. Discontinuation was defined as a gap of>45 days in insulin prescription coverage. A baseline was established using 12-month patient-level data prior to insulin initiation and the follow-up period was 6-24 months; all data were reported descriptively. Data were adjusted for confounders using Cox regression analysis for time to basal insulin discontinuation.

Results: Of 49,062 patients with T2DM identified, 5,159 (10.5%) experienced hypoglycaemia within the first 6 months of insulin use; 44% were identified using ICD-9-CM diagnosis codes and the remaining 56% using blood glucose values. Patients experiencing hypoglycaemia, versus those without hypoglycaemia, were older (mean age: 63 vs 60 years; P< 0.0001) with 49% aged≥65 years; had more comorbidities (Charlson Comorbidity Index: 1.21 vs 0.67; P<0.0001); had more experience of hypoglycaemia during the baseline period (18.9% vs 4.5%; P<0.0001); and a greater proportion had a health care utilization during the baseline period (inpatient visits: 20.1% vs 8.7%; P<0.0001). During the follow-up period, 68.1% of patients with hypoglycaemia experienced their first event in the first 3 months of treatment. In patients experiencing hypoglycaemia, versus those without hypoglycaemia, discontinuation of insulin therapy was higher at 6 months (58.7% vs 45.5%; P<0.0001) and at 12 months (68.1% vs 53.9%; P<0.0001). Data adjusted for confounders showed that patients with hypoglycaemia had almost twice the risk of discontinuation over 12 months compared with those without hypoglycaemia (hazard ratio 1.90; 95% CI 1.82-1.98; P<0.0001).

Conclusion: This study illustrates an incidence of hypoglycaemia as high as 10% in a US setting of patients with T2DM initiating basal insulin, and that these patients are at greater risk of discontinuation of their basal insulin therapy.

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Comparison of findings from the HAT study, the largest global hypoglycaemia study to date, with prior large real-world studies

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Background and aims: HAT (Hypoglycaemia Assessment Tool) was a non-interventional, 6-month retrospective and 1-month prospective real-world study to determine the extent of hypoglycaemia in 27, 585 people from 24 countries. The study outcomes were compared with similarly designed large real-world studies.

Materials and methods: A systematic literature search of PubMed (1995-2014) for population-based studies of people treated with insulin, excluding clinical trials and reviews, identified comparable reports of proportions of people experiencing hypoglycaemia or rates of events experienced. The study results that met the inclusion criteria were compared with prospective HAT data (see Table).

Results: Overall, severe and nocturnal hypoglycaemia rates varied widely between the studies identified and the HAT study (see Table). Estimated annual hypoglycaemia rates for Northern Europe and Canada in the HAT study (91.6 [T1DM]; 18.1 [T2DM]) were similar to or slightly higher than comparable studies identified in the search. However, in the HAT study, rates for overall hypoglycaemia in T2DM were higher for Latin America, Eastern Europe and Russia than for Northern Europe/Canada and lower for the Middle East and South East Asia. Rates for overall hypoglycaemia in T1DM in the HAT study were highest in Latin America and much lower in South East Asia. Similarly, severe and nocturnal hypoglycaemia rates for Northern Europe/Canada in the HAT study were comparable with other studies but differed from other regions in the HAT study. Comparable data from prior studies in non-US/European regions are sparse.

Conclusion: Findings from the HAT study revealed that more evidence from large, international, non-US/Europe-based real-world studies is needed, and highlighted that the rates of hypoglycaemia in some regions of the world may be higher than previously suggested by Europe and US-based studies. Reasons for these variations and optimisation of diabetes care in these regions are needed.

Study	Insulin therapy (duration, years)	Number of participants	Country/ region	Incidence, estimated annual rate (events PPY)					
				All/any hypoglycaemia		Severe hypoglycaemia		Nocturnal hypoglycaemia	
				T1DM	T2DM	T1DM	T2DM	T1DM	T2DM
HAT study	Any insulin (>1)	7106 (T1DM) 18,518 (T2DM)	Global	73.3	19.3	4.9	2.5	11.3	3.7
GAPP2	Basal or basal-bolus (1–33)	2915	Global		37.7				9.7
HAT study	Any insulin (>1)	1797 (T1DM) 3352 (T2DM)	Northern Europe/ Canada	91.6	18.1	3.4	1.3	12.9	3.7
Ostensen 2014	Any insulin (<2–>10)	1631 (T1DM) 2196 (T2DM)	Europe	91	20.3–35.4*	0.7	0.1–0.2*		
PREDICTIVE	Initiating insulin determir	7087 (T1DM) 12,720 (T2DM)	Europe	47.5	9.2	3.0	0.8	13.8	3.4
DIALOG	Any insulin	1358 (T1DM) 1776 (T2DM)	France	76.6	19.5	2.4	1.2	8.5	2.4
GAPP2	Basal or basal-bolus (1–20)	322	UK		26.7–35.5				
GAPP2	Basal or basal-bolus (mean 5)	156	Canada		45.0				13.2
Kulzer 2014	Any insulin (mean 14.8 [T1DM]/ 7.0 [T2DM])	207 (T1DM) 407 (T2DM)	Germany	85.3	31.7–32.8				
Kristensen 2012 (HypoAna)	Any insulin	3861 (T1DM)	Denmark	113.4–138.2*		1.09–1.47*			
Hypoglycaemia Study Group	Any insulin (<5–>15 [T1DM]/ 0–>5 [T2DM])	107 (T1DM) 274 (T2DM)	UK	29.0–35.5†	4.1–10.2†	1.1–3.2†	0.1–0.7†		
HAT study	Any insulin (>1)	3052 (T1DM) 6218 (T2DM)	Eastern Europe	66.9	23.7	4.5	2.2	9.8	4.0
HAT study	Any insulin (>1)	427 (T1DM) 1469 (T2DM)	Latin America	93.9	19.7	10.8	3.7	17.7	4.3
HAT study	Any insulin (>1)	997 (T1DM) 2842 (T2DM)	Middle East	66.2	15.4	6.7	2.4	10.9	2.8
HAT study	Any insulin (>1)	511 (T1DM) 726 (T2DM)	Russia	69.2	28.1	5.3	2.3	12.5	6.4
HAT study	Any insulin (>1)	224 (T1DM) 3811 (T2DM)	South East Asia	17.5	14.7	2.0	3.4	5.5	3.0
A1chieve	Initiating insulin alone/+ OADs	4100	China		1.35		0.0		0.22
GAPP2	Basal or basal-bolus	347	Japan		32.8				2.4
PRESENT	Initiating biphasic insulin aspart	3559	India		1.5		0.05		0.6

PPY, per person-year
*Several rates reported, depending on regimen
†Several rates reported, depending in duration of diabetes
The countries included in the HAT regions were: Austria, Canada, Denmark, Finland, Germany, The Netherlands and Sweden (N Europe/Canada); Bulgaria, Croatia, Czech Republic, Hungary, Poland, Romania, Serbia and Montenegro, Slovakia and Slovenia (Eastern Europe); Argentina and Mexico (Latin America); Israel, Lebanon and Saudi Arabia (Middle East); Russia; and India and Malaysia (SE Asia)

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Seasonal variations of severe hypoglycaemia in patients with type 1 diabetes and type 2 diabetes in a German population

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Background and aims: Severe hypoglycemia (SH) is associated with a critical prognosis. In addition to its acute life-threatening potential, SH may increase cardiovascular and all-cause mortality irrespective of diabetes type. Our aim was to highlight seasonal changes in SH and the corresponding clinical circumstances in patients with type 1 diabetes (T1DM) and type 2 diabetes (T2DM) in a German population under the conditions of real life.
Materials and methods: Prospective population-based observational trial. All SH occurred between 2007 and 2014 in a large tertiary care hospital in a rural area (200.000 inhabitants) were captured. SH was defined as a symptomatic event requiring treatment with intravenous glucose or administration of glucagon and being confirmed by a blood glucose measurement of <50 mg/dl. Seasons were scaled according to meteorological conditions. Statistical significant differences between diabetes groups were calculated using the Student's t-test and chi-square test. Furthermore one-way anova with post-hoc LSD-test was used to investigate significant differences between the seasons within a diabetes group.
Results: A total of 1080 episodes of SH in 747 patients were registered: 37.5% of cases were related to T1DM, 51.7% to T2DM, 3.2% to pancreoprivic diabetes and 7.6% occurred in non-diabetic individuals. The 405 cases of SH in T1DM comprised 206 subjects. The 558 cases of SH in T2DM occurred in 453 subjects. In T1DM 50.1% and in T2DM 16.1% of hypoglycemic episodes were related to only 31 respectively to 23 patients who experienced ≥3 SH. Most tested basic parameters (e.g. age, HbA1c, Charlson comorbidity index, creatinine-clearance, impaired awareness of hypoglycaemia) between patients with T1DM and T2DM were statistically significant (all p<0.001). In cases with T1DM we observed significantly increased prevalence of SH in spring (27.7%) and summer (28.6%) vs. autumn (21.5%; p=0.04 and p=0.02 respectively) and winter (22.2%; p=0.04 vs. summer). In contrast, there was a more balanced and not significant seasonal distribution of SH in subjects with T2DM (winter 25.3%, spring 26.0%, summer 25.1%, autumn 23.5%). Surprisingly, we found no significant variation in seasonal HbA1c and insulin dose in subjects with T1DM. However, at the scene of emergency there were significant lower initial blood glucose levels in spring (29.2± 11.1 mg/dl) vs. autumn (32.7±10.1 mg/dl) and a significant lower frequency of blood glucose self-measurement per week in spring (30.1± 11.4) vs. summer (33.0±12.9). In cases with T2DM we revealed a significant higher HbA1c in spring (7.0±1.6) compared to autumn (6.6± 1.0).
Conclusion: Individuals with T1DM and SH clearly distinguish from those with T2DM and SH. We found an increased frequency of SH in patients with T1DM in spring and summer which was probably due to short-term impacts like more physical activity or possibly higher alcohol consumption during these warmer seasons. Better seasonal adaptation of insulin therapy in subjects with T1DM could be an appropriate approach to prevent SH. For the cohort of mostly multimorbid geriatric patients with T2DM there was no seasonal effect for the risk of SH.

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Incidence, predictive factors and direct costs of severe hypoglycaemia: results from the prospective HYPO.15 study

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Background and aims: Severe hypoglycaemia (SH) is the most serious side effect of insulin therapy and has been associated with an increased risk of mortality. However, few data are available concerning its incidence and/or its direct cost. The observational, prospective, multicenter, French HYPO.15 study assessed the incidence of SH in patients with type 1 or type 2 diabetes.
Materials and methods: Emergency care units of 9 French University Hospital consecutively enrolled all patients with SH (diagnosed according to ADA criteria) during at least 3 months. The primary endpoint was frequency of confirmed SH. Secondary endpoints included predictive factors of SH and estimation of direct costs, including costs of emergency care and hospitalizations' costs.
Results: We included a total of 811 patients. Incidence of SH was estimated to 0,63 SH/100 patients/year (20 433 SH/year in France). Characteristics of the 811 patients were: median age 61,3±21,1 years (59,3 years in men vs. 64,0 years in women; p=0,002); duration of diabetes<5 years for 10,3% and duration of diabetes>10 years for 75,9%; previous history of SH during the past year in 62%. SH happened at home for 86,4% of the patients, during the morning (7 h-13 h) for 31,8% of them (23,8% in the afternoon; 22,7% in the evening and 21,7% during the night). 92,4% of the patients used insulin therapy, alone in 82,3% and together with oral anti-diabetics (OAD) in 10,1%. Patients with insulin therapy alone were younger (58,1 yrs vs 72,7 yrs for OAD + insulin and 76,1 yrs for OAD alone; p<0,0001). Sulfonylureas were used by 51% of patients with OAD alone, but only in 20% of patients with OAD + insulin (p=0,0005), whereas use of repaglinide was similar in both groups (38%). Median glycemia was 37±17 mg/dL. Fasting (43%), infection (26%) and treatment misuse (17%) were the most frequent etiologies of SH in our cohort.

55,5% of patients stayed at home after intervention of emergency care unit, 25,6% were brought to the hospital for a short monitoring and 15,6% were hospitalized for more than one night. Rate of hospitalization depends of treatment and was increased for patients with OAD alone (44, 8% vs 16,9% for OAD + insulin and 10,4% for insulin alone; p<0,05). Direct cost of SH was estimated to 47 834 978 € for 20 433 SH per year in France, which represents a mean cost by SH of 2 341 €.

Conclusion: HYPO.15 is the first prospective study giving the high incidence and the direct costs of confirmed SH in patients with type 1 and type 2 diabetes in France, underlying the importance of optimizing patients' treatment regimens and education. Previous history of SH and male gender appears to be the main predictive factors for SH.

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Regional variation in patient definitions and rates of hypoglycaemia: findings from the HAT study of 27,585 people with type 1 and type 2 diabetes on insulin

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Background and aims: Hypoglycaemia is an important concern for people with diabetes, but how individuals understand and define hypoglycaemia remains unclear. The Hypoglycaemia Assessment Tool (HAT) study was a non-interventional assessment of self-reported hypoglycaemia. This *post-hoc* analysis aimed to determine patient knowledge of hypoglycaemia and associated reporting.

Materials and methods: HAT was conducted in 24 countries using questionnaires and diaries (28-day prospective period) in 27, 585 adults with type 1 (T1DM) or type 2 diabetes (T2DM) using insulin for ≥12 months. The patient diary defined confirmed hypoglycaemia as a blood glucose (BG) level <3.9 mmol/L. Patients recorded in the diary if hypoglycaemia was based on BG levels, symptoms of hypoglycaemia or both.

Results: Most respondents (96.8% and 85.6% of patients with T1DM and T2DM, respectively) knew the ADA/EASD hypoglycaemia definition (see Table). However, in some regions, many patients actually identified hypoglycaemic events from symptoms alone. Reported hypoglycaemia rates varied substantially between regions, with SE Asia having a relatively high rate of unconfirmed symptomatic hypoglycaemia. Rates of severe hypoglycaemia also varied widely between regions, both for T1DM and T2DM, with lower rates in N Europe and Canada.

Conclusion: Regional variations in patient definition of hypoglycaemia may contribute to the global variations in rates of hypoglycaemia reported in the HAT study. Discrepancies between patient definitions in different countries and guidelines may highlight a need to redefine the criteria of hypoglycaemia.

	Global N=8022 N=19,563	N Europe/ Canada N=2388 N=3877	Eastern Europe N=3135 N=6389	Latin America N=531 N=1660	Middle East N=1124 N=3073	Russia N=618 N=737	SE Asia N=226 N=3847
Number of patients who knew the ADA/EASD definition of hypoglycaemia at baseline visit, %*	96.8 85.6	95.8 83.4	98.1 92.2	95.8 80.1	96.5 86.8	98.0 93.4	91.6 76.7
% of patients who defined hypoglycaemia in the patient diary on the basis of:							
Symptoms only	26.8 35.6	14.7 25.6	28.7 34.1	30.4 37.8	32.2 42.5	44.5 49.1	44.8 39.8
Blood glucose measurements only	3.9 7.2	4.2 6.2	3.8 6.7	2.8 8.3	3.9 6.3	3.2 6.1	6.7 10.1
Either	20.2 14.9	21.6 12.4	20.3 14.5	12.7 11.0	20.5 14.8	20.7 15.8	18.0 20.1
Both	49.1 42.3	59.5 55.8	47.1 44.7	54.2 42.9	43.5 36.4	31.7 29.1	30.4 29.9
Mean (SD) blood glucose level below which patients define a hypoglycaemia event, mmol/L	3.38 (0.75) 3.60 (0.81)	3.27 (0.71) 3.50 (0.79)	3.37 (0.72) 3.59 (0.80)	3.47 (0.71) 3.57 (0.77)	3.52 (0.83) 3.67 (0.83)	3.56 (0.77) 3.81 (0.87)	3.14 (1.04) 3.66 (0.85)
Rate of hypoglycaemia (any),† PPY (95% CI)	73.3 (72.6, 74.0) 19.3 (19.1, 19.6)	91.6 (90.0, 93.2) 18.1 (17.6, 18.7)	66.9 (65.8, 67.9) 23.7 (23.2, 24.1)	93.9 (90.6, 97.3) 19.7 (18.9, 20.6)	66.2 (64.4, 68.1) 15.4 (14.9, 15.9)	69.2 (66.8, 71.6) 28.1 (26.8, 29.6)	17.5 (15.6, 19.6) 14.7 (14.2, 15.1)
Rate of confirmed symptomatic hypoglycaemia,† PPY (95% CI)	51.1 (50.5, 51.7) 11.3 (11.1, 11.5)	63.9 (62.6, 65.3) 11.1 (10.7, 11.5)	50.2 (49.3, 51.2) 19.4 (19.0, 19.9)	62.8 (60.1, 65.6) 11.0 (10.4, 11.6)	36.9 (35.6, 38.4) 7.1 (6.8, 7.5)	48.4 (46.4, 50.5) 16.4 (15.4, 17.5)	6.0 (4.9, 7.3) 3.2 (3.0, 3.4)
Rate of unconfirmed‡ symptomatic hypoglycaemia,† PPY (95% CI)	4.3 (4.1, 4.5) 1.9 (1.9, 2.0)	4.0 (3.6, 4.3) 0.9 (0.7, 1.0)	5.6 (5.3, 5.9) 1.5 (1.4, 1.7)	5.4 (4.6, 6.2) 1.3 (1.1, 1.5)	2.3 (1.9, 2.6) 0.8 (0.7, 0.9)	2.1 (1.7, 2.6) 0.4 (0.3, 0.6)	3.2 (2.4, 4.1) 4.7 (4.5, 5.0)
Rate of severe hypoglycaemia,† PPY (95% CI)	4.9 (4.7, 5.1) 2.5 (2.4, 2.5)	3.4 (3.1, 3.7) 1.3 (1.2, 1.5)	4.5 (4.3, 4.8) 2.2 (2.1, 2.4)	10.8 (9.7, 12.0) 3.7 (3.3, 4.1)	6.7 (6.1, 7.3) 2.4 (2.2, 2.6)	5.3 (4.7, 6.0) 2.3 (2.0, 2.8)	2.0 (1.4, 2.8) 3.4 (3.2, 3.7)

*Percentages in this table are based on the number of patients with evaluable data
†Data are rate of hypoglycaemia in the 28-day prospective period of the study
‡Patient experienced symptoms of hypoglycaemia but did not provide a blood glucose measurement
ADA, American Diabetes Association; EASD, European Association for the Study of Diabetes; N, Northern; PPY, estimated number of events per patient year; SE, South East
Type 1 diabetes
Type 2 diabetes

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Self-reported hypoglycaemia and mortality in type 2 diabetes patients treated with insulin in the diabetes care system West-Friesland, Netherlands

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Background and aims: Tight glucose control trials and observational studies based on medical records have suggested that objectively measured hypoglycaemic events are associated with cardiovascular events and mortality in patients with type 2 diabetes (T2D). However, little is known about self-reported hypoglycaemia, as experienced in everyday life, and the associated risk on mortality in the general T2D population in usual care. Further, no observational studies have focused on the association between hypoglycaemia and mortality in insulin users. Our aim was to study the association between self-reported mild and severe hypoglycaemia and mortality in T2D patients in usual care treated with insulin.

Materials and methods: Demographics, clinical characteristics and mortality data were obtained from 1,667 T2D patients treated with insulin in the Diabetes Care System West-Friesland, the Netherlands. Hypoglycaemia was self-reported, including complaints like dizziness, dreaming, feeling restless, headache when getting out of bed, hunger, mood swings, palpitations, snoring, night sweats, tingling sensations around the mouth, and trembling. Mild hypoglycaemia was defined as events not requiring help from others, while severe hypoglycaemia was defined as events requiring help from other persons (either medical or non-medical). Logistic regression analysis was used to analyse the association between hypoglycaemia and mortality during follow-up. In addition, generalized linear models with an offset variable were used to allow for differences in follow-up duration.

Results: During a median follow-up of 1.9 years, 804 participants (48%) reported no hypoglycaemia, 744 (45%) reported only mild and 119 (7%) reported severe hypoglycaemia. During follow-up, 98 patients (5.9%) died. Reporting only mild hypoglycaemia was significantly associated

with lower mortality risk during follow-up (OR: 0.48, 95%CI 0.29-0.81), while reporting severe hypoglycaemia was not significantly associated with mortality (OR: 0.77, 95%CI: 0.33-1.80), compared to reporting no hypoglycaemia. These results were adjusted for age, sex, diabetes duration, HbA1c levels, hypertension, smoking, sulfonylurea use, and micro-/macro-vascular complications. Sensitivity analyses showed an OR of 1.38 (95%CI 0.31-6.11) for patients reporting severe hypoglycaemia requiring medical assistance, and 0.57 (0.23-1.46) for patients reporting severe hypoglycaemia, but not requiring medical assistance. Further, no significant interaction was found between mild and severe hypoglycaemia during follow-up. Finally, allowing for differences in follow-up duration did not lead to different results.

Conclusion: We observed that insulin-treated type 2 diabetes patients reporting mild hypoglycaemia had a lower mortality risk during a median follow-up of 2 years compared to patients reporting no hypoglycaemia. A possible explanation for this finding might be the concept of hypo-unawareness. If this finding is confirmed in other studies, it may be reassuring for T2D patients and their physicians to know that experiencing hypoglycaemic sensations not requiring medical help is associated with reduced short-term mortality risk.

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Increase in hospital admissions, length of hospital stay and outpatient visits in diabetes patients one month and one year following severe hypoglycaemic events events

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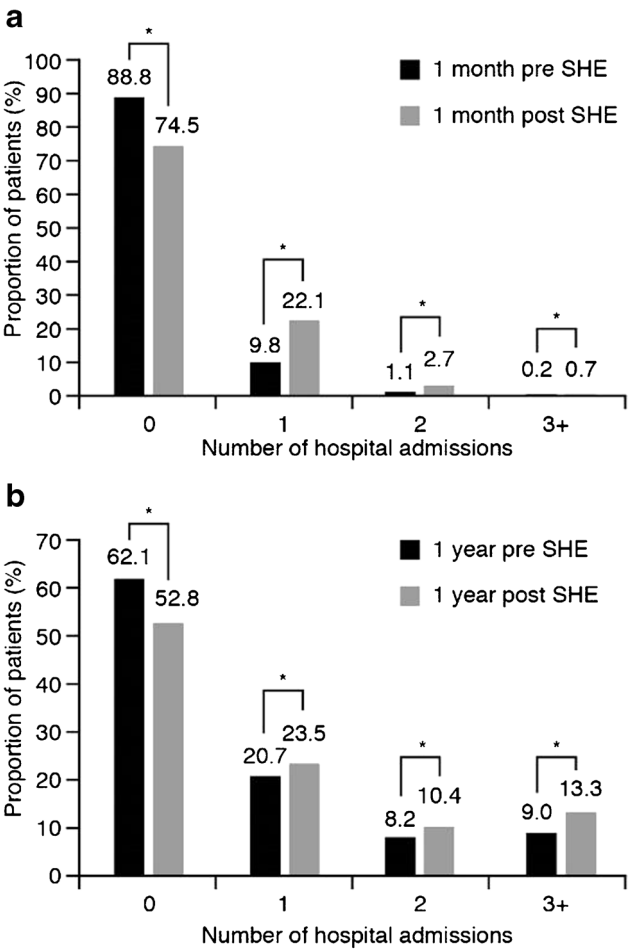
Background and aims: Severe hypoglycaemia is a burden for the patient and payer alike. In this study, we aimed to quantify the resource use associated with a severe hypoglycaemic event (SHE) in patients with diabetes using the database of Maccabi Healthcare Services (MHS), a large Health Management Organization in Israel.

Materials and methods: This retrospective, non-interventional study compared resource use 1 month and 1 year pre and post SHE. Data collected by the MHS database included overall as well as detailed health utilisation data per patient. Patients were included if they had a diagnosis of diabetes and experienced a SHE, as identified by diagnosis codes in the MHS database, from 2005-2014.

Results: From 2005-2014, a total of 4376 patients were eligible for inclusion in the study, out of approximately 100,000 patients with diabetes in the MHS registry. The study population consisted of 6.4% T1D, 75.2% T2D and 18.4% with diabetes type undefined. Mean characteristics of the study population were age 64.5 years, HbA1c 7.4%, BMI 29.5 kg/m^2, 47.9% male, 42.9% insulin-treated and 60.5% were treated with oral drugs that may cause hypoglycaemia (sulphonylureas or meglitinides). Diabetes duration (years) was <5 in 20.4%, 5-9 in 45.2% and 10+ in 34.4% of cases. At baseline (time of SHE), 42% of patients had a cardiac comorbidity and 73% had hypertension. Hospital admissions increased significantly (P<0.001) in the month following a SHE (Figure). Of those admitted to hospital (n=1418), the mean duration of stay was significantly (P<0.001) longer during the month post (5.59 days) vs pre SHE (4.37 days). In this period, outpatient visits increased by 46% (12557 vs 18441, P<0.001). The greatest increases were in visits to endocrinologists (+169%), nurses (+70%) and dieticians (+66%). The number of hospital admissions also increased in the 1 year post vs the 1 year pre SHE (Figure), corresponding to a 38% increase in hospitalisations (P< 0.001), and there was a 13% increase in outpatient visits (P<0.001) during this period. The mean duration of hospital stay increased from 4.81 to 6.82 days during the 1 year pre vs post SHE (P<0.001), however it is recognised that other factors could influence the data over the 1 year time period.

Conclusion: This real-world data analysis indicates an increased use of healthcare services, including more frequent and prolonged hospital admissions and outpatient visits after a SHE.

Proportion of patients who had hospital admissions pre and post SHE at a) 1 month and b) 1 year.



*P<0.001 for pre SHE vs post SHE time period.
SHE, severe hypoglycaemic event.

Supported by: Novo Nordisk A/S

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Incidence of severe hypoglycaemia in 2006 and 2011 in consideration of the medication in Germany: analysis based on health insurance data

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Background and aims: New antihyperglycaemic agents are advertised to offer effective glycaemic control while reducing the risk of hypoglycaemia. The aim of this study was to analyze the development of the incidence of severe hypoglycaemia from 2006 to 2011 in

consideration of the medication, based on AOK (largest health insurance in Germany) routine data.
Materials and methods: All adult insured persons with diabetes mellitus type 2 (extrapolated to the German population: 6.6 million in 2006 and 7.9 million in 2011) were screened for severe hypoglycaemia (identified by ICD 10 coding) which requires hospitalization or emergency use. Antihyperglycaemic agents were identified by ATC-Code and Defined Daily Dose (DDD) of the respective medication was calculated.
Results: There was a slight increase in the incidence of severe hypoglycaemia in 2006 (0.04%, n=28.892) to 2011 (0.05%, n=33.741). Persons with severe hypoglycaemia were older (69.7y vs. 73.8y in 2006 and 70.1y vs. 74.4y in 2011) and 46.5% in 2006 and 56.2% in 2011 had nephropathy. In 2006 8.3% (n=2.412 of 28.892) of all persons with severe hypoglycaemia had sulfonylureas as monotherapy and 8.9% (n=565.862 of 6.3 Mio.) of the persons without severe hypoglycaemia (DDD 214 with vs. 248 without severe hypoglycaemia). In 2011 the prescription of sulfonylureas as monotherapy was significantly lower, 4.4% (n=1.471 of 33.741) of persons with as well as without severe hypoglycaemia (n=330.175 of 7.5 Mio.) (DDD 252 with vs. 309 without severe hypoglycaemia). A combination therapy of biguanides and sulfonylureas had 17% (n=4.926 of 28.892) of the persons with and 12.7% (n=804.757 of 6.3 Mio.) of the persons without severe hypoglycaemia in 2006 (DDD of this combination therapy was 480 with vs. 577 without severe hypoglycaemia). In 2011 this combination therapy applied 14.2% (n=4791 of 33.741) of the persons with severe hypoglycaemia and 10% (n=749.878 of 7.5 Mio.) of the persons without severe hypoglycaemia (DDD 518 vs. 664). A combination therapy of short and long acting human insulin used 17.1% (n=4.941 of 28.892) of the persons with and 4% (n=254.588 of 6.3 Mio.) of the persons without severe hypoglycaemia in 2006 (DDD 481 vs. 563) and 14.4% (n=4.851 of 33.741) of the persons with and 3.4% (n=257.357 of 7.5 Mio.) of the persons without severe hypoglycaemia in 2011 (DDD 491 vs. 592). A combination therapy of short and long acting analogue insulin was present in 6.1% (n=1.761 of 28.892) of the persons with and 1.3% (n=81.898 of 6.3 Mio.) of the persons without severe hypoglycaemia in 2006 (DDD 558 vs. 589) and 12.4% (n=4.200 of 33.741) of the persons with and 2% (n=153.014 of 7.5 Mio.) of the persons without hypoglycaemia in 2011 (DDD 570 vs. 628).
Conclusion: Comparing 2006 to 2011 showed a significant reduced prescription of sulfonylureas as monotherapy, a slightly reduced use of human insulin and a significantly increased prescription of analogue insulin. Despite the changes in drug prescription there was a slight increase in overall incidence of severe hypoglycaemia from 2006 to 2011.

PS 090 Hypoglycaemia: recognition and avoidance

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Continuous Subcutaneous Insulin Infusion (CSII) and fear of hypoglycaemia in patients with type 1 diabetes: five years of study

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Background and aims: Fear of hypoglycemia is one of the factors associated with poor adherence to diabetologic treatment. 1) To assess the effect of CSII in fear of hypoglycemia in patients with type 1 diabetes (DM1) after 5 years of treatment. 2) Analyze the relationship of fear of hypoglycemia with other variables.
Materials and methods: The sample consisted of 72 patients with DM1 and CSII. Patients were evaluated clinically, metabolic and psychologically at baseline, 6 months and annually. Fear of hypoglycemia was assessed with the Fear of Hypoglycemia Scale (FH-15). In addition, quality of life (DQOL), depressive symptoms (BDI-II) and anxiety (STAI) were assessed. The analyses were performed using Student's t test and the relationship between variables was analyzed with the Pearson correlation coefficient. SPSS version 15.0 was used.
Results: At baseline, 66.7% of the sample showed fear of hypoglycemia. In fact, a significant decrease in prevalence from baseline score at 6 months ($p < 0.001$) and a year of treatment ($p < 0.001$) occurs. Over 5 years of treatment, there is a statistically significant decrease in scores of fear of hypoglycemia in all assessments made with respect to the initial score, except for two years. A significant negative relationship between fear of hypoglycaemia and number of self-controls ($p=0.008$) at 5 years was found. On the other hand, between fear of hypoglycaemia and quality of life was found a positive and significant correlation at baseline ($p < 0.001$) at 6 months ($p < 0.001$), 1 year ($p < 0.001$), 2 years ($p=0.025$), 3 years ($p < 0.001$), 4 years ($p < 0.001$) and 5 years ($p < 0.001$). There is also a significant positive correlation between fear of hypoglycaemia and depressive symptoms at baseline ($p=0.019$), at 3 years ($p=0.005$) and 4 years ($p < 0.001$).
Conclusion: Preliminary results of this study show some evidence of improvement experienced by patients with DM1 treated with CSII for 5 years in the fear of hypoglycemia. Also found relationships between this variable, greater depressive symptoms, poorer quality of life and fewer self-monitoring. The results of this study have demonstrated the importance of this variable and suggest that it is necessary to assess the fear of hypoglycemia in these patients by specific protocols.
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Insulin degludec/insulin aspart lowers fasting plasma glucose and rates of confirmed and nocturnal confirmed hypoglycaemia independent of disease duration

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Background and aims: Longer diabetes duration is linked with the need for intensification of insulin therapy. The novel co-formulation of 70% insulin degludec (IDeg) and 30% insulin aspart (IAsp) has been shown to provide effective glycaemic control with reduced rates of hypoglycaemia in individuals with type 2 diabetes (T2D). This *post-hoc* pooled analysis evaluated the efficacy of IDegAsp vs biphasic insulin aspart 30 (BIAsp 30) and IDeg OD + IAsp in improving glycaemic control and reducing risk of hypoglycaemia in subjects with T2D stratified according to disease duration (≤10 or >10 years).

Materials and methods: Data were pooled from five 26-week treat-to-target and phase 3a/b clinical trials in subjects with T2D in the IDegAsp clinical development programme. Baseline HbA_{1c} was similar between all five studies. Comparators from individual trials were pooled and included BIAsp 30 twice-daily (BID) or IDeg OD + IAsp (2-4 injections). End of trial (EOT) HbA_{1c}, fasting plasma glucose (FPG) and rates of confirmed and nocturnal confirmed hypoglycaemia and insulin dose were stratified according to disease duration (≤10 years or >10 years). For each separate group, continuous variables were analysed using ANOVA and event rates were analysed using negative binomial regression.

Results: There was no difference in EOT HbA_{1c} between IDegAsp and comparator in subjects with disease duration of ≤10 years (IDegAsp, n=430; comparator [BIAsp 30 and IDeg OD + IAsp], n=298) or >10 years (IDegAsp, n=681; comparator [BIAsp 30 and IDeg OD + IAsp], n=399) (Table 1). Significant reductions in EOT FPG were observed with IDegAsp vs comparator (BIAsp 30 and IDeg OD + IAsp) in subjects with a disease duration of ≤10 years (6.1 vs 6.9 mmol/L, respectively, >10 years (5.8 vs 6.9 mmol/L, respectively, $p<0.0001$). IDegAsp vs comparator (BIAsp 30 and IDeg OD + IAsp) was associated with greater reductions in rates of confirmed hypoglycaemia ($p<0.05$) and nocturnal confirmed hypoglycaemia ($p<0.0001$) in subjects with T2D, independent of disease duration. EOT total daily insulin dose was significantly lower for IDegAsp BID vs BIAsp30 BID (60.4 vs 73.8 U, $p<0.0001$) and IDegAsp BID vs IDeg OD + IAsp (93.8 vs 123.3 U, p10 years but not in the subjects with T2D duration ≤10 years.

Conclusion: No differences were observed in HbA_{1c} when stratified according to disease duration. IDegAsp was associated with significant reductions in FPG and rates of confirmed- and nocturnal confirmed hypoglycaemia. IDegAsp improves glycaemic control at a significantly lower total daily insulin dose irrespective of disease duration. The lower total daily insulin dose in subjects with T2D duration >10 years treated with IDegAsp vs comparators warrants further investigation.

Table 1. Trial endpoints stratified by duration of type 2 diabetes

Endpoint	IDegAsp	Comparator (BIAsp 30 BID and IDeg OD+ IAsp)	Treatment contrast p value
HbA_{1c} (%)*			
≤10 years	6.9	6.9	-0.07, p=ns
>10 years	7.0	6.9	0.10, p=ns
FPG (mmol/L)*			
≤10 years	6.1	6.9	–0.82, p<0.0001
>10 years	5.8	6.9	–1.04, p<0.0001
Confirmed hypoglycaemia*			Rate ratio
(Events/100 PYE)			
≤10 years	624	1024	0.61, p=0.0002
>10 years	908	1201	0.76, p=0.0013
Nocturnal confirmed hypoglycaemia*			Rate ratio
(Events/100 PYE)			
≤10 years	61	194	0.31, p<0.0001
>10 years	97	204	0.48, p<0.0001
Total daily insulin dose (U)*		BIAsp 30 BID	
≤10 years	76.9	77.8	–0.9, p=ns
>10 years	60.4	73.8	–13.4, p<0.0001
Total daily insulin dose (U)*		IDeg OD + IAsp	
≤10 years	100.0	104.8	–4.8, p=ns
>10 years	93.8	123.3	–29.5, p=0.0048

Data are from full analysis set, last observation carried forward
*Estimated
BIAsp30, biphasic insulin aspart 30; BID, twice-daily; OD, once-daily; PYE, patient years of exposure
Data are pooled from BOOST: INTENSIFY PREMIX I, BOOST: INTENSIFY ALL, BOOST: Start Twice Daily, BOOST: SIMPLE vs STEP-WISE and BOOST: TWICE DAILY vs BB, unless otherwise specified.

Supported by: Novo Nordisk A/S

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Sustained glycaemic control and less hypoglycaemia over 1y with new insulin glargine 300 U/ml vs glargine 100 U/ml in Japanese type 2 diabetes mellitus people on basal insulin + OAD(s) (EDITION JP 2)

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Background and aims: In EDITION JP 2, Japanese people with T2DM managed with basal insulin plus OAD(s) receiving new insulin glargine 300 U/ml (Gla-300) achieved comparable glycaemic control with less hypoglycaemia over 6 months when compared with glargine 100 U/ml (Gla-100).

Materials and methods: Participants continued Gla-300 or Gla-100 for an additional 6 months.

Results: 222 participants completed the 12-month study (107 [88.4%] with Gla-300 and 115 [95.8%] with Gla-100). From baseline to month 12, HbA_{1c} and FPG decreased with both Gla-300 (mean [SD] change −0.28 [0.84] % and −0.7 [3.1] mmol/l) and Gla-100 (mean [SD] change −0.33 [0.79] % and −1.0 [2.4] mmol/l). At month 12, the mean daily Gla-300 dose was 25 U/day (0.36 U/kg/day) and mean daily Gla-100 dose was 21 U/day (0.30 U/kg/day), with no change in dose observed between months 6 and 12. Over 12 months, the percentage of participants experiencing ≥1 nocturnal confirmed (≤3.9 mmol/l [≤70 mg/dl]) or severe hypoglycaemic event was lower with Gla-300 compared with Gla-100 (38.3% vs 52.5%; relative risk 0.73; 95% CI: 0.55 to 0.97). Consistently fewer nocturnal confirmed (≤3.9 mmol/l [≤70 mg/dl]) or severe hypoglycaemic events per participant-year were seen with Gla-300 compared with Gla-100 (2.09 vs 5.30; rate ratio 0.41; 95% CI: 0.18 to 0.92). Severe hypoglycaemia was rare, with only 3 and 2 events reported with Gla-300 and Gla-100, respectively. Mean (SE) weight change over the 12-month period was −0.7 (0.2) kg with Gla-300 and 0.5 (0.2) kg with Gla-100. No between-treatment differences in the number of adverse events were seen.

Conclusion: Japanese people with T2DM managed with basal insulin plus OAD(s) showed sustained glycaemic control with less nocturnal confirmed (≤3.9 mmol/l [≤70 mg/dl]) or severe hypoglycaemia over 12 months with Gla-300 compared with Gla-100. Gla-300 was well tolerated over the 12-month study period.

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Supported by: Sanofi

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The efficacy of degludec in improving hypoglycaemia in type 1 diabetic patients

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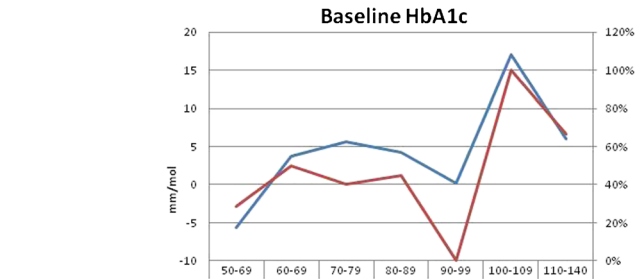
Background and aims: Insulin Degludec (Tresiba) is a recently launched ultra-long acting insulin with a steady and stable pharmokinetic profile with minimal intra and inter-day variability, with a glucose lowering effect. Over 1,578 patients have been treated in phase three trials but there are few data available relating to the real world benefits in clinical practice.
Materials and methods: Data from patients attending our diabetes centre between 07/02/2013 and 09/10/2013 who were switched from their usual basal insulin to insulin Degludec were included in this study. Laboratory results, together with clinical notes from general practice and outpatient clinic correspondence were reviewed.
Results: 57 patients were analysed with 40% of males & 60% of females. Basal insulin that had been used pre-Degludec includes Glargine in 77.1% & Detemir in 15.8%. Hypurin, Insulatard, Insulin Pump & Glimepiride, each represented 1.8%. In the graph as illustrated, a trend of improvement in hypoglycaemia had been observed in the mean HbA1c values between the 60-90 mmol/l. A more significant improvement in both the mean HbA1c values and percentage of patients with improvements in their hypogycaemic controls were observed in the group of patients with initial mean HbA1c of 100-140 mmol/l. In the initial pre-degludec group of 100-120 mmol/l, improvements of 100% of hypoglycaemic events and reduction of an average of 17 mmol/l, suggested that perhaps Tresiba is ideal in improving the glycaemic control and also in reducing hypoglycaemic events in this group of patients. Degludec that had been initiated for the group of both mixed hyperglycaemia and hypoglycaemia were noted in 3 patients. The mean initial HbA1c for this group was at 89 mmol/l. This increased to 96 mmol/l with no further reported improvements in hypoglycaemia in this group. The number of patients in this group was small and in addition, other factors need to be considered for this rise in HbA1c in this particular group of patients.
Conclusion: Our results confirms the benefits of Degludec over previously available basal insulin being translated into clinical practice, whereby modest improvements in glycaemic control are associated with significant reduction in hypoglycaemia rates. Thus, Degludec is a useful new addition to the basal insulin range & is most useful in Type 1 diabetes with hypoglycaemia as a frequent problem.



	50-69	60-69	70-79	80-89	90-99	100-109	110-140
Mean Improvement in HbA1c (mmol/l)	-5.7	3.8	5.7	4.2	0.2	17	6
Percentage of patients with improvements in hypoglycaemia (%)	29%	50%	40%	45%	0%	100%	67%

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Glycaemic control and hypoglycaemia burden in patients with type 2 diabetes initiating basal insulin in Europe

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Background and aims: Many patients with T2DM fail to achieve the ADA/EASD recommended target of HbA_{1c} ≤7%, despite numerous treatment options. Hypoglycaemia (HG) is the main barrier to effective glucose control for people on insulin, impacting long-term treatment adherence/persistence. This study evaluated short- and long-term glycaemic target attainment and HG incidence, specifically in patients with T2DM initiating insulin using a basal insulin (BI)-only regimen.
Materials and methods: This was an observational, retrospective longitudinal analysis of electronic medical records (EMRs) from five European countries (Germany, Italy, France, Spain and UK) extracted from the Cegedim Strategic Data Longitudinal Patient Database. Insulin-naïve patients aged ≥30 years with T2DM initiating BI with or without OADs between Jan 2007 and Sep 2014 were included. Patients had ≥1 HbA_{1c} measurement pre- and post BI initiation and data available for ≥1 year pre- and ≥2 years post BI initiation. Occurrence of HG was defined either as a recorded diagnosis of HG or a fasting plasma glucose of ≤3.9 mmol/l (70 mg/dl) during an office visit. A multivariate logistic regression model assessed baseline characteristics, 3-month glycaemic control (for patients with an HbA_{1c} value 2-4 months post BI initiation) and HG for long-term (3-24 months post BI initiation) glycaemic control and HG.
Results: A total of 12,141 patients were included (mean age across countries: 65-69 years). Most patients did not achieve HbA_{1c} target ≤7%, at both 3 months (range not at target: 70.2-91.9%) and 24 months (range not at target: 66.4-82.7%) post BI initiation. Those with suboptimal control at 3 months were less likely to reach target at 24 months ($p \leq 0.001$). A similar proportion of patients experienced HG over 24 months in Germany, Spain and France (~8-9%); HG was higher in Italy (~16%) and lower in the UK (~4%). HG during the initial 3-month period was predictive ($p \leq 0.02$) of longer-term risk (between 3-24 months) (**Table**).
Conclusion: Across five European countries, a consistent pattern of short- and long-term suboptimal glycaemic control emerged: the majority of patients initiating BI failed to reach HbA_{1c} target ≤7% in the first 3 months, or after 2 years' follow up. Pre- and post-index glycaemic control varied between countries. Approximately 9% of patients in all countries experienced HG in the first 2 years of initiating BI, with the exception of a higher rate in Italy and a lower rate in the UK. Treatment response and HG incidence by 3 months post BI initiation are predictive of longer-term glycaemic control and HG risk, respectively. The true burden of HG is likely underestimated because only EMR-captured episodes or HG during an office visit were analysed. These results support the need for interventions that both improve glycaemic control and reduce the risk of HG.

Table: Country-specific results of patients achieving target glycaemic control (HbA_{1c} ≤7%) and percentage experiencing hypoglycaemia (HG) at 3 and 24 months post basal insulin (BI) initiation

Country	Pre-index HbA_{1c} value (%)	Pts at HbA_{1c} target of ≤7% at 3 months (%)	Pts at HbA_{1c} target of ≤7% at 24 months (%)	Odds ratio for long-term risk of not achieving target if target is not achieved in the first 3 months (p-value)	Pts who had HG during 1 year pre-index (%)	Pts who had HG in first 3 months (%)	Pts who had HG over 24 months (%)	Odds ratio for long-term risk of HG if HG is experienced in the first 3 months (p-value)
Germany (N=4064)	8.4	29.8	33.6	3.71 (<0.001)	2.9	1.8	8.7	6.72 (<0.001)
Italy (N=1228)	9.1	20.0	27.4	5.22 (<0.001)	7.7	2.4	15.5	4.69 (<0.002)
France (N=2264)	9.0	19.6	27.6	5.04 (<0.001)	9.0	1.6	8.2	7.84 (<0.001)
Spain (N=1117)	9.2	20.4	23.9	3.50 (<0.001)	1.9	1.7	9.1	4.58 (0.020)
UK (N=3468)	9.9	8.1	17.3	5.51 (<0.001)	5.0	0.9	4.4	19.67 (<0.0001)

HG, hypoglycaemia; Pts, patients

Supported by: Sanofi

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Silent hypoglycaemic episodes among well controlled type 2 diabetic patients treated with sulfonylureas

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Background and aims: Hypoglycaemia is a relevant cardiovascular risk factor. The aim of our study was to assess the frequency of silent hypoglycaemic episodes among well-controlled type 2 diabetic patients treated with sulfonylurea.
Materials and methods: 29 type 2 diabetic patients (16 men, 13 women) with HbA1c ≤7% treated with sulfanylurea and/or metformin without manifest symptoms of hypoglycaemia during the past two year were involved in the study (mean age: 70.94±8.92 years, mean duration of diabetes mellitus 13.88±8.42 years, mean HbA1c was 5.48±0.82%). A 120-hour long (5 days) glucose monitoring using a Continuous Glucose Monitor System (CGMS) device (Medtronic iPro2®) was performed. Patients were asked to document four self-monitored capillary blood glucose levels each day for calibration of the monitor, as well as to record meal times and exercise. Hypoglycaemia symptoms were also recorded. According to the EASD/ADA recommendation, glucose concentration of ≤3.9 mmol/l were classified as hypoglycaemia. The number of hypoglycaemic events ≤3.9 mmol/l and, additionally, the number of hypoglycaemic episodes <3.1 mmol/l were determined. Hypoglycaemic episodes were defined as a glucose value that persisted for at least 15 minutes with or without symptoms.
Results: Twenty nine patients were monitored for a mean period of 5744.72±1406.28 min. The mean interstitial glucose (IG) concentration was 7.07±1.12 mmol/l. The minimal IG was 2.2 mmol/l, while the maximal IG concentration was 19.3 mmol/l. 18/29 (62.01%) patients had a total number of 65 silent hypoglycaemic episodes (interstitial glucose ≤3.9 mmol/l), the mean 3.88±2.05 episodes per patients. The total duration of these hypoglycemic episodes was 374.5±462.97 min. 8/29 (27.6%) patients had hypoglycaemic episodes of interstitial glucose ≤3.1 mmol/l. The mean frequency of these episodes was 0.88±1.01 per patient. The total number of episodes was 15. The total duration of hypoglycemic episodes with glucose values ≤3.1 mmol/l was 72.5± 120.04 min. None of our patients recorded symptoms of hypoglycaemia. CGMS measurements were generally well tolerated.
Conclusion: We demonstrated that silent hypoglycemia is quite common in well-controlled patients with type 2 diabetes treated with a sulfonylurea with or without metformin. As a key finding, all registered hypoglycaemic episodes were silent. CGMS is a useful clinical tool for detecting silent hypoglycaemia.

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Effects of liraglutide as add-on to insulin on counter-regulatory hormone responses, gastric emptying and glycaemic recovery during hypoglycaemia

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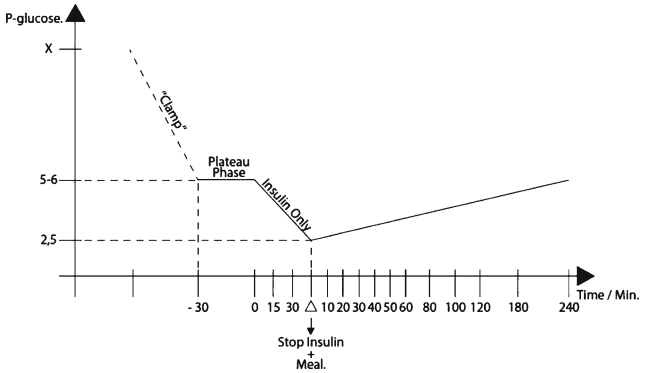
Background and aims: There is a growing body of evidence that GLP-1 receptor agonists (GLP-1 RA) could be beneficial as add-on to insulin in patients with type 1 diabetes (T1D). GLP-1 inhibits glucagon secretion and delays gastric-emptying (GE) during euglycaemia; however, these effects have not been fully elucidated during hypoglycaemia. The aim of this study was to investigate the counter-regulatory hormone responses and GE rate during hypoglycaemia in patients with T1D treated with liraglutide 1.2 mg or placebo for 12 weeks
Materials and methods: In total 20 T1D patients were randomized to 12 weeks treatment with once-daily liraglutide 1.2 mg or placebo as add-on to pre-existing insulin treatment. Before and after 12 weeks of treatment a hyperinsulinemic hypoglycaemic clamp was performed (figure). Before both experimental days glucose was monitored using continuous glucose monitor (CGM). In case of glucose measurements <3.5 mmol/l (CGM or glucose meter) within 24 hours of the study, study procedures were postponed. On both occasions patients were studied at normoglycaemia (plasma glucose target: 5-6 mmol/l), followed hypoglycaemia (approx. 2.0 mmol/l) and during glycaemic recovery after a liquid meal (containing 600 kcal and 1.5 g of acetaminophen) served at hypoglycaemia (figure). Blood samples for analysis of counter-regulatory hormone responses, p-glucose, and p-acetaminophen were drawn.
Results: Baseline characteristics were similar between groups: (liraglutide; placebo, mean±sem): HbA1c 77±4; 71±2 mmol/mol, BMI 24.9±0.8; 23.2±0.7 kg/m2, diabetes duration 20±3; 22±2 years, age 36 ±3; 37±2. In the liraglutide group, no counter-regulatory differences between experimental days were seen in change in cortisol, glucagon or growth hormone (GH) from normoglycaemia to maximum response following hypoglycaemia. However, at 12 weeks change from baseline in glucagon secretion tended to lower with liraglutide compared with placebo (liraglutide vs. placebo, iAUC0-240:1862±967 to 1290±993 vs. 2435 ±1239 to 2311±1295 pmol/l×min, p=0.4). The acetaminophen absorption test assessing rate of gastric emptying displayed no differences between treatments. Glycaemic recovery from hypoglycemia did not differ between groups
Conclusion: In patients with T1D liraglutide as add-on to insulin do not affect the counter-regulatory hormone responses during hypoglycaemia. However, a trend toward lower glucagon secretion was seen after liraglutide treatment. Foremost, gastric emptying was not delayed during hypoglycaemia and glycaemic recovery from hypoglycaemia was unaffected with liraglutide adjunct to insulin treatment in T1D



Clinical Trial Registration Number: NCT02092896
Supported by: Novo Nordisk

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Similar counter-regulatory hormone responses to hypoglycaemia with Basal Insulin peglispro (BIL) versus insulin Glargine (GL) in type 1 diabetes

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Background and aims: BIL is a novel PEGylated basal insulin with a flat pharmacokinetic and glucodynamic profile which has a hepato-preferential action resulting from reduced peripheral effects. This study assessed the effects of BIL and GL on the counter-regulatory response to and recovery from hypoglycaemia induced by human insulin infusion. In patients with type 1 diabetes, the glucagon response to hypoglycaemia is reduced over time and the glucose counter-regulatory response depends mainly on epinephrine. For this reason, the primary objective of the study was to assess the counter-regulatory response of epinephrine.
Materials and methods: This was an open-label, randomised, 2-period, crossover study. After once-daily, individualised, subcutaneous doses of BIL or GL for ≥14 days, 13 patients (mean [±SD] age, 41.0±10.4 years; duration of diabetes, 19.0±8.7 years) underwent a hypoglycaemic clamp by infusion of human insulin (0.5 mU/kg/min) to achieve sequential blood glucose (BG) targets of 72, 58 and 45 mg/dL (BG nadir) (4.0, 3.2 and 2.5 mmol/L). The amount of glucose infusion required to recover from 45 to 72 mg/dL (2.5 to 4.0 mmol/L) was also compared.
Results: There was no statistically significant difference between treatments in the counter-regulatory response of epinephrine (Table; see also norepinephrine, cortisol and growth hormone data). Mean baseline glucagon was higher following BIL than GL; this statistically significant difference was maintained during the clamp. There were no statistically significant differences between BIL and GL in the mean amount of glucose required to reach a BG concentration of 72 mg/dL (4.0 mmol/L) from 45 mg/dL (2.5 mmol/L).
Conclusion: BIL and GL showed similar counter-regulatory responses to and recovery from hypoglycaemia, indicating that the hepato-preferential action of BIL does not impair counter-regulation.

	LS means BIL	LS means GL	Difference of LS means (BIL-GL) (90% CI)
Epinephrine (pmol/L)			
- Baseline (preclamp)[a]	156.95	193.28	-36.34 (-152.22, 79.54)
- BG nadir (45 mg/dL [2.5 mmol/L])[b]	607.25	521.97	85.28 (-30.60, 201.16)
Norepinephrine (pmol/L)			
- Baseline (preclamp)[a]	1004.57	1146.51	-141.93 (-374.05, 90.19)
- BG nadir (45 mg/dL [2.5 mmol/L])[b]	1236.19	1441.51	-205.32 (-437.43, 26.80)
Cortisol (nmol/L)			
- Baseline (preclamp)[a]	205.62	257.48	-51.86 (-103.40, -0.31)
- BG nadir (45 mg/dL [2.5 mmol/L])[b]	336.24	331.33	4.91 (-46.63, 56.46)
Growth hormone (µg/L)			
- Baseline (preclamp)[a]	1.04	1.46	-0.42 (-3.00, 2.16)
- BG nadir (45 mg/dL [2.5 mmol/L])[b]	6.83	8.07	-1.24 (-3.82, 1.34)
Glucagon (pmol/L)			
- Baseline (preclamp)[a]	7.90	5.22	2.68 (1.86, 3.51)
- BG nadir (45 mg/dL [2.5 mmol/L])[b]	5.77	4.92	0.85 (0.03, 1.68)
Total glucose infusion (g)			
- for recovery from 45 to 72 mg/dL (2.5 to 4.0 mmol/L)	12.547	14.071	-1.524 (-3.617, 0.568)

Abbreviations: BG = blood glucose; BIL = insulin peglispro; CI = confidence interval; GL = insulin glargine; LS = least squares
[a] Baseline was the mean of the 3 preclamp measurements (-30, -15, and 0 minutes)
[b] Target BG nadir was 45 mg/dL (2.5 mmol/L); actual mean (SD) nadir was 44.8 ± 1.6 mg/dL (2.49 ± 0.09 mmol/L) for BIL and 44.5 ± 1.7 mg/dL (2.47 ± 0.09 mmol/L) for GL.

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Supported by: Eli Lilly and Company

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Good glycaemic control with low incidence of hypoglycaemia: complete evaluation of data of routine care by electronic patient file

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Background and aims: Regular assessments of treatment quality is only practicable with easy data handling from routine-care integrating existing processes and treatment structures and replace paper documents and files. Since 1997 we conceived and continuously improved an electronic database and patient file, which is easy to use, and can locally be adapted to specific needs and pre-existing software structure. Exemplarily the quality of care of all patients with diabetes mellitus cared for at the outpatient department in the year 2011 is shown.
Materials and methods: A total of 1411 people with DM were seen in our outpatient department. DM1 292 (49,5% females, 50,2y, time since diagnosis 21,5y, BMI 27,2 kg/m^2) and DM2 1118 (with insulin n=730, 43,6% females, age 68,5y, time since diagnosis 18,4y, BMI 33,69 kg/m^2; without insulin n=388, 48,6% females, age 64,6y, time since diagnosis 8, 4y, BMI 30,75 kg/m^2). HbA_{1c} was DCCT adjusted (mean of healthy persons 5,05%). All patients took part in a structured patient education programme in 2011 or earlier.
Results: All people with DM1 had an intensified insulin therapy with the basal-bolus schema (74%) or a therapy with insulin pump (26%). 50% used short-acting human insulin and 50% short-acting insulin analogues, NPH-insulin 30,5%, long-acting insulin analogues 31,8% and combined human insulin 5,5%. Mean blood pressure office was 135/81 and patient self 130/77, mean HbA_{1c} 7,5%. The incidence of severe hypoglycaemia (glucose or glucagone injection) was 0,11/pat/y and the frequency of non-severe hypoglycaemia was 1,76/pat/wk. 316 (44%) of the people with DM2 on insulin had a conventional therapy (CT) with human insulin and 404 (56%) a multiple injection therapy (MIT) with human or analogue insulin. The therapy was combined with metformin in 42,2% of the cohort, in 0,4% with sulfonylureas, 2,7% with DPP4-inhibitors and 1,9% with other medications. Mean blood pressure office was 145/83 mmHg and patient self 135/77, mean HbA_{1c} 7,5%. The incidence of severe

hypoglycaemia (glucose or glucagone injection) was 0,02/pat/y and the frequency of non-severe hypoglycaemia was 0,21/pat/wk. People with DM2 without insulin were treated in 71% with oral antidiabetic agents (58% metformin, 22% sulfonylureas, 7% DDP4-inhibitors, 4% other drugs (glinides, GLP1-analogues)). Mean blood pressure office was 144/88 and patient-self 133/78, mean HbA_{1c} 6,6%. The incidence of severe hypoglycaemia was 0% and non-severe hypoglycaemia 0,05/pat/wk.

Conclusion: The easily retrieved data from routine care of a tertiary university outpatient clinic displayed a very good metabolic control within the target of near normoglycaemia. The incidence of severe hypoglycaemias under insulin therapy was low, even in the group of DM1. Severe hypoglycaemias in patients with DM2 on insulin were very rare, subjects without insulin did not report severe hypoglycaemic events. Mild hypoglycaemias occurred rarely in people with DM1 and very rarely in people with DM2, even on insulin therapy. Blood pressure self-control is considerably lower than office blood pressure well in the target range.

PS 091 Basal insulin therapy

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A single dose study in healthy subjects demonstrating pharmacokinetic and pharmacodynamic equivalence between MK-1293 and EU- and US-approved innovator insulin glargine

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Background and aims: MK-1293 is a follow-on/biosimilar insulin glargine under development using EU- and US-approved innovator insulin glargine as the benchmark comparator.

Materials and methods: This double-blind, randomized, three-period balanced crossover euglycemic clamp study in healthy male subjects (age: 18-45 yrs; BMI: 18.5-25.0 kg/m2) was conducted to demonstrate pharmacokinetic (PK) and pharmacodynamic (PD) equivalence between MK-1293, innovator insulin glargine procured in the US ('US-approved innovator insulin glargine') and EU ('EU-approved innovator insulin glargine'). In each treatment period, subjects received single 0.4 units/kg S.C. doses of MK-1293, US-approved innovator insulin glargine, or EU- approved innovator insulin glargine in random sequence followed by a 24-hour assessment period for PK sampling and euglycemic clamping (clamp target 80 mg/dL [4.44 mmol/L]). An LC-MS/MS assay was used to quantify concentrations of the major circulating active glargine metabolite (M1) as well as parent glargine and the M2 metabolite.

Results: Data for the primary PK and PD endpoints are presented in the table. For all three treatment comparisons, the 90% confidence intervals for the geometric mean ratios for the primary PK endpoints and the arithmetic mean ratios for the primary PD endpoints were within 0.80 to 1.25, which was the pre-specified equivalence criterion for both PK and PD endpoints. All treatments were well tolerated.

Conclusion: This study demonstrated PK and PD equivalence between MK-1293, US-approved innovator insulin glargine, and EU-approved innovator insulin glargine, thereby supporting the potential for MK-1293 as a follow-on/biosimilar insulin glargine.

	MK-1293/US-approved innovator insulin glargine	MK-1293/EU-approved innovator insulin glargine	EU-approved innovator insulin glargine/US-approved innovator insulin glargine
Primary PK Endpoints			
AUC_{0-24h} of M1[a]	0.98 (0.93, 1.04)	0.97 (0.92, 1.02)	1.01 (0.97, 1.05)
C_{max} of M1[a]	1.01 (0.96, 1.07)	1.00 (0.95, 1.05)	1.01 (0.96, 1.06)
Primary PD Endpoints			
GIR AUC$_{0-24h}$	1.01 (0.92, 1.10)	0.96 (0.89, 1.04)	1.05 (0.97, 1.14)
GIR AUC$_{0-12h}$	0.96 (0.84, 1.09)	0.91 (0.82, 1.02)	1.05 (0.93, 1.19)
GIR AUC$_{12-24hr}$	1.03 (0.95, 1.12)	0.98 (0.91, 1.05)	1.05 (0.97, 1.14)
GIR_{max}	0.96 (0.87, 1.06)	0.91 (0.84, 0.99)	1.05 (0.95, 1.17)

[a]M1 metabolite accounts for ~90% of the total circulating active glargine and was therefore the primary PK analyte
PK endpoint results presented as geometric mean ratios and 90% confidence intervals
PD endpoint results presented as arithmetic mean ratios and 90% confidence intervals
GIR= glucose infusion rate; GIR_{max}= maximal glucose infusion rate

Supported by: Merck & Co., Inc.

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Superior reduction of HbA_{1c} in a double-blind, randomised study of basal insulin peglispro (BIL) vs insulin glargine (GL) in patients with type 1 diabetes: IMAGINE 3

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Background and aims: Basal insulin peglispro (BIL) is a novel basal insulin with a flat pharmacokinetic profile which has a hepato-preferential action resulting from reduced peripheral effects.

Materials and methods: In this Phase 3, 52-week, blinded study of BIL vs GL, 1114 adults with T1D (39% female) and HbA1c <12% (<108 mmol/mol) (mean 7.9% [62 mmol/mol]) were randomised 3:2 to bedtime BIL or GL with prandial insulin lispro. An electronic diary facilitated data capture and insulin dosing decisions.

Results: At 52 weeks, BIL vs GL provided superior reduction of HbA1c (difference -0.22% [-2.42 mmol/mol]; 95% CI: -0.32%, -0.12% [-3.47, -1.36 mmol/mol]). More patients on BIL achieved HbA1c <7% (<53 mmol/mol). With BIL, patients had less glucose variability, a 47% lower rate of nocturnal hypoglycaemia (SMBG ≤3.9 mmol/l [70 mg/dl]), and weight loss. The total hypoglycaemia rate was 11% higher with BIL and may be associated with daytime bolus insulin dosing; severe hypoglycaemia rates were similar. While total insulin dose was similar, BIL patients used more basal and less bolus insulin. With BIL, LDL-C and triglycerides (TG) increased and HDL-C decreased. More BIL patients had ALT ≥3× ULN (4.8% vs 2.0%, p=0.021) with no cases of Hy's law. Liver fat content (LFC, %), assessed by MRI in 182 patients from 2 studies of BIL vs GL in T1D, increased with BIL (5.7±0.3 vs 3.5±0.4, p <0.001). Patients on BIL were more likely to have injection site reactions (13.3% vs 0.2%, p<0.001).

Conclusion: In T1D, treatment with BIL vs GL gave superior HbA1c reduction, lower nocturnal hypoglycaemia, and weight loss, with increases in ALT, TG, LFC, and injection site reactions.

Outcomes at 52 Wks	GL (N=450)	BIL (N=664)	p-value[a]
HbA1c (%) (mmol/mol)[b]	7.6 ± 0.0 (60 ± 0.4)	7.4 ± 0.0 (57 ± 0.4)	<0.001
HbA1c <7% (% of patients)	26.1	35.3	<0.001
Fasting serum glucose (mmol/l) (mg/dl)[b]	9.5 ± 0.2 (172 ± 3.5)	7.9 ± 0.2 (142 ± 3.0)	<0.001
Body weight change (kg)[b]	1.2 ± 0.2	-0.6 ± 0.2	<0.001
Hypoglycemia rates:			
Nocturnal (events/patient/30 d)[c,d]	2.5 ± 0.1	1.3 ± 0.1	RR 0.53, <0.001
Total (events/patient/30 d)[d]	13.9 ± 0.4	15.3 ± 0.3	RR 1.11, 0.002
Severe (events/patient/100 y)[e]	22.5 ± 3.5	19.7 ± 2.7	RR 0.88, 0.520
Between-day glucose variability (mmol/l) (mg/dl)[b,f]	3.5 ± 0.1 (63 ± 1.5)	3.0 ± 0.1 (54.0 ± 1.2)	<0.001
Within-day variability (mmol/l) (mg/dl)[b,g]	4.0 ± 0.1 (71.7 ± 1.3)	3.7 ± 0.1 (66.4 ± 1.1)	0.002
Basal insulin dose (U)[b]	29.0 ± 0.7	37.3 ± 0.6	<0.001
Bolus insulin dose (U)[b]	35.4 ± 0.9	26.0 ± 0.8	<0.001
Total insulin dose (U)[b]	62.9 ± 1.4	61.9 ± 1.2	0.570
ALT (IU/l)[b]	23.4 ± 0.9	29.9 ± 0.8	<0.001
Lipids (mmol/l) (mg/dl)[b]			
TG	1.0 ± 0.0 (88 ± 2.6)	1.2 ± 0.0 (105 ± 2.2)	<0.001
HDL-C	1.5 ± 0.0 (60 ± 0.5)	1.5 ± 0.0 (58 ± 0.4)	0.021
LDL-C	2.6 ± 0.0 (101 ± 1.1)	2.7 ± 0.0 (106 ± 1.0)	0.002

[a]Between treatments; [b]Least squares mean±SE; [c]Bedtime to waking,10 PM to 10 AM; [d]Group mean±SE, 0-52 weeks; [e]Aggregated rate±SD, 0-52 weeks; [f]SD of 7d FBG at Wk 52; [g]SD of 2d 9-pt SMBG profile at Wk 52; ALT, alanine aminotransferase; RR, relative rate BIL/GL; ULN, upper limit of normal.

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Efficacy, patient-reported outcomes and safety of insulin degludec U200 compared with insulin glargine in patients with type 2 diabetes requiring high-dose insulin

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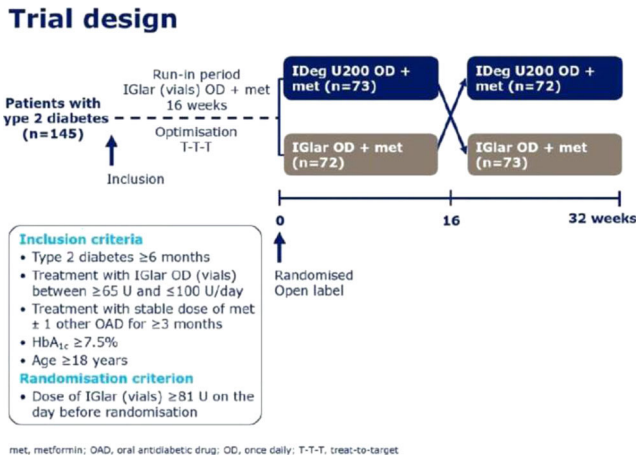
Background and aims: This was a 32-week, open-label, crossover, treat-to-target trial that compared the efficacy, patient-reported outcomes and safety of insulin degludec (IDeg) 200 U/mL with insulin glargine (IGlar) 100 U/mL, administered once daily (OD) after 16 weeks' run-in.

Materials and methods: Adults aged ≥18 years with type 2 diabetes (T2D) for ≥6 months, receiving IGlar OD (≥65-≤100 U/day) + metformin ± one other oral antidiabetic drug for ≥3 months, with a HbA1c ≥7.5% who required ≥81U insulin after a 16-week run-in were randomised 1:1 to IDeg/IGlar or IGlar/IDeg (Figure).

Results: After 16 weeks, noninferiority of IDeg to IGlar with respect to change in HbA1c was confirmed. Mean fasting plasma glucose was significantly reduced with IDeg vs. IGlar (-0.82 vs. -0.05 mmol/L; estimated treatment difference [ETD]: -0.77 mmol/L, p<0.05). For IDeg vs. IGlar, the rate of confirmed hypoglycaemia was significantly lower (estimated rate ratio [ERR]: 0.59, p<0.05) while nocturnal hypoglycaemia was numerically lower (ERR: 0.66, p=NS). Mean weight change was 0.42 kg (IDeg) vs. 1.04 kg (IGlar), p=NS. The IDeg delivery device was rated significantly better for function (ETD: 8.40, p<0.05) and less bother (ETD: 6.01, p<0.05) compared with the IGlar device, and more patients preferred IDeg treatment (54.5%) vs. IGlar (20.0%); 15.9% no preference and 2.8% didn't know.

Conclusion: IDeg U200 was effective in regulating glycaemic control as measured by HbA1c (noninferiority to IGlar confirmed) and is preferred by a numerically higher proportion of patients with T2D requiring high-dose insulin.



Clinical Trial Registration Number: NCT01570751
Supported by: Novo Nordisk A/S

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The efficacy and safety of LY2963016 insulin glargine in patients with type 1 and type 2 diabetes previously treated with insulin glargine

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Background and aims: LY2963016 insulin glargine (LY IGlar) and insulin glargine (IGlar) are insulin glargine products with identical primary amino acid sequences as well as similar pharmacokinetic and pharmacodynamic profiles. In 2 prospective, global, parallel, Phase 3 trials, LY IGlar had a similar efficacy and safety profile to IGlar with no clinically meaningful differences. To further assess the treatment effect and similarity of LY IGlar to IGlar, additional analyses were done in the subgroup of patients with type 1 diabetes mellitus (T1DM) or type 2 diabetes mellitus (T2DM) who reported prestudy treatment with IGlar (prior IGlar) in these 52-week open-label (ELEMENT-1) and 24-week double-blind randomised (ELEMENT-2) studies, respectively.
Materials and methods: In both studies, unit-to-unit dose conversions were used from prestudy IGlar to study LY IGlar or IGlar followed by treat-to-target titration (eg, HbA_{1c} <7.0%). The efficacy and safety of LY IGlar and IGlar were compared in the prior IGlar subgroup. Primary efficacy was defined as change in HbA_{1c} from baseline to the 24-week endpoint. Continuous data were analysed using ANCOVA. Treatment comparisons for hypoglycaemia rate were done using the Wilcoxon test. Fisher's exact test or chi-square test was used for categorical data.
Results: No significant treatment differences were observed for primary and other efficacy outcomes, including proportion of patients achieving endpoint HbA_{1c} targets, fasting plasma glucose, basal insulin dose, and prandial insulin dose (T1DM only) (Table), except for a significantly greater weight change from baseline observed in the LY IGlar group of patients with T1DM, which was not consistently observed in patients with T2DM and in the total T1DM population. For safety outcomes, a significant difference in the proportion of patients with detectable insulin antibodies at any time point of the study (overall) was noted in patients with T2DM but not for patients with T1DM. No significant difference was observed at the 52-week (T1DM) or 24-week (T2DM) endpoint (LOCF); median insulin antibody levels were low (<5%) and similar in the 2 treatment groups. Significantly fewer patients with T2DM treated with LY IGlar reported ≥1 serious adverse event, which was not seen in patients with T1DM and no treatment differences were observed for preferred terms. No significant treatment differences were observed for other safety outcomes, including total and nocturnal hypoglycaemia rates and incidences (endpoint and overall; blood glucose ≤3.9 mmol/l if available), incidence of treatment-emergent antibody response, and treatment-emergent adverse events.
Conclusion: In patients who reported prestudy treatment with IGlar in these 2 Phase 3 trials, patients randomised to LY IGlar had similar efficacy and safety outcomes as those randomised to IGlar.

Outcome Measure LSM (SE) Unless Otherwise Indicated		ELEMENT-1 (T1DM) Total population LY IGlar N=268[a]	ELEMENT-1 (T1DM) Total population IGlar N=267[a]	ELEMENT-1 (T1DM) Prior IGlar subgroup LY IGlar N=218	ELEMENT-1 (T1DM) Prior IGlar subgroup IGlar N=234	ELEMENT-2 (T2DM) Total population LY IGlar N=376[a]	ELEMENT-2 (T2DM) Total population IGlar N=380[a]	ELEMENT-2 (T2DM) Prior IGlar subgroup LY IGlar N=155	ELEMENT-2 (T2DM) Prior IGlar subgroup IGlar N=144
HbA_{1c} (%)	Baseline	7.76±0.07	7.79±0.07	7.70±0.07	7.78±0.07	8.35±0.06	8.31±0.06	8.13±0.09	8.12±0.09
	Change from Baseline (Week 24 LOCF)	-0.35±0.05	-0.46±0.05	-0.32±0.06	-0.42±0.06	-1.29±0.06	-1.34±0.06	-1.02±0.08	-1.01±0.08
	LSM Diff [95% CI] (Week 24 LOCF)	0.11 [-0.00, 0.22]		0.10 [-0.02, 0.22]		0.05 [-0.07, 0.18]		0.00 [-0.19, 0.19]	
Pts Reaching HbA_{1c} <7.0%, n (%)		81 (30)	67 (25)	70 (32)	59 (25)	180 (49)	197 (53)	63 (41)	58 (41)
Basal Insulin Dose/ Prandial Insulin Dose[b] (U/kg/day) Endpoint (LOCF)		0.38±0.01/ 0.37±0.02	0.36±0.01/ 0.37±0.02	0.38±0.01/ 0.38±0.02	0.37±0.01/ 0.38±0.02	0.50±0.03/ NA	0.48±0.03/ NA	0.61±0.03/ NA	0.55±0.03/ NA
Weight (kg)	Change from Baseline (LOCF)	0.7±0.3	0.4±0.3	1.0±0.3*	0.2±0.3	1.8±0.3	2.0±0.3	1.4±0.3	1.7±0.3
	LSM Diff [95% CI] (LOCF)	0.3 [-0.3, 0.9]		0.8 [0.2, 1.4]		-0.2 [-0.5, 0.2]		-0.3 [-1.1, 0.5]	
Total Hypoglycaemia Rate (events/pts/year), Mean (SD), Overall		77.0 (68.7)	79.8 (74.5)	77.4 (67.6)	78.7 (74.3)	21.3 (24.4)	22.3 (28.2)	20.8 (22.7)	21.5 (29.6)
Pts with Detectable Antibodies: n (%), Overall		107 (40)	105 (39)	82 (38)	92 (39)	56 (15)	40 (11)	29 (19)*	11 (8)
Insulin Antibody: % Binding (median), Endpoint (LOCF)		0.92	0.89	0.85	0.83	1.07	0.65	1.02	0.45

[a]Full Analysis Set; N numbers reflect maximum sample size; *Measured in ELEMENT-1 only; *p< 05; all other p-values > 05.
Diff = difference; IGlar = insulin glargine; LOCF = last observation carried forward (endpoint) and is up to 52 weeks for ELEMENT-1 unless otherwise indicated; LSM = least squares mean; LY IGlar = LY2963016 IGlar; n = number of patients in the specified category; N = number of patients in the Full Analysis Set in the specified population/subgroup; NA = not applicable; pts = patients; T1DM = type 1 diabetes mellitus; T2DM = type 2 diabetes mellitus; U = units

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Adherence to initiated basal insulin analogue treatment in type 1 and 2 diabetes

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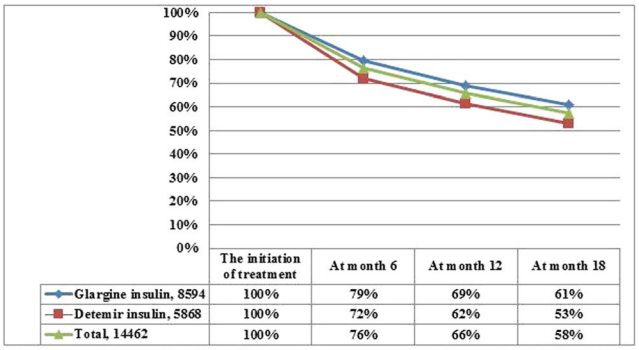
Background and aims: Poor medication adherence is common in diabetes potentially causing poor health outcomes and complications. The aim of this study was to analyze the discontinuation rate of initiated basal insulin analog in type 1 and type 2 diabetic patients in Finland.
Materials and methods: The data was obtained from the national reimbursement registry. Study population consisted of 14 462 diabetic patients (18% had type 1 diabetes) who started basal insulin analogs (insulin glargine or insulin detemir) in 2012. Patients were followed by their insulin purchases for 18 months after the initiation. The data was analysed with χ^2-test and logistic regression analysis. Logistic regression analysis was used to find out what variables (age, gender, type of diabetes, type of insulin analog) explain patient staying in the treatment.
Results: Type of insulin, gender, age and type of diabetes had statistically significant influence on patients' treatment adherence ($p<0.001$ for all). Overall 47% of patients starting insulin detemir and 39% starting insulin glargine patients discontinued their basal insulin treatment within 18 months of the initiation. Most of the patients stopped treatment within first 6 months after the initiation. In type 1 diabetic patients, 42% of insulin glargine patients and 57% of insulin detemir patients stopped the initiated treatment. In type 2 diabetic patients, 35% stopped insulin glargine and 38% insulin detemir. In only 15% of the patients discontinuing the initiated basal insulin, death or switch to other insulin or GLP-1RA explained the discontinuation suggesting non-adherence to insulin therapy from other reasons.
Conclusion: There is a considerable proportion of diabetic patients discontinuing their initiated basal insulin analog. Future studies are warranted to examine the detailed reasons for discontinuation.



	The initiation of treatment	At month 6	At month 12	At month 18
Glargine insulin, 8594	100%	79%	69%	61%
Detemir insulin, 5868	100%	72%	62%	53%
Total, 14462	100%	76%	66%	58%

% of patients who stay in initiated basal insulin treatment within 18 months of the initiation

Supported by: Sanofi

971

Superior HbA_{1c} reduction with Basal Insulin peglispro (BIL) vs insulin glargine in patients with type 2 diabetic patients previously treated with basal insulin: IMAGINE 5

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Background and aims: Basal insulin peglispro (BIL) is a novel basal insulin with a flat profile which has a hepato-preferential action resulting from reduced peripheral effects.
Materials and methods: This Phase 3, open-label, treat-to-target 52-week study in patients with type 2 diabetes (T2D) (HbA1c ≤9%) on basal insulin alone or with up to 3 oral antihyperglycaemic medications (OAMs), assessed noninferiority of BIL to insulin glargine (GL) in reducing HbA1c at 26 weeks (margin=0.4%) when added to prestudy OAMs. Patients were randomised 2:1 to BIL (N=307) or GL (N=159); 162 patients underwent MRI to assess liver fat content (LFC).
Results: Superiority of BIL vs GL was demonstrated for change in HbA1c at 26 and 52 weeks. In addition, more BIL patients reached HbA1c <7% and lab FSG was lower with BIL. BIL patients had a 60% rate reduction vs GL in nocturnal hypoglycaemia and more patients reaching HbA1c <7% without nocturnal hypoglycaemia over 52 weeks. BIL-treated patients had a lower total hypoglycaemia rate, higher basal insulin dose. BIL patients had higher triglycerides (TGs), lower LDL-C and HDL-C, no difference in non-HDL-C, and higher ALT and AST. More BIL patients had ALT≥3× ULN (2.3 vs 0%, p=.101), with no cases of Hy's law. LFC increased from baseline in patients on BIL vs GL with stable LFC from weeks 26 to 52.
Conclusion: In summary, in patients previously treated with conventional basal insulins, BIL provided superior reduction in HbA1c, less nocturnal and total hypoglycaemia, and higher TGs, ALT, and LFC compared to GL.

Outcome	26 Weeks GL	26 Weeks BIL	26 Weeks p-value[d]	52 Weeks GL	52 Weeks BIL	52 Weeks p-value[d]
HbA1c (%)	7.1 ± 0.06	6.6 ± 0.04	<.001	7.2 ± 0.06	6.8 ± 0.05	<.001
Baseline GL: 7.4 ± 0.06 | Baseline BIL: 7.4 ± 0.05 | LSM Diff [95% CI]	-0.52 [-0.67, -0.38]			-0.44 [-0.60, -0.29]		
HbA1c <7%, n (%)	82 (52)	219 (73)	<.001	72 (46)	193 (64)	<.001
HbA1c <7% & no noct hypo, n (%)	29 (19)	121 (40)	<.001	16 (10)	105 (35)	<.001
FSG from laboratory (mmol/L)	6.6 ± 0.2	5.8 ± 0.1	<.001	6.4 ± 0.2	6.0 ± 0.1	.016
Body weight change (kg)[a]	0.94 ± 0.24	0.50 ± 0.17	.125	1.32 ± 0.32	0.69 ± 0.23	.106
Basal insulin dose (U/kg)	0.49 ± 0.01	0.57 ± 0.01	<.001	0.49 ± 0.02	0.58 ± 0.01	<.001
Total hypo rate[b]	2.0 ± 0.2	1.6 ± 0.1	.052	1.6 ± 0.2	1.2 ± 0.1	.031
RR, BIL/GL		0.79			0.77	
Noct hypo rate[b]	1.0 ± 0.2	0.4 ± 0.1	<.001	0.9 ± 0.1	0.4 ± 0.1	<.001
RR, BIL/GL		0.41			0.40	
Severe hypo[c]	1	0	--	2	0	.117
Triglycerides (mmol/L)	1.62 ± 0.06	1.91 ± 0.04	<.001	1.79 ± 0.06	1.96 ± 0.05	.026
LDL-C (mmol/L)	2.61 ± 0.05	2.49 ± 0.04	.062	2.58 ± 0.05	2.40 ± 0.04	.007
HDL-C (mmol/L)	1.22 ± 0.01	1.18 ± 0.01	.009	1.17 ± 0.01	1.13 ± 0.01	.018
Non-HDL-C (mmol/L)	3.34 ± 0.06	3.34 ± 0.04	.927	3.36 ± 0.06	3.29 ± 0.04	.283
ALT (IU/L)	26.6 ± 1.1	35.9 ± 0.8	<.001	26.4 ± 1.1	34.3 ± 0.8	<.001
AST (IU/L)	23.3 ± 0.8	28.7 ± 0.5	<.001	23.5 ± 0.8	27.7 ± 0.6	<.001
Liver Fat Content (%)	9.1 ± 0.7	15.1 ± 0.5	<.001	9.6 ± 0.8	14.9 ± 0.5	<.001

[a]from baseline; [b]events/patient/30 days; [c]number of patients with severe hypoglycaemia; [d]p-values are for between treatment differences
Data are LSM ± SE unless otherwise indicated. FSG, fasting serum glucose; hypo, hypoglycaemia; LSM, least squares mean; noct, nocturnal; RR, relative rate; ULN, upper limit of normal

Clinical Trial Registration Number: NCT01582451
Supported by: Eli Lilly and Company

972

Superior HbA_{1c} reduction with basal insulin peglispro (BIL) vs insulin glargine (GL) and preprandial insulin lispro in a double-blind study in type 2 diabetes patients: IMAGINE 4

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Background and aims: Basal insulin peglispro (BIL) is a novel basal insulin with a flat activity profile which has a hepato-preferential action resulting from reduced peripheral effects.
Materials and methods: In this 26-week, Phase 3, blinded, treat-to-target trial, 1369 patients with T2D (HbA1c ≥53 mmol/mol [>7.0%] and <108 mmol/mol [<12.0%] on ≥1 insulin injection/day) were randomised to bedtime BIL (N=691) or GL (N=678). Patients could continue metformin. A wireless electronic diary system facilitated communication of SMBG, hypoglycaemia, and insulin dosing.
Results: At Week 26, patients on BIL vs GL demonstrated superior HbA1c reduction (baseline: 69 mmol/mol [8.4%]; Week 26: 50 vs 53 mmol/mol [6.8% vs 7.0%]; treatment difference -2.30 mmol/mol [-0.21%], p<.001). BIL-treated patients had lower FSG (7.0 vs 7.3 mmol/l [125 vs 132 mg/dl], p=.015), and more reached HbA1c <7% (63% vs 53%, p<.001). The improved glycaemic control with BIL vs GL was achieved with a 45% lower rate of nocturnal hypoglycaemia and more patients reaching HbA1c <53 mmol/mol (<7%) without nocturnal hypoglycaemia over 26 weeks (24% vs 12%, p<.001). Total hypoglycaemia relative rate was 1.10 (BIL/GL, p=.053); severe hypoglycaemia rate and incidence did not differ between treatments. With BIL, basal insulin dose was 11% higher, but bolus and total insulin doses were similar at 26 weeks. The BIL group had greater reduction in within-day and between-day fasting and 9-point SMBG variability, and less weight gain (change from baseline, 1.3 vs 2.2 kg, p<.001). BIL treatment was associated with higher triglycerides (TG, change from baseline: 0.27 vs -0.04 mmol/l [24 vs -3 mg/dl], p<.001), lower HDL-C (change from baseline: -0.04 vs -0.01 mmol/l [-2 vs 0 mg/dl], p<.001), and similar LDL-C, with no difference in cardiovascular or other serious adverse events. At Week 26, ALT mean change from baseline was 7.6 vs -0.6 IU/l (p<.001); more BIL patients had ALT ≥3× ULN (1.9% vs 0.9%, p=0.16), with no cases of Hy's law. There were no significant differences in efficacy or safety in patients with anti-BIL treatment-emergent antibody responses.

Conclusion: In patients with T2D, BIL vs GL, in combination with insulin lispro, provided improved glycaemic control with less nocturnal hypoglycaemia, less weight gain, and increases in TG and ALT.
Clinical Trial Registration Number: NCT01468987
Supported by: Eli Lilly and Company

973

Liver enzyme and Liver Fat Content (LFC) results from Basal Insulin peglispro (BIL) clinical trials in type 1 and type 2 diabetes

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Background and aims: BIL, a basal insulin analog with a flat pharmacokinetic profile, has reduced peripheral activity resulting in hepatopreferential action. To monitor hepatic safety, liver enzymes and LFC were measured in patients (pts) with T1D and T2D during BIL trials.
Materials and methods: Alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), and total bilirubin (TBL) were measured at 0, 4, 8, 12, 16, 26, 39, 52, 65, and 78 weeks (wks), depending on study duration, and 4 wks following study basal insulin withdrawal. Data were integrated for studies comparing BIL with insulin glargine (GL) in pts with T1D (1 Phase 2 and 2 Phase 3 trials; 1026 BIL and 676 GL) and T2D (1 Phase 2 and 3 Phase 3 trials; 2194 BIL and 1464 GL). LFC was measured via magnetic resonance imaging (MRI) in 3 subsets of pts (N given in table) at 0, 26, and 52 wks: 1) T1D, 2) insulin naïve (IN) T2D pts, and 3) T2D pts previously treated with insulin (PTI). A small number of T1D pts had MRI at 78 wks (26 BIL, 11 GL).
Results: In T1D and T2D pts, mean ALT increased from baseline in BIL pts and was higher than in GL pts at all time points to 78 wks (all $p<.001$) and decreased after discontinuation of BIL. Similar results were observed for AST, but no clinically meaningful changes in mean TBL or ALP occurred. More pts taking BIL had ALT≥3X upper limit of normal (ULN) than GL but TBL≥2X ULN was similar for BIL vs GL. AST≥ 3X ULN was more common in BIL than GL pts for T2D (1.2% vs 0.4%, $p=.009$) but not for T1D. No patient had ALT≥3X ULN with a TBL≥2X ULN that could not be attributed to another cause; thus Hy's Law criteria were not met. Of the pts taking BIL with ALT≥3X ULN (44 in T1D, 44 in T2D), all pts with T1D and 40 (90.9%) with T2D returned to or trended toward baseline while continuing BIL (37 in T1D, 22 in T2D) or after discontinuing BIL (7 in T1D, 18 in T2D), consistent with hepatic adaptation. Four T2D pts had insufficient follow-up to adequately assess the ALT trend. In T2D IN pts, LFC did not change with BIL but decreased significantly with GL. In T1D and T2D PTI pts, LFC increased significantly with BIL but no change occurred with GL. In T1D pts at 78 wks, LFC remained higher in BIL than GL (6.1%±0.5% in BIL, 2.9%±0.8% in GL; $p=.001$). More BIL than GL pts had LFC≥6% (ULN). The correlation between the change in ALT and change in LFC at 52 wks was statistically significant in T1D, T2D IN, T2D PTI pts taking BIL ($R=0.37$ to 0.47; all $p<.001$); it was also significant in pts taking GL in T2D IN ($R=0.29$; $p=.029$) but not in T1D or T2D PTI pts.
Conclusion: No acute, severe, hepatocellular drug-induced liver injury was apparent with BIL treatment for up to 78 wks. LFC was significantly higher in T1D and T2D pts treated with BIL compared to GL for treatment up to 78 wks. In T2D IN pts, this difference was due to a decrease in LFC with GL. In T1D and T2D PTI pts, the difference was the result of an increase in LFC with BIL. Further research is needed to understand and characterize the potential effects, if any, of changes in LFC observed with BIL treatment in Phase 3 trials.

	Baseline		26 weeks			52 weeks		
	GL	BIL	GL	BIL	p-value	GL	BIL	p-value
T1D, integrated, N[a]	661	1005	537	809	---	505	730	---
ALT (IU/L)	21.8±0.4	21.6±0.4	22.6±0.7	30.5±0.6	<.001	22.2±0.8	29.4±0.7	<.001
% with ALT ≥ 3X ULN	Overall at any time post-baseline: 1.51% GL, 4.37% BIL, p=.002							
AST (IU/L)	22.9±0.4	22.8±0.3	23.4±0.7	25.0±0.5	.063	23.4±0.7	24.6±0.6	.164
LFC, N	64	118	64	118	---	57	107	---
LFC (%)	3.4±0.4	3.0±0.3	3.0±0.3	5.4±0.2	<.001	3.5±0.4	5.7±0.3	<.001
% with LFC ≥6%[b]	9.4	8.5	7.8	25.4	.008	14.0	28.0	.05
T2D, integrated, N[a]	1452	2169	1225	1767	---	583	1105	---
ALT (IU/L)	28.0±0.4	28.3±0.3	26.7±0.4	33.6±0.3	<.001	27.1±0.5	34.5±0.3	<.001
% with ALT ≥ 3X ULN	Overall at any time post-baseline: 0.62% GL, 2.03% BIL; p<.001							
AST (IU/L)	23.8±0.3	24.2±0.2	23.5±0.3	27.1±0.2	<.001	24.0±0.3	28.0±0.2	<.001
T2D IN, N for LFC	56	112	55	109	---	47	94	---
LFC (%)	12.7±1.1	13.3±0.8	9.3±0.6	12.3±0.4	<.001	10.0±0.7	12.6±0.5	.002
% with LFC ≥6%[b]	78.6	73.2	69.1	77.1	.343	66.0	74.5	.325
T2D PTI, N for LFC	52	110	52	108	---	44	92	---
LFC (%)	10.0±1.1	10.4±0.8	9.1±0.7	15.1±0.5	<.001	9.6±0.8	14.9±0.5	<.001
% with LFC ≥6%[b]	55.8	66.4	50.0	86.1	<.001	47.7	82.6	<.001

All values are least squares mean ± standard error of actual measurement unless otherwise noted.
[a] N=number of patients with ALT measured at visit.
[b] 6% was chosen as the ULN for LFC measured by MRI based on published literature.

Clinical Trial Registration Number: NCT01027871, NCT01049412, NCT01435616, NCT01468987, NCT0141779, NCT01454284, NCT01582451
Supported by: Eli Lilly and Company

PS 092 Basal insulin analogue: effects on hypoglycaemia

974

Is hypoglycaemia a modifiable patient risk in type 2 diabetes: A pooled analysis of insulin glargine 300 U/mL (Gla-300) vs 100U/mL (Gla-100) trials?

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Background and aims: Repeated episodes of hypoglycemia are associated with an increased risk of adverse clinical outcomes. Underlying patient characteristics may predispose to repeated hypoglycemia.
Materials and methods: We evaluated potential patient predisposition to documented hypoglycemia in pooled data (N=2488) from 3 T2DM clinical trials of Gla-300 vs Gla-100 and their 6-month extensions (N=1994). Study populations for the three studies are as follows: DIV>14 patients were removed due to missing baseline data. Monthly study data were analyzed with general estimating equations (GEE) to assess the impact of patient characteristics on risk of repeated hypoglycemic events over 12 months.
Results: Baseline characteristics were comparable between groups: mean age 59 years, BMI 35 kg/m^2, A1C 8.3%, diabetes duration 13 years, and Charlson Comorbidity Index (CCI) 0.56 for Gla-300 vs 0.60 for Gla-100. Less documented (≤70 mg/dL) symptomatic hypoglycemia was noted with Gla-300 vs Gla-100 (57.0% vs 63.3%, odds ratio=0.77, 95% CI: 0.65-0.90, P=0.001). Female gender (P<0.001), BMI ≤35 kg/m^2 (P<0.001), CCI >0.60 (P=0.017), and diabetes duration >10 years (P=0.001) were associated with an increased number of documented, symptomatic hypoglycemia events. Poisson regression showed a lower event rate for Gla-300 vs Gla-100 (4.4 vs. 5.2 events/year; rate ratio 0.84, 95% CI: 0.76-0.92, P<0.001) independent of patient characteristics. Furthermore, monthly percentage of patients experiencing documented hypoglycemia increased in Gla-100 but stayed unchanged in Gla-300 over CCI ($P_{Interaction}$=0.017).
Conclusion: Repeated episodes of documented hypoglycemia in T2DM are reflective of such patient characteristics as gender, BMI, diabetes duration and comorbidity. Gla-300 is associated with a lower event rate than Gla-100 through the 12 months period. In particular, increasing hypoglycemia risk due to patient burden of comorbidity appears to be mitigated with Gla-300 compared to Gla-100.
Supported by: Sanofi

975

Patient-level meta-analysis of 1y phase 3a EDITION type 2 diabetes mellitus studies: glycaemic control and hypoglycaemia with insulin glargine 300 U/ml (Gla-300) vs glargine 100 U/ml (Gla-100)

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Background and aims: EDITION 1, 2 and 3 assessed Gla-300 vs Gla-100 in people with type 2 diabetes (T2DM).
Materials and methods: A patient-level meta-analysis of 1-year data was conducted.
Results: Glycaemic control was sustained in both groups, with more sustained HbA_{1c} reduction for Gla-300 at 1 year. LS mean (SE) change from baseline in HbA_{1c} was −0.91 (0.03) % for Gla-300 and −0.80 (0.03) % for Gla-100 (LS mean difference [95% CI] between groups −0.10 [−0.18 to −0.02] %; p=0.0174). There was a reduced risk of confirmed (≤3.9 mmol/l [≤70 mg/dl]) or severe hypoglycaemia at any time (24 h) and during the night with Gla-300 vs Gla-100. Weight gain was less with Gla-300 vs Gla-100. The slight insulin dose difference at 6 months remained at 1 year.
Conclusion: Gla-300 was associated with more sustained glycaemic control, with greater up-titration of insulin dose, primarily in the first weeks, and without increased risk of any time and nocturnal hypoglycaemia or weight gain at 1 year vs Gla-100.

Table: Efficacy and safety of Gla-300 in a patient-level meta-analysis of 1-year data from the EDITION 1, 2 and 3[†] clinical trials

HbA_{1c} (%)			
mITT population		**Gla-300 (*N*=1239)**	**Gla-100 (*N*=1235)**
BL	*Mean (SD)*[a]	8.30 (0.91)	8.31 (0.90)
Change from BL to 1 year	*LS mean (SE)*[a]	−0.91 (0.03)	−0.80 (0.03)
	LS mean difference (95% CI)[a]	−0.10 (−0.18 to −0.02)	
	p-value for LS mean difference	p=0.0174	

Confirmed (≤3.9 mmol/l) or severe hypoglycaemia		**Nocturnal hypoglycaemia (00:00–05:59 h)**		**Hypoglycaemia at any time (24 h)**	
Safety population		**Gla-300 (*N*=1242)**	**Gla-100 (*N*=1246)**	**Gla-300 (*N*=1242)**	**Gla-100 (*N*=1246)**
BL to 1 year	*% people ≥1 event*	38.7	45.7	73.1	77.8
	Relative risk[b] *(95% CI)*	0.85 (0.77 to 0.92)		0.94 (0.90 to 0.98)	
	Events/participant-year	2.0	2.4	13.7	14.1
	Rate ratio[c] *(95% CI)*	0.82 (0.67 to 0.99)		0.97 (0.87 to 1.09)	

Weight (kg)			
Safety population		**Gla-300 (*N**=1242)**	**Gla-100 (*N**=1246)**
BL	*Mean (SD)*	99.9 (22.9)	99.9 (21.7)
1 year	*Mean (SD)*	100.9 (23.1)	101.2 (22.1)
Change from BL to 1 year	*Mean (SD)*	1.19 (5.32)	1.52 (4.18)
	p-value for LS mean difference[d]	0.0117	

Insulin dose (U/kg/day)			
mITT population		**Gla-300 (*N**=1239)**	**Gla-100 (*N**=1235)**
BL	*Mean (SD)*	0.49 (0.30)	0.50 (0.31)
Change from BL to month 6	*Mean (SD)*	0.34 (0.25)	0.25 (0.23)
Change from BL to 1 year	*Mean (SD)*	0.39 (0.29)	0.27 (0.27)

[†]EDITION 1, 2 and 3 included people with type 2 diabetes on basal + mealtime insulin, basal insulin + oral antihyperglycaemic drugs, and no prior insulin, respectively; **N* represents the maximum number of participants available for analysis in each group; [a]based on Mixed Model for Repeated Measurements (MMRM) analyses; [b]based on Cochran-Mantel-Haenszel method stratified by randomisation strata of screening HbA_{1c} (<8.0 and ≥8.0%) and study; [c]based on overdispersed Poisson regression model; [d]based on Analysis of Covariance (ANCOVA). BL, baseline; CI, confidence interval; LS, least squares; mITT, modified intention-to-treat; SD, standard deviation; SE, standard error.

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Supported by: Sanofi

976

Sustainable effects of basal insulin have favourable outcomes on fasting blood glucose control in patients with type 2 diabetes

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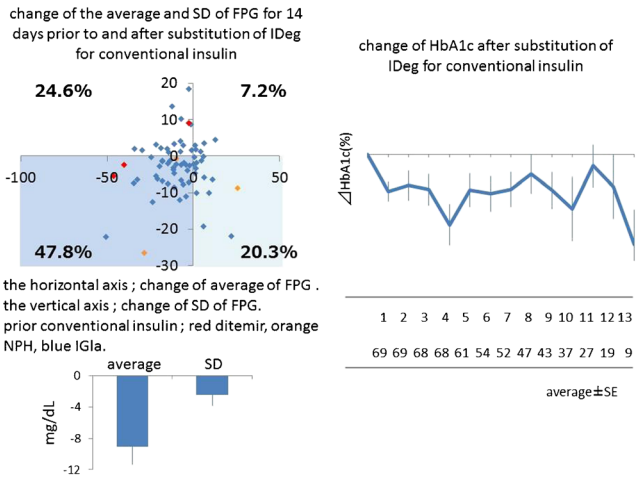
Background and aims: Recently, basal insulin formulations of longer effect have been developed. We investigated the difference in the sustainable effect of longer acting formulations on blood glucose control in patients with Type 2 diabetes. We compared insulin degludec (IDeg) therapy which has the longest effect of 42 hours with a conventional basal insulins (NPH, ditemir or glargine (IGla)) therapies of which the effect is less than 24 hours.
Materials and methods: We conducted two different retrospective analyses, namely; 1) A comparison study of the newly introduced IDeg versus conventional insulin, the duration needed to achieve the target FPG and the quantity of insulin needed in both groups were analysed. and 2) In substitution study of IDeg for conventional insulin, the average and variability (SD) of FPG for 14 days prior to and after both switches were analysed.

Results: 1) Out of 332 patients with T2DM, 114 patients (60 males, 64.4 ±9.8 years old, disease duration of 7.6±7.4 years, HbA1c 10.1±2.1%, BMI 26.7±3.7, FPG 201±52 mg/dl) were newly administered IDeg once-daily, and 218 patients (95 males, 57.4±13.0 years old, disease duration of 5.6±5.6 years, HbA1c 10.4±1.8%, BMI 27.1±4.6, FPG 200±56 mg/dl) were newly administered conventional therapy once-daily or twice a day. Patients newly treated with IDeg achieved the target FPG earlier (6.7±6.9 days) than those with conventional insulin IGla (8.9 ±11.7 days) ($p<0.01$). Total doses of insulin IDeg (104.4±75.3 unit) needed to achieve the target FPG tended to be less than those of IGla (122.5±93.4 unit) (NS).2) Sixty-nine patients undergoing conventional therapy were switched to IDeg for more than 30 days and then switched back to conventional therapy. For the 14 days before and after both switches, the average variability (SD) of FPG were analyzed. After switching from conventional therapy to IDeg therapy, both the average and SD of FPG decreased in 47.8% of patients (the average decreased in 72.4% and the SD of FPG decreased in 68.1%), and the amount of insulin was reduced by 10%. Nocturnal hypoglycemia occurred in 1% and 5% of patients undergoing IDeg and conventional therapy,repectively.
Conclusion: Longer active basal insulin therapy, i.e. IDeg, provides more effective and safer control of blood glucose in T2DM. It might also be expected to reduce medical costs.



Supported by: JAPAN VASCULAR DISEASE RESEARCH FOUNDATION

977

Older people with type 2 diabetes: glycaemic control and hypoglycaemia risk with new insulin glargine 300 U/ml (Gla-300)

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Background and aims: In people with T2DM, a patient-level meta-analysis of EDITION 1, 2 and 3 has shown Gla-300 provides comparable glycaemic control with less hypoglycaemia over 6 months vs insulin glargine 100 U/ml (Gla-100).
Materials and methods: In this post hoc analysis, we investigated these outcomes, as well as an extended and clinically defined window of nocturnal hypoglycaemia (22:00 h to pre-breakfast SMPG) and composite endpoints, in those aged ≥65 years (n=659).
Results: Gla-300 showed comparable glycaemic control to Gla-100 (LS mean [95% CI] difference in HbA_{1c} change from baseline to month 6: 0.00 [−0.14 to 0.15] %). There was less confirmed (≤3.9 mmol/l [≤70 mg/dl]) or severe hypoglycaemia during the night irrespective of the nocturnal window analysed and a trend towards fewer any time (24 h) events for Gla-300 vs Gla-100. More Gla-300-treated participants reached HbA_{1c} targets or had HbA_{1c} reduction ≥0.5% without confirmed (≤3.9 mmol/l [≤70 mg/dl]) or severe nocturnal hypoglycaemia. Those on Gla-300 were 55-70% more likely to achieve HbA_{1c} <7% with no nocturnal hypoglycaemia (significant using both windows).
Conclusion: The comparable glycaemic control plus hypoglycaemia benefit of Gla-300 is confirmed in a potentially more vulnerable subgroup aged ≥65 years, with more people reaching HbA_{1c} targets without hypoglycaemia on Gla-300 vs Gla-100. The nocturnal hypoglycaemia benefit of Gla-300 was also confirmed when an extended window (22:00 h to pre-breakfast SMPG) was considered.

Table: Hypoglycaemia and responders to composite endpoints in people with T2DM aged ≥65 years, over 6 months in a patient-level meta-analysis of EDITION 1, 2 and 3 clinical trial data

Percentage of participants with ≥1 confirmed (≤3.9 mmol/l) or severe hypoglycaemic event, n (%) (safety population)

Hypoglycaemia	Gla-300 (n=327)	Gla-100 (n=332)	RR	95% CI
Any time of day (24 h)	234 (71.6)	257 (77.4)	0.93	0.85 to 1.01
Nocturnal (00:00–05:59 h)	104 (31.8)	149 (44.9)	0.70	0.57 to 0.85
Clinically defined nocturnal (22:00 h–pre-breakfast SMPG*)	191 (58.4)	239 (72.0)	0.81	0.73 to 0.91

Responders to composite endpoints, n (%) (modified intention-to-treat population)

		Gla-300 (n=326)	Gla-100 (n=329)	RR	95% CI	p-value
HbA_{1c} <7 %	No hypoglycaemia at any time of day (24 h)	36 (11.0)	26 (7.9)	1.45	0.90 to 2.33	0.127
HbA_{1c} <7.5 %		49 (15.0)	37 (11.2)	1.35	0.91 to 2.01	0.127
HbA_{1c} change ≥0.5 %		54 (16.6)	42 (12.8)	1.28	0.88 to 1.85	0.192
HbA_{1c} <7 %	No nocturnal hypoglycaemia (00:00–05:59 h)	83 (25.5)	57 (17.3)	1.55	1.16 to 2.07	0.003
HbA_{1c} <7.5 %		125 (38.3)	89 (27.1)	1.46	1.17 to 1.82	0.001
HbA_{1c} change ≥0.5 %		138 (42.3)	105 (31.9)	1.31	1.07 to 1.60	0.007
HbA_{1c} <7 %	No clinically defined nocturnal hypoglycaemia (22:00 h–pre-breakfast SMPG*)	56 (17.2)	35 (10.6)	1.70	1.16 to 2.50	0.006
HbA_{1c} <7.5 %		80 (24.5)	48 (14.6)	1.72	1.25 to 2.37	0.001
HbA_{1c} change ≥0.5 %		83 (25.5)	52 (15.8)	1.60	1.17 to 2.18	0.003

*Hypoglycaemia occurring between 22:00 h and the time of a pre-breakfast self-monitored plasma glucose (SMPG) measurement.
Median time of pre-breakfast SMPG was 07:45 h (interquartile range [IQR] 07:00–08:30 h).
CI, confidence interval; RR, relative risk.

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Supported by: Sanofi

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Comparison of glycaemic variations in Japanese patients with type 1 diabetes receiving insulin detemir vs insulin degludec as assessed by Continuous Glucose Monitoring (CGM)

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Background and aims: Japanese patients with type 1 diabetes receiving the long-acting soluble insulin preparations insulin degludec (Deg) and insulin detemir (Det) were compared for glycemic variation by using continuous glucose monitoring (CGM).
Materials and methods: A total of 12 patients with type 1 diabetes (males/females, 4/8) were enrolled in the study (mean age, 51.8±12.0 (SD) years; BMI, 22.1±2.5 kg/m2; and HbA1c, 7.8±0.6%). The patients received one insulin preparation and were crossed over to the other for comparison. Of these, 2 patients received 1 Det injection, and 10 received 2 Det injections per day. The timing of Deg injection was set to fit the lifestyle of each patient and the amount administered was determined by

each patient in consultation with their doctors. Each patient was fitted with a CGM device in the outpatient clinic and 24-hour data were collected for comparison after they had had the same test meals. During each period, blood glucose levels were monitored with the CGM for at least 3 days. Mean glucose levels, standard deviations of glucose (SD), time in hypoglycemia (200 mg/dL), range of postprandial glucose increase, time to peak glucose values (time to peak glucose values after each meal and mean time to peak glucose values after 3 meals), and area under the concentration-time curve for glucose (AUC, > 200 mg/dL) were compared between those given Det and those given Deg by using paired t-test. All statistical analyses were performed by using SPSS 22.0. The present study was approved by the Ethics Committee of our university.

Results: Those receiving Det versus Deg had a mean glucose level of 183.3±46.2 mg/dL versus 144.5±37.2 mg/dL (P=0.025) and an SD of 77.4±20.6 mg/dL versus 59.6±17.5 mg/dL (P=0.004), indicating that the glucose values were significantly lower during degludec administration. Those receiving Det versus Deg had a SD during nighttime (midnight to 6:00 AM) was 34.5±27.6 mg/dL versus 16.5±10.4 mg/dL, with their nighttime SD being significantly lower while on Deg (P=0.048). No significant differences were seen in time in hypoglycemia (200 mg/dL) while on Det versus Deg. Their mean preprandial blood glucose level before breakfast was 165.3±83.6 mg/dL versus 102.3±72.3 mg/dL while on Det versus Deg (P=0.010) and their mean postprandial peak level after breakfast was 284.5±60.6 mg/dL versus 200.3±48.2 mg/dL while on Det versus Deg (P=0.004), with the values significantly lower while on Deg. No significant differences were seen at lunch or dinner times. Their AUC (>200 mg/dL) was 44836±36118 versus 16993±18932 while on Det versus Deg, with the AUC being significantly lower while on Deg (P= 0.007).

Conclusion: Type 1 diabetic patients receiving insulin detemir or insulin deguldec were compared for glycemic variability by using CGM. Results suggested that insulin deguldec appears to be superior to insulin detemir in stabilizing blood glucose levels from late at night through breakfast.

979

Similar HbA_{1c} reduction and hypoglycaemia with variable time and fixed time dosing of basal insulin peglispro (BIL) in type 1 diabetes: IMAGINE 7

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Background and aims: Basal insulin peglispro (BIL) is a novel basal insulin with a flat profile which has a hepato-preferential action resulting from reduced peripheral effects. The flat profile suggests some variation in dosing intervals will not affect measures of glycaemic control. This Phase 3 crossover study was designed to assess whether variable dose timing was noninferior (margin=.4%) to fixed dose timing for HbA1c after 12 weeks of BIL treatment in patients with type 1 diabetes.

Materials and methods: During the lead-in phase, 212 patients received BIL (evening dosing) for 12 weeks. Of those patients, 182 completed the lead-in phase and were randomised to two 12-week crossover treatment periods comparing fixed time dosing (evening) and variable time dosing (dosing intervals were 8±2 hours to 40±2 hours, with morning dosing on Monday, Wednesday, Friday, and evening dosing on Tuesday, Thursday, Saturday, Sunday).

Results: Patients were mostly Caucasian (96%), approximately 43 years old with diabetes duration of about 20 years. During the 12-wk lead-in, mean ± SD HbA1c decreased from 7.5%±.8 to 6.8%±.7; fasting serum glucose (FSG) decreased from 9.0±3.9 mmol/L (162±69 mg/dL) to 6.9± 3.3 mmol/L (123±59 mg/dL). During the randomised phase, variable timing and fixed timing groups had similar HbA1c, FSG, glucose variability, total and nocturnal hypoglycaemia, and basal and bolus insulin doses (Table). Self-monitored fasting blood glucose was .46 mmol/L (8 mg/dL) lower (LS mean difference) with fixed time dosing compared to variable time dosing (p=.014). Treatment-emergent adverse events (AEs) and serious AEs were similar for both groups. Over 36 weeks, LS mean weight decreased 1.2±.3 kg (p<.001) and mean triglycerides, ALT, and AST increased significantly (all p<.001) but trended toward baseline levels by 4 weeks after BIL discontinuation. No patients met criteria for Hy's law. Twenty patients (9.4%) experienced injection site reactions including injection site swelling and lipohypertrophy.

Conclusion: We conclude that BIL allows 8±2 to 40±2 hour dosing intervals with similar HbA1c compared to fixed time dosing in patients with type 1 diabetes.

Outcomes at Primary Endpoints

		12-Week Treatment Following Randomization		
		Fixed Timing (N=177)	Variable Timing (N=180)	Between Group p-value[a]
HbA1c, %		6.87 ± .04	6.93 ± .04	.095
HbA1c, mmol/mol		52 ± .4	52 ± .4	.095
Fasting serum glucose (laboratory)	mg/dL	122 ± 4.3	120 ± 4.3	.831
	mmol/L	6.75 ± .24	6.69 ± .24	
Fasting blood glucose (SMBG)	mg/dL	131 ± 2.9	140 ± 2.9	.014
	mmol/L	7.29 ± .16	7.75 ± .16	
Within-day variability (SMBG)	mg/dL	47 ± 1.4	48 ± 1.4	.791
	mmol/L	2.63 ± .08	2.65 ± .08	
Between-day FBG variability	mg/dL	42 ± 3.3	36 ± 2.2	.105
	mmol/L	2.34 ± .18	2.01 ± .12	
Basal insulin dosage, unit/kg		.50 ± .01	.51 ± .01	.570
Bolus insulin dosage, unit/kg		.20 ± .01	.20 ± .01	.996
Total hypoglycaemia rate[b]		10.5 ± .67	10.4 ± .62	.947
Nocturnal hypoglycaemia rate[b]		1.5 ± .13	1.3 ± .11	.060
Severe hypoglycaemia rate[c]		25.7 ± 7.90	20.0 ± 8.56	.639

		Safety During 36-Week BIL Treatment (N=212)		
		Baseline[d]	Change from Baseline	p-value[e]
ALT, IU/L		22.38 ± 12.00	10.59 ± 1.55	<.001
AST, IU/L		22.17 ± 8.81	3.91 ± .76	<.001
HDL-cholesterol	mg/dL	63 ± 19	-6 ± .8	<.001
	mmol/L	1.64 ± .49	-.15 ± .02	
LDL-cholesterol	mg/dL	96 ± 27	4 ± 1.6	.007
	mmol/L	2.47 ± .70	.11 ± .04	
Triglycerides	mg/dL	75 ± 43	26 ± 3.8	<.001
	mmol/L	.85 ± .49	.30 ± .04	

LS Mean values ± SE, unless otherwise indicated
[a] Between group comparison
[b] Group mean ± SE, events/subject/30 days
[c] Aggregate rate ± SE, events/subject/100 years
[d] Mean ± standard deviation
[e] Comparison with baseline value

Clinical Trial Registration Number: NCT01792284
Supported by: Eli Lilly and Company

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Reduced nocturnal hypoglycaemia with basal insulin peglispro (BIL) compared to insulin glargine (GL): pooled analyses of 5 randomised controlled trials

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Background and aims: Nocturnal hypoglycaemia is a significant safety concern and limits optimisation of basal insulin dosing to achieve glycaemic goals.

Materials and methods: We compared the novel basal insulin analog BIL, with its flat pharmacokinetic profile and hepato-preferential action, to GL for glucose control in 5 international studies in 3 patient groups

(type 2 diabetes [T2D] basal only, T2D basal-bolus, and type 1 diabetes [T1D]). We conducted integrated analyses to assess HbA1c change and hypoglycaemia events (SMBG ≤53 mmol/l [≤70 mg/dL]) across studies.
Results: Patients (N=4927) were randomised to bedtime BIL or GL in 26-, 52- and 78-week treat-to-target trials. In all patient groups, patients treated with BIL met statistical superiority in the primary outcome of reduction in HbA1c, and had 36%–45% lower nocturnal hypoglycaemia rates. Total hypoglycaemia rates were not significantly different in T2D patients. In T1D patients, total hypoglycaemia rates were higher with BIL and associated with higher rates of daytime hypoglycaemia following bolus insulin administration. There were no statistically significant treatment differences in severe hypoglycaemia rates in the integrated analyses. For symptomatic hypoglycaemia events, the mean SMBG values were not different with BIL vs GL. Continuous glucose monitoring in a subset of T1D and T2D patients showed similar mean duration of individual hypoglycaemia events by treatment groups.
Conclusion: Treatment with BIL compared to GL was associated with greater HbA1c reductions and fewer nocturnal hypoglycaemia events in patients with T1D or T2D.
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Basal Insulin peglispro (BIL) provides better HbA_{1c} control with less nocturnal hypoglycaemia than NPH when used in combination with oral agents in type 2 diabetic patients: IMAGINE 6

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Background and aims: Basal insulin peglispro (BIL) is a novel basal insulin with a flat profile which has a hepato-preferential action resulting from reduced peripheral effects.
Materials and methods: This Phase 3, open-label, treat-to-target (TTT) study assessed if BIL was non-inferior (margin=0.4%) to NPH in reducing HbA1c in insulin naïve patients with type 2 diabetes when added to prestudy oral agents (26-week endpoint).
Results: Patients were randomised to bedtime NPH (n=213) or BIL (n=428; n=213 morning and n=215 bedtime dosing). HbA1c at endpoint was lower for BIL vs NPH (6.8 vs 7.1%; treatment difference [95% CI]: -0.37% [-0.50, -0.23%]; p<0.001). A greater proportion of BIL patients achieved HbA1c <7% (66.3 vs 44.7%; p<0.001) and HbA1c <7% without nocturnal hypoglycaemia (39.1 vs 12.6%; p<0.001). Weight gain did not differ between groups; insulin doses were higher in BIL patients (BIL: 0.40 vs NPH: 0.35 U/kg; p=0.015). Nocturnal hypoglycaemia rates (events/patient/30 d) were lower for BIL vs NPH (0.31 vs 0.61; p< 0.001) and total hypoglycaemia rates were similar (BIL: 1.46 vs NPH: 1.73; p=0.092). No significant differences between groups were observed at endpoint for any lipid variables. At endpoint, ALT increased with BIL, but patients with ALT ≥3X ULN did not differ between groups, with no cases of Hy's law. Injection site reactions were infrequent.
Conclusion: In this TTT study, BIL treatment showed clinically relevant improvements in glycaemic control and a significant reduction in nocturnal hypoglycaemia compared to NPH, consistent with a hepato-preferential action and reduced peripheral activity.

Outcomes after 26 Weeks of Treatment

Outcome	NPH		BIL		Between Treatment (BIL vs NPH)	
	Baseline	26 Weeks	Baseline	26 Weeks	Difference or RR	p-value[b]
HbA1c (%)	8.5±0.07	7.1±0.06	8.5±0.05	6.8±0.04	-0.37	<0.001
HbA1c Change from Baseline	-1.4±0.06		-1.7±0.04			
HbA1c <7%, n (%)	8 (3.8)	89 (44.7)	16 (3.8)	258 (66.3)	NA	<0.001
HbA1c <7% and no nocturnal hypo, n (%)	7 (3.3)	25 (12.6)	13 (3.1)	152 (39.1)	NA	<0.001
FSG from laboratory (mmol/L)	9.8±0.18	6.6±0.12	9.7±0.13	6.3±0.09	-0.33	0.027
Body weight change from baseline (kg)	NA	2.34±0.22	NA	2.02±0.16	-0.32	0.239
Basal insulin dose (U/kg)	NA	0.35±0.02	NA	0.40±0.01	0.05	0.015
Total hypo rate[a]	0.49±0.15	1.73±0.13	0.22±0.06	1.46±0.09	RR: 0.84	0.092
Nocturnal hypo rate[a]	0.14±0.07	0.61±0.07	0.14±0.05	0.31±0.04	RR: 0.51	<0.001
Severe hypo, n (%)	0	0	0	2 (0.5)	NA	>0.999
Triglycerides (mmol/L)	1.9±0.07	1.7±0.07	1.9±0.05	1.9±0.05	0.16	0.082
Total-C (mmol/L)	4.4±0.07	4.5±0.05	4.5±0.05	4.5±0.04	-0.02	0.751
LDL-C (mmol/L)	2.4±0.06	2.6±0.04	2.5±0.04	2.5±0.03	-0.07	0.177
HDL-C (mmol/L)	1.2±0.02	1.2±0.01	1.2±0.01	1.2±0.01	-0.02	0.224
Non-HDL-C (mmol/L)	3.3±0.07	3.4±0.05	3.3±0.05	3.3±0.04	-0.01	0.868
ALT (IU/L)	29±1.0	26±0.9	27±0.7	33±0.7	7.4	<0.001
AST (IU/L)	24±0.7	23±0.6	23±0.5	26±0.5	3.1	<0.001
Pts with ALT≥3X ULN, n (%)	6 (2.8)		5 (1.2)		NA	0.195
Pts with injection site reactions, n (%)	3 (1.4)		3 (0.7)		NA	0.404

[a] BIL treatment refers to combined morning and bedtime BIL dosing.
[b] p-values are for between treatment differences.
[c] Group mean events/patient/30 days.
[d] Number of patients with severe hypoglycaemia.
Data are LS Mean ± SE unless otherwise indicated.
FSG=fasting serum glucose; LS Mean=least squares mean; NA=not applicable; RR=relative rate BIL/NPH; hypo=hypoglycaemia; Pts=patients; ULN=upper limit of normal.

Clinical Trial Registration Number: NCT01790438
Supported by: Eli Lilly and Company

PS 093 Closing the loop

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Clinical performance of a new integrated insulin pump system in adult patients with type 1 diabetes

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Background and aims: The Accu-Chek® Insight diabetes therapy system combines a glucose meter with integrated bolus advice and extended data management capabilities with an insulin pump. The use of the new system was evaluated in a multicenter clinical trial conducted in Austria and the United Kingdom.
Materials and methods: Individuals with type 1 (T1) or type 2 (T2) diabetes mellitus (DM) ≥18 years of age, and for ≥6 months on intensive insulin therapy were eligible for this 6-month study of the new system. All individuals received training on the use of the new system. HbA_{1c} results were evaluated in a central laboratory. Pump signals were captured through data uploads using engineering software. User acceptance of the new system was assessed by means of a self-report questionnaire using an 11-point Likert Scale.
Results: 90 individuals with T1DM were included in the intention-to-treat analyses: age 41.8 (18-75) years, BMI 27.1 (19-41) kg/m^2, HbA_{1c} 7.8 (5.8 - 10.8) %, T1DM since 23 (1-49) years. 76 individuals had been previously on insulin pump therapy, and 14 on multiple daily injection (MDI) therapy. 81 individuals completed the study, 9 individuals discontinued the study or were withdrawn. Insulin pump experienced users maintained their mean
baseline HbA_{1c} levels (7.76±1.00%) at Month 3 (7.58±0.93%) and Month 6 (7.73±0.96) after switching from other insulin pump systems, most often the Accu-Chek® Combo system, to the Accu-Chek® Insight system. Mean HbA_{1c} levels decreased in previous MDI users from 7.96±1.20% at baseline to 7.45±1.22% at Month 3 and 7.46±1.08% at Month 6. At month 6, the mean change from baseline was significantly different from 0: -0.50, 95% CI [-0.94; -0.06]. Two episodes each of severe hypoglycemia and diabetic ketoacidosis were reported. Most frequently recorded errors were electronic for the pump, and battery life time related for the meter. User acceptance of the new system was high, with highest ratings given for the ease of use of the insulin pump.
Conclusion: In adults with type 1 diabetes, the new Accu-Chek® Insight diabetes therapy system was effective and safe to use under routine practice conditions. Glycemic control improved when switching from MDI therapy, and was maintained in previous insulin pump users. User acceptance of the new system was high.
Clinical Trial Registration Number: NCT02105103
Supported by: Roche Diagnostics

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Continuous glucose monitoring improved glycaemic control in patients with type 1 diabetes during 52 weeks of insulin pump therapy as well as with basal-bolus insulin regimen

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Background and aims: Insulin pump therapy (CSII) improves glycemic control of patients with diabetes, both individually and in combination with continuous glucose monitoring (SAP - Sensor Augmented Pump). However, the efficacy of long-term use of real-time continuous glucose monitoring (RT-CGM) in patients on multiple daily injections (MDI) is less described. The aim of the study was to compare the efficacy of long-term use of RT-CGM on glycemic control in patients treated with CSII or MDI in comparison to more common schemes based on classical self-monitoring of blood glucose (SMBG).
Materials and methods: In this prospective study, 52 patients with type 1 diabetes were followed up for 1 year (age 36±13 years, diabetes duration 15±8 years, HbA1c 68±10 mmol/mol). Patients were divided into 3 groups with comparable baseline parameters. 17 patients started to use RT-CGM. 18 patients initiated insulin pump therapy alone (without RT-CGM) and 17 patients in a control group continued on MDI and SMBG only. In the first group with RT-CGM, 10 patients started to use insulin pump and sensors (SAP) and the remaining 7 continued with MDI (MDI + RT-CGM). Prerequisite for participation in the first group was the willingness to use sensors>70% of the time. At the baseline, all 52 patients were monitored by blinded CGM and underwent a structured education according to type of their future treatment. In addition, groups of patients without RT-CGM were monitored for a week by blinded CGM every 3 months and at the end of the study. The main endpoints were reduction of HbA1c, glycemic variability expressed by the standard deviation of blood glucose (SDT) and the incidence of hypoglycemia (% of time below 3.9 mmol/L). Statistical analysis was performed by nonparametric tests (Kruskal-Wallis and ANOVA-repeated measurement).
Results: After a year, the group of patients on RT-CGM had significantly lower HbA1c (67±11 vs. 55±11 mmol/mol; $p<0.0001$), and both subgroups of patients, with SAP and with MDI + RT-CGM, showed comparable improvement in HbA1c. Insulin pump therapy alone (without RT-CGM) also led to reduction of HbA1c (68±9 vs. 63±8 mmol/mol; $p<0.05$), while in the control group just on MDI significant decrease of HbA1c was not reached (67±9 vs. 64±10 mmol/mol; NS). Importantly, any treatment strategy using RT-CGM was superior to insulin pump therapy alone (HbA1c: 55±11 vs 63±8 mmol/mol; $p<0.05$). As compared to the baseline, glycemic variability was lower both, in the group with RT-CGM (SDT: 4.0±0.7 vs. 3.0±0.5; $p<0.0001$) as well as in patients with insulin pumps alone (SDT 3.8±0.7 vs. 3.4±0.6; $p<0.01$). Moreover, reduction of time spent in hypoglycemia was observed only in patients with RT-CGM (8±4 vs. 6±3%; $p<0.01$).
Conclusion: The usage of RT-CGM for 70% of the time or more resulted in significant HbA1c reduction in patients with insulin pumps as well as in patients on MDI. This improvement was greater than the improvement with insulin pumps alone. The combination of RT-CGM and MDI can be a suitable alternative to the SAP system for some patients.
Supported by: The research project of Charles University (Prvouk 25)

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Diabetes control in type 1 diabetes mellitus patients treated with continuous subcutaneous insulin infusion vs multiple daily insulin injections in short and long term follow up

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Background and aims: Type 1 Diabetes Mellitus (T1DM) is a chronic disease that requires continuous training of patients in order to optimize their blood glucose levels and achieve the goals set for glycated hemoglobin A1c (HbA1c). Patients are often treated with either Continuous Subcutaneous Insulin Infusion (CSII) or Multiple Daily Insulin Injections(MDII). The aim of this study was to investigate the outcome in lowering HbA1c and fasting blood glucose (FBG), as well as the result in body weight (BW) of T1DM patients treated with either CSII or MDII.
Materials and methods: A retrospective cohort including 291 T1DM patients who were followed in the Diabetes Center of our Department between 2008 and 2014. Two groups were included: 196 patients started on treatment with CSII and 95 treated with MDII. All CSII patients were previously treated with MDII. Both groups received intensive training on how to monitor their blood glucose and how to control both hyper and hypoglycemia. Wilcoxon test was used to calculate each group at baseline vs. one-year (FU1, short-term) and five-year (FU5, long-term) follow up and Mann-Whitney test was used to compare CSII vs. MDII at baseline, one-year and five-year follow up due to not normally distributed variables. Statistical significance was defined when $p<0.05$.
Results: The patients' age ranged from 20 to 68 years old; diabetes duration since diagnosis ranged from 4 to 48 years. At baseline, there was a statistically significant difference in BW between the two groups, CSII 74.0 kg (64.0-82.5) and MSII 69.0 kg (58.0-78.5), p=0.043. HbA1c was significantly reduced from baseline both at one-year as well as at five-years follow up in patients on CSII ($p<0.001$ for both). HbA1c was reduced significantly in patients on MDII at one-year follow up 7.1% (6.3-8.3), compared to the baseline value 8.0% (6.9-9.3),p=0.013, but not at five-year follow up 7.1% (6.5-7.9), p=0.181.All other variables were not significantly different. The reduction of HbA1c, FBG and the BW difference between the two groups were not significantly different (Table 1).
Conclusion: Our study demonstrates that both CSII and MDII have comparable results in improving diabetes control both in short and long-term follow up, which could be attributed to the intensive training program applied in this T1DM patient cohort.

Table 1		CSII	MDII	p (sign)	test
BSL	HbA1c (%)	8.0 (7.3-9.1)	8.0 (6.9-9.3)	0.474	Mann Whitney test
	FBG (mg/dl)	166(115-242)	174(103-257)	0.977	
	BW (kg)	74.0 (64.0-82.5)	69.0 (58.0-78.5)	**0.043**	
FU1	HbA1c (%)	7.2 (6.6-7.9)	7.1 (6.3-8.3)	0.981	
	FBG (mg/dl)	156(101-192)	127(106-158)	0.562	
	BW (kg)	78.0 (60.5-86.1)	69.0 (58.0-78.5)	0.939	
FU5	HbA1c (%)	7.0 (6.75-8.0)	7.1 (6.5-7.9)	0.407	
	FBG (mg/dl)	134(105-175)	147(94-180)	0.939	
	BW (kg)	71.0 (63.3-87.3)	73.5 (64.0-93.3)	0.693	
P (sign)	BSL vs FU1	HbA1c (**p<0.001**) FBG (p=0.144) BW (p=0.112)	HbA1c(**p=0.013**) FBG (p=0.328) BW (p=0.663)	BSL = baseline FU1= one-year follow up FU5 = five-year follow up FBG = fasting blood glucose BW= body weight	
	BSL vs FU5	HbA1c (**p<0.001**) FBG (p=0.225) BW (p=0.508)	HbA1c(p=0.298) FBG (**p=0.031**) BW (p=0.753)		
	FU1 vs FU5	HbA1c (p=0.181) FBG (p=0.500) BW (p=0.990)	HbA1c p=0.213) FBG (p=0.937) BW (p=0.332)		
		Wilcoxon Test			

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Cost savings associated with CSII therapy compared to MDI in a Swedish setting

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Background and aims: The aim was to estimate the potential clinical and economic benefits of insulin pump therapy (CSII) compared to multiple daily injection (MDI) therapy in a real life setting in Sweden.
Materials and methods: The model used a Markov framework based on a recent meta-analysis where real life data from Uppsala Academic Hospital was used to define baseline cohort characteristics. A 100 patient cohort was modelled over a four year period. Key events modelled were: Severe Hypoglycemic events (SH), Diabetic Ketoacidosis (DKA) and death. Cost of treatment and the costs associated to address acute complications in the Swedish health care setting were retrieved from published literature.
Results: The total cost of CSII for the patient population (including societal cost) over 4 years was 21 365 608 SEK and the total cost of MDI treatment was 44 042 816 SEK. This suggests a total cost saving of 22 677 208 SEK when CSII is selected vs MDI. By avoiding events such as DKA and SH followed associated benefits with the mortality risk of these specific events. In the 4 year time horizon selected, 8 deaths were avoided by using CSII therapy.
Conclusion: The greater cost of the CSII treatment is offset by the reduced costs related to acute complications being avoided due to better control compared to MDI treatment.

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Efficacy and safety after 1 year of insulin pump therapy in type 2 diabetes: the OpT2mise Study continuation phase

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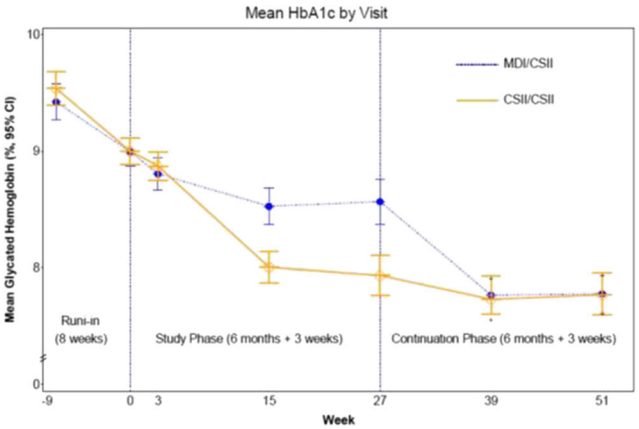
Background and aims: The impact of continuous subcutaneous insulin infusion (CSII) administered to type 2 diabetes patients failing to respond to multiple daily injections (MDI) is still debated. The OpT2mise study was a multicenter, randomized, controlled trial comparing CSII vs MDI in insulin-using patients with T2D.
Materials and methods: Subjects using MDI basal bolus regimen with rapid and slow-acting insulin analogs at a total daily dose (TDD)>0.7 U/kg/d, with persistent hyperglycemia (A1c ≥8%) after a 8-week optimization period, were randomly assigned to CSII or to continue MDI in the "study phase" (SP) of 6 months. In another 6-month "continuation phase" (CP), CSII patients continued pump therapy while MDI subjects crossed to CSII. Of 331 subjects randomized in the study phase (45.6% women, age 56.0±9.6 yr, BMI 33.4±7.3 kg/m2, diabetes duration 15.1±8.0 yr, A1c 9.0±0.8%), 291 completed the 12 months study.
Results: At 6 months, CSII subjects had achieved significantly greater A1c reduction than MDI subjects (-1.1±1.2% vs. -0.4±1.1%, $p<0.001$)- and this effect was maintained until 12 months in the CSII subjects. MDI subjects crossing to CSII after the 6-month SP showed during the CP a -0.8% A1c reduction, with a final A1c similarly dropping at 7.8% in both arms. SP response rate (A1c <8.0%) was higher in the CSII arm (CSII 55% vs. MDI 28%) but by end of the CP, both groups had achieved

similar response rates of 57%. In the overall patient population, the decrease in A1c at 12 months was independent of diabetes duration, body mass index (BMI), education level, MoCA score, and number of blood glucose self-assessment(SMBG) performed per day. Frequency of SMBG declined similarly in both groups throughout both study phases from a mean of 4.0/day to the 12-month result of 3.4/day for MDI and 3.6/day for CSII subjects. At the end of the SP, TDD was 20.4% lower in the CSII subjects compared to the MDI subjects. During the CP, MDI subjects crossing to CSII showed a 19.0% TDD reduction and finally TDD was equivalent in both groups. There was no between-groups difference in weight gain during the whole study period (CSII : +2.1±5.2 and MDI : + 2.3±4.9 kg). No ketoacidosis occurred during the whole study period in both groups. One patient in the CSII subjects and 2 in the MDI subjects experienced severe hypoglycemia during the whole study phase.

Conclusion: OPT2MISE demonstrates that CSII provides a significant advantage in glycemic control over MDI with a safe and consistent effect in long-term treatment, proving the durability of CSII impact on glucose control during a 1 year period of treatment.



Clinical Trial Registration Number: NCT01182493

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Twelve-week unsupervised day-and-night closed loop insulin delivery during free daily living in adults with type 1 diabetes: a multicentre randomised cross-over study

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Background and aims: Closed loop studies in outpatient settings have shown promising results in an up to one week day-and-night application. Prolonged day-and-night home use has not been investigated. In a 12-week open-label prospective multinational three-centre randomised crossover study, we evaluated the efficacy and safety of unsupervised day and night closed-loop insulin delivery compared to sensor augmented pump (SAP) therapy at home settings under free living conditions.

Materials and methods: Twenty-five adults with type 1 diabetes on insulin pump therapy (13 male, age 40.0±9.4 years, HbA1c 8.5±0.6%, duration of diabetes 20.9±9.3 years) underwent 5 weeks of training and optimisation on SAP, followed by two 12-week periods of SAP and day-and-night closed-loop utilising a model predictive control algorithm to direct insulin delivery overnight and between meals and prandial insulin delivery based on standard bolus wizard. The order of interventions was random with a 4 week washout between study periods. The primary outcome was time when sensor glucose were in the target range between 3.9 and 10.0 mmol/l. Analyses were by intention to treat.

Results: The proportion of time when sensor glucose was in target range between 3.9 and 10.0 mmol/l was significantly increased during closed-loop compared to SAP (P<0.001; Table). Mean glucose and time spent above target range were significantly reduced during closed loop, while time spent below target was low and comparable during both interventions. Hypoglycaemia exposure measured by AUC <3.5 mmol/l was reduced during closed loop (P=0.004). Glycaemic variability measured by standard deviation of sensor glucose was modestly reduced during closed-loop (P=0.001). Reduction in mean glucose and time spent above target range during closed loop was brought about without changing the total daily insulin delivery (P=0.44).

Conclusion: Prolonged home use of unsupervised day-and-night closed loop under free living conditions in adults with type 1 diabetes is feasible demonstrating improved glucose control and reduced exposure to hypoglycaemia without increasing total daily insulin requirements.

Table: Glucose control based on sensor glucose during day-and-night closed-loop insulin delivery and sensor augmented pump therapy over 12 weeks in 25 participants (mean ± SD, median (IQR))

	Closed-loop (n=25)	*Sensor augmented pump (n=25)*	*P^*
Time spent at glucose levels (%):			
3.9 to 10.0 mmol/l	67.4 ± 10.2	56.1 ± 15.3	<0.001
>10.0 mmol/l	29.8 ± 11.2	40.4 ± 17.4	<0.001
<3.9 mmol/l	2.7 (1.3, 4.3)	3.0 (1.1, 5.2)	0.15
Mean glucose (mmol/l)	8.7 ± 1.0	9.5 ± 1.6	<0.001
SD of glucose (mmol/l)	3.4 ± 0.6	3.6 ± 0.6	0.001
$AUC_{<3.5}$ mmol/l (mmol/l x min)	6.9 (1.8, 13.7)	11.0 (3.2, 23.0)	0.004
Total daily insulin dose (U)	48.2 (42.1, 55.2)	47.1 (41.1, 52.7)	0.44

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Psychological aspects of evening and night closed-loop insulin delivery under free living conditions: a 2 months cross-over trial

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Background and aims: Closed-loop glucose control or Artificial Pancreas (AP) research is currently at a level that allows assessment of psychological aspects and patients' perspective. In this study we aimed to assess the acceptance of long-term use of a current AP system and its impact on fear for hypoglycaemia and treatment satisfaction.

Materials and methods: In a multicentre, randomized cross-over trial, 35 adults with Type 1 Diabetes using an insulin pump were included. Sensor Augmented Pump (SAP) therapy was used day and night during the control period, while SAP was replaced by closed-loop glucose control from dinner until wake-up in the intervention period. Both intervention periods lasted 8 weeks, were preceded by a run-in and divided by 4 weeks wash-out. The AP acceptance questionnaire, Hypoglycaemia Fear Survey II (HFS-II) and Diabetes Treatment Satisfaction Questionnaire (DTSQs) were administered throughout the study and semi structured interviews were conducted after study completion in a subset of

patients (n=8). Normality of data distribution was confirmed, paired t-tests were used for analysis.

Results: Three drop-outs resulted in 32 patients available for analysis, 56.3% female, mean age 48, mean pump treatment duration 10.2 years and a total daily insulin dose of 0.54 u/kg/day. The total AP perspective score was 69.14 (SD 14.71 [95% CI 63.54, 74.73], 76.82% of 90 points max score), indicating a positive attitude towards the AP. Perceived usefulness (42.10, SD 8.73 [95% CI 38.78, 45.42], 77.97% of max score of 54) and perceived ease of use (9.62, SD 2.03 [95% CI 8.85, 10.39], 80.17% of max score of 12) were the highest scoring items in the AP questionnaire. Themes frequently mentioned by patients in the interview were 'positive effects at work', 'improved blood glucose', 'less worries about blood glucose', but also 'frequent alarms, interfering with sleep and social life', 'technological issues', mainly connectivity problems and 'demand for an all-in-one device'. No significant difference between AP and SAP was found in fear of hypoglycaemia (as reflected by the HFS-II mean total score, range 0-4, reduction 0.15 vs. 0.06, p 0.32, difference 0.09 [95% CI -0.09, 0.27]) or worries about hypoglycaemia (as reflected by the HFS-II worry subscale, range 0-4, reduction 0.19 vs. 0.12, p 0.53, difference 0.07 [95% CI -0.15, 0.28]). DTSQs results showed similar treatment satisfaction scores (range 0-36) for the AP and SAP of 28.01 (SD 7.06 [95% CI 25.40, 30.67]) and 28.17 (SD 5.19 [95% CI 26.23, 30.10]).

Conclusion: Our data indicates that current acceptance of the AP is reasonably good. Positive effects of the AP include perceived improved blood glucose and better performance at work, shadowed by frequent technological hassles impacting factors such as nocturnal usability. Patients are equally satisfied with AP and SAP use, without difference in improvement of fear for hypoglycaemia. Current AP technology is promising but diminishing the frequent hassles is needed.

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Evaluation of HbA_{1c} in subjects with type 1 diabetes with and without insulin pump transferred from paediatric to adult diabetes care through an adolescence unit between 2002 and 2014

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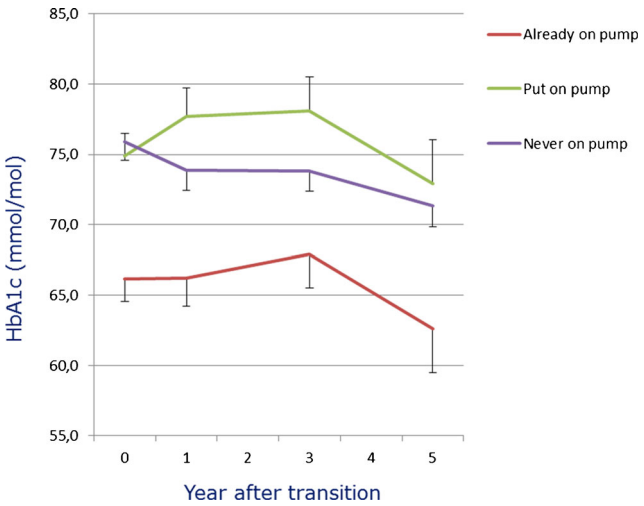
Background and aims: We wanted to evaluate the HbA1c development in adolescent patients with type 1 diabetes (T1D) transferring from a paediatric to an adolescence clinic between 2002 and 2014 with a particular focus on the use of insulin pumps; continuous subcutaneous insulin infusion (CSII). Over time we have observed a decrease in HbA1c at the service and wanted to investigate to what extent this decrease was due to the increased use of CSII in children and adolescents with T1D.

Materials and methods: We included adolescent patients transferred from paediatric departments to our adolescence clinic between 2002 and 2014; in total 480 subjects, 253 males and 227 females in the study. Average age at transfer was (mean ± SD): 18.6±1.5 and 18.8±1.6 years, and diabetes duration 6.6±4.8 and 7.9±5.0 years for males and females, respectively. We divided the cohort into three groups: CSII at transfer (C, n=96), receiving CSII during follow-up (R, n=53) and multiple daily injections (M, n=331).

Results: The HbA1c level of patients transferring from pediatric services to our adolescence center has successively and significantly decreased since 2005 up until today (e.g. from 81,1±25,5 mmol/mol in 2005 (n=53) to 69,0±18,4 in 2014 (n=54); p<0.001 for the whole study period by One Way Anova.In parallel, the use of CSII has increased from 0 to 40% of patients during the study period (p<0.0001). Baseline HbA1c was significantly lower in the C compared to the R and M groups (66.1±2.2, 74.9±2.9, and 75.9±1.2, respectively; p=0.001). Patients already on CSII were older at transfer (19.3±0.2, 18.8±0.2, and 18.5±0.1, respectively; p<0.0001), and had a significantly longer duration of diabetes (9.8±0.5, 8.4±0.7, and 6.3±0.3 years, respectively; p<0.0001). The 53 patients receiving CSII during follow-up were characterized by a significant decrease in Hb1Ac between the first and forth year after transfer (from 78,5+22.9 to 72,8+22,4 mmom/mol; p=0.04 (n=27). The HbA1c development as a function of time after transfer is illustrated in Figure 1. Over the five year follow-up period there was a non-significant decrease in the HbA1c of patients already on CSII at transfer. Among the other patients there was a slight increase in HbA1c in those subsequently put on pump. Their HbA1c levels decreased parallel to the other patients during the follow-up. Patients considered not in need of CSII either before or after transfer were characterized by a continuous decrease in HbA1c which was significant after five years (p=<0.01).

Conclusion: Adolescence patients on CSII transitioning from paediatric to adult diabetes care appear to have a lower HbA1c compared to patients on MDI. CSII users trended towards being female and having a longer duration of diabetes. Patients put on CSII post-transition also experience benefits in terms of HbA1c lowering.



PS 094 The rising costs of diabetes

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The impact of hypoglycaemia on health care use and costs in US patients with type 2 diabetes mellitus newly-initiated on basal insulin

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Background and aims: Insulin use is associated with an increase in hypoglycaemia, which may have a negative impact on insulin therapy persistence, target HbA_{1c} achievement, and health care costs. This retrospective cohort study, of US patients, assessed health care use and costs in those with type 2 diabetes mellitus (T2DM) who experience hypoglycaemia after initiating insulin.
Materials and methods: The MarketScan™ claims databases were used to identify adult patients with T2DM who initiated basal insulin therapy (defined as having no insulin prescription≥12 months before starting insulin glargine, insulin detemir, or NPH insulin) between January 2007 and March 2013. Hypoglycaemic events were identified by health care encounters with an ICD-9-CM diagnosis code of 250.8× during the first 6 months of basal insulin use. Data were assessed for 12 months prior (baseline) and 12 months after insulin initiation. Differences in baseline demographic and clinical characteristics between patients who had hypoglycaemia during the first 6 months following initiation with basal insulin therapy and those who did not were compared using t-tests for continuous variables, and Chi-squared tests for dichotomous variables. Outcomes were adjusted for differences in demographics, clinical characteristics and baseline health care utilization, and were adjusted using regression analyses. Logistic regression was used to model inpatient admissions and visits to the emergency department (ED). Health care costs were modelled with a generalized linear model.
Results: Of the 134,934 patients identified, 7,371 (5.5%) had hypoglycaemia during the first 6 months; there were significant differences in baseline characteristics between patients (Table). At 12 months, more patients with hypoglycaemia, versus those without, had hospitalizations and ED visits, and greater total and diabetes-related health care costs (Table).
Conclusion: Hypoglycaemia was seen in 5.5% of US patients with T2DM within 6 months after basal insulin initiation, and was associated with greater costs and health care resource use.

Table. Parameters of patients who initiated basal insulin.

	Hypoglycaemia in the first 6 months (n = 7,371)	No hypoglycaemia in the first 6 months (n = 127,563)	*P*-value
Age, mean (SD), years	59.9 (13.7)	57.4 (13.0)	< 0.001
Female, n (%)	3,184 (43.2)	58,630 (46.0)	< 0.001
Baseline hypoglycaemia, n (%)	2,377 (32.2)	5,046 (4.0)	< 0.001
Charlson Comorbidity Index, mean (SD)	1.36 (1.88)	0.94 (1.61)	< 0.001
Outcomes			
Any hospitalization, %	35.3	23.3	< 0.001
Any ED visit, %	51.3	32.3	< 0.001
Annual total all-cause health care costs, per patient, US $	28,253	19,459	< 0.001
Annual total diabetes-related health care costs, per patient, US $	13,458	7,668	< 0.001
Average quarterly incremental total cost, per patient, US $	7,787	5,181	< 0.001
Average quarterly incremental diabetes-related costs, per patient, US $	3,835	2,076	< 0.001
Insulin discontinuation based on a gap of ≥ 45 days in insulin prescription coverage, n (%)	5,495 (74.5)	93,529 (73.3)	0.020

Supported by: Sanofi US, Inc.

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Health economic impact of hypoglycaemia in a global population of patients with insulin-treated diabetes

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Background and aims: HAT (Hypoglycaemia Assessment Tool) was a non-interventional, multicentre, 6-month retrospective and 1-month prospective study of hypoglycaemic events in 24 countries.
Materials and methods: Questionnaires and patient diaries (28-day prospective period) were completed by adults with type 1 (T1DM) or type 2 diabetes (T2DM) and using insulin for ≥12 months (N=27,585).
Results: Patient responses to hypoglycaemia had both direct and indirect economic impact with regional variations in their extent. Direct economic impact included more frequent blood glucose self-monitoring (reported by 69.7% [T1DM] and 60.9% [T2DM] of patients), increased hospitalisation (T1DM 2.1%; T2DM 3.4% of patients) and increased medical contact in the month following hypoglycaemia (T1DM 3.8%; T2DM 6.8% of patients). Indirect economic impact included loss of productivity due to absence from work or study; 3.9% (T1DM) and 6.2% (T2DM) of patients took leave from work following hypoglycaemia. Regional differences included higher hospital admissions in Latin America vs global data (5.2 and 6.8% vs 2.1 and 3.4% of patients for T1DM and T2DM, respectively) and more clinic visits for southeast (SE) Asia vs global data (5.7 and 12.6% vs 3.8 and 6.8%). However, increased blood glucose monitoring was lower for SE Asia vs global data (53.2 and 37.5% vs 69.7 and 60.9% for T1DM and T2DM, respectively).
Conclusion: This study shows that hypoglycaemia has a significant but variable impact on the economics of diabetes healthcare globally.

Patient response to hypoglycaemia	Global	N Europe/ Canada	Eastern Europe	Latin America	Middle East	Russia	SE Asia
Type 1 diabetes Type 2 diabetes	N=8022 N=19,563	N=2388 N=3877	N=3135 N=6369	N=531 N=1660	N=1124 N=3073	N=618 N=737	N=226 N=3847
Increased blood glucose monitoring, %†	69.7	62.1	75.7	65.2	61.2	80.8	53.2
	60.9	57.2	71.9	53.5	57.3	75.6	37.5
Admitted to hospital, %†	2.1	2.1	1.2	5.2	3.6	1.3*	3.6*
	3.4	2.9	2.1	6.8	3.8	1.2*	6.2
Extra clinic visits, mean (SD) number of visits, %†‡	1.6 (1.0); 3.8	1.5 (1.1); 2.4	1.3 (0.7); 3.0	1.6 (0.9); 2.8*	1.9 (1.2); 7.9	1.5 (1.0); 0.6	2.3 (0.6); 5.7*
	1.5 (1.1); 6.8	1.4 (1.1); 3.2	1.3 (0.6); 5.1	2.9 (2.2); 5.7	1.5 (0.8); 7.0	1.4 (0.9); 12.3	1.4 (0.6); 12.6
Extra telephone contact with medical personnel, mean (SD) number of contacts, %†‡	2.0 (1.6); 8.8	1.5 (0.9); 3.6	2.0 (2.0); 9.2	2.0 (1.5); 10.7	1.8 (0.9); 11.7	2.3 (2.0); 17.1	1.8 (0.8); 8.6*
	1.7 (1.2); 16.0	1.5 (0.8); 7.3	1.6 (1.0); 14.2	1.7 (1.1); 21.4	1.9 (1.0); 21.7	2.1 (1.7); 17.3	1.9 (1.3); 22.3
Taken sick leave from work or studies, mean (SD) days; % of patients in work/study†‡	2.0 (2.3); 3.9	2.6 (4.3); 2.5	2.3 (2.2); 2.5	2.8 (2.7); 3.3*	1.7 (1.1); 10.1	2.0 (NC); 0.3*	1.4 (0.5); 25.9
	1.8 (2.1); 6.2	1.0 (0.0); 0.9*	2.6 (5.0); 2.5*	3.0 (2.5); 6.1*	1.5 (1.0); 8.2	NC; 0	1.6 (1.0); 14.6
Arrived late to work or studies, mean (SD) days; % of patients in work/study†‡	1.6 (1.1); 5.9	1.6 (1.5); 4.5	1.5 (0.9); 4.3	1.7 (1.2); 7.8*	1.8 (1.3); 11.2	1.3 (0.7); 5.8	1.1 (0.3); 19.8*
	1.4 (1.2); 6.6	1.6 (1.0); 2.7	1.2 (0.5); 2.7*	1.9 (2.8); 7.1*	1.5 (1.1); 10.3	1.3 (0.5); 4.5*	1.3 (0.7); 11.8
Left work or studies early, mean (SD) days; % patients in work/study†‡	1.5 (1.0); 4.6	1.3 (1.0); 2.3	1.5 (0.9); 4.0	1.6 (1.0); 5.3*	1.6 (1.1); 7.7	1.4 (0.8); 5.0*	1.6 (1.2); 19.0*
	1.5 (1.6); 7.1	1.0 (0.0); 0.6	1.8 (3.1); 4.9*	1.1 (0.4); 6.6*	1.6 (1.3); 8.3	1.4 (0.7); 10.0*	1.3 (0.7); 12.4

*n<20; †Data are prevalence of patient response to hypoglycaemia in the 28-day prospective period of the study; ‡Percentages are based on the number of patients with evaluable data; N, Northern; NC, not calculated; SD, standard deviation; SE, southeast

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Impact of the Framingham Offspring Study (FOS) vs Kaiser Permanente NorthWest (KPNW) prediction equations for diabetes mellitus in economic modelling of type 2 diabetes mellitus

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Background and aims: The short- and intermediate-run consequences of overweight and obesity patterns are well-known, including elevated risk of T2DM, micro- and macrovascular complications, and premature mortality. Given worrisome population weight trends in most western countries, estimation of long-term consequences is necessary to allocate health care resources for primary and secondary intervention efficiently. Because trial data of sufficient duration are seldom available, allocation of economic resources is often based on economic simulation modelling. Prediction equations for developing T2DM are a key driver of model outcomes, but the impact of choice of equation is unknown. The aim of this study is to evaluate the performance of two published risk equations in predicting outcomes in the data they were estimated with and in an external study and to compare predictions over the long horizons typical of health economic modelling.

Materials and methods: The Economic and Health Outcomes Model of Weight Management (ECHO-WM), a long-term stochastic microsimulation model, was used to estimate the conversion rate to T2DM for three cohorts of non-T2DM patients matching baseline characteristics of the Framingham Offspring Study (FOS), Kaiser Permanente Northwest (KPNW), and the Diabetes Prevention Study (DPP). ECHO-WM includes both the FOS and the KPNW T2DM equations, yielding six simulations. Key outcomes included the cumulative incidence of T2DM at 8 years for the FOS and KPNW patient cohorts and 10 years for the DPP patient cohort and 25-year cumulative incidences of T2DM and micro- and macrovascular events.

Results: The FOS equation better matched the observed FOS cumulative incidence of 5.1% (5.5% vs. 13.1%) and the KPNW risk equation better matched the observed KPNW cumulative incidence of 16.5% (16.6% vs. 7.2%). The differences were maintained at 25-years. The incidence of microvascular complications followed differences in predicted T2DM, though predicted rates of macrovascular events and life-years were similar. Quality-Adjusted Life-Years were lower with the KPNW equations. Both risk equations underestimated the ~51.8% incidence of T2DM over 10-years in the placebo arm of the enriched (impaired glucose tolerance) DPP cohort (13.8% and 28.4% incidence rates when using the FOS and KPNW equations, respectively).

Conclusion: Simulations using an economic model support the internal validity of the FOS and KPNW equations. External validity, however, was limited and the simulations lend support to concerns that recalibration of the FOS (and by extension the KPNW) equations is necessary for extrapolation to different patient populations. The FOS and KPNW risk equations provide accurate predictions of the risks in patient populations similar to that which they were estimated from, but may be inaccurate when applied to different patients. Until more accurate prediction equations are available, it is advisable to include multiple risk equations in economic models and use the most appropriate in any given application.

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Vildagliptin is cost-effective in real-world: economic evaluation evidence from EDGE study

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Background and aims: Therapeutic benefits of vildagliptin have been extensively studied and characterised in randomised controlled trials (RCTs). It is also commonly accepted that RCTs are largely unable to reproduce real-life observations. Therefore, evaluation of outcomes in real-world clinical practice would allow a full understanding of vildagliptin usefulness as an oral antidiabetes therapy alternative. EDGE (Effectiveness of Diabetes control with vildaGliptin and vildagliptin/mEtformin) was a worldwide prospective, non-interventional, 'real-life' observational study that compared the effectiveness and tolerability of vildagliptin-based dual combinations with other oral antidiabetes drugs in patients uncontrolled with monotherapy. Unlike sulphonylureas (SUs), vildagliptin was able to provide glycaemic control targets at the same levels as observed in RCTs. However, if this translates to cost-effectiveness in real-world, is yet to be established. Therefore, the objective of this study is to evaluate the cost-effectiveness of vildagliptin+metformin compared with generic SUs+metformin in patients with type 2 diabetes mellitus (T2DM) uncontrolled with metformin, from a real-life perspective.

Materials and methods: Cost-effectiveness was assessed by building a patient-level simulation model based on risk equations from the United Kingdom Prospective Diabetes Study Outcomes (UKPDS) model. The results were generated by simulating a cohort of 10,000 patients for a lifetime time horizon. The model predicted micro- and macro-vascular complications and mortality in yearly cycles. The base case compared metformin+vildagliptin with metformin+SUs. The patients could move on to metformin+basal insulin if they had inadequate glycaemic control (using a threshold of HbA1c >7.5%). Effectiveness parameters for the two strategies were derived from the EDGE study. Cost estimates were based on UK direct medical costs. Both costs and benefits were discounted at 3.5%. The annual therapy costs used were: £110.51, £410.53 and £806.84 for metformin+SUs, metformin+vildagliptin and insulin glargine+metformin, respectively. Hypoglycaemia rates and utility decrements were derived from the literature.

Results: Over the lifetime time horizon, fewer non-fatal diabetes-related adverse events (AEs) were observed in patients treated with metformin+vildagliptin compared to metformin+SUs treated patients that led to a differential AE cost of £190 (£783 versus £973). Nonetheless, addition of vildagliptin compared with SUs led to increased drug acquisition costs that were counter-balanced with reduced costs of AEs, managing morbidities, and monitoring patients. Metformin+vildagliptin yielded an incremental gain of 0.03 quality-adjusted life years (QALYs) and a mean per patient increase in total cost of £548, giving an incremental cost-effectiveness ratio (ICER) of £18,801 per QALY.

Conclusion: In clinical practice, treatment with metformin+vildagliptin resulted in lower incidence of diabetes-related AEs compared to metformin+SUs. Additionally, an increase in health benefits was also observed. Together, these results demonstrate that metformin+vildagliptin is a cost-effective treatment strategy for T2DM in real-life setting.

Supported by: Novartis

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Cost-effectiveness of metformin plus vildagliptin versus metformin plus sulphonylurea for the treatment of type 2 diabetes: a real-life perspective

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Background and aims: Metformin plus vildagliptin has been previously shown to be cost-effective when compared with metformin plus sulphonylurea (SU) in the Portuguese setting. However, the cost-effective analysis was based on data from a head-to-head randomised controlled trial (RCT) thereby, potentially limiting the external validity and generalisability. EDGE (Effectiveness of Diabetes control with vildaGliptin and vildagliptin/mEtformin) was a worldwide, prospective, non-interventional, real-life observational study that compared the effectiveness and tolerability of vildagliptin-based dual combinations with other oral antidiabetes drugs in patients with type 2 diabetes mellitus uncontrolled on monotherapy. Unlike SUs, vildagliptin was able to provide glycaemic control targets at the same levels as observed in RCTs. However, the cost-effectiveness of vildagliptin in the real-world is yet to be established. Therefore, the aim of this analysis was to determine the cost-effectiveness of metformin plus vildagliptin using the EDGE data and to compare these estimates with the previously published cost-effectiveness results based on the RCT data in the Portuguese setting.
Materials and methods: Cost-effectiveness was assessed by building a patient-level simulation model based on risk equations from the United Kingdom Prospective Diabetes Study Outcomes (UKPDS) model. The results were generated by simulating a cohort of 10,000 patients for a lifetime time horizon. The model predicted microvascular and macrovascular complications and mortality in yearly cycles. The base case compared metformin plus vildagliptin with metformin plus SU. The patients could move on to metformin plus basal insulin if they had inadequate glycaemic control (HbA1c >7.5%). Effectiveness parameters for the two strategies were derived from the EDGE study. Cost estimates were based on direct medical costs only. Costs and benefits were discounted at 5.0%. The annual therapy costs used were: €76.70 and €662.74 for metformin plus SU and metformin plus vildagliptin, respectively. Hypoglycaemia rates and utility decrements were derived from the literature.
Results: Compared with the cost-effectiveness estimates based on the RCT data, average quality adjusted life years (QALYs) increased from 5.63 to 5.73 for metformin plus vildagliptin and 5.74 to 5.77 for metformin plus SU. Average total costs decreased from €13,052 to €12,073 for metformin plus vildagliptin and from €14,310 to €12,544 for metformin plus SU. The decrease in cost for metformin plus vildagliptin was mainly driven by reduction in costs due to complications and adverse events, namely hypoglycaemia. Overall, based on the EDGE data, treatment with metformin plus vildagliptin yielded an incremental cost-effectiveness ratio (ICER) of €11,072 per QALY. This value is within the same range observed for cost-effectiveness assessment based on the RCT data.
Conclusion: This analysis confirms that the cost-effectiveness of metformin plus vildagliptin extends beyond controlled clinical trials, and continues to be cost-effective in real-life settings.
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Cost-effectiveness of second-line therapies in real-world setting: an economic evaluation of the EDGE study using patient level data

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Background and aims: The observational, non-interventional EDGE study showed that a DPP-4 inhibitor, vildagliptin, is efficacious in patients with type 2 diabetes mellitus who have suboptimal glycaemic control on metformin monotherapy in the real-world setting, confirming the results of previous randomised clinical trials (RCTs). Simultaneously the effectiveness of second-line sulphonylurea (SU)-based regimens was decreased in EDGE vs. the results attained in RCTs. Cost-effectiveness evaluations are typically pharmaco-economic modelling based on RCT data, however, there is a growing trend towards the complementary use of real-world patient level data (PLD). Consequently, we sought to perform a health economic evaluation based on data from EDGE study using an established diabetes outcomes model and PLD drawn from the cohort worldwide.
Materials and methods: The IMS Core Diabetes Model (CDM) was used to evaluate the lifetime costs and outcomes of two different regimens: metformin+vildagliptin compared to metformin+SU. Annual therapy costs applied were for metformin+SU: £106.79 (year 1), then £110.51 (year 2+) and for metformin+vildagliptin: £410.53 (year 1), then £412 (year 2+). Therapy escalation at HbA1c of 8.5% was modeled assuming insulin glargine+metformin (cost £899.51; year1, then £806.84; year 2+). Published network meta-analysis data were used to populate the CDM with hypoglycaemia rates. Multivariate regression analysis of PLD output from CDM was undertaken using R version 2.15.2. UK costs (£UK) and health benefits were discounted at 3.5%.
Results: Overall predicted life expectancy (LE) and quality-adjusted life expectancy (QALE) were 16.24 and 11.01, respectively. In multivariate analysis, adjusting for baseline characteristics, metformin+vildagliptin was associated with an increase in LE (0.8 years, $p<0.001$) and QALE (0.12 years, $p<0.001$) compared to metformin+SU. Gains in LE and QALE favouring metformin+vildagliptin were driven by an 8.6% and 6.8% reduction in the cumulative incidence of major microvascular and cardiovascular complications. Total costs were £28,512 (metformin+SU) and £27,507 (metformin+vildagliptin); this cost saving favouring metformin+vildagliptin driven by reduced complications and a longer time to insulin intensification compared to metformin+SU.
Conclusion: In the real-world setting, compared to SUs, vildagliptin was estimated to be associated with lower overall costs and additional health benefits. To our knowledge, this is the first report showing the potential of cost savings achievement with use of vildagliptin in detriment of low acquisition cost SUs. These data further highlight the potential role of real-world observational data in assessing health economic value.
Supported by: Novartis

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Healthcare utilisation and costs following initiation of insulin treatment in type 2 diabetes: a long-term follow-up in clinical practice

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Background and aims: Initiation of insulin is often chosen in type 2 diabetes mellitus (T2DM) patients when blood glucose control with other antidiabetics fail. However, long term changes in resource use and costs in primary and hospital care have not been reported before, important

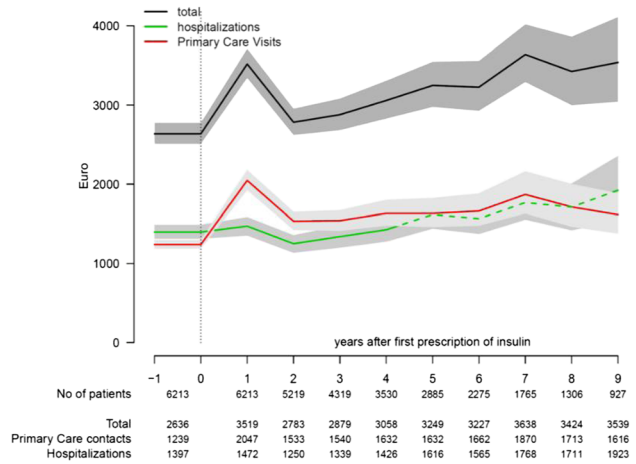
when planning type 2 diabetes health care utilization. The aim of this study was to describe how T2DM patients were initiated on insulin, and describe long-term changes in healthcare utilization and costs before and after insulin initiation.

Materials and methods: T2DM patients newly initiated on insulin were identified in primary health care records from 84 primary care centers (almost 10% of all primary care centers in Sweden), between 1999-2009. The index-date was set to date of first prescription with insulin and patients and at least a second insulin prescription within the following 15 months. Healthcare resource use data, excluding pharmaceuticals, were extracted from electronic primary care medical records in primary care and mandatory national patient register covering in- and outpatient care. The mean healthcare costs per patient are reported as annual and cumulative costs. Patients had to be on insulin treatment during follow-up and were censored when no renewed prescription of insulin was registered within one year.

Results: During a median of 3 years (maximum 9 years) follow-up; 6,213 T2DM patients (18,639 patientyears) were initiated, in combination or as monotherapy, on short-, medium-, mixed medium long and long acting insulin, 3%, 51%, 27% and 19% respectively. Patients initiated on insulin were on average 64 years, 59% men, had HbA1c 7.6%, body mass index, 29 kg/m2, blood pressure 140/80 mmHg and LDL 2.7 mmol/l. Mean per patient annual healthcare costs were € 3,519 (95% CI 3,342-3,713) the first year after insulin initiation, € 2,783 (95% CI 2621-2955) the second year, and € 3,539 (95% CI 3,042-4,111) in year 9 (2012 unit cost values), see figure. Mean per patient cumulative healthcare costs were € 29,316 (95% CI 27,833-31,106) at 9 years of follow-up. Higher costs were seen in primary care compared with hospital care, € 15,245 (95% CI 14,122-16,578) and € 14,071 (95% CI 13,226-15,043) respectively. The 2,776 patients with HbA1c >7.5% at insulin initiation had lower mean per patient healthcare costs before insulin start compared with the 2,770 patients with HbA1c ≤7.5%, € 2,325 (95% CI 2,189-2,482) and € 2,931 (95% CI 2,717-3,168) respectively.

Conclusion: Initiation of insulin in type 2 diabetes patients were followed by increased costs in both primary and hospital care and higher costs were generated in primary care. Patients with lower HbA1c at insulin start had surprisingly higher initial costs compared to those with higher HbA1c, indicating two different clinical situations when the treatment decision was made.

Figure: Annual costs of primary- and hospital care visits one year prior to insulin prescription and up-to 9 years follow-up



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Cost-effectiveness of simple insulin infusion: Sweden

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Background and aims: Continuous subcutaneous insulin infusion (CSII) in people with type 2 diabetes (T2DM) has proven to improve glycemic control (HbA1c) and reduce insulin dosage compared to multiple daily injections (MDI). However, CSII has not been widely adopted in T2DM due to costs, complexity and training requirements. New devices that provide simple CSII reduce complexity and training requirements. This analysis assessed the cost effectiveness in Sweden of simple insulin infusion (SII) compared to MDI in people with T2DM not in glycemic control.

Materials and methods: The UKPDS Outcomes Model was used to project long-term cost-effectiveness over 40 years, based on results of recently published studies and direct costs for Sweden. Costs and outcomes were discounted at 3%. Cost-effectiveness was pre-defined in relation to per capita gross domestic product (GDP) with Incremental Cost Effectiveness Ratios (ICERs) below 1X, respectively 3X GDP per capita per life year gained, defined as highly 'cost-effective', respectively 'cost-effective'.

Results: Our analysis showed discounted 0.3 life year gained on average and lifetime discounted savings on complication costs and insulin reductions of SEK 88,352. Based on projected direct costs and life expectancy, a simple CSII device will be highly cost-effective in Sweden at a price of SEK 36 per day and cost-effective at a price up to SEK 77. This implied an ICER at 1, 3 times per capita GDP per life year gained for the two cases, respectively. Cost effectiveness would be further supported if indirect costs (i.e. costs of premature mortality and sick leave) were included. These estimates were very robust to sensitivity analyses on both reductions in HbA1c and dose effects.

Conclusion: For people with T2DM not in glycemic control on MDI, simple CSII is highly cost-effective at a daily cost in Sweden of SEK 36 or less and cost-effective at a daily cost of SEK 77 or less in Sweden.

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PS 095 Gestational diabetes: pregnancy outcomes

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The relative importance of maternal body mass index and glucose levels for the prediction of large for gestational age births

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Background and aims: The risk of gestational diabetes mellitus (GDM) increases substantially with increasing maternal body mass index (BMI). GDM and maternal obesity are independently associated with adverse neonatal outcomes, in particular large for gestational age (LGA) births, which in turn increase the risk of complications among both the newborns and the mother. The aims of the present study were to evaluate the relative contribution of maternal body mass index and glucose levels on the prediction of LGA births in women giving birth in southern Sweden during 2003-2005, and to investigate whether the prediction could be further improved by adding information on maternal characteristics.
Materials and methods: Information on 10 974 pregnancies was retrieved from a population-based perinatal register. A 75-g oral glucose tolerant test (OGTT) was performed in the 28th week of pregnancy for determination of the 2-h plasma glucose concentration. Maternal BMI was calculated from weight and height measured during the first trimester. The prediction of LGA was assessed by receiver-operating characteristic (ROC) curves, with LGA defined as birth weight>+2 SD of the mean. The data set was divided into a development and a validation set. Using the development set, predictive maternal variables were identified by multiple logistic regression analysis. The predictive ability of the model was then validated in the test sample.
Results: Based on the total data set, the area under the ROC curve (AUC) of the 2-h glucose level to predict LGA was 0.54 (95% confidence interval (CI) 0.48-0.60), indicating poor performance. Factors associated with LGA in the final multiple regression analyses were BMI (per 1-step increase), 2-h glucose concentration (per 1 mmol/L increase), smoking and parity 1 (less than 2-3 reference): odds ratio (OR) (95% CI) were 1.10 (1.08-1.13), 1.09 (1.01-1.18), 0.29 (0.16-0.52) and 0.51 (0.40-0.67), respectively. Using the validation data base, the AUC for the developed model was 0.69 (95% CI 0.66-0.72), which was identical to the AUC retrieved from a model not including 2-h glucose, and larger than a model including 2-h glucose but not BMI (AUC 0.63, 95% CI 0.60-0.67).
Conclusion: Both the 2-h glucose level of the OGTT and maternal BMI had a significant impact on the risk of LGA births but the relative contribution was higher for BMI. The data support the importance of targeting healthy body weight in young women and to closer monitor weight during pregnancy as a strategy in reducing the risk of excessive fetal growth.
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Gestational weight gain is associated with obstetric and neonatal outcome in morbid obese mothers with gestational diabetes

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Background and aims: To study the effect of gestational weight gain (GWG) on pregnancy-induced hypertensive disorders (PIHD), caesarean deliveries, neonatal ponderal index (NPI) and macrosomia in morbid obese women with gestational diabetes (GDM) and to evaluate the best upper limit of weight gain in these women.
Materials and methods: Observational retrospective study in 2747 women with GDM (NDDG criteria) between 1986 and 2012. Multiple pregnancies, pregestational diabetes and secondary causes of hyperglycaemia were excluded. A number of 118 (4.3%) consecutive GDM women being morbidly obese pre-pregnancy (BMI≥35 kg/m2) were included. GWG was measured as the difference between last prenatal weight and self-reported pre-pregnancy weight. Associations between GWG and PIHD (140 and/or 90 mmHg±proteinuria ≥300 mg/24 hours at >20 weeks' gestation), caesarean deliveries, NPI (neonatal birthweight / p50 for gestational age and sex) and macrosomia (neonatal birthweight ≥4000 g) were assessed. Spearman correlations or Mann Whitney U tests were used in bivariable comparisons. The effect of GWG on the associated outcomes ($p < 0.05$) was adjusted for pre-pregnancy BMI and glycaemic control (average of monthly HbA1c) in multiple stepwise forward linear or binary logistic regression models. For each model constructed, GWG threshold values to predict an outcome were examined on the basis of sensitivity (S) and specificity (E) values under the ROC curve.
Results: Median GWG was 4.5 (IQ 1.3-8.2) (range -18 to 17.5) kg. Median pre-pregnancy BMI was 37.4 (IQ 35.8-40.4) (range 35.0 to 52.1) kg/m2. Concerning other risk factors for GDM, mean age was 32 ±5 yr, 80 (67.8%) women had family history of diabetes, 12 (10.2%) had GDM in a previous pregnancy and 15 (12.7%) previous macrosomia. Forty three (36.4%) women were nulliparous. Gestational age at GDM diagnosis was 19.8±6.0 weeks (in 48 (40.7%) cases before 24 weeks). GDM class was A1 in 71 (60.1%) and A2 in 47 (39.9%). Mean average HbA1c was 5.3±0.5%. PIHD occurred in 41 (34.7%) women and caesarean delivery in 49 (41.5%). Mean NPI was 1.03±0.17 and the rate of macrosomia was 12 (10.2%) of the newborns. GWG was 6.5 (IQ 2.7-11) in PIHD vs. 4.0 (0.9-6.6) kg in non-PIHD women ($p=0.027$). GWG ≥8 kg was very specific to predict PIHD (S=40.0%, E=90.0%). GWG was not associated with caesarean delivery. GWG and NPI were positively correlated ($r=0.227$, $p=0.017$). GWG was related to macrosomia: 7.2 (4.4-14.0) vs. 4.4 (1.0-7.5) kg ($p=0.044$). GWG ≥5.5 kg correctly classified macrosomic newborns (S=77.8%, E=61.5%) and GWG≥11 kg was highly specific (S=44.0%, E=99.0%) to detect macrosomia.
Conclusion: Morbid obese women with GDM and GWG ≥8 kg are at a high risk of PIHD. We suggest an upper GWG threshold of 5.5 kg in morbid obese women in order to reduce macrosomia.

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Triglyceride-glucose index is related to neonatal overgrowth in mothers with gestational diabetes

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Background and aims: Diabetes during pregnancy is associated with abnormalities in protein and lipid metabolism in addition to those of glucose metabolism. A positive correlation between maternal serum triglyceride (TG) level and newborn birthweight has been reported. The TyG index, the product of fasting plasma glucose (FPG) and TG has been suggested as a surrogate of insulin resistance in healthy subjects. Our objective is to determine whether maternal TyG index is associated with newborn birthweight at term.
Materials and methods: A number of 223 consecutive women diagnosed with gestational diabetes mellitus (GDM) between 2009 and 2015 were followed. Multiple pregnancies were excluded. FPG and serum TG concentrations were determined at 27±5 (1) and 34±2 (2) weeks of gestation. The TyG index was calculated as Ln[Fasting TG (mg/dL)*FPG (mg/dL)]/2. At term, neonatal weight was registered and fetal ponderal index (FPI) calculated. Infants with birthweight above the

90thpercentile were classified as LGA, based on gestational age and sex-adjusted birthweight from Spanish neonatal growth charts. Pearson's correlation coefficients between TyG index, birthweight and FPI were computed, respectively. T-test were applied to calculate TyG mean differences between LGA and non-LGA neonates. A p-value <0.100 was considered statistically significant.

Results: Women's mean age was 35.1±4.6 years. Pre-pregnancy BMI was 25.8±5.4 kg/m2. Gestational weight gain was 9.2±5.7 kg. Gestational age at diagnosis was 24.9±4.7 weeks. Mean FPG of oral glucose tolerance test and HbA1c were 90.5±13.2 mg/dl and 5.2±0.4%, respectively. FPG1 was 85.2±15.9 mg/dL, median TG1 175 (144-227) mg/dL and TyG1 4.8±0.2; FPG2 was 82.0±10.9 mg/dL, TG2 205 (150-254) mg/dL and TyG2 4.6±0.2 (p =0.000, 0.001 and 0.008, respectively). Gestational age at delivery was 38±1 weeks with a birthweight of 3126 ±504 g and a FPI 0.98 (0.92-1.06), identifying 17 (7.6%) newborns as LGA. Maternal TG1, TG2 and TyG1 levels were not correlated with birthweight and FPI and were not different in LGA newborns. TyG2 index was positively correlated with birthweight (r=0.221, p=0.023) and FPI (r=0.219, p=0.027). Mean TyG2 index was higher in LGA newborns (4.9±0.2 vs. 4.8±0.2, p=0.073).

Conclusion: The TyG index is a simple parameter of insulin resistance that can be calculated from routine laboratory data and, according to our results, it is related with birthweight. Even in well-controlled GDM pregnancies, this index at 32-36 weeks of gestation is related to neonatal overgrowth.

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Differences in characteristics and pregnancy outcomes between insulin- and diet-treated women with gestational diabetes

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Background and aims: The predictors for insulin use and the difference in pregnancy outcomes between insulin- and diet-treated women with gestational diabetes (GDM) remain unclear in different populations. Our aim was to evaluate the difference in characteristics and pregnancy outcomes between insulin- and diet-treated women with GDM in Belgian patients.

Materials and methods: Retrospective analysis of the medical files from 01-01-2010 till 31-12-2013 of women with GDM diagnosed with the Carpenter & Coustan criteria attending two large obstetrical clinics, one in a university and another in a non-university hospital. The prevalence of GDM in these centers at the time of the study was respectively 3.3% and 5.1%. Clinical and biochemical characteristics associated with insulin use were analyzed. Multivariable logistic regression was used to adjust for confounders such as age, BMI, weight gain, ethnicity and multiparity. For women attending the university hospital, indices of insulin sensitivity (the Matsuda index and the reciprocal of the homeostasis model assessment of insulin resistance, 1/HOMA-IR) and an index of beta-cell function, the Insulin Secretion-Sensitivity Index-2 (ISSI-2) were calculated.

Results: Over a 4 year period, 601 women were identified with GDM, with a mean age of 31.9±4.8 years and of whom 32.2% were overweight and 22.9% were obese at first prenatal visit. 24.2% needed insulin. Compared to diet-treated women, women on insulin more often had an ethnic minority background (33.3% vs. 21.6%, p=0.004), more often had a history of GDM (21.5% vs. 10.4%, p=0.002), were more often multiparous (59.3% vs. 47.6%, p=0.044) and were diagnosed with GDM earlier in pregnancy (weeks 25.3±4.9 vs. 27.1±3.7, p<0.0001). When undergoing an oral glucose tolerance test (100 g OGTT) women treated with insulin had a higher fasting glycaemia (97.6±18.8 vs.87.7±10.3, p< 0.0001), a higher 1 hour glycaemia (197.7±30.1 vs.184.5±25.8, p< 0.0001), a higher 2 hour glycaemia (185.2±28.5 vs. 175.0±22.8, p< 0.0001) and more often 3 and 4 abnormal values (58.1% vs. 37.8%, p< 0.0001 and 24.8% vs. 7.7%, p<0.0001), with higher HbA1c levels at the time of the OGTT (5.5±0.6 vs 5.2±0.5, p<0.0001). ISSI-2 (1.3±0.5 vs. 1.7±0.5, p<0.0001) and 1/HOMA-IR [0.01 (0.001-0.002) vs. 0.02 (0.01-0.03), p=0.027] were significantly lower in women on insulin. Women on insulin more often received corticoids in preparation of preterm delivery (11.0% vs. 2.4%, p<0.0001). Insulin did not prevent adverse outcomes, as women on insulin had higher rates of large-for-gestational age infants (LGA) (28.5% vs. 13.1%, p<0.0001) and more cesarean sections (44.1% vs. 27.0%, <0.0001), remaining significant after adjustment for confounders.

Conclusion: Compared to diet-treated women with GDM, women treated with insulin have a higher metabolic risk profile, impaired beta-cell function and lower insulin sensitivity. Rates of LGA and cesarean sections were higher in insulin-treated women.

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How to improve worse pregnancy outcomes in immigrant pregnant women such as in comparable Italian ones by insulin treatment and/or education

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Background and aims: Immigration of foreign workers and families to northern Italy increased more and more in the past three decades: at the same time a growing incidence of gestational diabetes (GDM) has been detected in our Diabetes Unit, owing to immigrant pregnant women more than to italian ones. Pregnancies have been more frequent in foreign women (29.7% while foreigners percentage is only 9.6% of the whole population) and the overall incidence of GDM in the last five years has been much higher in the immigrant (22.4%) than in the italian pregnant women (10.6%). Aim of this study was to compare maternal features and pregnancy outcsomes in 2 cohorts of immigrant and italian pregnant women with GDM, according to diagnostic criteria of IADPSG (International Workshop Conference on Gestational Diabetes) Consensus Panel (2008), enrolled from july 2010 to december 2014. Moreover we investigated whether an insulin treatment or an intensive educational program could improve the outcomes in two subgroups of immigrant women.

Materials and methods: 182 immigrant and 168 italian pregnant women were compared for age, body mass index (BMI) before pregnancy, gestational age at diagnosis of GDM, HbA1c at diagnosis and before delivery, need of insulin treatment, weight gain, timing and mode of delivery and, as fetal outcomes, birth weight and number of LGA (Large for Gestational Age) infants. The immigrant pregnant women were a multiethnic population, to which an iso- or slightly hypocaloric diet was prescribed and training to self blood monitoring performed. Eventually two subgroups of immigrant pregnant women, 48 and 51 respectively, were randomly allocated to insulin treatment, analogue aspart in three doses before meals, or to an intensive educational program for six weeks, HbA1c and weight gain being evaluated.

Results: Immigrant and italian pregnant women were well-matched for age (33.2+/-5.8 vs 39.2+/-4.8 years, p=ns) and pre-pregnancy BMI (28.6+/-5.5 vs 27.7+/-6.4 kg/sq.m, p=ns). No difference was found in HbA1c at diagnosis, need of insulin treatment (21.4 vs 19.7%, p=ns) and mode of delivery: cesarean section 44.9 vs 43.8%, p=ns (currently performed in 28.3% of pregnancies in our country). The immigrant women had a more advanced gestational age at diagnosis (27.5+/-5.1 vs 24.3+/-4.8 weeks, p<0.01) but an earlier one at delivery (38.3+/-1.6 vs 39.5+/-

1.7 weeks, p<0.05), a higher pre-delivery HbA1c (5.8+/-0.4 vs 5.2+/-0.4%, p=0.03) and a greater weight gain (12.14+/-3.87 vs 8.57+/-3.68 kg, p<0.01). Birth weight (3676+/-652 vs 3423+/-455 g, p<0.01) and rate of LGA infants (8.1 vs 12.3%, p<0.05) were higher in newborns of immigrant women. The outcomes of two subgroups on insulin or intensive education did not show any significant difference after six weeks: a comparable decrease of HbA1c (-1.1+/-0.3 vs -0.9+/-0.2%, p=ns)) and a similar weight gain (2.1+/-0.4 vs 2.0+/-0.5 kg, p=ns).
Conclusion: The immigrant pregnant women and the comparable italian ones had similar pre-pregnancy features but different outcomes, likely due to socio-economic factors and cultural barriers, in spite of the same given access to our health care system. In the two random subgroups on insulin therapy or intensive education, we surprisingly found the two treatments being both and likewise effective.

1003

Gestational diabetes mellitus after gastric bypass surgery
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Background and aims: The number of obese women in childbearing age is increasing and the most common and effective weight loss treatment is gastric bypass (GBP). Overweight is a risk for developing Gestational Diabetes Mellitus (GDM) and therefore all pregnant women who have undergone GBP are screened for GDM in our region. The aim of this study was to compare eventual differences in demographics and pregnancy outcome among women who had undergone a GBP and developed GDM during pregnancy against those who did not develop GDM.
Materials and methods: Since 2012 all women in our region who have undergone GBP are screened for GDM with a three day plasma glucose profile. The regular oral glucose tolerance test is not performed because of the risk for dumping symptoms. During 2012-2014 there were 73 pregnant women who had undergone a GBP earlier and not developed GDM during pregnancy and these were compared against 11 women who had undergone a GBP and also developed GDM.
Results: When comparing pregnant women who had undergone GBP against the other pregnant women who had undergone GBP and also developed GDM, there was no significant difference between the groups regarding age of the mother, ethnicity, family history of diabetes, smoking habits, education level, years after GBP surgery, first weight/height/BMI of the mother during pregnancy, the mother's weight gain during pregnancy and weight at delivery, gestational length, birth weight/length of the child, Apgar score at 1, 5 and 10 minutes of the child, weight of placenta and number of caesarian deliveries.
Conclusion: No characteristic pattern was found for those who developed GDM during pregnancy after GBP surgery. Due to the small number of observations it is important to continue screening all pregnant women who have undergone GBP for GDM.

PS 096 Gestational diabetes: predictors and consequences

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Gestational diabetes: metabolic risks of adult women in respect to birth weight
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Background and aims: Metabolic disorders such as obesity, insulin resistance and other components of metabolic syndrome (MetS) are associated with birth weight (BW). Low BW is associated with higher risk of developing type 2 diabetes mellitus, the mechanism is not clear. In this study, we evaluated the association between BW and anthropometric as well as biochemical components of MetS in women with a history of special diabetic condition - gestational diabetes mellitus (GDM). In part of the tested women, we evaluated metabolic changes over the years.
Materials and methods: All 456 women involved in the study gave birth at least once. Depending on the course of pregnancy, they were divided into gestational diabetics (GDM, n=376) and controls (C, n=80). Anthropometric characteristics of BMI, WHR, waist, hips, and abdomen circumferences were evaluated. Glucose metabolism was defined by basal glucose, insulin, proinsulin, C-peptide and glucagon. These parameters were also monitored 7 times during the 3-hour oGTT. Lipid profile included total cholesterol, HDL-CH, LDL-CH, TG and FFA. Furthermore, blood pressure, uric acid, thyroid hormones, and liver enzymes were assessed. 150 women from this cohort (100 GDM and 50 C) were completely re-examined after at least 5 years. Statistical analysis: Mann-Whitney test, Repeated Measures ANOVA (NCSS 2004).
Results: From all the components of MetS tested in the study, BW was systematically associated solely with glucose metabolism: In GDM group, low BW (below 25th percentile) was associated with higher postchallenge insulinemia during oGTT (AUC I7 p=0.002), C-peptide levels (AUC C-pep7 p=0.0001), and with lower indices of insulin sensitivity (Matsuda p=0.02, Cederholm p=0.002) compared to medium (25th-75th percentile) and high (above 75th percentile) BW categories. Re-examinations in the GDM group indicate this association lasts over the years. Repeated measures ANOVA confirms BW as a significant factor for glucose processing. In the group of controls only borderline significance between low BW and higher postchallenge glucose levels (AUC Glc7 p=0.04) was found.
Conclusion: From the evaluated biochemical and anthropometric components of MetS in adult women we proved the association of low BW with glucose processing, in particular among women with a history of GDM. Low BW GDM women reveal significantly higher postchallenge insulin secretion and, accordingly, lower peripheral insulin sensitivity. This association persists long after delivery.
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1005

Hypovitaminosis D in 1st trimester may predict gestational diabetes
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Background and aims: Pregnancy is nature's gift that should be nurtured over nine months with no adverse outcome. Gestational Diabetes Mellitus affects 3 to 10% of pregnancies, depending on the population studied. The progressive increase of insulin resistance observed in pregnancy

contributes to the pathophysiology of gestational diabetes mellitus (GDM). There is controversy whether vitaminD deficiency contributes to abnormal glycemic regulation in pregnancy

Materials and methods: We tested the associations between first trimester 25-hydroxyvitamin D3(25OHD3) levels and the risk of developing GDM in a large hospital-based prospective cohort of pregnant women. Participants (n=200) were seen at first (6-13 weeks) and second (24-28 weeks) trimesters for blood samples. Each participant was selected in their first trimester and demographic parameters were recorded. Height in cms, weight was measured in kg's. BMI was calculated as weight (kg)/ height (m2). Family history of Diabetes, Obstetric score for gravidity, history of previous abortion and previous history of GDM was recorded. Fasting blood sugar was measured and in those >92 mg/dl HbA1c is done and those with >6.5% were excluded. Those with FBS value of 140 mg/ dl, Oral Glucose tolerance test (OGTT) was done and diagnosed as GDM or NGT based on ACOG criteria.

Results: Based on ACOG criteria, 18 participants (9%) developed GDM. 25(OH) Vitamin D level was deficient in 190 participants (95%) and sub group analysis revealed 25(OH) Vitamin D3 level of <20 nmol/l in 48% of participants. On logistic regression analysis for GDM risk factors, Advancing age, Increased BMI and 25(OH) Vitamin D3 level of <20 nmol/l were significantly associated with risk of GDM. 25(OH) Vitamin D3 level <20 nmol/l was found to be significantly associated with increased risk of GDM however our study found this risk was evident only in advanced maternal age (29.07±1.68).

Conclusion: Severe Hypovitaminosis D was present in 48% of study participants. Pregnant females with very low vitamin D (<20 nmol/l) are at odds of 2.45 in relation to increased risk of development of Gestational Diabetes mellitus. In our study we found that advanced maternal age in parallel with simultaneous very low vitamin D level may contribute to insulin resistance and increased risk of GDM.

Table 1: Family history of Diabetes, Gravidity and history of abortion in relation to development of GDM among those with 25(OH) Vitamin D3 level<20nmol/l.

Study variable		GDM		Chi square value	p
		Yes N(%)	No N(%)		
Family History of diabetes mellitus	Yes	25 (73.5%)	9 (26.5%)	5.972	0.015*
	No	57 (91.9%)	5 (8.1%)		
Gravidity	Primigravida	30 (90.9%)	3 (9.1%)	1.128	0.27
	Multigravida	52 (82.5%)	11 (17.5%)		
History of abortion among multigravida (n=113)	Yes	12 (70.6%)	5 (29.4%)	2.308	0.12*
	No	40 (87.0%)	6 (13%)		

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Maternal stress predicts insulin resistance in pregnancy

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Background and aims: Endometrial environment affects maternal metabolism and fetal growth during pregnancy.It is not known yet to what extent maternal stress could affect maternal glucose metabolism and fetal growth. Aim was to investigate the association of maternal stress and fasting stress hormones (CRH and cortisol) with insulin resistance and fetal growth.

Materials and methods: The investigation was approved by the local ethical committee and it has been performed in accordance with the ethical standards laid down in the Helsinki Declaration. Consent has been obtained from each patient after full explanation of the purpose and nature of all procedures used. Eighty five pregnant, with no history of diabetes mellitus, primigravidae Caucasian women (mean ± SD); age: 27.7± 3.6 years; pre-pregnancy BMI 26±2.1 kg/m^2 were recruited during the second trimester of pregnancy from an Obstetrics and Gynecology outpatient clinic of a university hospital.Women were seen randomly in the clinic at the second and third trimester and had their weight measured, a 75gr OGTT with insulin and glucose measured, HOMAR values were estimated and fasting (9:00 am) serum CRH, cortisol and IL6 were measured. Three women were diagnosed with gestational diabetes and were withdrawn from the study. During the visits they had fetal ultrasound (US) measurements of abdominal and head circumference. A specialist clinical psychiatrist interviewed them with STAI state and trait stress questionnaires and scored them.The STAI state questionnaire is composed of 20 questions about how the individual feels at that particular period. The STAI trait questionnaire is composed of 20 questions about how the person usually feels.A p-value of <0.05 was considered to be statistically significant.

Results: In the second trimester maternal cortisol (r=0.53), CRH (r= 0.35) and STAI state score (r=0.82) were positively associated with HOMAR (p<0.05). Best predictor of HOMAR was STAI state score (p=0.01, beta=0.88) among STAI trait score, CRH, IL6 and cortisol levels and maternal weight. Best predictor of US fetal abdominal circumference was serum cortisol (p=0.028, beta=0.638) among STAI state and trait scores, CRH and IL6 levels and maternal weight. In the third trimester cortisol (r=0.65) and STAI state score (r=0.35) were asssociated with HOMAR and the latter was its best predictor (p=0.013, beta=0.83) among STAI trait score, maternal weight and CRH, IL6, cortisol levels.

Conclusion: Maternal stress, as assessed by STAI state score which shows how the woman feels at that particular period studied, seems to affect insulin resistance during pregnancy. The stress hormone cortisol is associated with fetal abdominal circumference. Stress assessment by STAI state score at initial stages of pregnancy could be a potential risk factor tool of insulin resistance and possibly fetal growth in the outpatient clinic.

1007

Selective screening for gestational diabetes development based on risk factors

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Background and aims: Gestational diabetes (GDM) development is one of the most common complications during pregnancy. It is speculated that OGTT should be performed only on women with high risk for GDM. The aim of the present study was to assess the predictive value of risk factors for GDM established by selective screening and to identify subgroups of women at a higher risk of developing GDM.

Materials and methods: 1300 pregnant women between 24 and 28 weeks of gestation were recruited for the purposes of the study. They have undergone standard OGTT as part of screening program for GDM in Clinical Center of Endocrinology. For GDM diagnosis we have use the IADPSG criteria and logistic regression analysis for identifying significant risk factors.

Results: We found 29.1% (378) prevalence of GDM. The application of the selective screening criteria would result in the execution of an oral glucose tolerance test (OGTT) in 55.1% of women and 24 (24.0%) cases

of GDM would not be detected due to the absence of any risk factors. The presence of relatives with diabetes type 2 increase 1,394 (p<0,034) risk for GDM development. Furthemore, the presence of I and II line relatives with diabetes type 2 additionally increase the risk (OR 2.523 (p<0.001). Body mass index (BMI) between 25 and 29.9 kg/m2 before pregnancy increases the risk 1.629 (p30kg/m2 4,162 (p<0.0001). Women above 30 years of age have significantly higher risk (OR 2.245, p0.371). Other significant risk factors were higher random blood sugar (OR 3,883 (p< 0.0001) and the need of assisted reproduction as method for conception (OR 2.123, p<0.001).

Conclusion: The lack of established national screening program necessitates the active search for pregnant women at high risk for GDM development. Identifying major risk factors will categorize the women, who will benefit from performing OGTT and thus how reducing their risks, if having GDM.

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1008

The growth patterns in children born to mothers with gestational diabetes mellitus

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Background and aims: The aim of the study was to assess if gestational diabetes mellitus (GDM) influences the pregnancy outcomes and the growth patterns in children.

Materials and methods: Our prospective study cohort consisted of 261 children born to mothers with GDM and 153 control children born to non-diabetic mothers. In both groups children's birth weight, and length were assessed. Children were matched for large for gestational age (LGA) (birth weight >90th percentile for gestational age), appropriate for gestational age (AGA) (birth weight between the 10th and 90th percentile for gestational age), small for gestational age (SGA) (birth weight <10th percentile for gestational age) status. Children anthropometric measurements were made at 12, 18, 24, and 30 months after their birth. BMI was calculated. The overweight was diagnosed at BMI between 90-96 percentile, obesity between 97-100 percentile. The WHO child growth standards were implemented. The bioethics committee agreement was obtained.

Results: Among 261 children born to mothers with GDM, 80.5% were AGA, 10% SGA, 9.6% LGA; in control group - 75.2% AGA, 5.9% SGA, and 19% LGA. Birth weight of the children in GDM pregnancies was 3.30±0.53 kg comparing to the control children 3.42±0.54 kg, p=0.01. No weight and BMI differences were found between groups in the 12th month of life. The weight >90 percentile at this period was observed in 37.7% GDM children, comparing to 32.4% in control group. BMI between 90-96 percentile was found in 15.63% and between 97-100 percentile in 15.63% GDM children comparing to 18.6% and 18.6% respectively in control group. At the age of 18 months the weight of children born to GDM mothers was >90 percentile in 54.8% comparing to the control group in 29%, p=0.04; No changes were found in BMI. At the age of 24 and 30 months we found no differences in BMI between both groups.

Conclusion: We found that children who were LGA at birth were less often born to GDM mothers after implementation of dietary regiment comparing to general population. GDM did not influence the growth pattern in first 36 months of live.

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1009

A randomised trial of the effects of prenatal education of overweight or obese pregnant women to prevent childhood overweight: the ETOIG study

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Background and aims: We aimed to evaluate whether pre and perinatal education of overweight or obese pregnant women would reduce childhood overweight.

Materials and methods: Four French centers included before 20 weeks of gestation 268 pregnant women who were overweighed or obese before pregnancy (BMI 32.5±5.4 kg/m2, obesity 62%, age 30.4±5.0 years). They were randomized in a control group (n=136: routine care including at least one dietary visit) and an interventional group (n=132). This intervention which included 2 individual and 4 collective dietary counseling at 18, 26, 33 weeks of gestation and 2 months after delivery aimed to educate the future mother for infant and maternal nutritional aspects, without weight objectives. The primary objective was postnatal catch-up growth from birth to two years (>0.67 DS), which is associated with obesity in childhood.

Results: Events during pregnancy were similar in both groups, including incident gestational diabetes mellitus, maternal gestational weight gain and birth weight. The rate of postnatal catch-up growth was similar in interventional and control groups: in intention to treat (59.1 vs 60.3% respectively, p=0.84), in available data (AD n=206) and in per-protocol population (PP n=177). Disappearance of maternal overweight 2 years after delivery was more frequent in the interventional group (AD, n=149: 12.9 vs 3.8%, p=0.04; PP, n=128: 10.7 vs 4.2%, p=0.18). The children were less likely with BMI>19 kg/m^2 (corresponding to the 97eme percentile) when they were 2 years old (AD, n=204: 0 vs 6.8%, p=0.014; PP, n=176: 0 vs 6.4%, p=0.03).

Conclusion: Intervention based on education and nutritional counseling in women with overweight / obesity, starting at more than three months of gestation, has no effect on postnatal catch-up growth but appears to prevent overweight in mothers and obesity in children two years after delivery.

Clinical Trial Registration Number: NCT00804765

1010

Should HbA_{1c} be used for the diagnosis of glucose tolerance disorders in women with a history of gestational diabetes mellitus?

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Background and aims: Women with a history of Gestational Diabetes Mellitus (GDM) present an increased risk for Diabetes Mellitus 2 (DM2). Thus it is recommended to perform an oral glucose tolerance test (OGTT) after delivery. Recently use of A1C has been proposed as a simpler and faster method to diagnose glucose tolerance disorders. The aim of this study is to investigate whether A1C measurement can replace OGTT in the detection of pre-diabetes and diabetes in women with a history of GDM.

Materials and methods: 1336 women with a history of GDM after delivery were studied. All women had been evaluated through an OGTT and a simultaneous A1C measurement. ADA criteria were used for the assessment of dysglycemia. Sensitivity and specificity of A1C were measured for the prediction of diabetes and pre-diabetes; Cohen's coefficient of agreement (k) was also calculated. ROC analysis was performed to

evaluate the sensitivity and specificity of A1C for detection of abnormal glucose tolerance.

Results: Based on OGTT, 725 (54.3%) women were normal, Impaired Fasting Glucose (IFG)=406(30.4%), Impaired Glucose Tolerance (IGT)=48(3.6%), IFG+IGT=74 (5.5%) and 83 women presented with DM2 (6.2%). On the contrary, using A1C as a criterion of dysglycemia 1150 (94.1%) women were normal, while 49 (4.0%) had an impaired A1C and 23 (1.9%) were diagnosed as DM2. Sensitivity of A1C for the diagnosis of pre-diabetes was 5.3% in comparison to OGTT, specificity was 99.2%, while for the diagnosis of DM2 the percentages were 29.6% and 100% respectively. The consistency in classifying abnormal glucose tolerance between A1C and OGTT was 59.7% (Cohen's coefficient of agreement k=0.116). Performing a ROC curve, the optimal value of discriminating ability of A1C was 5% for the prediction of pre-diabetes and diabetes.

Conclusion: The study showed that A1C measurement has a very low sensitivity in detecting pre-diabetes and diabetes in women with a history of GDM. Hence, we suggest that the ADA proposed thresholds cannot be used as an alternative to OGTT in this regard.

1011

A model for individual prediction of diabetes up to five years after gestational diabetes mellitus

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Background and aims: Gestational diabetes mellitus (GDM) is a major risk factor for subsequent diabetes. A cumulative diabetes incidence of 30-50% within five to ten years after GDM has been described. In southern Sweden universal screening with a 75-g OGTT during pregnancy has been applied since 1995. Using the WHO criteria from 1999 to define GDM we have previously reported a diabetes incidence of 6% 1-2 years after delivery. The aim of the present study was to identify risk factors associated with diabetes development based on a 5-year follow-up of these women and to develop a prediction model to be used on an individual basis when counselling women after GDM.

Materials and methods: Five years after GDM a 75-g OGTT was performed in 362 women, excluding women already diagnosed with diabetes at the 1-2 year follow-up visit or later (n=45). All but 21 women had results from follow-up at 1-2 years, while 84 women were lost from that point. Predictive variables were identified by multiple logistic regression analysis. The performance of the developed model was assessed in receiver operating characteristic (ROC) curves.

Results: Five years after GDM, 28/362 (8%) women were diagnosed with diabetes whereas 187/362 (52%) had normal glucose tolerance (NGT). Among the latter, 139/187 (74%) also had NGT at 1-2 year follow-up. In univariate regression analysis, using NGT at 1-2 and 5 years as the reference, diabetes at 1-2 year follow-up or later (n=73) was clearly associated with easily assessable clinical variables, such as BMI at 1-2 year follow-up, 2-h OGTT glucose concentration during pregnancy and non-European ethnicity ($p<0.001$). A prediction model based on these variables, resulting in 86% correct classifications with an area under the ROC curve of 0.91 (95% CI 0.86-0.95), was applied in a function sheet line diagram illustrating the individual effect of weight on diabetes risk.

Conclusion: The results highlight the importance of BMI as a potentially modifiable risk factor of diabetes after GDM. For motivational purposes, a patient information function sheet illustrating the effects of weight versus diabetes risk is proposed as a model to be used on an individual basis, see Figure. As an example, a European woman with a height of 1.75 m, a 2-h OGTT plasma glucose concentration in pregnancy of 9.8 mmol/l and a weight of 90 kg one year after pregnancy would have a 60% risk of diabetes, declining to 20% with a weight loss of 20 kg.

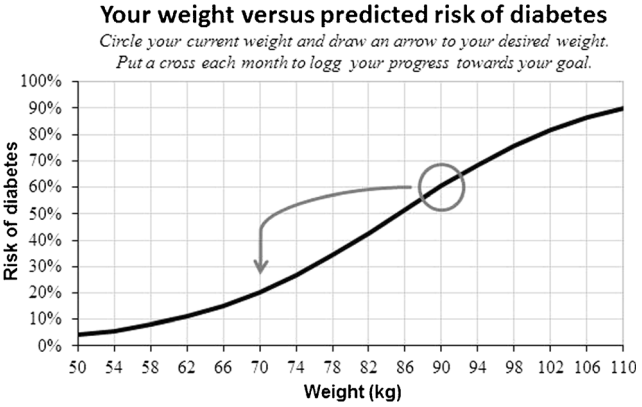

Figure. The prediction model applied in a function sheet line diagram of individual risk of diabetes up to five years after gestational diabetes versus weight. The individual example is depicted in the conclusion of the abstract. To confirm understanding and enforce active engagement by the woman, an appeal of interaction with the diagram is added.

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PS 097 Pathophysiology of gestational diabetes

1012

Metabolic changes during oral glucose tolerance test in healthy pregnancy and gestational diabetes mellitus revealed by LC-MS-based metabolomics

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Background and aims: Gestational diabetes mellitus (GDM) is manifested by changes in such metabolites as lysophospholipids, bile acids, fatty acids and amino acids. However, its pathogenesis and mechanisms responsible for complications caused by GDM are still not entirely clear. In order to evaluate which metabolic pathways are affected by glucose load metabolomics was applied. Serum samples obtained during oral glucose tolerance test (OGTT) were fingerprinted by LC-QTOF-MS. Among different tools used in metabolomics, LC-MS is characterized by the highest sensitivity and dynamic range therefore this method was chosen to perform the experiment. The aim of the present study was to examine serum metabolic changes in response to glucose load in patients with GDM and healthy pregnant women.
Materials and methods: The study group contained 129 pregnant women who underwent a 75 g-OGTT between 24-28 weeks of gestation. Fasting, 1 h and 2 h serum samples were collected from each participant during OGTT. Patients were divided into two groups: primary set (n=67) and validation set (62), both consisted of GDM and healthy pregnant women (control). Serum samples were fingerprinted using LC-QTOF-MS. Data was collected in ESI (+) and ESI (-) mode (50-1,000 m/z). Prior to statistical analysis, quality assurance protocol was applied to keep only metabolic features with good repeatability. Statistical analysis was performed independently in both sets and afterward only statistically significant metabolites (p-value <0.05), common in both sets, were selected for MS/MS analysis to confirm their identification. Depending on the data distribution, paired t-test or Wilcoxon signed rank test were used and obtained p-values were corrected by application of Benjamini-Hochberg false discovery rate test.
Results: Around 140 metabolic features changed significantly after glucose load. The differences concerning about a hundred of them were observed irrespective of the presence of GDM, while other changes were significant only in the control group or patients with GDM. The list of metabolites that differed significantly at 2 h of OGTT in comparison to the fasting state in both groups included dicyclohexylurea (+85%, p<10-7) and several fatty acids (e.g. eicosadienoic +120%, p<10-4; stearolic -48%, p<10-4; oleic -55%, p<10-8). A significant decrease (14-21%, p< 0.03) in phosphatidylethanolamine and sphingomyelin at 2 h of OGTT was observed only in patients with GDM. On the other hand, in the GDM group we did not observe any significant changes in several metabolites, which increased markedly at 2 h of OGTT in the control group (e.g. lysophospholipids [12-20%, p<0.01] and choline [58%, p<0.01]).
Conclusion: Our results suggest that glucose load causes mainly the derangement of lipid metabolism. The changes in fatty acids were observed irrespective of the presence of GDM, whereas choline containing lipids and choline itself were affected in a different way in control and GDM samples. However, further investigations are needed to evaluate the role of observed changes in the metabolism of choline and choline containing lipids in the development of GDM and its complications.
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Testing for genetic associations in gestational diabetes mellitus (GDM): preliminary results from the EFSD New Horizons study

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Background and aims: To identify maternal single nucleotide gene polymorphisms (SNPs) associated with GDM or influencing fasting and 2 hours glucose levels at OGTT.
Materials and methods: A set of 77 SNPs was selected based on their previously reported contribution to beta-cell dysfunction or genesis, incretin effect, K+-channel function, amyloid formation, zinc transport, insulin resistance, obesity, IGF system, glucose homeostasis, neuronal regulation of appetite, energy balance or immunoregulation. The majority of these SNPs were previously reported to be associated with either type 2 diabetes or with a metabolic trait. Thus far 149 pregnant women with GDM, 269 non-diabetic pregnant women and 154 neonates born to mothers with GDM and 274 neonates born to non-diabetic mothers were enrolled. This study was conducted in two Hungarian and one Austrian centers. Genomic DNA was isolated from a total of 846 samples using a magnetic bead based robotized approach (Hamilton Robotics, Magna Starlet). PCR-based KASP™ genotyping (FRET based) assay (LGC Genomics) was used for the bi-allelic discrimination of SNPs. Logistic regression (logit) risk model and subsequent algorithms written in "R" statistical programming language were used to assess Odds Ratios, p-values and to detect potential gene-gene interactions. Maternal gene variants were also assessed for association with fasting and 2 hours plasma glucose levels at routine OGTT (24-28th gestational week).
Results: Carrying a G allele of the rs10830963 of the melatonin receptor 1B (MNTR1B) or a G allele of the rs6832769 of the clock circadian regulator (CLOCK) genes were most significantly associated with the binary trait of GDM (OR=1.708-1.56, p<10-5). In qualitative trait analysis we found that the rs10830963 in MTNR1B was consistently associated with increased fasting and 2 hours glucose levels. The rs10871777 (G allele) of the melanocortin 4 receptor (MC4R) was associated with an increase in 2 hours plasma glucose values at OGTT. In contrast, the A allele of the rs1143634 of the interleukin 1 beta (IL1B) gene was associated with decreased fasting glucose levels. We identified significant gene-gene interactions between MC4R and CLOCK gene variants (rs1087177-rs6832769 and rs17782313-rs6892769 with OR=2.09, p=1.4×10-3 and OR=2.11, p=1.1×10-3, respectively) and between rs10830963 of MNTR1B and rs4712526 of the CDK5 regulatory subunit associated protein 1-like 1 (CDKAL1) gene (OR=2.17, p=1.7×10-3) when interactions were analyzed for GDM binary trait.
Conclusion: Our preliminary data suggest that a few SNPs could be associated with GDM or qualitative traits. Surprisingly, the identified gene polymorphisms are likely to participate in neuronal regulation of appetite, circadian rhythm, energy balance, immunoregulation or are with unknown function.
Supported by: EFSD

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The role of hepassocin in gestational diabetes mellitus

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Background and aims: Gestational diabetes mellitus (GDM) is hyperglycemia that is first detected during pregnancy and does not meet the criteria for manifest diabetes. GDM is associated with the development of preeclampsia, the birth of a big baby, emergency cesarean section, birth trauma, and neonatal hypoglycemia. In addition, it is a risk factor for obesity, type 2 diabetes and cardiovascular diseases in the mother and her offspring. However, there is no suitable biomarker for the screening of GDM that leads to the diagnosis of this condition is delayed. Recently, a novel physiological function of hepassocin was found that increased hepassocin levels activated ERK1/2 to induce insulin resistance; however, the role of hepassocin in GDM is still unknown. Thus, the aim of this study is to investigate the role of hepassocin in GDM.
Materials and methods: Fifteen subjects for each group including normal control and GDM groups were enrolled. GDM was diagnosed by 75-g oral glucose challenge test screening at 24-28 weeks of gestation. The plasma samples were collected to examine hepassocin concentrations by enzyme-linked immunosorbent assay. In addition, the placenta was also collected for the expression of hepassocin by reverse transcriptase-polymerase chain reaction, and western blots, as well as immunoflurorescent staining. 3A-sub-E trophoblasts were used to evaluate the physiological effect of hepassocin in placenta. The effect of hepassocin on insulin-induced glucose uptake was determined by L6 myoblasts.
Results: The expression of hepassocin was detected in the placenta, and the placental expression level of hepassocin was increased in GDM as compared with control group. The result from immunoflurorescent staining indicated that hepassocin was mainly expressed in the trophoblasts of placenta. In addition, interleukin-6 treatment induced the expression of hepassocin in 3A-sub-E trophoblasts. The serum concentrations of hepassocin were significantly increased in patients with GDM (N=15 for each group) ($P<0.01$). Treatment with hepassocin in trophoblasts significantly increased the migration of trophoblast through an ERK1/2-dependent pathway. In addition, hepassocin disrupted insulin signaling to decrease insulin-induced glucose uptake in L6 myoblasts.
Conclusion: Inflammation in the placenta of GDM increased the expression of hepassocin and the elevated hepassocin in circulation further led to the insulin resistance in skeletal muscle, and affected glucose utility. In addition, hepassocin increased the migration of trophoblasts and this effect might be correlated with the increased weight of placenta in GDM. Thus, we provided a novel mechanism in the development of GDM by hepassocin.

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Oxidative stress and gestational diabetes: the impact on placenta, perinatal outcomes and postpartum evolution

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Background and aims: The simultaneous existence of a placental oxidative stress and maternal-placental interactions during Gestational Diabetes Mellitus [GDM] are still not well understood. The aim of the study was to evaluate the levels of oxidative stress markers and antioxidants in women con GDM, and to assess the impact on the placenta oxidative status and perinatal outcomes.
Materials and methods: One hundred twenty six pregnant women (63 with GDM, 63 controls) were enrolled and 41 cases and 21 controls were recruited for the following-up study. Markers oxidative stress and antioxidants status were measured during 24th and 29th week of gestation and twelve months after delivery; moreover, 6 placenta from healthy pregnant women and 6 from women with GDM were studied.
Results: In the postpartum period, we found significant increases of levels of Lipoperoxides [LPO] ($p=<0,001$) and catalase ($p=<0,001$) and significant declines of levels of glutathione peroxidase [GPX] ($p=0,003$) in both (GDM and controls). In addition, in cases had lower levels of glutathione transferase [GST] ($p=0,003$) and in controls had lower levels of superoxide dismutase [SOD] ($p=<0,001$), respectively. In order to investigate the influence of clinical and metabolic variables on postpartum period, multiple regression analysis was performed and the levels of oxidative stress and antioxidant markers postpartum were significantly associated with the GDM. We observed that the levels of GPX ($p=0.012$) and LPO ($p=0.04$) were significantly related to the percentage of Caesarean deliveries, while the percentage of macrosomia was related to the levels of GPX ($p=0.01$). In GDM placenta a significant increase was noted in the levels of carbonyl protein ($p=<0,05$), LPO ($p<0,05$) and reduced antioxidants enzymatic activities of SOD ($p=<0,05$) and catalase ($p=<0,05$) compared to controls.
Conclusion: We concluded that increased oxidative stress and reduction in antioxidant defense mechanisms appeared in the mother and GDM placenta. These maternal and placental oxidant/ antioxidant imbalances could lead to maternal and fetal complications and should be carefully considered.

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Pregnancy induces an insulin resistance promotin hepatic ceramide phenotype in mice

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Background and aims: Temporary insulin resistance (IR) develops in women during pregnancy, in order to ensure appropriate supply of nutrients to the growing fetus. This physiological adaptation can develop into a pathological insulin resistance and overt gestational-diabetes mellitus (GDM). Understanding the mechanism behind the normal pregnancy-induced IR is an important tool to elucidate key molecular mechanisms leading to GDM. Altered ceramide-metabolism has been implicated in the development of type-2 diabetes mellitus (T2DM). In particular ceramide species produced by ceramide synthase 6 (CS6) have been identified as potential inducers of hepatic IR. We therefore hypothesise that pregnancy induced IR is caused by altered ceramide metabolism, with a higher flux through CS6 and increased production of ceramide species with the ability to induce IR.

Materials and methods: Fasting glucose and insulin was analysed in age-matched non-pregnant (n=12) and pregnant mice (gestations day 18, n=10). Hepatic lipids were extracted and fractionated into different lipid classes. Ceramide content and composition was analysed using liquid chromatography-MS. Ceramide species were identified based on exact mass, fragmentation pattern and retention time. Quantification was performed using internal standard and external standard curves. Pairwise comparisons were performed using Mann-Whitney U-test. Ceramide composition was assessed by principal component analysis (PCA) and differences between principal components for pregnant (P) and non-pregnant (NP) dams was also analysed using Mann-Whitney U-test.
Results: Fasting insulin (NP: 0.18±0.13 ng/mL, P: 0.45±0.13 ng/mL, $p<0.01$), but not fasting glucose (NP: 7.7±1.1 mM, P: 8.4±0.9 mM, $p=0.19$), was increased at gestation day 18, compared to the non-pregnant dams. Total hepatic ceramide concentration was elevated in pregnant dams (NP: 4.9±0.6 mg/g tissue, P: 8.1±0.9 mg/g tissue, $p<0.0001$), while hepatic TAG-levels were unaffected. The PCA analysis of ceramide profile strongly separated the pregnant from the non-pregnant dams along PC1 ($p<0.0001$), which explained 46% of thevariation in the data. The loading plot revealed that ceramide in pregnant dams were characterized by relatively shorter saturated acyl groups (C16:0 and C18:0), i. e. those synthesized by CS6, while the non-pregnant dams were characterized by the longer-chained, C22:0, C24:0 and C24:1, ceramides, synthesized by ceramide synthase 2. Total hepatic ceramide correlated to QUICKI (Spearman r=0.-57, p=0.03), but the correlation to ceramide composition (given as PC1) was stronger (Spearman r=-0.70, p=0.003.
Conclusion: In late stage of pregnancy, the hepatic ceramide profile is characterized by ceramide species that are known drivers of insulin resistance, while it in the non-pregnant state is characterized by ceramide structures that have been shown to attenuate IR in high-fat fed mice. This suggests that pregnancy-induced IR can partly be explained by altered metabolic fluxes through the different ceramide synthases pathways, resulting in altered ceramide composition. This interpretation is supported by the strong the correlation between ceramide composition and QUICKI.
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A role for kisspeptin in regulating beta cell mass during pregnancy
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Background and aims: Whilst effects of kisspeptin on islet function have previously been studied the physiological role remains unclear. The extremely high levels of kisspeptin released by the placenta suggest a possible role during pregnancy, characterised in the islets by a rapid and reversible increase in beta-cell mass. This study therefore examined the effects of kisspeptin on beta cell mass in pregnant and non-pregnant mice.
Materials and methods: Control or pregnant mice were implanted with subcutaneous osmotic minipumps containing either saline, kisspeptin (50 nmol/day) or the kisspeptin antagonist kisspeptin-234 (200 nmol/day) and were given water containing 1 mg/ml BrdU for 10 days (from day 8-18 of pregnancy). Immunohistochemical staining of pancreas sections for BrdU and insulin followed by morphometric analysis was used to assess beta-cell proliferation, beta-cell hypertrophy and islet mass.
Results: Kisspeptin administration in non-pregnant mice had no effect on beta-cell proliferation, assessed by the extent of BrdU labelling, compared to untreated non-pregnant mice (non-pregnant: 7.83±0.72%, non-pregnant + kisspeptin: 8.65±1.18%, percentage of BrdU positive beta-cells, n=6). Kisspeptin also had no significant effect on beta-cell hypertrophy in non-pregnant mice (non-pregnant: 107.8±12.3 μm^2/cell, non-pregnant + kisspeptin: 116.3±8.9 μm^2/cell, beta-cell size, n=6). However, administration of kisspeptin-234 in pregnant mice significantly attenuated the increased beta-cell proliferation associated with pregnancy (pregnant: 21.42±1.19%, pregnant + kisspeptin-234: 16.91±1.07%, percentage of BrdU positive beta-cells, n=6, $p<0.05$). Similarly, blocking endogenous placental kisspeptin attenuated pregnancy related beta-cell hypertrophy (pregnant: 194.3±11.5 μm^2/cell, pregnant + kisspeptin-234: 162.7±13.1 μm^2/cell, beta-cell size, n=6, $p<0.05$).
Conclusion: These data indicate that endogenous kisspeptin increases beta-cell proliferation and hypertrophy, supporting a role for placental kisspeptin in regulating the increase in beta-cell mass during pregnancy. The lack of significant effect of kisspeptin on beta-cell mass in non-pregnant mice suggests that this is likely to be an effect specific to the pregnant environment and may involve other factors.
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The role of long non-coding RNAs in beta cell mass expansion during mouse pregnancy
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Background and aims: Pregnancy is accompanied by a remarkable pancreatic islet β-cell mass expansion, whereas progression of type 2 diabetes (T2D) correlates with β-cell mass loss. Long non-coding RNA (lncRNA) is a novel RNA species with emerging evidence highlighting its multiple regulatory functions in biology and diseases. Strikingly, lncRNA tissue distribution suggests a higher degree of specificity compared to coding genes in particular cell types, such as in human islets. The aim of this study is to identify functionally important lncRNAs in β-cell expansion during pregnancy that are β-cell specific, and therefore, putative candidate drug targets or biomarkers.
Materials and methods: We performed a microarray analysis on islets isolated from non-pregnant and pregnant mice at gestational day 14.5 (peak β-cell proliferation). Bioioinformatic approaches were applied to compare a public RNA-seq dataset with our microarray data to identify differentially expressed lncRNAs during pregnancy. The differential expression of candidate lncRNAs were verified using real time RT-PCR in a mouse beta-cell line (MIN6c4) and isolated primary mouse islets treated with prolactin to mimic pregnancy and induce β-cell proliferation.
Results: We identified a small number of lncRNAs that were differentially expressed in pancreatic islets during pregnancy from RNA-seq analysis ($P<0.01$). Most of them displayed a similar expression pattern in the microarray analysis. As an initial approach, we validated the expression of 5 novel lncRNAs in prolactin-treated MIN6c4 cells and mouse islets. Mn-Lnc03 and Mn-Lnc04 were markedly induced by prolactin in both MIN6c4 cells and mouse islets: Mn-Lnc03 expression was dose-dependently induced by 24-hour treatment of prolactin 200 ng/mL ($P<0.05$) and 500 ng/mL ($P<0.001$) in MIN6c4 cells and by 24-hour ($P<0.01$) and 48-hour ($P<0.001$) treatment of prolactin 500 ng/mL in mouse islets; similarly, Mn-Lnc04 expression was dose-dependently induced by 24-hour treatment of prolactin 200 ng/mL ($P<0.001$) and 500 ng/mL ($P<0.001$) in MIN6c4 cells and by treatment of prolactin 200 and 500 ng/mL for 24 hours ($P<0.05$) and 48 hours ($P<0.01$) in mouse islets.
Conclusion: These findings suggest that pregnancy is associated with an altered expression of specific lncRNAs in islets, which may play a regulatory role in β-cell mass expansion. Although their functions in beta-cell proliferation and human relevance are currently not known, our data suggests that novel lncRNAs that could be drug targets or biomarkers are indeed regulated by pregnancy-induced adaptation in the islet.

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INS^{C93S} transgenic pigs: a novel large animal model for gestational diabetes

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Background and aims: The prevalence of gestational diabetes mellitus (GDM) is steadily increasing. GDM has a negative impact on foetal development and can trigger intrauterine programming of diseases like obesity or diabetes in the offspring's later life. Due to major differences in foetal development, mouse models are not sufficient to address these questions. In contrast, prenatal development of pigs mimics the human situation more closely. We evaluated *INS*^{C93S} transgenic pigs characterised by a pre-diabetic condition (i.e. fasting normoglycaemia, reduced glucose tolerance and decreased beta cell mass) as a novel model of GDM.
Materials and methods: *INS*^{C93S} transgenic pigs express the *INS*^{C93S} coding sequence under the control of porcine *INS* regulatory sequences. *INS*^{C93S} transgenic (n=3) and wild-type (WT) sows (n=4) were oestrus synchronized and artificially inseminated with sperm of the same boar. Hyperinsulinaemic-euglycaemic clamps (HIC) were performed within the third trimester of pregnancy. At gestation day 98±1.5, sows were necropsied and foetuses were recovered. From all foetuses blood samples were taken, and body weight (BW) and crown-rump length (CRL) data were recorded. In addition, pancreas was sampled from a random subset of foetuses of both groups of pregnant sows and processed for stereological analysis. From *INS*^{C93S} transgenic sows only the WT foetuses were studied. Effects on foetal outcomes were analysed using the General Linear Models (GLM) procedure (SAS), taking sow genotype and fetal sex as fixed effects, and sow body weight and litter size as covariates into account. Data are presented as mean ± SEM.
Results: HIC showed a 35% reduced insulin sensitivity in pregnant (n=4) compared to non-pregnant (n=3) WT sows (p=0.028), and insulin sensitivity of pregnant *INS*^{C93S} transgenic sows was reduced to the same extent. While fasting blood glucose (FBG) levels were not different between the two sow groups at the beginning of pregnancy, *INS*^{C93S} transgenic sows exhibited significantly higher FBG levels at gestation day 50 (71±2.3 vs. 50.3±3.3 mg/dl, p<0.01) and even more at gestation day 95 (106.7±9.7 vs. 55.5±4.3 mg/dl, p<0.001) compared to pregnant WT sows. WT foetuses (n=14) recovered from *INS*^{C93S} transgenic dams revealed significantly higher blood glucose levels than those (n=44) from WT sows (45.4±3.9 vs. 33.7±1.1 mg/dl, p<0.01). While foetal BW was not significantly affected by maternal GDM, the relative CRL (CRL/BW$^{0.33}$) was significantly increased in foetuses from *INS*^{C93S} transgenic vs. WT sows (2.75±0.08 vs. 2.68±0.03; p<0.01). Additional analyses of foetal insulin and metabolite levels as well as pancreas stereology are ongoing.
Conclusion: Like humans, pigs develop reduced insulin sensitivity in the late gestational period. In pre-diabetic *INS*^{C93S} transgenic sows pregnancy-induced metabolic stress resulted in a gradual elevation of blood glucose levels to a clinically relevant extent which also projected to the corresponding fetuses. The increased relative CRL in fetuses from *INS*^{C93S} transgenic sows suggests effects on foetal growth which deserve organ specific analysis in future studies. This model is an important tool to systematically analyse the molecular and biochemical changes induced by GDM in the mother, fetuses and offspring.
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PS 098 Care of the pregnant woman with diabetes

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Haemoglobin A_{1c} levels in the periconception period with type 1 diabetes and the risk of adverse neonatal outcomes: a systematic review and dose-response meta-analysis

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Background and aims: Poor peri-conceptional (i.e., in the preconception period or during first trimester of pregnancy) glycemic control significantly increased adverse neonatal pregnancy outcomes in type 1 diabetes mellitus (T1DM). Glycemic target in this period is controversial. We aimed to evaluate and quantify the potential dose-response association between peri-conceptional hemoglobin A1c (HbA1c) levels and the relative risk (RR) of congenital malformations and fetal loss in women with T1DM.
Materials and methods: A systematic review and dose-response meta-analysis of prospective cohort studies or nested case-control studies were performed. Relevant articles in English were identified through searching PubMed and EMBASE databases up to November 2014. The references of relevant articles were reviewed to identify potential publications. Studies that were stratified by HbA1c with measures of RR, and 95% confidence intervals (CI) for congenital malformations or fetal loss in T1DM compared with non-diabetes were included.
Results: We reviewed 3958 abstracts and extracted 7 articles with data for 4153 individuals. A total of 124 congenital malformations events from 6 prospective cohort trials, and 88 fetal loss events from 5 prospective cohort trials were observed. There was a linear dose-response relationship between the peri-conceptional HbA1c of women with T1DM and the relative risk of congenital malformations and fetal loss as shown in figure 1. The RR of congenital malformations was 1.61[0.58-4.49], 2.14[0.55-8.32], 3.07[1.23-7.65] and 3.29[0.58-18.71], and the RR of fetal loss was 1.54[0.61-3.91], 2.09[0.48-9.11], 3.54[0.94-13.33] and 6.23[1.65-23.47], when the maternal HbA1c during periconception was 5.5%, 5.9%, 7% and 10%, respectively.
Conclusion: The risk of congenital malformation and fetal loss both increased with maternal HbA1c level. If it is achievable without significant hypoglycemia, HbA1c levels should be maintained as close to normal as possible in the periconception period.

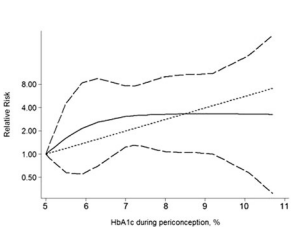

Fig 1a Dose-response analyses between peri-conceptional HbA1c and RR of congenital malformations

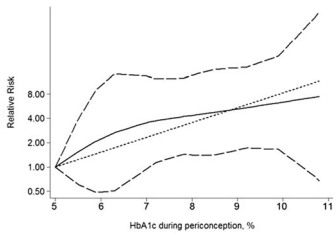

Fig 1b Dose-response analyses between peri-conceptional HbA1c and RR of fetal loss

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Lower levels of placental growth hormone in early pregnancy in type 1 diabetic women with large for gestational age infants

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Background and aims: Early growth delay may occur in the first trimester of pregnancy in women with diabetes. Insulin-like Growth Factor-I (IGF-I) levels are higher in women with fetuses smaller than expected from gestational age compared with women with normal-size fetuses. Placental growth hormone (GH) levels in late pregnancy have previously been reported to be associated with fetal growth. We evaluated whether levels of placental GH and IGF-I are associated with development of large for gestational age (LGA) infants in pregnant women with type 1 diabetes.
Materials and methods: Observational study of 107 consecutive pregnant women with type 1 diabetes (median duration 16 years (range 1-36), HbA_{1c} 6.6% (4.9-10.5) in early pregnancy). At 8, 14, 21, 27 and 33 weeks blood was sampled for measurements of placental GH, IGF-I and HbA_{1c}. Weight gain in early pregnancy was calculated as the difference between weight at 14 weeks and pre-pregnancy weight. Total gestational weight gain was calculated as the difference between the last weight measured before delivery and the self-reported pre-pregnancy weight. LGA was defined as birth weight >90th percentile.
Results: Throughout pregnancy the levels of placental GH were similar in 51 (48%) women delivering LGA infants (birth weight 3930 g (3295-5620)) compared with the remaining women (3198 g (2040-3880)) except at 8 weeks where placental GH levels were lower in the women with LGA infants (1.1 ng/ml (0.1-4.3) vs. 1.7 (0.3-11.7), p=0.02). IGF-I levels during pregnancy were similar (p=0.61) in women with and without LGA infants. Gestational age at first blood sampling was similar in women with and without LGA (60 days (37-89) vs. 62.5 (42-94), p=0.26). Placental GH levels at 14 weeks correlated negatively with weight gain in early pregnancy (r=-0.29, p=0.006). Total gestational weight gain correlated positively with birth weight (r=0.28, p=0.003). LGA was more frequent in women with parity ≥1 (34 (67%) vs. 17 (33%), p=0.001), but was not associated with pre-pregnancy BMI. As predictors of LGA infants multivariate logistic regression analysis identified placental GH levels at 8 weeks (OR 0.4 (95% CI: 0.2-0.9), p=0.03; i.e. a doubling of placental GH levels implied a 60% lower risk of having an LGA infant), total gestational weight gain (1.7 (1.1-2.7), p=0.04; i.e. a 5 kg increase in gestational weight gain implied a 70% higher risk of having an LGA infant) and parity ≥1 (3.5 (1.5-8.3), p=0.004; i.e. parity ≥1 implied a 3.5 fold risk of having an LGA infant compared with primiparous women).
Conclusion: Unexpectedly, elevated levels of placental GH or IGF-I were not associated with the development of LGA infants in this cohort of women with type 1 diabetes. Women delivering LGA infants had lower placental GH levels in early pregnancy. Growth factors and maternal weight gain in early pregnancy may play an important role for healthy fetal growth.

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Maternal lipids are associated with large for gestational birth weight in women with type 1 diabetes: results from a prospective single-center study

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Background and aims: Despite improvement in diabetes care over the years, incidence of large for gestational age (LGA) is still very high even in a well controlled type 1 diabetic mothers. Aside from the already studied effects of glycemia, it has been suggested that maternal lipids also play an important role in influencing fetal growth. However, data on the association of lipids and LGA in women with T1DM is scarce. Therefore, the aim of our study was to determine whether changes in maternal lipids might contribute to high rates of LGA in this population.
Materials and methods: Prospective single-center study on a population of 171 women with T1DM admitted to the perinatal center for women with diabetes between June 2012 and December 2014. Anthropometric, clinical and laboratory data (HbA1C, total cholesterol, HDL, LDL, triglycerides) were collected during 3 planned hospitalizations: in the first trimester (<12th week), in mid-pregnancy (20th-24th weeks) and before delivery (34th-39th weeks). All multivariate models were adjusted for maternal age, duration of diabetes, BMI, gestational weight gain, parity (mulitpara vs. primipara), mode of insulin therapy (CSII vs. MDI) and HbA1c.
Results: Fifty- seven women were excluded from the study group due the following: multiple pregnancy, spontaneous abortions, preeclampsia, and delivery in a different hospital/loss of follow-up, giving a group of 114 women included in the final analysis (LGA=30; AGA=84). In the multivariate logistic regression analysis lower HDL concentration in the first trimester was significantly associated with LGA (p=0.01). Similar association was observed for the HDL concentrations in mid-pregnancy (p=0.04) and before delivery (p=0.03). Higher triglycerides concentrations in the first trimester (p=0.02) and before delivery (p=0.008) were associated with increased risk of LGA. There was no association between second trimester TG and LGA. Both total cholesterol and LDL were not associated with LGA. Higher HbA1c was associated with LGA in the second trimester (p=0.01) and before delivery (p=0.02). There was no association between first trimester HbA1c and LGA.
Conclusion: Decreased HDL and increased triglycerides during pregnancy might contribute to the development of LGA in women with type 1 diabetes. HbA1c seems to be a significant predictor of LGA, however, only in the second part of pregnancy.
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Diabetic autonomic neuropathy in type 1 diabetic pregnant women

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Background and aims: Pregnant women undergo several metabolic and circulatory changes to meet fetal needs. Diabetic autonomic neuropathy (DAN) is a common complication of type 1 diabetes, involving cardio vascular system. Primary aim: To evaluate the pregnancy outcome of two groups of women with type 1 diabetes with and without autonomic diabetic neuropathy. Secondary aim: Longitudinal evaluation of time and frequency domain indexes and 24 h ambulatory blood pressure monitoring (24hABPM) in pregnancy.
Materials and methods: Diabetic autonomic neuropathy was diagnosed before pregnancy, according to 'SID diabetic neuropathy study group' reccomandations. 22 normotensive type 1diabetic women at enrollment, 7 with DAN (N+) e 15 without DAN (N-) underwent 24 hour Holter ECG recording (24 h ECG) and 24hABPM at the 1st and the 3rd trimester. We

analyzed time (SDNN, SDANN, SDNN INDEX, PNN50%) and frequency (LF, HF and LF- HF ratio) domain indexes derived from 24 h ECG and 24 hour blood pressure profiles. Statistics: IBM SPPS ver.20 t test, chi square and Mann Whitney.

Results: N+ showed a longer diabetes duration than N- (N+=20.2±3,9 vs N- =12.7±5,8 years , p=0.007) with a higher prevalence of microalbuminuria (N+=28.5% vs N-=0%, p=0,04) and retinopathy (N+=100% vs N-=6,6%, p=0,01). Despite HbA1c levels were similar during pregnancy, post prandial blood glucose levels were higher at lunch time in N+ at the 1st trimester (N+=150,6±45,9 vs N-=112,9±26,5 mg/dl, p=0,04). The prevalence of hypertension during pregnancy was not significantly higher in neuropathic women (N+= 42,8% vs N-=33,3%, p=0,66) even though two cases of pre-eclampsia were only observed in N+ with microalbuminuria. At 1st and 3rd trimester, 24 hour systolic and diastolic blood pressure values were not significantly higher in N+. At 3rd trimester N+ were all 'non dipper' with a systolic delta day-night lower than N- (N+=4 (3-5) vs N-=8,0 (5-9) mmHg, p=0,04). Time domain indexes were lower in N+ at the 1st (SDNN index N+=78,2±21,5 vs N-=102,6±18,8 milliseconds, p=0,01, SDANN index N+=69,4±23,4 vs N-=94,1±22,7 milliseconds, p=0,04, PNN50 (N+=3,5 vs N-=11,1%, p=0,04) and at the 3rd trimester (SDNN index N+=69,7±25,1 vs N-=95,4±31,7 milliseconds, p=0,04, SDANN index N+=61,7±27,2 vs N-81,4±30,2 milliseconds, p=0,04, PNN50 index N+ =2,5 vs N-=6,0%, p=0,04). We did not find any difference in frequency domain indexes. N+ women delivered not significantly earlier than N-.

Conclusion: Diabetic autonomic neuropathy is associated with higher post prandial blood glucose values at the first trimester and worse blood pressure levels throughout gestation. Differences in vagal indexes between neuropatic and non neuropatic women were definitely unmasked in late pregnancy.

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Diabetic ketoacidosis in type 1 diabetic pregnancy: a case series review

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Background and aims: Diabetic ketoacidosis (DKA) in pregnancy is a rare but serious medical and obstetrics emergency associated with a high risk of fetal demise (9-35%) and maternal mortality (4-15%). Factors predisposing to development of DKA in pregnancy include reduced buffering bicarbonate capacity due to hyperventilation in 2nd and 3rd trimester, increased insulin resistance induced by pregnancy hormones, increased insulin requirements in later stages of pregnancy, emesis gravidarum and increased carbohydrate absorption due to progesterone effect on gut mobility. The most common precipitants include infection, first presentation of type 1 diabetes (T1DM), omission of insulin, hyperemesis gravidarum, steroids for fetal lung maturation or severe emotional stress. Ketonaemia, dehydration and acidosis cause reduced utero-placental blood flow and increased affinity of maternal haemoglobin to oxygen which can lead to fetal hypoxia. The aim of this case series was to review the presentations of DKA in pregnancy in patients with T1DM in our institution over the last 5 years.

Materials and methods: The electronic notes of 87 women with T1DM seen in local antenatal service with 96 separate pregnancies between 1/4/2010 to 31/3/2015 were reviewed. 5 of 87 (5.75%) women had 7 distinct episodes of DKA in pregnancy. Demographic data on each woman was collected alongside information on pre-pregnancy HbA1c, diabetes complications, degree of metabolic derangement on admission, DKA trigger and outcomes of pregnancy.

Results: 7 episodes of DKA occurred in 5 women in 6 pregnancies over 5 year period. One woman had 2 episodes of DKA in each of the 1st and 2nd trimesters of her pregnancy. Average pre-pregnancy HbA1c was 103 mmol/mol. 3/5 women had pre-pregnancy retinopathy and one woman had overt diabetic nephropathy. The majority of pregnancies (4/6) were unplanned and 4/6 women did not attend pre-pregnancy counselling clinic. DKA precipitants were influenza, mumps, dental abscess, UTI, vomiting, gastritis and omission of insulin. DKAs occurred with equal frequency in 1st trimester (3 episodes) and 3rd trimester (3 episodes) and one DKA occurred in 2nd trimester. Average blood glucose at presentation was 17 mmol/l with two DKA episodes presented with only mildly elevated glucose levels (12.7 and 8.9 mmol/l). Average pH at presentation was 7.23. Those who had blood ketones measured on admission had levels ranging between 3.7-6.9 mmol/l. In one woman the DKA episode was associated with fetal demise on admission at 9 weeks gestation. 4 women delivered by emergency caesarean section and their babies required admission to neonatal intensive care unit due to prematurity or neonatal hypoglycaemia.

Conclusion: Higher risk of DKA in pregnancy despite intensive antenatal input seems to be associated with suboptimal HbA1c prior conception and past history of DKA admissions prior to pregnancy. Our case review demonstrated that pregnant women with DKA presented predominantly to medicine. Cases presented with lower than expected glucose levels with one woman presenting with a level of 8.9 mmol/l, which might present a diagnostic challenge. One woman presented with fetal death on admission but no fetal deaths occurred after treatment of DKA was initiated which highlights importance of rapid diagnosis and rigorous treatment. All babies were delivered via emergency caesarean section and required specialist baby unit care which would suggest that DKA in pregnancy is a risk factor for these outcomes.

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The clinical significance of overt diabetes diagnosed in pregnancy

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Background and aims: To analyze maternal characteristics, pregnancy and neonatal outcomes in women diagnosed of overt diabetes (ODM) during pregnancy and to compare these results with those of women diagnosed with gestational diabetes mellitus (GDM)

Materials and methods: A prospective study of women who gave birth in our hospital between January 2011 and October 2014 was carried out. All women underwent screening for GDM using the two-step approach between the 24th and the 28th week of pregnancy. Those with risk factors for GDM underwent testing during the first trimester. The diagnosis of GDM was based on the recommendations of the National Diabetes Data Group. A1c levels were determined in those diagnosed of GDM. ODM was diagnosed in those who met the ADA criteria for diabetes (fasting glucose ≥126 mg/dl and/or a glycosylated hemoglobin≥6.5%) but who had not a previous diagnosis of diabetes mellitus.

Results: During the study period 567 women were diagnosed of GDM and 40 fulfilled the criteria for ODM. Maternal, gestational and newborn characteristics of both study groups are shown in the Table.

Conclusion: In this patient cohort, overt diabetes in pregnancy significantly increased the risk of adverse pregnancy outcomes such as premature birth, induced delivery, caesarean section and large for gestational age babies, when compared with GDM. The majority of women diagnosed of ODM belong to ethnic minority groups and so special attention to these women should be paid in order to achieve early detection and treatment.

Maternal characteristics and pregnancy and neonatal outcomes

	DMG	T2DM	p
	N=567	N=40	
Maternal characteristics			
Age (years ± SD)	32.8 (±5.2)	36.3 (±4.8)	<0.001
Previous BMI (Kg/m² ± SD)	27.2 (±5.6)	30.4 (±5.4)	0.001
Ethnicity n (%)			
• Caucasian	244 (43.1%)	2 (7.3%)	<0.001
• Latin american	80 (14.1%)	5 (12.2%)	
• Indopakistan	108 (19.0%)	21 (51.2%)	
• Moroccan	69 (12.1%)	5 (12.2%)	
• Asian	66 (11.7%)	6 (14.6%)	
• African	0 (0%)	1 (2.4%)	
Previous DMG n (%)	98 (17.3%)	14 (35.9%)	0.004
Previous macrosoma n (%)	62 (10.9%)	6 (13.9%)	0.313
Nulliparous n (%)	26 (45.7%)	10 (24.4%)	0.008
Pregnancy outcomes			
Stillbirth n (%)	0 (0%)	2 (4.9%)	0.004
Onset of labor (%)			
• Spontaneous delivery	282 (49.7%)	8 (20.0%)	0.001
• Induced delivery	227 (40.0%)	25 (62.5%)	
• Elective Cesarean section	58 (10.3%)	7 (17.5%)	
Type of delivery (%)			
• Eutocic delivery	298 (52.6%)	17 (42.4%)	0.007
• Instrumented delivery	158 (27.8%)	6 (15.2%)	
• Urgent caesarean section	111 (19.6%)	17 (42.4%)	
Neonatal outcomes			
Preterm birth (%)	38 (6.7%)	9 (23.1%)	0.001
SGA n (%)	9 (1.6%)	0 (0%)	0.428
LGA n (%)	84 (14.8%)	16 (41.0%)	<0.001

PS 099 Diabetic neuropathy: emerging concepts

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The prevalence of early somatic neuropathy in children and adolescents with type 1 diabetes and its association with the persistence of GAD-65 and IA-2 autoantibodies

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Background and aims: Diabetic neuropathy (DN) is a complication of type 1 diabetes mellitus (T1D) with some well established risk factors and others that remain to be identified. We tried to evaluate the prevalence of early somatic neuropathy in children and adolescents with T1D and its association with the persistence of autoantibodies against glutamic acid decarboxylase (GADA) and islet antigen-2 (IA-2A).
Materials and methods: A cross-sectional study was conducted in a hospital-based cohort of pediatric T1D patients (n=85, mean (SD) age: 12.3±3.4 years). Peripheral neuropathy was assessed with nerve conduction studies (NCS). GADA and IA-2A titers were measured in samples collected prior to electrophysiological testing.
Results: Among the study population, 34.1% had at least one abnormal electrophysiological parameter in NCS, although usually asymptomatic. The highest rates of abnormalities were detected in the sensory fibers of the peroneal nerve. These patients were not different from the rest of the study group in terms of age, diabetes duration or glycaemic control. Among our patients with a mean (SD) T1DM duration of 5.4±3.3 years, 62.4% had positive GADA, 58.8% positive IA-2A and 41.2% double antibody positivity. Abnormal NCS correlated neither with GADA nor with IA-2A levels. However lower sensory nerve action potential (SNAP) in the peroneal nerve, which was indicative of early axonal dysfunction, was observed in patients with GADA (8.3±3.0 mV vs 10.2±4.2 mV, p= 0.046) or IA-2A (7.8±3.9 mV vs 10.6±4.0 mV, p=0.002) positivity.
Conclusion: Impaired indices of subclinical peripheral sensory neuropathy were present among one third of the T1DM children and adolescents, with no difference in diabetes duration or glycaemic control. GADA and IA-2A seem to be involved in the development of axonal degeneration, in a pathway which remains to be identified.

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Is IGT-associated sensory neuropathy driven only by glycaemia?

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Background and aims: Traditional cardiovascular risk factors play an important role in the development of neuropathy in diabetes. In the present study, we aimed to investigate risk factors of sensory neuropathy in patients with impaired glucose tolerance (IGT) and healthy volunteers.
Materials and methods: Seventy-five people with impaired glucose tolerance and 40 age and gender matched healthy volunteers underwent a

detailed clinical examination and neurological assessment. Sensory nerve function was assessed by using Neurometer (current perception threshold [CPT]) and Medoc devices. Autonomic neuropathy was detected by the five standard cardiovascular autonomic tests. Mean 24-h systolic and diastolic blood pressures were assessed by 24-hour ambulatory blood pressure monitoring.
Results: The prevalence of sensory neuropathy was 58% in subjects with impaired glucose tolerance. The Odds ratio (OR) was 11.23 (CI: 3.57-35.35). This association was independent from the presence of autonomic neuropathy or the measure of BMI, HbA1c, BP and HR. However, adjustment for fasting plasma glucose attenuated the association notably (OR: 6.75; CI: 1.33-34.27), and the significance was lost after adjustment for 120 min glucose level (OR: 3.76; CI: 0.26-54.10). Sensory neuropathy in patients with IGT showed a strong association with 120 min glucose level (OR: 1.78; CI: 1.20-2.63) in the entire population studied.
Conclusion: Our data suggest that glycemia (120 min glucose level) is a risk factor for sensory neuropathy in subjects with IGT which is independent of all other known cardiovascular risk factors. This new knowledge may be an important addition to risk reduction strategies.

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Reduced sudomotor function in adult patients with long lasting type 1 diabetes and peripheral neuropathy

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Background and aims: Diabetic neuropathy is the most common chronic complication of diabetes. It often remains undiagnosed for a long time and usually leads to chronic pain and foot ulcerations. Therefore, new accurate and noninvasive methods are needed for its early diagnosis. Sweat glands are innervated by small C-fibers. Dysfunction of the sweat glands is very early clinical manifestation of diabetic neuropathy. SUDOSCAN+ device is used to noninvasively assess the function of the sweat glands, measuring electrochemical skin conductance (ESC) through reverse iontophoresis. The aim of this study was to evaluate the sudomotor function in patients with long lasting type 1 diabetes.
Materials and methods: The study included 381 adult patients with type 1 diabetes (185 women and 196 men) aged 41 years (IQR: 32-51), with disease duration of 23 (IQR: 18-30) years. The study group was subdivided into two groups depending on the presence of peripheral neuropathy identified by standard methods. Neuropathy was diagnosed in patients with two or more of the following five elements: the presence of symptoms, lack of ankle reflexes, impaired sensation of touch, temperature and/or vibration. We measured ESC with SUDOSCAN+ on feet (Feet ESC) and hands (Hands ESC) in the study group and in the control group of 63 healthy volunteers sex and age matched. We also evaluated metabolic control of diabetes using HbA1c value and skin autofluorescence (AF) measured with AGE-Reader as well as the presence of late diabetic complications.
Results: Peripheral neuropathy was diagnosed in 45% of patients. Patients with peripheral neuropathy, compared to those without it, had lower Feet ESC [68 (IQR: 46-81) vs 83 (IQR: 76-87)μS; $p<0.001$] and lower Hands ESC [56 (IQR: 39-69) vs 69 (IQR: 59-77)μS, $p<0.001$]. We found a negative correlation between the result of ESC on feet and hands and patients' age ($Rs=-0.41$, $p<0.0001$; $Rs=-0.40$, $p<0.0001$), duration of diabetes ($Rs=-0.33$, $p<0.0001$, $Rs=-0.31$, $p<0.0001$), HbA1c level ($Rs=-0.12$, $p=0.015$, $Rs=-0.12$, $p=0.017$), skin AF ($Rs=-0.33$, $p<0.0001$, $Rs=-0.30$, $p<0.0001$) and positive correlation with the eGFR ($Rs=0.36$, $p<0.0001$, $Rs=0.30$, $p<0.0001$). Feet ESC and Hand ESC did not differ between patients with diabetes without neuropathy and controls [83 (IQR: 76-87) vs 82 (IQR: 78-86) μS), $p<0.94$ and 69 (59-77) vs 69 (60-79)μS, $p=0.57$]. In a multivariate logistic regression model electrochemical skin conductance was independently associated with the presence of peripheral neuropathy (Feet ESC OR 0.96; 95%CI: 0.95-0.98, $p<0.0001$ and Hands ESC OR 0.97; 95%CI: 0.95-0.98, $p=0.0001$). This association was independent from age, sex, duration of diabetes, HbA1c, skin AF and eGFR.
Conclusion: In adults with type 1 diabetes and peripheral neuropathy sudomotor function is markedly reduced. The longer duration of diabetes, worse metabolic control or reduced renal function, the greater sudomotor dysfunction is.
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Sudoscan as a tool for early screening of diabetic microvascular complications: experience of a French hospital

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Background and aims: The screening of microvascular complications in patients with type 2 diabetes is not optimal, especially for neuropathy at early stage. As sweat glands are innervated by small autonomic C-fibers the measurement of sweat function has been proposed to evaluate peripheral small fiber neuropathy, which may occur very early. This study aimed to evaluate SUDOSCAN, an easy, fast, non-invasive and quantitative measurement of sweat function for the detection and follow-up of microvascular complications of diabetes.
Materials and methods: During this study involving 189 patients with type 2 diabetes, the following tests were performed: a clinical examination including monofilament testing (n=154), blood tests including MDRD clearance (Modification of the Diet in Renal Disease) measurement, ophthalmologic examination (n=113), SUDOSCAN with hands and feet conductance measurements (μS). A general risk score for autonomic neuropathy based on previous studies was also calculated. Results were expressed as mean ± SD and comparisons were made by a Student test.
Results: The population had a mean age of 60±16 years, 49% were men, HbA_1c 9.0±1.3%. Feet conductances of patients having Fasting blood glucose>7.2 mmol/L were significantly lower (70±15 vs 74±16 μS, $p=0.03$). Patients with MDRD clearance<90 ml/min had significant lower conductances for hands and feet (Figure). Patients with urinary albumin excretion (UAE)≥15 mg/L had significant lower feet conductances (69±17 vs 75±14 μS, $p=0.002$). Lastly, patients who presented abnormalities to monofilament testing had lower conductances for hands (57±16 vs 65±16 μS, $p=0.004$), and feet (65±20 vs 75±11 μS, $p=0.003$). Same tendency was observed in patients with retinopathy as compared to patients without (69±16 vs 74±14 μS, $p=0.13$). Global autonomic risk score using 35 as threshold had a sensitivity of 78% and a specificity of 54% to detect at least one microvascular complication. No adverse event or discomfort has been reported during or following SUDOSCAN recording.
Conclusion: This study showed lower conductances values for diabetic patients who presented abnormalities to monofilament test, assessing big myelinisated fibers. These data also suggest that the conductances measured by the device correlate well with renal microvascular alterations as represented by both decreased MDRD clearance and increased UAE. These results indicate that SUDOSCAN device could be used for early detection and monitoring of both diabetic neuropathy and nephropathy.

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Thalamic γ-aminobutyric acid in diabetic neuropathy

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Background and aims: The thalamus forms a major component of the ascending sensory pathway to the brain, the function of which may play an important role in patients with diabetic neuropathy (DN). Previous Magnetic Resonance (MR) studies have demonstrated thalamic metabolite involvement on Proton MR Spectroscopy (H-MRS). One such metabolite, γ-Aminobutyric acid (GABA), is an inhibitory neurotransmitter. This study assessed thalamic GABA in-vivo using specialised novel H-MRS editing techniques in patients with and without DN.
Materials and methods: 44 type 2 diabetes (T2DM) Caucasian subjects (14 Painful-DPN, 15 Painless-DPN and 15 No-DPN) and 15 healthy volunteers without diabetes (HV) underwent detailed clinical and neurophysiological assessments. T2DM subjects were divided into three groups based on the neuropathy composite score [NIS(LL)+7 and Douleur Neuropathique 4 score(DN4). All groups were age-matched. Subjects underwent localised H-MRS at 3 Tesla to assess GABA relative to unsuppressed water and creatine using a single-voxel, spin-echo, spectral editing technique (MEGA-PRESS; echo time=68 ms) centred over thalami. GABA resonance signal was obtained relative to that of parenchymal water
Results: Analysis of Variance revealed differences in group mean GABA/H20 ratios [ANOVA $p<0.01$; Painless-DPN 1.47(sd=0.23), Painful-DPN 1.61(0.33), HV 1.75(0.25) and T2DM with No-DPN 1.84(0.38)]. Post-hoc comparisons indicated significantly lower mean GABA/H20 in Painless-DPN compared to No-DPN ($p<0.005$) and significantly lower mean GABA/H20 in Painless-DPN compared with HV ($p<0.05$).
Conclusion: This study containing a well-sized, well-characterised cohort clearly demonstrates lower relative levels of the 'H-MRS-visible' inhibitory neurotransmitter GABA in the thalamus of patients with DPN. Previous published pilot data from 7 diabetics with neuropathy and 7 non-diabetics reported lower GABA in the posterior insula in the context of DN. Our current larger sample size indicates significantly lower GABA within the thalamus often considered the sensory gateway to the brain. A further understanding of the cerebral neuronal excitatory/inhibitory balance inferred from this H-MRS technique may help determine the mechanistic basis of central nervous system involvement in pain perception associated with DN.
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Neuroinflammation and neurodegeneration in the prefrontal cortex during diabetic neuropathic pain

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Background and aims: Diabetic neuropathy (DN) is associated with structural and functional changes of nervous system, including areas involved in central pain control system, as the prefrontal cortex (PFC), which can lead to chronic excruciating pain. Neuroinflammation and synaptic plasticity have been associated to chronic pain conditions, with interplay between increased glial activation, pro-inflammatory mediators overexpression and neurodegeneration. Whether such factors contribute in the impairments detected in PFC in painful DN is still unknown. This study intended to evaluate neuroinflammation and neurodegeneration in PFC using an animal model of diabetic neuropathic pain - the streptozotocin (STZ)-diabetic rat.
Materials and methods: Rats were rendered diabetic by an intraperitoneal injection of streptozotocin (STZ). Control (CTR) rats were received vehicle solution. Ten weeks post-induction, the animals were sacrificed and the interest brain areas were removed and processed by western blot to quantify expression of Iba1 (microglial activity marker), IL1β and TNFα (pro-inflammatory factors) and synaptophysin (synaptic integrity marker). Volumes and neuronal density was determined by stereological methods in PFC areas, namely in the anterior cingulated cortex (ACC), prelimbic cortex (PL) and infralimbic cortex (IL). Glycaemic values and behaviour pain responses to mechanical stimuli were evaluated before sacrifice in order to confirm the diabetic neuropathic pain condition. Data were compared by independent sample t test and presented as mean ± SEM. Statistical significance was settled as $p<0.05$.
Results: The STZ-diabetic rats presented hyperglycaemia (CTR: 151.3± 43.8; STZ: 580.5±19.5; mgdl-1, $p<0.05$) and decreased mechanical thresholds (CTR: 14.8±0.5; STZ: 8.5±0.2; grams, $p=0.002$). We observed an increase of Iba1 expression in STZ-diabetic rats (CTR: 0.8± 0.1; STZ: 2.3±0.2; optical units, $p=0.002$), which was accompanied by an increase of IL1β (CTR: 1.2±0.1; STZ: 2.0±0.3; optical units, $p=0.041$) and TNFα (CTR: 0.8±0.1; STZ: 1.2±0.05; optical units, $p=0.048$) levels. Synaptophysin expression was significantly decreased (CTR: 1.0±0.04; STZ: 0.7±0.07; optical units, $p=0.009$). A significant reduction was observed in the estimated volume of ACC in diabetic animals (CTR: $3.3\times10^{7}\pm1.3\times10^{6}$; STZ: $2.8\times10^{7}\pm6.0\times10^{5}$; μm3, $p=0.007$) accompanied by a clear reduction in neuronal density (CTR: $14.8\times10^{10}\pm2.1\times10^{10}$; STZ: $8.1\times10^{10}\pm3.8\times10^{10}$; neurons/μm2), albeit non-significant ($p>0.05$).
Conclusion: Diabetes induces neuroinflammation and neurodegeneration in PFC. These changes may contribute to diabetic neuropathic pain, point for the need of strategies to prevent it.
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GLP-1 analogue has anti-inflammatory effects at the spinal cord of diabetic rats

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Background and aims: Diabetic neuropathy is a common complication of diabetes, often associated with chronic pain. Several studies pointed to changes at the spinal cord as possible mechanisms underlying the diabetic neuropathic pain (DNP). Neuroinflammation emerges as a possible cause of diabetes-induced spinal dysfunction. Incretin drugs, namely glucagon-like peptide 1 (GLP1) analogues, emerge as promising drugs as they were shown to present anti-inflammatory and neuroprotective effects in neurodegenerative diseases. Moreover, the GLP1 receptor was shown to be expressed by CNS immune cells, the microglia, at the spinal dorsal horn, reinforcing the possible effect of GLP1 in mediating spinal inflammatory responses. The aim of present study was to evaluate the anti-inflammatory effects of Liraglutide (LIRA, GLP1 analogue) in the spinal cord of rats with DNP.
Materials and methods: Diabetes was induced by injection of streptozotocin (i.p., STZ), whereas control rats (CTR) received vehicle solution. One week after diabetes induction, STZ-injected rats initiated a treatment with LIRA (1 g/kg s.c.) (STZ+LIRA) or were maintained untreated for 10 weeks (STZ-untreated). Blood glucose and mechanical hypersensitivity were evaluated after injections and at 4 and 10 weeks post-injections. The L4-L5 spinal segments were removed and processed

for western blot to quantify Iba1 (microglial activation marker), GFAP (astroglial activation marker) and interleukin 1β (IL1β) and tumor necrosis factor alpha (TNFα). Behavioral data was compared by Two Way ANOVA with repeated measures and molecular data was compared by One Way ANOVA, followed by Tukey post-hoc test for multiple comparisons. Statistical significance was settled as $p<0.05$. Results are presented as mean ± SEM.

Results: All STZ animals presented increased blood glucose concentration at 4 (CTR: 106.3±9.7; STZ-untreated: 462.0±37.2; STZ+LIRA: 378.0±20.5, mg/dl) and at 10 weeks post-induction (CTR: 151.3±43.8; STZ-untreated: 580.5±19.5; STZ+LIRA: 574.9±84.1, mg/dl). The treatment with LIRA significantly reduced mechanical hypersensitivity at 10 weeks of diabetes (STZ-untreated: 9.8±0.4; STZ+LIRA: 11.9±0.7, grams), but the mechanical hypersensitivity thresholds were maintained significantly lower than in control animals (CTR: 14.9±0.5; STZ+LIRA: 11.9±0.7, grams). A significant increase in the expression of IL1β (CTR: 0.8±0.1; STZ-untreated: 1.9±0.5, optical units) and TNFα (CTR: 0.9±0.1; STZ-untreated: 2.9±0.9, optical units) was detected in the spinal cord of STZ-untreated rats. The levels of IL1β were significantly reduced by the treatment with LIRA (STZ+LIRA: 0.5±0.1, optical units), whereas a non-significant decrease in TNFα expression was detected (STZ+LIRA: 1.6±0.6, optical units). The STZ+LIRA rats presented increased expression of Iba1 (CTR:0.8±0.1; STZ-untreated: 0.9±0.1; STZ+LIRA: 1.2±0.2, optical units). The expression of GFAP was dramatically decreased in STZ-untreated rats (CTR: 0.9±0.1; STZ-untreated: 0.2±0.1, optical units) and was not affected by the treatment with LIRA(STZ+LIRA: 0.3±0.1, optical units).

Conclusion: This study shows that diabetes induces neuroinflammation in the spinal cord, which may be reduced by the treatment with LIRA. The anti-inflammatory effects of LIRA at the spinal cord may concur for the attenuation of diabetes-induced mechanical hypersensitivity.

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AICAR-transformylase/ cyclohydrolase mediates glucotoxic complications in C. elegans and can be treated in an AMP-kinase dependent manner

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Background and aims: As the expression atic-1, which codes for an enzyme that catalyzes the last two steps of purine de-novo synthesis (AICAR-transformylase/ cyclohydrolase) was found to be upregulated under high-glucose conditions in C. elegans, it was hypothesized that ATIC itself might be causally involved in mediating glucotoxic effects.

Materials and methods: Nematodes were cultured under standard and high glucose (HG) conditions, leading to glucose concentrations in whole animal extract of 5.5 mM and 13 mM, respectively, and treated with 1 mM AICAR. The dependency of ATIC was evaluated by using specific ATIC RNAi. Further, AMPK (aak-2), iron/ manganese superoxide dismutase (sod-3) and glyoxalase-1 (glod-4) mutant strains were used to evaluate downstream mechanisms. Life span, neuronal structure (microscopy of the pan neuronal GFP-expressing strain NW1229) and neuronal function (video analysis) were determined as well as accumulation of reactive oxygen species (ROS) and advanced glycation end-products (AGEs). Furthermore, concentrations of AICAR were measured.

Results: Under high glucose conditions the expression of atic-1 is increased 3.1-fold ($p=0.006$) compared to standard conditions. If the expression of atic-1 is abrogated by specific RNAi, which leads to an increase of its substrate AICAR (16-fold), HG mediated shortening of lifespan (from 11.0±0.5 days to 14.4±0.9 days, $p<0.0001$) as well as structural and functional neuronal damage (from 0.12±0.02 mm/s to 0.24±0.01 mm/s, $p=0.012$) are prevented. This goes along with a normalization of ROS and AGEs. The normalization of mean lifespan (and neuronal function?) by atic-1 RNAi is dependent on AMP-K as this effect is not seen in aak-2 mutant animals (life span from 9.5±0.3 days to 9.6±0.4 days). Conversely, ATIC overexpression shortens lifespan (from 36 days to 30 days) and negatively affects neuronal structure (0.25 damage scores to 0.75 damage scores). Corresponding to the effect of ATIC RNAi, exogenous AICAR treatment could prevent deleterious effects on neuronal structure (from 1.76±0.2 damage scores to 0.84±0.13 damage scores, $p=0.016$) and function (from 0.09±0.04 mm/s to 0.25 mm/s, $p=0.03$) and lifespan (from 14.8±0.9 days to 19.9±0.7 days) in high glucose cultured C. elegans. Additional treatment with the antioxidant butylated hydroxyanisole (BHA) and the complex-1 inhibitor rotenone could not further improve the effect of AICAR treatment on normalizing lifespan (+AICAR: 16.9±0.8 days, +BHA: 16.5±1.1 days and +rotenone: 16.3±1.1 days). Finally, the life prolonging effect of AICAR under HG conditions is independent on glod-4 (from 13.8±0.3 days to 18.3±0.9 days, $p=0.001$) and sod-3 (from 14.4±0.6 days to 17.9±0.6 days, $p=0.0012$) but dependent on AMP-K (aak-2) (from 13.8±0.5 days to 14.0±0.8 days, $p=0.438$).

Conclusion: The overexpression of atic-1 seen under high glucose conditions in C. elegans is contributing to the deleterious effects on lifespan and the neuronal system. This can be reversed by the use of specific atic-1 RNAi or treatment with AICAR in an AMP-K dependent manner.

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PS 100 Diabetic neuropathy: identification and evaluation

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Validation of Neuropathy Symptoms Score (NSS) and Neuropathy Disability Score (NDS) in clinical evaluation of peripheral neuropathy in diabetes mellitus

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Background and aims: About one third of diabetic people are at risk of foot ulceration because of loss of protective sensation due to peripheral Neuropathy. The symptoms of neuropathy & the conventional assessment of it with Cotton wool, Vibrating tuning fork, pin prick and hot and cold sensation can give a qualitative diagnosis. Biothesiometer can quantify & pick up early cases of DPN & is an important diagnostic tool in clinical practice

Materials and methods: Present pilot study was designed to find out the prevalence of Diabetic peripheral neuropathy (DPN) incidence by bed side evaluation using NDS & NSS as per "Young et al" criterion & then to study its co-association with other diabetic complications 855 Type -2 diabetes patients who got newly enrolled at our Diabetes Centre" between July 2013 to Dec 2014 were evaluated clinically by neuropathy symptom score (NSS) & Neuropathy Disability score (NDS) as suggested by "Young et al". Peripheral Neuropathy was diagnosed clinically if sum of NDS + NSS was >10 135 patients out of total 855 patients who had NSS 5-6 (mild symptoms) or NDS 5-6 but if sum of NDS + NSS was>10 were then evaluated by biothesiometry to validate this score . VPT>15 volts was taken as cut off for mild DPN & VPT >25 volts was considered as significant DPN. Detailed Diabetes Profile including their clinical profile namely Age, Sex, Mean duration of Diabetes, their personal habits smoking, dietary habits were evaluated. These patients were also evaluated for presence of Microabluminuria, Retinopathy, PVD and Dyslipidaemia etc.

Results: 86 patients had monofilament impairment, 49 patients had normal monofilament test. 96 patients were detected to have DPN by VPT (60 severe, 36 mild to Moderate). Hence, applying NDS+NSS >10 as per " Young et al criterion " could pick up early DPN in 96 out of 135 (sensitivity of 71.1% & specificity of 90%) This has a +ve predictive value of 57.14% & –ve predictive value of 94.32% as validated & documented by biothesiometer. Prevalence of DPN in total 855 patients evaluated clinically by NSS & NDS later tested by biothesiometry was found to be 15.4%.

Conclusion: Neurological examination like NSS & NDS can be an important bed side tool in the clinics for early diagnosis of DPN with a sensitivity of 71.1% & specificity of 90%. It is simple, acceptable, reproducible & validated as per our study. DPN has a strong co-association with other complications like DR (44.1% Vs 19.2%) and Microalbuminuria 61.3% Vs 38%.

COMPLICATIONS TABLE 2

Microalbumin uria	61.3%	38 %
Dyslipidaemia	36.4 %	28 %
Retinopathy	44.1 %	19.2%
VPT > 15 volts	26.6% (N=36)	4.8%
VPT > 25 volts	44.4% (N=60)	5.2%
Prevalence of P-Neuropathy (Baye's Theorem)	15.4%	

TABLE 3

	NSS+NDS > 10 N=135 (Young et al)	NSS+NDS < 10 N=720	Total
VPT+ve	96	72	168
VPT−ve	39	648	687
	135	720	855
Sensitivity	96/135 = 71.1%		
Specificity	648/720 = 90%		
+ve Predictive Value	57.14%		
-ve Predictive Value	94.32%		

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Can a nurse successfully diagnose distal diabetic polyneuropathy?

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Background and aims: Distal symmetrical polyneuropathy (DPN) is one of the most frequent chronic complications of diabetes. Early diagnosis of DPN is of paramount importance for the prevention of diabetic foot syndrome. We conducted a study aiming at assessing whether a nurse using a Diabetic Neuropathy Symptom (DNS) score can accurately diagnose DPN. DNS is a simple 4-item questionnaire evaluating the 14-day frequency of gait abnormalities and sensory disturbances (pain, tingling and numbness) in lower limbs; answering 'yes' to one of the questions reflects the presence of DPN.

Materials and methods: In a single outpatient diabetes clinic 118 patients (21 with type 1, and 97 with type 2 diabetes) were first met by nurse, who administered DNS. Then they were seen by a diabetologist who performed thorough medical examination aiming at assessing the presence of DPN. The specialist was blinded to the results of nurse-conducted DNS.

Results: 71 (60%) patients replied positively to at least one of four questions in DNS, while the diabetologist confirmed the presence of DPN in 80 (68%) patients, including all who were tested positive by a nurse. Thus, the sensitivity of DNS was 0.89 (95%CI 0.84-0.89), and specificity reached 1.00 (0.95-1.00). According to the results of the nurse-conducted DNS, the most frequent complaint was tingling (31%), then numbness (27%), pain (24%) and gait abnormalities (18%). The significant risk factors for positive DNS result were disease duration, HbA1c, smoking, and recent (in the previous 3 months) change in diabetes treatment. DPN was confirmed by a diabetologist in 71% of the patients with diabetes duration >15 years and in 42% of those who had diabetes for less than 5 years, in 90% patients with HbA1c >9.0% and in 34% of those with HbA1c <7%, in 69% of ever smokers and in 46% of those who never smoked, and in 84% of those whose diabetes therapy was modified in previous 3 months and in 17% of those whose treatment remained stable recently (all differences $p<0.05$). Interestingly, self-declared alcohol intake level was not related to DNS results.

Conclusion: In conclusion, DNS can be a very effective tool to diagnose DPN when used by a nurse working on her own. Engaging nursing staff into DPN assessment may help diagnose the condition at its early stage.

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The first demonstration that the Norfolk QOL-DN scale establishes safety in painful diabetic peripheral neuropathy

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Back ground and aims: PACE was a Phase III, randomised, controlled, open-label, 52-week, safety study that assessed the safety and efficacy of repeat treatment with the capsaicin 8% patch plus standard of care (SOC), versus SOC alone in patients with painful diabetic peripheral neuropathy (PDPN) (N=468). The Norfolk QOL-DN scale was the primary safety endpoint and it was used to assess patient-reported functional consequences of potential impairment in small-fibre nerve function that may be due to capsaicin treatment. Secondary safety endpoints included an objective utilization of the Utah Early Neuropathy Scale (UENS) and quantitative sensory perception testing.
Materials and methods: As PACE is the first study in which the Norfolk QOL-DN scale was a safety endpoint, patient level analyses were performed to determine correlation with other safety endpoints. An additive (Gaussian) regression model, adjusted for repeated measures using an AR(1) error structure, including additional random effects and covariates was utilised. Relationship strength was assessed using F-tests.
Results: Treatment with capsaicin improved quality of life in parallel with improved UENS and sensory function. This relationship between change in Norfolk QOL-DN total and UENS total scores was observed in patients in all treatment arms ($p<0.0001$), with a 1.0 unit decrease in UENS corresponding to a 0.4 unit decrease in Norfolk. In all treatment arms, improvement in sharp, heat, cold and vibration sensation scores were correlated with improvement in Norfolk total ($p\leq0.01$ for all) and UENS total ($p\leq0.0006$ for all) scores, but there were no relationships with deep-tendon reflex. Improvement in the Norfolk small fibre subscale score was correlated with improvement in UENS pinprick subscale and sharp sensation score in patients in all treatment arms ($p<0.001$ for both). In addition, improvement in Norfolk large fibre subscale score was correlated with improvement in UENS total ($p<0.0001$), UENS large fibre subscale ($p=0.0025$) scores, as well as improved cold, heat and sharp sensation scores ($p\leq0.004$ for all) in all arms. In contrast, deterioration in Norfolk total and small fibre subscale, UENS total and sharp sensation scores were all found if an increase in average daily pain occurred in patients in any arm ($p\leq0.001$ for all).
Conclusion: In patients with PDPN treated with either capsaicin 8% patch plus SOC or SOC alone, a consistent correlation was observed between assessments with the Norfolk QOL-DN scale, UENS scale and sensory perception testing. These findings suggest that the Norfolk QOL-DN scale is sensitive to changes in small-fibre nerve function and is a relevant safety endpoint in treating neuropathic pain in PDPN.
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One-leg standing time correlates with peripheral and cardiac autonomic nerve function in Japanese patients with type 2 diabetes

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Background and aims: Patients with diabetes show impaired physical functional capacity and peripheral nerve function. Some evidence suggests that exercise may prevent and improve peripheral nerve dysfunction in diabetic patients. However, information is lacking regarding the relationship between poor physical fitness and peripheral nerve dysfunction, along with possible risk factors for diabetic neuropathy, in Japanese patients with type 2 diabetes.
Materials and methods: The current study analyzed data collected from 299 Japanese patients with type 2 diabetes: 212 males (71%); mean age, 51±14 years; mean duration of diabetes, 8±8 years; hemoglobin (Hb) A1c, 10.4±2.5%; and normal cardiovascular response to exercise as determined by the double Master two-step test. Patients were excluded if they had a history of coronary heart disease, cerebrovascular disease, peripheral artery disease or psychiatric disorder. Peripheral sensory and motor nerve functions were evaluated by nerve conduction studies on the sural sensory nerve and tibial motor nerve, respectively. The coefficient of variation for normal R-R intervals (CVRR) at rest and during deep breathing was also used to assess cardiac autonomic nerve function. Furthermore, we examined body composition, muscle strength (knee extension), leg muscle quality (knee extension strength per unit leg muscle mass), functional balance (one-leg standing time with eyes open), agility performance (whole-body reaction time) and cardiopulmonary fitness (maximal oxygen uptake) in these patients. To identify independent correlates of peripheral nerve dysfunction, stepwise multiple regression analysis was performed. Covariates were age, sex, duration of diabetes, height, total body mass, fat mass, skeletal muscle mass, weight loss rate (maximal body weight minus body weight at entry divided by maximal body weight), blood pressure, brachial-ankle pulse wave velocity, ankle brachial index, carotid plaque, retinopathy, insulin use and levels of HbA1c, fasting plasma glucose, serum C-peptide, lipids, albumin and creatinine as well as urinary excretion of albumin and C-peptide. Highly skewed data were log-transformed.
Results: Multiple regression analysis showed that one-leg standing time correlated independently with sural sensory nerve conduction velocity, sural sensory nerve action potential, tibial motor nerve conduction velocity, minimal F-wave latency, compound muscle action potential and CVRR during deep breathing. Whole-body reaction time also correlated with sensory nerve conduction velocity and sensory nerve action potential. In addition, knee extension strength as percentage of body weight and in kilograms and leg muscle quality were related to motor nerve conduction velocity, minimal F-wave latency and CVRR at rest, respectively.
Conclusion: Based on these results, poor physical fitness as indicated by decreased one-leg standing time, increased whole-body reaction time and decreased knee extension strength correlated with impaired peripheral and cardiac autonomic nerve function in Japanese patients with type 2 diabetes. In particular, decreased one-leg standing time was most predictive of peripheral nerve dysfunction and thus offers a novel risk factor for diabetic neuropathy in these patients.
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Atrophy of both extensor digitorum brevis muscle may be a useful sign for diagnosis of diabetic symmetric polyneuropathy in Japanese diabetic men

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Background and aims: Though atrophy of small muscles of feet has been known as a cause of diabetic foot lesions, diagnostic significance of small muscles atrophy on diabetic symmetric polyneuropathy (DPN) has not been established. We aimed to evaluate the validity and reliability of observation of typical small muscle as extensor digitorum brevis muscle (EDB) EDB atrophy for diagnosis of DPN. Firstly, we examined the relations between EDB atrophy and neurological findings in the regional population-based Japanese subjects (Study I). Secondly, we investigated

the relations between EDB atrophy and quantitative neurological findings in the hospital-based diabetic patients (Study II).
Materials and methods: Study I. 367 non diabetic persons (128 male, 239 female) who received medical screening program of Katsuragi-Town were subjected. Subjective symptoms (numbness in toes and sole, pain in feet, paresthesia in feet), vibratory perception at both medial malleolus (VP; using C-128 tuning fork), bilateral Achilles tendon reflexes (ATR) and EDB atrophy were evaluated. We judged EDB atrophy as positive when both EDB could not be identified by inspection and palpation in the position of toe extension. We also interviewed about Seiza habit (Japanese sit-down style with the buttocks on top of the ankles). Study II. In 193 diabetic patients (109 male, 84 female), neurological examination as same as Study I were examined. Additional 8 objective nerve function tests such as: 4 parameters of nerve conduction study, 2 parameters of coefficient of variation of ECG R-R intervals, fall in systolic blood pressure during head-up tilt and quantitative vibratory perception threshold were evaluated. The relationships among these findings were analyzed.
Results: Study I. Prevalence (%) of EDB atrophy and Seiza habit in female (46, 67) were significantly higher than those in male (14, 26), respectively. Prevalence of EDB atrophy in positive Seiza habit subjects was significantly higher than that in Seiza habit negative subjects (53 vs 29, p=0.001). Therefore, female and male were separately analyzed. Only in male, EDB atrophy was significantly associated with ATR reduction and VP impairment. Study II. EDB atrophy was observed in 49% of women and in 27% of men. Only in male, EDB atrophy was significantly more prevalent in diabetic compared to non-diabetic subjects (27 vs 14, p=0.011). In male diabetic patients, all items of 8 quantitative nerve tests were significantly associated with EDB atrophy. In female, only 4 items were associated with EDB atrophy. We evaluated the predictive power of EDB atrophy for diagnosing the probable DSPN of Toronto consensus in diabetic men. The sensitivity, specificity and positive predictive value of EB atrophy to determine probable DSPN were 48%, 92% and 83%, respectively.
Conclusion: EDB atrophy was also seen in non-diabetic subjects, but EDB atrophy was significantly associated with peripheral neuropathy. EDB atrophy was more frequent in female than male. The gender difference seemed to depend the lifestyle such as Seiza habit. In diabetic men, EDB atrophy clearly reflected the DPN and EDB atrophy indicated the presence of DPN in approximately 90% of probability.

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Initial findings in the relationship between diabetic peripheral neuropathy and microvascular reactivity in the foot

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Background and aims: Microvascular dysfunction is common in people with diabetes resulting in diabetic nephropathy, retinopathy and neuropathy of both the large and small fibre nerves. In the periphery it contributes to diabetic ulceration via both peripheral neuropathy and changes to microvascular function. Post-occlusive reactive hyperaemia is a measure of microvascular reactivity (vasodilation capacity) that has been implicated in the diabetic foot complications. This study aimed to investigate the relationship between large and small fibre neuropathy and the post-occlusive reactive hyperaemia response in the diabetic foot.
Materials and methods: Diabetic participants were recruited from podiatry clinics for this cross-sectional study. They underwent testing for large fibre neuropathy (vibration perception threshold and monofilament detection), small fibre neuropathy (temperature and pain perception) as well as post occlusive reactive hyperaemia at the hallux (using laser Doppler). Correlations between presence of large and small fibre neuropathy, post-occlusive reactive hyperaemia parameters (time to peak and peak as a % of baseline) and demographics characteristics including sex, HbA1c, age, height, weight and duration of diabetes were performed. Binary logistic regressions were performed on factors associated with the presence of large fibre neuropathy and small fibre neuropathy.
Results: Eighty-eight participants were included in the analysis. Significant but weak correlations were observed between presence of large fibre neuropathy and age (r=0.20; p<0.05), height (r=-0.35.; p<0.05), and time to peak (r=-0.21; p<0.05). Correlations were observed between small fibre neuropathy and height (r=0.34; p<0.05), and time to peak (r=0.24; p<0.05). Binary logistic regression analyses demonstrated presence of large fibre neuropathy was associated with several demographic factors including increasing age (OR of 1.08 (95%CI: 1.02-1.15, p<0.05) and greater height (OR of 1.1 (95%CI: 1.04-1.15, p<0.05) and time to peak perfusion after occlusion (OR of 1.02 (95%CI: 1-1.04, p<0.05). Presence of small fibre neuropathy in taller people (OR of 1.09 (95%CI of 1.03-1.15, p<0.05) and those with an increased time to peak perfusion after occlusion (OR1.02, 95%CI 1-1.03).
Conclusion: Greater height is associated with increased likelihood of the presence of both large and small fibre peripheral neuropathy. An increased time to peak following occlusion was also associated with increased likelihood of both types of neuropathy suggesting microvascular dysfunction measured by post occlusive reactive hyperaemia (specifically increased time to peak) is associated with higher likelihood of the presence of peripheral neuropathy.

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Association between biomarkers of subclinical inflammation and nerve conduction in individuals with newly diagnosed diabetes: German Diabetes Study

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Background and aims: Biomarkers of subclinical inflammation are associated with distal sensorimotor polyneuropathy, but their relationship to electrophysiological measures has not been assessed. Therefore, the aim of this study was to investigate associations between inflammation-related biomarkers and nerve conduction velocity (NCV) in the German Diabetes Study (GDS).
Materials and methods: Motor and sensory NCV was assessed in 513 patients with newly diagnosed type 1 and type 2 diabetes (161 T1D and 352 T2D; mean age 47±14 years, 64.5% male, BMI 29.5±6.4 kg/m^2, time since diagnosis of diabetes 6±3 months). NCV sum scores were calculated using z-transformed NCV for motor (N. medianus, N. ulnaris, N. peronaeus) and sensory (N. medianus, N. ulnaris, N. suralis) conduction. Associations between biomarkers of subclinical inflammation (C-reactive protein [CRP], interleukin [IL]-6 and IL-18, total and high-molecular-weight [HMW] adiponectin) and NCV sum scores were estimated using multiple linear regression models adjusted for time since diagnosis of diabetes, HbA1c, waist circumference, height, hypertension, cholesterol, smoking, physical activity, medication and history of myocardial infarction and/or stroke.
Results: In persons with T2D serum IL-6 and CRP were inversely associated with motor NCV (p=0.011 and p=0.052, respectively), and both total and HMW adiponectin were inversely associated with motor and sensory NCV (p between 0.001 and 0.033). In persons with T1D, total and HMW adiponectin showed positive associations with motor NCV (both p=0.02), but not with sensory NCV. Serum IL-18 was neither associated with motor nor sensory NCV in T1D and T2D.
Conclusion: Our results indicate that IL-6 may play a role in slowed motor NCV, but not in slowed sensory NCV in individuals with recent-

onset T2D. The lack of associations for CRP and IL-18, in contrast to IL-6, points to a differential rather than general association between inflammation-related biomarkers and NCV. The fact that associations between adiponectin and NCV appear to depend on diabetes type is intriguing and may reflect differences in the pathogenesis of both diabetes types.

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The centre of sub-basal corneal nerve fibres can best reflect alterations in the blood glucose concentrations in diabetes mellitus

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Background and aims: Small fibre neuropathy belongs to one of the most common long-term complications of diabetes mellitus (DM). In the assessment of diabetic neuropathy (DN), visualization and quantification of small nerve fibre damage is hitherto only possible by skin biopsy, thus, an invasive diagnostic tool. Another method, in vivo corneal confocal microscopy (IVCCM) is currently a matter of investigation to be established as a non-invasive procedure. The aim of this study was to elucidate the impact of streptozotocin (STZ) induced hyperglycaemia and subsequent insulin treatment on corneal und intraepidermal nerve fibre changes in thy1-YFP mice.
Materials and methods: Neuronal yellow fluorescent protein expressing B6.Cg-Tg(Thy1-YFP)16Jrs/J mice were injected by multiple low-dose injection of STZ (diabetic group n=15) or an equal volume of sodium citrate buffer (control group n=10). After induction of DM at day 20, half of the diabetic animals were treated with insulin releasing pellets. By using IVCCM, the subbasal nerve plexus in the centre of the cornea was examined at day 10, 20, 40 and 80. Corneal nerve fibre length (CNFL in mm/mm^2) was quantified by NeuronJ. At the endpoints, day 20 and 80, mice were euthanized for tissue extractions. Using fluorescence microscopy ex vivo, the nerve fibres from the periphery and the centre of the cornea were examined as well as skin biopsies taken from the plantar surface of the hind paw. Intraepidermal nerve fibre density (IENFD in number/mm) was determined according to the guidelines of the European Federation of Neurological Societies (EFNS).
Results: STZ-treated mice showed hyperglycaemia (23.87±0.87 vs. 8.36 ±0.44 mmol/l; p<0.0001), a significant increase in HbA1c values (46.89 ±1.36 vs. 22.09±1.42 mmol/mol; p<0.0001) and a decrease in body weight. Insulin treatment resulted in a normalization of blood glucose concentration (8.24±1.72 vs. 6.26±0.29 mmol/l; p<0.29), HbA1c values (26.78±1.94 vs. 20.00±1.17 mmol/mol; p<0.02) and body weight. However, higher levels of advanced glycation end products (AGEs) (24.62± 1.32 vs. 16.07±1.73 ng/ml; p<0.005) were observed at day 80. At day 20, the decrease of in vivo CNFL inversely correlated with hyperglycaemia. Ex vivo analyses revealed changes in the centre of the cornea, but not in peripheral parts. At day 80, in insulin treated mice recovery of central CNFL was observed. IENFD showed no significant changes to the diabetic group or age-matched controls at any time point.
Conclusion: Our study demonstrated that acute hyperglycaemia triggers subbasal corneal nerve fibre damage, whereas intraepidermal nerves remained unaffected. We hypothesize a higher susceptibility of the cornea to advanced glycation end products. However, after insulin therapy and blood glucose normalization, corneal nerve fibres start to regenerate. Additionally, it could be shown by ex vivo fluorescence analyses that nerve fibre damage and repair in DM take place from the centre of the cornea with a tendency to the inferior whorl. Integration of IVCCM in the diagnosis of DN in addition to quantitative sensory tests seems to be helpful, but needs further investigation to be a valuable tool.

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Nocturnal antihypertensive treatment restores 24 hour blood pressure profile in type 1 diabetic patients with autonomic neuropathy

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Background and aims: Cardiac autonomic neuropathy (CAN) and elevated nocturnal blood pressure are independent risk factors for cardiovascular disease in patients with diabetes and associations between CAN, non-dipping of nocturnal blood pressure and coronary artery disease have been demonstrated. The objective of the present study was to test the efficacy of bedtime dosing (BD) versus morning dosing (MD) of the angiotensin converting enzyme (ACE) inhibitor enalapril on 24 h blood pressure profile in patients with type 1 diabetes.
Materials and methods: In a randomised, double-blind, two-way cross-over study, 24 normoalbuminuric patients with long-term type 1 diabetes with CAN were treated for 12 weeks with either MD or BD of 20 mg enalapril, followed for 12 weeks of switched treatment regimen. During each treatment period, two 24 hour ambulatory blood pressure measurements were performed. The systolic blood pressure (SBP) and diastolic blood pressure (DBP) were measured automatically every 20 min during daytime (between 07 and 23 h) and once every hour during nighttime (between 23 and 07 h). SBP, DBP and mean arterial pressure (MAP) during daytime and nighttime and the dipping percentage was calculated using Spacelabs Healthcare Ambulatory Blood Pressure Report Management System version 3.0.0.9. Furthermore two computed tomography (CT) scans were performed after each treatment period measuring the left ventricle mass (LVM) and the left ventricle volume (LVV). The mean age of the patients were 60 +/- 7 (SD) years, 40% were males, BMI was 24.5 +/- 4.0 (SD), HbA_{1C} was 62 +/- 7 (SD) mmol/mol, creatinine was 66 +/- 13 (SD) μmol/L and the diabetes duration was 36 +/- 11 (SD) years. Sixty percent of the patients were treated with antihypertensive drugs (median 2 drugs) before the study.
Results: Nighttime SBP dipping increased 2.4 (0.03 - 4.9) % (p<0.05) with BD of enalapril compared with MD. Also, there was seen a trend towards a MAP dipping increase of 2.2 (-0.1 - 4.5) % (p=0.07) and a nighttime MAP reduction of 1.7 (-3.6 - 0.2) mmHg (p=0.07) with BD compared with MD. Daytime SBP and DBP were not significantly different between the two treatment regimens. A small, but insignificant reduction in pulse pressure was observed. No significant difference was found on LVM or LVV between the two treatment regiments. No adverse events were registered during nighttime treatment of blood pressure and no drop outs occurred during the study.
Conclusion: This study demonstrates for the first time that high risk long-term type 1 diabetic patients with autonomic neuropathy favourably and without risk can be treated with an ACE inhibitor during night. The potentially beneficial effect on long-term cardiovascular risk remains to be determined.

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Diabetic nephropathy lesions associate with cardiovascular autonomic neuropathy in Pima Indians with type 2 diabetes

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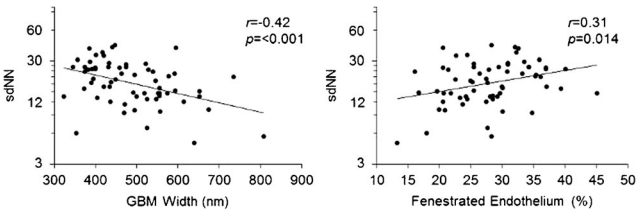
Background and aims: Cardiovascular autonomic neuropathy (CAN) and diabetic kidney disease (DKD) are thought to be linked. The association of CAN with renal structure is unknown. To identify the renal lesions that associate with CAN in Pima Indians with type 2 diabetes (T2D).

Materials and methods: Sixty-three Pima Indians (27% men) with T2D underwent kidney biopsy at baseline. Masked unbiased random sampling morphometric methods were used to measure renal structural parameters of DKD. Glomerular filtration rate (GFR; iothalamate), urinary albumin/creatinine ratio (ACR), and other clinical variables were measured at an examination within a median of 46 days of the biopsy. CAN was assessed after a mean follow-up of 9 years by standard cardiovascular reflex testing (deep-breathing test) and heart rate variability studies. Measures of CAN analyzed, included the expiration/inspiration ratio (E/I) and the standard deviation of the normal R-R intervals (sdNN). Associations of CAN with structural variables were assessed by Spearman's correlations and by multiple linear regression after adjusting for age, sex, diabetes duration, HbA1c, ACR and GFR. E/I and sdNN were log-transformed for linear regression modeling due to skewed distributions.

Results: Mean age at kidney biopsy was 46±9 years, diabetes duration 16±6 years, mean HbA1c 9.1±1.9%, mean GFR 156±57 ml/min, and median ACR 25 mg/g (IQR=9-68 mg/g). At follow-up, 95% of participants had CAN (E/I<1.17, median=1.04, IQR=1.03-1.08). E/I correlated positively with percentage of fenestrated endothelium (FE; r=0.26, p=0.040) and negatively with mesangial fractional volume (VvMes; r=-0.39, p=0.002) and glomerular basement membrane (GBM) width (r=-0.40, p=0.001). sdNN (median=18 ms, IQR=11-27 ms) correlated positively with glomerular filtration surface density (Sv; r=0.35, p=0.005) and negatively with VvMes (r=-0.34, p=0.007) and GBM width (r=-0.42, p=0.001). Neither E/I nor sdNN correlated with ACR or GFR. In linear regression analyses adjusted for age, sex, diabetes duration, HbA1c, ACR, and GFR, E/I was positively associated with FE (r=0.33, p=0.011) and negatively associated with VvMes (r=-0.32, p=0.018) and GBM width (r=-0.28, p=0.034). sdNN was positively associated with FE (r=0.31, p=0.019) and Sv (r=0.41, p=0.001) and negatively associated with VvMes (r=-0.28, p=0.035) and GBM width (r=-0.42, p=0.001). Adjusted associations between sdNN and morphometric variables are shown in Figure

Conclusion: Pima Indians with T2D and DKD have a high prevalence of CAN, which is more severe in those with more severe renal structural damage.



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Hypoxia as a reversible cause of cardiovascular autonomic dysfunction in patients type 2 diabetes and impaired renal function

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Background and aims: Cardiovascular autonomic dysfunction, characterized by an imbalance between sympathetic and parasympathetic activities, is common in patients with diabetes mellitus. Although this is conventionally considered to be an organic, irreversible disorder, it has already been demonstrated in patients with type 1 diabetes that baroreflex sensitivity (BRS) could be corrected by deep breathing, thus suggesting a functional component to the disorder. In this study we tested this hypothesis in the type 2 diabetic patients with or without renal impairment.

Materials and methods: Twenty-six patients affected by type 2 diabetes were enrolled (age 61.1±0.8 years, Mean±SEM; diabetic duration 10.5±2 years, BMI 29.9±0.7 kg/m2, GFR, [CKD-EPI formula]: 68.1±5.5 ml/min (range 18-105 ml/min). Six patients presented microalbuminuria and five macroalbuminuria. Twenty-four healthy subjects (age 58.5±1.0 years) were enrolled as controls. BRS was obtained from recordings of RR interval and systolic blood pressure fluctuations during spontaneous and slow, deep (6 breaths/min) controlled breathing. Then, the entire protocol was repeated in hyperoxia (breathing 5 L/min oxygen).

Results: During normal breathing diabetic patients presented higher HR and lower BRS compared with the control participants (5.5±0.6 vs 11.8±2.0 ms/mmHg, p<0.005). Deep breathing and oxygen administration significantly increased arterial saturation, reduced HR and increased BRS in both the diabetic patients and the control group and (to 9.6±1.9 and 15.3±2.3 ms/mmHg, respectively, P<0.05, hyperoxia vs normoxia). Twelve diabetic patients presented chronic kidney disease (CKD), defined by the presence of proteinuria and reduced GFR. CKD patients compared with non-CKD subjects showed a longer duration of diabetes and a poorer glycaemic control, with a significant inverse correlation between diabetes duration and GFR values (r2=0.20, p=0.02). However, also this group of patients presented a significant improvement of BRS during slow breathing and hyperoxia (p<0.05 vs spontaneous breathing in normoxia).

Conclusion: Our results show that autonomic dysfunction present in type 2 diabetic patients is further aggravated in CKD. However, this condition can be partially reversed by increasing oxygen supply, suggesting a possible role of hypoxia in the origin of diabetic complications. This finding supports the hypothesis that interventions known to relieve tissue hypoxia, like physical activity, may be useful in the prevention and management of complications in diabetic patients. The severity of autonomic dysfunction and response to oxygen seen in CKD call attention on hypoxia as a reversible pathogenic factor of the renal damage in diabetic patients.

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Erectile dysfunction among Bangladeshi diabetic men

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Background and aims: Erectile dysfunction (ED) is an important impediment to quality of life of men. ED is approximately, three times more common in diabetic than non-diabetic men, and diabetic men develop ED earlier than age matched non-diabetic subjects. Glycemic control and other factors may contribute in developing and or deteriorating ED. The

aim of the study was to determine the prevalence of ED and its risk factors in type 2 diabetic (T2DM) men in Bangladesh.
Materials and methods: During 2013-2014, 3980 diabetic men aged 30-69 years were interviewed at the out-patient departments of seven diabetic centers in Dhaka by using the validated Bengali version of the questionnaire of the international index of erectile function (IIEF) for evaluation of baseline erectile function (EF). The indexes indicate a very high correlation between the items and the questionnaire is consistently reliable. Data were analyzed with Chi-squared (χ^2) test using SPSS software. P≤0.05 was considered significant.
Results: Out of 3790, ED was found in 2046 (53.98%) of T2DM men. The prevalence of ED was increased with age from 10.5% in men aged 30-39 years to 33.6% in those aged over 60 years (P<0.001). In comparison with patients with reported diabetes lasting≤5 years (26.4%), the prevalence of ED was less than in those with diabetes of 6-11 years (35.3%) and of 12-30 years (42.5%, P <0.001). ED increased significantly in those who had poor glycemic control. The prevalence of ED in patients with good, fair and poor glycemic control was 22.8%, 42.5% and 47.9% respectively (P=0.004). Treatment modalities (medical nutrition therapy, oral agents, insulin and insulin plus oral agents) had significant association with ED and its severity (P<0.001).
Conclusion: Prevalence of ED is very high among T2DM men in Bangladesh and can be reduced the burden by improving glycemic status. Glycemic control, duration of diabetes, treatment modalities, increasing age are associated with ED.

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Low gastrointestinal symptom burden in a Swedish population based cohort with insulin dependent diabetes mellitus

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Background and aims: Long-standing diabetes mellitus (DM) is afflicted with a wide variety of complications that may cause symptoms from multiple organ systems including the gastrointestinal (GI) tract. Data from other centers have claimed that autonomic neuropathy affects up to 30-40% of patients with long-standing disease and as many as 75% of patients attending DM clinics report significant GI symptoms The aim of our study was to understand the extent to which a defined cohort of Swedish patients with DM suffers from GI symptoms.

Materials and methods: Patients with insulin dependent DM type 1 (IDDM1) and type 2 (IDDM2) who attended the diabetic outpatient clinics at two county hospitals were included in the study. They were sent the Patient Assessment of upper GastroIntestinal Symptom Severity Index (PAGI-SYM) questionnaire for assessment of GI symptom severity which consists of 20 questions answered by use of a six-point Likert response scale, ranging from 0 (none) to 5 (very severe). The Gastroparesis Cardinal Symptom Index (GCSI) can be calculated by use of the first nine questions of PAGI-SYM resulting in scores for total symptom burden, nausea/vomiting, postprandial fullness/early satiety and bloating. Hospital anxiety and depression (HAD) scale was used for psychological assessment and the short form (SF) 36 questionnaire summary measures for physical and mental health. For those who answered the questionnaires (one reminder to non-responders), clinical information was retrieved from medical records regarding complications related to long-standing DM such as manifestations from large vessel atherosclerosis (ischemic heart disease and stroke), peripheral neuropathy, retinopathy and nephropathy.
Results: We included 754 patients (mean age 48 years (range18-82 years). The questionnaire was answered by 436 patients (response rate 57.8%), and 363 (83.3%) of these had IDDM1 and 73 (16.7%) had IDDM2. The duration of insulin treatment was 21±13 years. The mean GCSI total score in the cohort was 0.7±0.8. Females with IDDM had significantly higher GCSI total score (0.853 vs. .622; p=0.003), GCSI nausea/vomiting score (0.42 vs. 0.28; p=0.019) and GCSI bloating score (1.2 vs. 0.81; p=0.001) compared to males. There was a strong correlation(p=0.001) between SF-36,HAD and the GCSI total, nausea/vomiting and bloating scores. There was no significant difference between sexes for GCSI postprandial fullness/early satiety score. There was no significant difference in GCSI scores comparing patients with IDDM1 and IDDM2, also not within sexes. Patients with one or more complications related to long-standing DM reported GI symptoms to the same extent as those with no complications and more complications was not associated with higher GCSI scores.
Conclusion: Females with IDDM reported more GI symptoms compared with males. Although the total GI symptom burden in our study corresponded to "very mild" there was a highly significant correlation between the GCSI subscores; total, nausea/vomiting, bloating and mental and physical health and anxiety and depression. Furthermore it appears that the number complications to long standing DM do not per se imply an increase in reported GI symptoms.

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Cardiovascular autonomic neuropathy predicts development of diabetic foot ulcer in patients with type 2 diabetes mellitus

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Background and aims: We investigated potential risk factors that might influence the development of diabetic foot ulcers in patients with type 2 diabetes.
Materials and methods: From January 2003 to December 2004, a total of 1,534 patients with type 2 diabetes were consecutively enrolled. Significant diabetic foot ulcer was defined as a full-thickness break in the epithelium with a minimum width of 5 mm. Patients were checked every 3-6 months for status of diabetic foot ulcer, or amputation during the follow-up periods. A cardiovascular autonomic function test (AFT) was performed to diagnose cardiovascular autonomic neuropathy (CAN) using heart rate variability parameters. We used a Cox proportional hazard regression analysis to test associations between diabetic foot ulcer and potential explanatory variables.
Results: The median follow-up time was 9.7 years. The mean age was 55.6±10.8 years, and the duration of diabetes was 8.3±7.0 years. During the follow-up periods, 135 patients (8.8%) developed new ulcer, and 26 patients (1.7%) underwent an amputation. The patients in the diabetic foot ulcer group had a longer duration of diabetes (p<0.001), higher baseline HbA_{1c} levels (p<0.001), higher rates of smoking (p=0.001), albuminuria (p<0.001), retinopathy (p<0.001), and received more insulin treatment (p<0.001). A Cox hazard regression analysis revealed that the development of diabetic foot ulcer was significantly associated with the presence of cardiovascular autonomic dysfunction (normal vs early CAN, HR 2.06, 95% CI 0.95-4.45; p=0.066; normal vs definite CAN, HR 2.50, 95% CI 1.18-5.26; p=0.010) after adjusting for sex, age, diabetic duration, mean HbA_{1c}, albuminuria, retinopathy, and treatment of insulin.
Conclusion: The development of diabetic foot ulcers was independently associated with cardiovascular autonomic dysfunction in patients with type 2 diabetes.

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Short-term beat-to-beat QT-interval variability in patients with impaired glucose tolerance

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Background and aims: Determination of short-term QT-interval variability (QTV) is a non-invasive method for assessment of proarrhythmic risk. The aim of the present study was to evaluate the QTV in patients with impaired glucose tolerance (IGT).
Materials and methods: 18 IGT patients (age: 63.0±2.7 years, BMI: 31.4±1.5 kg/m2, fasting glucose: 6.0±0.1 mmol/l, 120 min postload glucose: 9.0±0.2 mmol/l, HbA1c: 5.9±0.1%; mean±SEM) and 18 healthy controls (age: 56.1±2.2 years, BMI: 27.3±1.3 kg/m2, fasting glucose: 5.2±0.1 mmol/l, 120 min postload glucose: 5.5±0.3 mmol/l, HbA1c: 5.4±0.1%) were enrolled into the study. ECGs were recorded, processed, stored and analyzed off-line using a computer based analyzing system. The RR and QT intervals were expressed as the average of 31 consecutive beats. The ventricular repolarization was characterized by uncorrected QT-interval and QT intervals corrected by Bazett (QTc-b), Fridericia (QTc-f) and Hodges (QTc-h) formula. The spatial repolarization inhomogenity was specified by QT dispersion (QTd). To evaluate the short-term temporal instability of beat-to-beat repolarization, Poincare' plots of the QT intervals were constructed, where each QT value is plotted against its former value. QTV was calculated using the following formula: $QTV=\sum|QTn+1-QTn|\ (30\times\sqrt{2})$-1. Autonomic function was assessed by means of five standard cardiovascular reflex tests using the same analyzing system.
Results: Comparison of the two groups (IGT vs control) revealed no difference in the uncorrected QT (410±10.2 ms vs 409±11.5 ms), QTc-b (431±5.8 ms vs 427±6.8 ms), QTc-f (424±6.3 ms vs 421±7.8 ms), or QTc-h (424±6.9 ms vs 420±8.2 ms) intervals. There was no difference in the QTd between IGT and control individuals (43.8±3.0 ms vs 43.1± 5.6 ms). The QTV, however, was increased in the IGT patients relative to those for the control group (4.95±0.16 ms vs 3.82±0.23, p=0.0004).
Conclusion: The short-term QT variability may be elevated in patients with impaired glucose tolerance. This enhanced temporal QT variability may early indicate the increased instability of cardiac repolarization.
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Changes in vago-sympathetic activity and its influence on heart rate and insulin response to oral glucose load

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Background and aims: We showed that cardiac parasympathetic activity is often altered in non diabetic obese patients and similarly in metabolically healthy obese patients. The aim of this study was in obese patients with a metabolically healthy phenotype to examine the influence of the changes in vago-sympathetic activity on heart rate and insulin response to oral glucose load (OGTT).
Materials and methods: We recruited 24 patients, aged 34±13 years, BMI 42.2+/-6.9 kg/m^2, with only one criterion of the metabolic syndrome (hyperglycemia in only one patient) in addition to large waist circumference. An oral glucose tolerance test (OGTT, 75 g) was performed. Composite indexes of insulin secretion (insulinogenic index=IGI= Δinsulinemia(T0-T30)/Δglycemia(T0-T30), and oral disposition index= ODI=IGI/insulinemia) were calculated. Cardiac parasympathetic activity (HF-HR), sympatho-vagal balance (LF/HF-HR), and vascular sympathetic activity (LF-SBP) were evaluated by spectral analysis of heart rate and systolic blood pressure (SBP) variations (Finapress®), and plasma epinephrine and norepinephrine levels were measured at fasting and during the OGTT.
Results: Patients were separated in two groups according to cardiac parasympathetic activity at fasting: high or low (HF-HR>or<median value: 2.78). During OGTT the patients with high parasympathetic activity at fasting maintained a higher HF-HR ($p<0.001$) despite a transient decrease after insulin peak. These patients had lower LF/HF-HR at fasting which remained lower during OGTT ($p=0.019$). Epinephrine and norepinephrine response did not differ significantly between the two groups. A significant acceleration of heart rate occurred during OGTT in the two groups ($p=0.002$) but heart rate was lower both at fasting and during OGTT in the patients with high parasympathetic activity ($p=0.03$). High parasympathetic activity was associated with higher ODI ($p=0.035$).
Conclusion: Among obese patients with metabolically healthy phenotype those with a higher parasympathetic activity keep a higher parasympathetic activity after glucose intake. This profile accounts for a lower heart rate at fasting and after glucose load and seems to contribute to a greater insulin secretion.

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Why not to use the handgrip test in the assessment of cardiovascular autonomic neuropathy?

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Background and aims: The cardiovascular autonomic reflex tests (CARTs) have been considered as gold standard in the assessment of cardiovascular autonomic neuropathy (CAN). However, only four of the five tests have been currently suggested to be performed - sympathetic function should be evaluated by the orthostatic hypotension (OHT) test while measuring diastolic blood pressure (BP) response to sustained handgrip is omitted in recent guidelines. Aim of our study was to assess the relationship of handgrip test with the other tests in detecting CAN as well as to identify the factors having impact on the handgrip test results in diabetic patients.
Materials and methods: Our study involved 353 patients with diabetes (age: 60,2±7,4 years; female: 57,2%; BMI: 29,3±2,1 kg/m2; diabetes duration: 15,6±9,9 years; HbA1c: 8,2±1,9%; with type 1 diabetes: 18, 1%). CAN was assessed by five CARTs: the deep breathing test, Valsalva ratio, 30/15 ratio, handgrip and OHT test. CARTs based on heart rate changes mainly reflect parasympathetic while those based on BP response to manoeuvres explore sympathetic function. Connection between results of the handgrip and the other CARTs as well as clinical parameters of the study population were analysed using Kendall monotony coefficient (γ) and Spearman's correlation (ρ). Dichotomous variables were analysed using $\chi 2$-test.
Results: Definite diagnosis of CAN according to recent guidelines as well as abnormal results of the deep breathing, Valsalva ratio, 30/15 ratio,

handgrip test and OHT test were present in 49,3%, 86,1%, 35,1%, 4,5%, 22%, and 32,3%, respectively. Results of the handgrip test did not show any association with results of the deep breathing test (p=0,897) and 30/15 ratio (p=0,357), as they did not do with the results of the partially sympathetically controlled Valsalva ratio, either (p=0,781). An association between handgrip and OHT failed to be proven (p=0,833). Sensitivity, specificity, positive and negative predictive value of the handgrip test versus definite diagnosis of CAN were 22,9%, 78,8%, 51% and 51,3%, respectively. The results of handgrip test did actually show a significant negative association with the presence of hypertension (γ=-0,315, p=0,009), whereas the diastolic BP elevation during the manoeuvre was inversely related to the baseline diastolic BP (ρ=-0,283, p=0,013). In multiple logistic regression analysis, the significant inverse association between abnormality of the handgrip test and hypertension was independent of age, gender, BMI, diabetes duration, type of antihypertensive medication, insulin treatment as well as presence of sensory loss and neuropathic pain medication. OHT was associated with the severity of the parasympathetic impairment (γ=0,303, p<0,0001) and with Valsalva ratio abnormality (γ=0,342, p<0,0001). Presence of hypertension had no effect on the results of OHT test (p=0,97).

Conclusion: Our data may provide evidence that the handgrip test should no longer be part of the cardiovascular autonomic testing being highly dependent of the hypertensive status and baseline blood pressure. The putative mechanism for the inverse association between abnormal results of the handgrip test and hypertension could be an exaggerated exercise pressor reflex related to increased sympathetic cardiovascular activation in patients with diabetes and hypertension.

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Integrated cardiovascular/respiratory control in type 1 diabetes

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Background and aims: Diabetic autonomic dysfunction and ventilatory control abnormalities both increase cardiovascular risk. Cardiovascular (baroreflex) and respiratory (chemoreflex) control mechanisms were studied separately in diabetes, but their reciprocal interaction had never been assessed. We hypothesized that prevalent autonomic neuropathy would depress both chemoreflexes and the arterial baroreflex mechanisms, whereas prevalent autonomic imbalance through sympathic activation would depress the baroreflex but enhance chemoreflex mechanisms.

Materials and methods: In 46 type-1 diabetic subjects (33±1 yr, 22 female) and in 103 age-matched controls we measured RR interval, continuous noninvasive blood pressure (Colin®), minute ventilation, oxygen saturation (SaO2) and end-tidal carbon dioxide (CO2) at rest and during progressive normoxic hypercapnia (from baseline to 55 mmHg) and progressive isocapnic hypoxia (from baseline down to 80% SaO2). Baroreflex sensitivity was estimated by a battery of 6 standardized methods (based on spectral analysis and spontaneous sequences of RR interval and Systolic Blood pressure variabilities), hypercapnic and hypoxic chemoreflex sensitivities were obtained by the slopes of the linear regressions linking minute ventilation to CO2 increase and to SaO2 decrease, respectively. Autonomic neuropathy was evaluated by a standard battery of cardiovascular reflex tests (deep breathing, lying-to-standing, handgrip, Valsalva manoeuvre, blood pressure changes to tilting).

Results: Mild signs of autonomic neuropathy were present in 26/46 diabetics (average score 1.42±0.14, group N+), and absent in 20/46 patients (group N-). Baroreflex sensitivity was reduced in the entire diabetic group (9.8±0.9 vs controls: 13.4±0.8 ms/mmHg, p<0.01), and particularly in group N+ (8.5±0.9 ms/mmHg, p<0.01), whereas although reduced, it was not significantly different in N- diabetics (11.7±1.6 ms/mmHg, p:ns). The hypercapnic chemoreflex was significantly increased in the entire diabetic group (1.86±0.19 vs 1.41±0.13 L/min/mmHgCO2 in controls, p<0.05), and particularly in N+ diabetics (2.18±0.28 L/min/mmHgCO2, p<0.05). Conversely, the hypoxic chemoreflex was slightly depressed in the entire diabetic group (0.73±0.9 vs controls: 0.91±0.07 L/min/%SaO2, p:ns), and equally reduced in N+ and N- groups.

Conclusion: Thus, a reduced sensitivity to hypoxia seems a primary factor leading to reflex sympathetic activation (enhanced hypercapnic chemoreflex and depression of baroreflex), hence suggesting a functional origin of autonomic abnormalities in initial diabetes.

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Parasympathetic autonomic dysfunction rather than central afferent impairment is the early sign of neuropathy in recently diagnosed type 2 diabetes

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Background and aims: It is well known that hyperglycemia has a long-term harmful effect on nerve fibres, but the shortest period of diabetic exposure leading to the appearance of different neuronal dyfunctions is poorly documented. The aim of this study was to characterize the presence and severity of autonomic and central nerve function in patients with recently diagnosed type 1 or type 2 diabetes (DM).

Materials and methods: 40 patients with type 1 or type 2 DM were involved (type 1: n=21, age: 28.8±2 years, duration of DM: 2.1±0.5 months; type 2 DM: n=19, age: 48.4±3.9 years, duration of DM: 3.3±0.8 months; mean±SE). Age and sex matched subjects served as controls. Cardiovascular reflex tests (CRT) were applied for the assessment of autonomic neuropathy (AN). The afferent central pathways were measured by analysis of the latencies of the four waves (I-IV/V) of brainstem auditory-evoked potentials (BAEP).

Results: The results of the CRT-s and the latencies of BAEP did not differ from controls among recently diagnosed type 1 DM patients. The overall AN score was higher in recently discovered type 2 DM than in controls (AN score: 2.16±0.5 vs 0.33±0.1, p<0.01; diabetic vs control). The heart rate response to breathing was lower in type 2 DM patients than in controls (17±2.1 vs 23.1±2.3 min., p<0.05) and a negative correlation was found between Valsalva ratio and the wave V. latency of BAEP (r=-0.61, p<0.01). There was a non-significant tendency of longer latencies of all BAEP waves in type 2 DM patients than in controls.

Conclusion: The autonomic and central neuronal function seems to be intact among type 1 diabetic patients with short duration. Early to moderate impairment of parasympathetic cardiovascular autonomic function was found in patients with recently diagnosed type 2 diabetes. The more abnormal values of a parasympathetic test correlated with the latency of a wave of the auditory-evoked potentials. Our data support that autonomic parasympathetic dysfunction rather than central afferent impairment should be considered as the early sign of neuropathy in recently diagnosed type 2 diabetes.

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Main determinants of autonomic dysfunction in subjects with normoglycaemia and newly-diagnosed type 2 diabetes

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Background and aims: Cardiovascular autonomic dysfunction (CAD) is a serious and common complication of diabetes associated with increased cardiovascular morbidity and mortality. As it occurs even at the early stages of glucose intolerance and insulin resistance the present study aims to outline main predictors of CAD in subjects with normal glucose tolerance (NGT) and newly-diagnosed type 2 diabetes (NDT2D) with or without metabolic syndrome (MetS).
Materials and methods: A total of 200 subjects - 108 females and 92 males (mean age 49.8±10.3 years, mean BMI 31.1±5.8 kg/m2), divided into two age- and BMI-matched groups according to their glucose tolerance: 100 with NGT and 100 with NDT2D, and subdivided into 4 groups according to the presence of MetS; and into two groups according to the presence of CAD - 42 with CAD and 158 controls, were enrolled. Glucose tolerance was studied during OGTT. Anthropometric indices, blood pressure (BP), HbA1c, serum lipids and hsCRP were measured. ECG for calculating QTc interval was performed. AGEs were evaluated by skin autofluorescence (AGE-Reader). Body composition was estimated by impedance analysis (InBody 720). Cardiovascular autonomic function (CAF) was assessed by ANX-3.0 using frequency-domain analysis at rest and during deep breathing, Valsalva and standing from a seated position. Statistical analysis was performed using SPSS v.20.0.
Results: A higher prevalence of CAD in NDT2D as compared to NGT - 27/100 (64.3%) vs 15/100 (35.7%), OR 1.4 (95%CI: 1.05 to 1.84), p= 0.037 was observed. Despite the trend toward lower autonomic power in the subgroups with MetS, the difference was not statistically significant. CAF showed a significant negative correlation with age, visceral fat area, total body fat, systolic BP, AGEs, and QTc interval in both groups with NDT2D and NGT. Although significantly elevated levels of triglycerides, total cholesterol, BP, HbA1c, fasting glucose, AGEs, QTc interval and age were found in the group with CAD in comparison to controls, after performing logistic regression analysis, $\chi 2(4)=26.385$, $p<0.0001$ with R2 of 0.021, correctly classifying 81.0% of cases, only increasing age - OR 1.06 (95%CI: 1.02 to 1.1), p=0.005 and the presence of hypertension - OR 2.8 (95%CI: 1.2 to 6.7), p=0.019 were associated with an increased likelihood of CAD - AUC 0.762 (95%CI: 0.682 to 0.842), $p<0.0001$.
Conclusion: Our results demonstrate increased frequency of CAD in NDT2D independently of the presence of metabolic syndrome. Hypertension and age seem to be the main predictors of CAD in the early stages of impaired glucose metabolism.
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Vitamin B12 deficiency is associated with cardiovascular autonomic neuropathy in patients with type 2 diabetes

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Background and aims: Cardiovascular autonomic neuropathy (CAN) is a severe diabetic complication associated with increased cardiovascular mortality. The risk of CAN in diabetes 2 patients could be additionally increased by vitamin B12 deficiency which is known to be a risk factor for peripheral neuropathy and has also been associated with markers of CAN in non-diabetic patients. Type 2 diabetes patients are prone to B12 deficiency by the use of metformin and possibly proton pump inhibitors (PPIs). We aim to be the first to investigate the possible cross-sectional association between serum levels of Vitamin B12 and CAN in patients with type 2 diabetes.
Materials and methods: 512 type 2 diabetes patients (mean diabetes duration 10.0 years, mean age 58.7 year, 34.9% men, 14.5% with CAN) had serum vitamin B12 measured and were screened for CAN using 3 standard cardiovascular reflex tests (CARTS): deep breathing test (E:I-ratio), lying-to-standing test (30/15) and the Valsalva maneuver. 5 minute resting heart rate (5 min RHR) was also measured. CAN was diagnosed if at least 2 of 3 CARTS were abnormal. Associations between vitamin B12 and outcomes were assessed by using logistic and linear regression models adjusting for age and sex.
Results: We found associations between levels of serum vitamin B12 and the E:I-ratio, an early marker of CAN. A 25 pmol/l increase of vitamin B12 was associated with an increase in E:I-ratio of 0.22% (P=0.038, 95%Cl 0.01-0.43) . No significant associations between 5 min RHR, the 30/15 test, the Valsalva maneuver or the CAN diagnosis were found possibly be due to lack of statistical power.
Conclusion: Serum vitamin B12 is associated with early CAN in patients with type 2 diabetes prompting the need for annual vitamin B12 screening in high risk patients e.g. those treated with metformin and/or PPIs.
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Patients and health care provider related delay in the treatment of diabetic foot ulcers

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Background and aims: Diabetic foot ulcer is a life threatening complication and very costly both for the health care system and the patient. Prompt treatment is necessary to reduce the risk of deep infections and amputations. Time from ulcer to treatment is an important determinant of treatment outcome. The aim was to study factors that influence healing time in patients with diabetic foot ulcers seen in a multidisciplinary outpatient clinic.
Materials and methods: Newly referred patients between Sep. 9th 2013 and March 9th 2014 were identified and primarily analysed for time between ulcer to contact, and contact to appointment in relation to various baseline and outcome variables. Data are shown as mean±standard deviation (SD). Non-parametric statistical analyses were used, and a p-value below 0.05 was considered statistically significant.
Results: 118 patients presenting 193 ulcers were identified: Age 65.9± 1.1 years (89 [75%] males), diabetes duration 27.8±1.3 years (47 type 1 diabetes [40%]), BMI 29.8±0.5 kg/m2, HbA1c 66.9±1.4 mmol/mol, total cholesterol 4.6±0.1 mmol/L, LDL-cholesterol 2.3±0.1 mmol/L, HDL-cholesterol 1.3±0.05 mmol/L, triglycerides 2.3±0.2 mmol/L, and blood pressure 137±1.8/74±1 mm Hg. 21% were smokers and 29% had a previous history of amputation; more common among males (35%) than females (10%), p=0.01. Total loss of vibration sense >50 volt was seen in right 59% and left foot 66%, and only 17% showed no signs of retinopathy. 29 patients were normoalbuminuric (26%), 47 micro- (42%) and 37 had macro-albuminuria (33%). 45% had lacking palpable peripheral pulses.Time before contacting the service (contact delay time) was 17± 2 days, and wound healing time was 8±0.6 weeks (n=164). Patients were offered an appointment within 24 hours for 165 of the wounds (86%), and within a week for another 11%. There was no difference in contact delay time between males and females (p=0.6). Wound healing time was positively correlated with contact delay time (r=0.2, p=0.01) which was also the only independent predictor of wound healing time as judged by a multiple regression analysis including relevant co-variates such as age, gender, diabetes duration, metabolic control, co-morbidities and complications, although the model explained less than 10% of the variability in healing time (r2=0.09, p=0.02).
Conclusion: Diabetic foot ulcer healing in a multidisciplinary setting is dependent on patient lag time to contact the clinic. Further investigation as to the underlying reasons and remedies for this delay is warranted in order to further improve the long term prognosis of diabetic foot ulcers.

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Patients' adherence to customised diabetic insoles as objectively assessed by a temperature sensor

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Background and aims: Customized diabetic insoles reduce the mechanical stress by re-distributing pressure to the plantar tissue. Thus, customized diabetic insoles are an effective means to prevent the reoccurrence of neuropathic diabetic foot ulcerations. However, the efficacy of these insoles is highly dependent on patients' adherence. By recommendation, patients should wear their customized diabetic insoles as much as possible for the prevention of diabetic foot problems. However, adherence data often rely on self-report since objective parameters are not available. The aim of this study was to objectively assess patients' adherence with a temperature sensor directly incorporated into their insoles.
Materials and methods: In a pilot study, the cut-off value for optimal temperature was determined that differentiates between wearing and not wearing footwear. For this purpose, a ROC analysis was conducted that yielded an area under the curve of .996 (p<.0001). A cut-off value of 25°Celsius was determined that achieved a sensitivity of 95.3%, a specificity of 99.8%, a positive predictive value of 98.7%, and a negative predictive value of 99.2%. In the main study, temperature sensors were incorporated into the specialized diabetic insoles of 26 patients with type-2-diabetes and diabetic foot syndrome (age: 67.5±10.8 yrs.; 35% female; BMI: 30.3± 4.7 kg/m^2; diabetes duration: 10.4±6.8 yrs.; HbA1c: 7.7±0.6%).
Results: On average, data from 133.5 days per patient could be analysed. Patients wore their diabetic footwear (temperature>25°C) on an average (median) of 3.4 hours per day (inter-quartile-range (IQR): 0.5 - 6.9 hours/ per day). On an average (median) of 51% of days, patients did not wear their diabetic footwear (IQR: 16.9 - 81.8%).
Conclusion: Wearing time of diabetic insoles and other specialized diabetic footwear can be objectively and validly assessed by temperature sensors. This study offers objective data regarding patients' adherence to their customized diabetic insoles. Nearly every second day, patients did not wear their insoles at all. Results of this study indicate that the utilization of specialized diabetic footwear is suboptimal in order to prevent re-ulcerations and other diabetes foot problems. Future studies should examine how the adherence of patients with a high risk for foot ulcerations can be enhanced, e.g. by patient education or technological assistance or reminders.
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The ABCDEF classification of perfusion in the diabetic foot

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Background and aims: The diabetic ischaemic foot is difficult to assess and treatment is often delayed. There is no consensus on a standardised assessment of foot perfusion in patients presenting with diabetic foot ulceration. A new classification system that grades the varying degrees of perfusion in the diabetic foot is needed to match assessment with management in individual patients. The aim was to create a new classification that reflects the spectrum of perfusion.
Materials and methods: We recruited consecutive patients attending the diabetes foot clinic with an ulcer in one or both feet. We measured brachial and toe blood pressure (TBP) and derived the toe brachial index (TBPI) in both feet. We then measured transcutaneous oxygen tension ($TcP0_2$), at the dorsum of both feet in the supine position and on the forearm, and derived the baseline $TcP0_2$ Index as a ratio of $TcP0_2$ on the foot compared to the arm. If the foot $TcP0_2$ was ≥40 mmHg, we then measured it after a provocation test of 30^o leg elevation for 5 minutes. If the supine $TcP0_2$ was <40 mmHg, we measured it after an oxygen challenge (inhalation of 100% oxygen for 10 min), to assess the restoration of the $TcPO_2$ Index after oxygen inhalation. All measurements were done with the PeriFlux System 5000.
Results: We studied 236 limbs in 130 patients. Hundred and seven limbs had a TBPI of ≥0.7 and baseline $TcP0_2$ Index of 0.84±0.19 (Mean ± SD), indicating that these limbs had good perfusion and were graded as Group

A. There were 129 limbs with TBPI <0.7 of which 91 had $TcPO_2 \geq$ 40 mmHg; 46/91 of these limbs had a sustained $TcPO_2$ that did not fall on leg elevation and were graded as Group B, but 45/91 limbs had a fall of ≥10 mmHg on elevation and were graded as Group C. TBP was significantly greater at 75±25 mmHg in Group B compared with 56±18 mmHg in Group C [p<0.001]. Thirty eight of the 129 limbs with TBPI <0.7 had $TcPO_2$<40 mmHg. Eleven of the 38 limbs responded to the oxygen challenge, with restoration of their $TcP0_2$ Index (1.06±0.15) and were graded as Group D, but 27/38 did not respond to oxygen challenge. Sixteen of the 27 were graded as Group E, with a baseline $TcPO_2 \geq$ 10 mmHg but <40 mmHg and the remaining 11 were graded as Group F, having a baseline $TcPO_2$<10 mmHg. TBP were not significantly different between Group E and F, 48±21 vs 40±28 mmHg respectively [p= 0.203], but Groups E and F were distinguished by their response to oxygen inhalation, the $TcPO_2$ Index after oxygen inhalation being 0.55 ±0.15 in Group E vs 0.26±0.25 in Group F [p<0.001].

Conclusion: We have classified 6 grades of perfusion, using a 3 step approach of TBPI, $TcPO_2$ and a provocation test. Group A: TBPI ≥0.7, Group B: TBPI <0.7 $TcPO_2 \geq$40 mmHg sustained on elevation, Group C: TBPI <0.7, $TcPO_2 \geq$40 mmHg falling on elevation, Group D: TBPI <0.7, $TcPO_2$<40 mmHg with restoration of $TcPO_2$ Index on oxygen challenge, Group E: TBPI <0.7, TcPO2<40 mmHg but >10 mmHg with failure to restore $TcPO_2$ Index and Group F: TBPI <0.7, with failure to restore $TcPO_2$ Index and TcPO2<10 mmHg. This study has derived a novel classification of diabetic foot perfusion, namely the ABCDEF classification, which objectively stratifies the diabetic foot into 6 groups, thus facilitating the standardisation of assessment and management of the diabetic foot.

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Toe-brachial index is associated more strongly with outcome of chronic diabetic foot ulcers than ankle-brachial index in patients with type 2 diabetes

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Background and aims: Chronic diabetic foot ulcers remain a significant problem in individuals with diabetes and precede 84% of all diabetes-related lower-leg amputations. Such ulcers are responsible for a prolonged period of hospitalization and co- morbidities caused by infected diabetic foot ulcers. Both micro- and macroangiopathy strongly contribute to the development and delayed healing of diabetic wounds. Peripheral arterial disease (PAD) can be diagnosed noninvasively by segmental blood pressure measurement and calculating an ankle-brachial index (ABI) or toe-brachial index (TBI). The toe vessels are less susceptible to vessel stiffness, which makes the TBI useful. The objective of this study was to determine whether ankle-brachial index or toe-brachial index can influence outcome of chronic diabetic foot ulcers in patients with type 2 diabetes.

Materials and methods: We recruited a total of 89 type 2 diabetic patients (58 men and 31 women) with chronic nonhealing diabetic foot ulcers with Wagner grade 1 or 2 ulcers that are ≥2 cm in largest diameter at diagnosis for more than 1-month duration. The age was 67.4± 15.9 years, and the diabetes duration was 15.8±12.3 years. Anthropometric, clinical, and laboratory data were measured. All patients were seen biweekly for debridement, offloading, and other treatments during the initial 8 weeks. All patients subsequently underwent ABI and TBI testing. ABI and TBI measurements were performed with the subject in a supine position, and were determined as the ratio of ankle or toe systolic blood pressure to the brachial systolic blood pressure, with both determined using an automatic device.

Results: At 8 weeks, 56 of the 89 ulcers had completely healed. The patients were assigned into healed group (n=56) or unhealed group (n= 33) according to clinical outcome of ulcer healing at 8 weeks. There were not significantly different in age, duration of diabetes, HbA1c, or initial wound size in diameter of the ulcer between the healed and unhealed groups. The healing time of foot ulcers in healed group was 4.3± 3.1 weeks (range 2.1-7.8). The TBI was significantly (P<0.05) lower in the unhealed group (0.58±0.21 cm/s) as compared with the healed group (0.71±0.19 cm/s). ABI was not significantly different between unhealed and healed group. Age (r=0.533; p<0.01), duration of diabetes (r=0.459; p<0.05) and ABI (r=0.725; p<0.001) were significantly correlated with TBI. But BMI, cholesterol levels, HbA1c, and systolic and diastolic BP were not correlated with TBI. Univariate analysis revealed that TBI was significantly correlated with healing rate of diabetic ulcers (r=0.296, p< 0.05) and duration of diabetes (r=0.403, p<0.05).

Conclusion: This study demonstrated that toe-brachial index is more strongly associated with the healing time of diabetic ulcers than ankle-brachial index. We suggest that the measurement of toe-brachial index may help to identify ulcers at risk of poor healing in chronic diabetic foot ulcers in patients with type 2 diabetes.

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Do ulcer characteristics and cardiovascular risk factors predict healing and mortality in people with diabetic foot ulcers in northern England?

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Background and aims: This population based study examines foot disease and non-foot disease associations with ulcer healing and mortality among people with incident diabetic foot ulcers in a 'real world' longitudinal cohort.

Materials and methods: In the city of Salford routine electronic clinical records are kept by podiatrists for all people with an incident diabetic foot ulcer. Where possible these records have been linked to complementary primary and secondary care electronic health records to identify patient characteristics, cardiovascular risk factors and death registrations to June 2013. Regression and survival models were created to examine factors associated with ulcer healing and mortality.

Results: The foot care records of 1728 out of 2977 Salford residents presenting with foot ulcers between 2001 and 2012 could be linked to their general health record to provide sufficient data for analysis. 55.1% of ulcers healed within 90 days. Age (OR 0.99 per additional year of age, 95% CI 0.98-0.99) and being male (OR 0.74 95% CI 0.60-0.92), increased depth (probing to bone) (OR 0.22 95% CI 0.08-0.60), rear foot location (OR 0.68 95% CI 0.49-0.92), peripheral vascular disease (OR 0.52 95% CI 0.39-0.69) and blood pressure below the current NICE recommended target for people with vascular disease (130/80) (OR 0.64 95% CI 0.51-0.79) were associated with a lower chance of healing in 90 days. Total cholesterol below 5 mmol/l was linked with ulcer healing (OR 1.20 95% CI 1.01-1.41) in 90 days, whereas kidney function was inversely related to the chance of healing in 90 days (OR compared to eGFR 90+ for eGFR 60-89 1.27 95% CI 1.01-1.60, eGFR 30-59 1.71 95 CI 1.19-2.47, eGFR <30 4.57 95% 0.52-39.92). Cellulitis, type of diabetes, HbA1c, body mass index and smoking were not associated with ulcer healing rates at 90 days. In this cohort mortality one year from initial presentation was 13.1% and 19.5% at two years. Increasing age (HR 1.10 per additional year of age, 95% CI 1.09-1.11) and being male (HR 1.31 95% CI 1.08-1.60) were associated with shorter survival. Peripheral vascular disease (HR 1.61 95% CI 1.30 - 1.99) and rear foot ulcers (HR 1.72 95% CI 1.35 - 2.20) were associated with shorter survival. Smokers had significantly higher mortality than those who had never smoked (HR 1.71 95% CI 1.30 - 2.25) whilst ex-smokers showed a non-significant raised risk of dying (HR 1.19 95% CI 0.97 - 1.47). Blood pressure below 130/80 was associated with higher mortality (HR 1.39 95% CI 1.15 -

1.69). Poor kidney function was associated with higher mortality but only reached statistical significance with very advanced disease (eGFr <15 (OR 9.71 95% 2.38-39.64). The depth of the incident ulcer and cellulitis, type of diabetes, HbA1c, body mass index and lipid profile were not significantly associated with differential mortality.

Conclusion: High risk characteristics for poor healing of diabetic foot ulcers identified in this record linkage extend beyond the well-recognised age, male sex, ulcer location and depth and peripheral vascular disease to dyslipidaemia, low blood pressure and chronic kidney disease. Similar factors plus smoking were associated with early mortality. In this analysis HbA1c, weight and cellulitis were not related to healing or death. More intensive vascular and renal management may be necessary to improve outcomes in people with diabetic foot ulcers.

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Prolonged hospital stay and increased readmission of lower limb cellulitis in type 2 diabetes patients

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Background and aims: Incidences of soft tissue infections in people with and without diabetes are increasing. Cellulitis, perhaps by damaging the lymphatic vessels, is prone to recur. However, there is little data on hospitalization rates of lower limb (LL) cellulitis, specifically in the diabetes population. We quantify the impact of this condition and assess the factors associated with prolong stay and readmissions.

Materials and methods: Retrospective case-control study conducted in an urban hospital, servicing a multi-ethnic population of nearly 0.4 million adults, including ~27000 (7%) with known type 2 diabetes (T2DM). Adults with T2DM and those without diabetes admitted with LL cellulitis between 2008-2013 were identified from discharge coding and medical records. Length of stay, patient demographics, HbA1c, presence of LL ulceration, and subsequent readmission with LL cellulitis >1 month from the index admission were recorded.

Results: T2DM patients were over-represented, with 719 patients accounting for 22% of the 4600 LL cellulitis admissions in the 6 year period. ~3% of the estimated T2DM population were admitted at least once during that time, compared to 0.8% of the non-diabetic population ($p<0.0001$). T2DM was associated with a significantly longer length of stay (median 5.3 vs 3.0 days, $p<0.001$), regardless of age, ethnicity and despite relatively good glycaemic control (mean HbA1c 61 mmol/mol). Ulceration commonly accompanied LL cellulitis, particularly in T2DM subjects (52% vs 18%, $p<0.001$), and increased the length of stay (T2DM: ulcer vs no ulcer: 7.9 vs 3.7 days, $P<0.0001$; control: ulcer vs non ulcer: 5.9 vs 2.9 days, $p<0.0001$). Amputation was almost exclusively performed in patients with ulceration, more commonly in T2DM patients (10% vs 0.1%, p<001), particularly in those with HbA1c >89 mmol/mol. The risk of readmission during the mean 37 months of follow-up was increased in the T2DM group compared with control (HR 1.73, 95% CI 1.4-2.4, $p<0.001$) and remained elevated even in the absence of ulceration (HR 1.67, 95% CI 1.2-2.4, $p<0.01$). Non-diabetic patients without ulceration were at the least risk of readmission (Figure). Ethnicity, age, HbA1c, and length of index admission were unrelated to the risk of readmission. We estimate that 3% of the total T2DM patients account for 1/3 of the 26364 cellulitis-related bed days and the average annual cost of €3.4 million during the study period.

Conclusion: This study highlights the huge economic and social burden of LL cellulitis admissions and its recurrent nature, posed by a small proportion of the population. Patient demographics and chronic glycaemic control do not predict readmission risk. Exploring other clinical factors that may influence recurrence and response to antibiotic prophylaxis in the T2DM population will enable targeting of high risk patients.

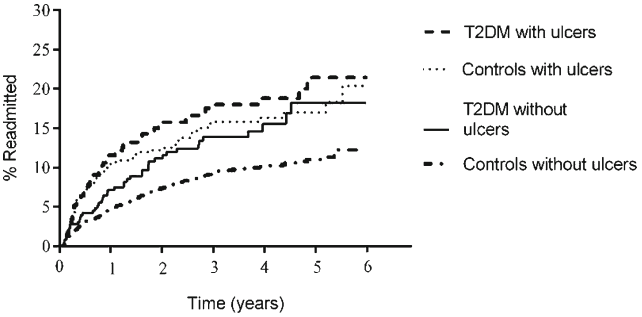

Figure 1. Proportion of patients readmitted over follow up period.

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Diabetic foot disease, self-care, and clinical monitoring in adults with type 2 diabetes: the Alberta's Caring for Diabetes (ABCD) cohort study

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Background and aims: Diabetic foot disease is a major cause of morbidity, disability and mortality, and contributes to increased healthcare cost. Throughout the world, up to 70% of leg amputations are performed on people with diabetes, the majority of which are preceded by a foot ulcer and considered preventable. Our aim was to examine the prevalence and predictors of foot disease, self-care and clinical monitoring in adults with type 2 diabetes (T2D) in Alberta, Canada.

Materials and methods: We used data from the baseline survey of a large prospective cohort of adults with T2D. Data were collected via a mailed self-administered questionnaire. Assessment of foot disease included self-reported peripheral neuropathy, peripheral vasculopathy, foot or leg ulcer/infection or gangrene/amputation. Foot self-care was assessed using the Summary of Diabetes Self-Care Activities, and clinical monitoring was assessed by patient-report of having feet checked for lesions or sensory loss by a health professional. Multivariable logistic regression was used to estimated odds ratios (OR) and 95% confidence intervals (CI) for potential predictors of diabetic foot disease, good foot self-care and clinical monitoring.

Results: Mean age of respondents (N=2040) was 64 (SD 10.6) years, 45% were female, and 91% Caucasian. Peripheral neuropathy was reported by 18% of the respondents, peripheral vasculopathy by 28%, ulcer/infection by 6%, and gangrene/amputation by 1.4%. Only 14% of respondents performed recommended foot self-care behaviours≥6 days/week, and only 41% and 34% had their feet clinically checked for lesions or sensory loss respectively. Predictors of increased risk of diabetic foot disease included longer diabetes duration, history of smoking, depressive symptoms, low self-efficacy, and a history of cardiovascular diseases. Predictors of good foot self-care included older age (OR 1.8; 95%CI 1.0, 3.1), female sex (1.4; 1.1, 1.8), longer diabetes duration (1.7; 1.3, 2.3), inadequate health literacy (1.4; 1.0, 2.0), and presence of hyperlipidemia (0.6; 0.5, 0.8). Predictors of clinical monitoring included female sex (0.8; 0.7, 0.9), residing in urban areas (1.6; 1.3, 2.0), longer diabetes duration (1.5; 1.2, 1.8), current smoking (0.7; 0.5, 0.9), presence of heart disease (1.4; 1.1, 1.8) or hyperlipidemia (1.2; 1.1, 1.5).

Conclusion: Peripheral neuropathy and vasculopathy were the most commonly reported foot problems in this population. Recommended foot self-care is generally infrequent, and clinical monitoring is performed for less than half of these patients, with significant variations by patient demographics and clinical presentation. We found it particularly interesting that smokers, who have a higher risk of developing foot disease, were less likely to receive a clinical foot exam compared to non-smokers. Our

results underline the importance of a detailed exploration of clinical monitoring of diabetic foot disease by care providers, and the development of standards, resources and tools for health care providers to enhance these preventive services.
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Long-term follow-up of a cohort with a history of diabetic foot ulcer in Austria

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Background and aims: Patients with previous diabetic foot ulcer are prone to re-ulceration and (re-)amputation. To evaluate the rate of foot related complications and mortality in a high-risk population of patients with diabetic foot syndrome over a decade.
Materials and methods: A cohort of 91 patients with recently healed diabetic foot ulcer were invited for follow-up 1, 6 and 11 years after inclusion. Patient characteristics at inclusion were: 65±11 years, 44 women, diabetes type 1 (n=6) or 2 (n=85), BMI 28.5±4.4 kg/m2, and HbA1c 8.4±1.6%. Patients presented with clinical signs of neuropathy (n=91), peripheral vascular disease (n=42), history of minor (n=25) or major (n=5) amputation, nephropathy (n=40) and retinopathy (n=53).
Results: Over the follow-up period ulceration recurred in 71 (78%) patients, time to first recurrence was 1.8±2.4 years (mean ± SD). 21 patients (23%) had to undergo a new amputation (minor n=19, major n=2), time to new amputation was 3.6±1.9 years. Over time 3 further major amputations were required in patients with an initial minor amputation. 33 of the initially included patients completed the follow-up period of 11.0±0.6 years. 58 (64%) patients died during the observational period, time until exitus was 5±3 years in this group. Causes of death were the following: 56.9% cardiovascular, 17.2% infections, 6.9% cancer, 19% other causes. Microvascular complications were not predictive for a composite of amputation or death, while peripheral artery disease was significantly predictive ($p<0.0001$).
Conclusion: Our data highlights the high recurrence rate of ulcerations and the poor longterm prognosis in high-risk patients with a diabetic foot syndrome. More research is urgently needed to improve the outcome of this patient cohort.

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Inflammatory cytokines role in extracellular matrix turnover induces in human skin fibroblasts by AGEs exposure

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Background and aims: AGEs accumulation in the skin affects extracellular matrix (ECM) turnover and triggers diabetes associated skin conditions and skin ageing. The receptor of AGEs (RAGE) has an essential contribution to cellular dysfunction driven by chronic inflammatory responses. TGF-β1 is also critical in dermal homeostasis and inflammation. We investigated the contribution of RAGE and TGF-β1 to the modulation of inflammatory response and EMC turnover in AGEs milieu, and the effects generated by their blockade in order to delineate a potential therapeutic strategy.
Materials and methods: Human skin fibroblasts (CCD-1070Sk) were exposed for 12 and 24 h to 50, 100 and 200 μg/ml glycated BSA (AGEs-BSA) and unglycated BSA (control). Anti-RAGE and anti-TGF-β1 blocking antibodies were applied to the 200 μg/ml AGEs exposed cells for 24 h. Secreted TGF-β1, IL-2, IL-4, IL-6, IL-8, granulocyte-macrophage colony-stimulating factor (GM-CSF), IFN-γ and TNF-α were determined by multiplex immunoassay, while RAGE, TGF-β1, collagen I and III and metalloproteinase (MMPs) protein levels were evaluated by western blot. The gene expression was quantified by real-time RT-PCR, and activity of MMPs was evaluated by gelatin zymography.
Results: The 12 h exposure to AGEs-BSA induced a dose dependent upregulation of RAGE, TGF-β1, collagen I and III, increasing the relative gene expression ratio (R) to 4.1, 3.1, 5.9, and 2.7, after exposure to 200 μg/ml AGEs-BSA, whereas their protein expression was affected to a lesser extent. Notably, after 24 h, the increases of collagen III gene and protein expression exceeded those of collagen I. MMP-2 was activated after 12 h of AGEs-BSA exposure, while MMP-9 activity was not detected. MMP-2 protein expression was induced in a dose dependent manner by AGEs-BSA exposure, with a maximal 2 fold increase after 12 and 24 h. While RAGE blockade diminished MMP-2 activation, TGF-β1 didn't significantly inhibit it. All cytokine levels increased after 12 h of exposure to AGEs-BSA in a dose dependent manner; IL-8 and TNF-α had the highest expression levels and increased over 1.7 fold, while after 24 h, TGF-β1, IL-6, GM-CSM and TNF-α increased over 2 fold. R of collagen I and III decreased after RAGE blockade to 0.14 and 0.52 compared to 200 μg/ml AGEs-BSA exposed cells, while TGF-β1 blockade only inhibited collagen III expression. IL-2, IL-8, and TNF-α protein levels were diminished by RAGE blockade, which also mildly decreased active TGF-β1 secretion. TGF-β1 blockade diminished IL-6 and GM-CSF levels in AGEs milieu, as opposed to IL-8 and TNF-α which were additionally increased.
Conclusion: The pro-inflammatory effect of AGEs on human skin fibroblasts involved both the activation of TGF-β1 and RAGE-NF-κB-TNF-α signaling axes. The activation of MMP-2 and over-expression of collagen I and III probably prompted an increased ECM turnover. Additionally, after 24 h, collagen III expression ratio dominance over collagen I could suggest diminished ECM mechanical stability. RAGE blockade markedly decreased collagen I level, and inhibited MMP-2 activation, while TGF-β1 blockade decreased collagen III expression and mildly inhibited MMP-2 activity, as it induced IL-8 expression. TGF-β1 blockade overall effect could contribute to restore the collagen I/III ratio, in favor of the former.
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Direct revascularisation based on angiosome model reduces major amputations and increases life expectancy in diabetic patients with critical limb ischaemia and diabetic foot

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Background and aims: The role of AM to guide revascularization procedures is debated. We evaluated whether direct or indirect revascularization, based on Angiosome model (AM), affects clinical outcomes in type 2 diabetic patients (T2DM) with critical limb ischemia (CLI) undergoing percutaneous trans-luminal angioplasty (PTA).
Materials and methods: We retrospectively evaluated 445 consecutive successful lower limb PTA performed in 370 T2DM patients (M/F: 257/113; age: 73.5±9.3 yrs; BMI: 27.4±4.8 Kg/m^2; diabetes duration: 21.4±12.8 yrs; HbA1c 7.8±1.6%) because of CLI and diabetic foot ulceration (DFU). Patients were divided into 2 groups: direct (DG - 266 pts, 72%) or indirect (IG - 104 pts, 28%) depending on whether the flow to the artery directly feeding the site of ulceration, as determined by AM, was successfully identified or not. No significant difference was apparent between groups regarding main clinical characteristics. Ulcer healing (HR), major amputation (MA) and death (D) rates were compared in the 2 groups during a follow-up of 18.9±12.4 months (range 0.7-43.2).
Results: HR was achieved in 68% of DG vs. 52% of IG (χ^2=9.6; p< 0.05). MA was needed in 11% of DG vs. 4% of IG (χ^2=9.4; p<0.02). Cumulative mortality rate during follow-up was 14% in DG and 27% in IG (χ^2=8.7; p<0.02).
Conclusion: Our data show that direct revascularization of arteries supplying the DFU site by means of AM is associated with higher rate of healing and lower risk of amputation and mortality as compared to indirect PTA procedure. These results support use of AM for PTA guiding in diabetic patients with DFU.

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Trends in lower extremity amputations over 5 years at a tertiary hospital

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Background and aims: Around 6000 people with diabetes have leg, foot or toe amputation each year in England. Up to 80% people die within 5 years of having an amputation. Amputations remain a common outcome of diabetic foot disease and have an impact on individuals economically, socially and psychologically. It has been estimated that a reduction of 50% late referrals to specialist foot teams could save £34 million (47 million Euros) a year by a reduction in amputations. We analysed all lower extremity amputations between 2009 and 2013 in our tertiary referral hospital and evaluated patient characteristics and trends in amputations.
Materials and methods: We performed a retrospective review of data on hospital discharges of patients who were coded as having had a lower extremity amputation from 2009 to 2013 in our hospitals which cover a relatively static population. Data were collected from the hospital coding activities from 1 January 2009 to 31 December 2013. For each amputation, diabetes status was ascertained by looking through our diabetes register and the pathology system. The demographic, admission details and relevant medical history were also collected.
Results: 30-40% of amputations occurred in patients with diabetes, comparable with other series. The total number of patients with diabetes in the region in 2011 was 49,645, 52,428 in 2012 and 59,611 in 2013, with a calculated mean incidence of 0.56/1000 diabetic population in 2011, 0.53/1000 diabetic population in 2012 and 0.55/1000 diabetic population in 2013 for major amputations. The figures for minor amputations were 1.13/1000, 1.28/1000 and 1.16/1000 diabetic population respectively. In our local population, the mean incidence over the 3 years was 1.74/1000 diabetic population for all foot amputations, 0.55/1000 for major amputations and 1.19/1000 diabetic population for minor amputations. The amputation rate per 1000 diabetic population in England over the same 3 year period was 2.6 for all amputations, 0.9 for major foot amputations and 1.7 for minor foot amputations.
Conclusion: Increasing numbers of people with diabetes in the population area is expected to lead to a rise in the number of lower extremity amputations. Our major and minor amputation rates have remained unchanged from 2011 to 2013. The trend is an increase in the number of minor amputations from 2009-2010 to 2011-2013. An early minor amputation can prevent a later major amputation. . Thus, minor amputations may reflect improved quality of care with earlier intervention; consequently preventing the progression from minor to major amputation.

Year	2009	2010	2011	2012	2013
Total major amputations	82	78	88	76	90
Below Knee Amputations	35	39	29	30	38
Above Knee Amputations	47	39	59	46	42
Number with diabetes	34	30	28	28	33
% with diabetes	41.5	38.5	31.8	36.8	36.7
Total minor amputations	56	64	88	86	89
Number with diabetes	44	46	56	67	69
% with diabetes	78.6	71.9	63.6	77.9	77.5

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Nationwide lower extremity amputation rates in patients with diabetes in secondary care in the Netherlands (DUDE-7)

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Background and aims: Patients with diabetes are at increased risk for lower extremity amputation as a consequence of micro- and microvascular complications. Almost one in five European patients with a diabetic foot ulcer ends up with a below knee amputation (Eurodiale). The estimated amputation rate estimates from the general diabetes population in the UK were 0.25% and in the Netherlands between 0.36 and 0.55% in at the end of the last century, treated in both primary and secondary care. The primary aim of this study was to estimate the annual incidence rate of amputations in secondary care treated patients with diabetes. The secondary aim was to estimate the amputation rate in secondary care treated patients diagnosed with diabetic retinopathy.
Materials and methods: The study population consisted of all patients for whom an insurance claim was made for treatment of diabetes in secondary care. Patients were selected from a nationwide database that collects data on insurance claims. Physicians are required to record Diagnosis-treatment-codes (DTC) for receiving reimbursement of hospital care using. Each DTC code contains information about the specialism that recorded the DTC, the diagnosis and type of treatment. From this database, patients with diabetes were selected and incidence rates of non-traumatic lower extremity amputation and retinopathy were selected between 2007 and 2011. The percentage of claims for amputations was identified for the whole study group and for the subgroup with retinopathy.

Results: Total number of secondary care treated patients with diabetes in the Netherlands increased from 132.499 to 137.049 between 2007 and 2011 (table 1). The annual rate of total non-traumatic lower-extremity amputation was 0,5% and remained stable over the years. The percentage of patients diagnosed with retinopathy by an ophthalmologist ranged from 13,7%% to 15,0%. Annual rate of lower extremity amputations was 1.2% for those with retinopathy, see table 1.

Conclusion: The Dutch annual incidence rates of non-traumatic lower extremity amputations in the Netherlands in secondary care patients with diabetes between 2007 and 2011 were comparable to the rates 20 years ago and higher compared to data from the UK (2005-2008), although the most recent UK data concerned primary and secondary care treated patients. The annual amputation rate in patients with retinopathy was higher, indicating a higher risk on lower extremity amputation when (other) microvascular complications are present.

	2007	2008	2009	2010	2011
Patients with diabetes (n)	132,499	134,027	134,877	138,486	137,049
Patients with retinopathy (% of total patients)	18,232 (13.8%)	19,879 (14.8%)	19,775 (14.7%)	20,742 (15.0%)	18,795 (13.7%)
Patients with lower extremity amputation (% of total patients)	573 (0.4%)	707 (0.5%)	663 (0.5%)	683 (0.5%)	629 (0.5%)
Patients with lower extremity amputations and retinopathy (% of all patients with diagnosis retinopathy)	157 (0.9%)	245 (1.2%)	252 (1.3%)	274 (1.3%)	253 (1.4%)

Table 1. Annual incidence rates of retinopathy and lower extremity amputations in patients with secondary care for their diabetes.

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Efficacy, quality of life and treatment satisfaction with capsaicin 8% patch versus standard of care in painful diabetic peripheral neuropathy

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Background and aims: PACE was a Phase III, randomised, controlled, open-label, 52-week, safety study that assessed the safety and efficacy of repeat treatment with the capsaicin 8% patch plus standard of care (SOC) versus SOC alone in painful diabetic peripheral neuropathy (PDPN). Secondary efficacy, quality of life (QoL) and treatment satisfaction results are presented.

Materials and methods: Patients (N=468) with mean [SD] duration of PDPN 4.4 [3.6] years, glycosylated haemoglobin 7.4% [1.0], average daily pain 5.6 [1.3] and European quality of life in 5 dimensions visual analogue scale (EQ-5D VAS) 54.6 [14.7], were randomised (1:1:1) to capsaicin 8% patch 30 min plus SOC (n=156), capsaicin 8% patch 60 min plus SOC (n=157), or SOC alone (n=155). Capsaicin arms received 1-7 treatments with at least 8-week intervals. Results for the secondary endpoints BPI-DN pain severity index, BPI-DN pain interference index, EQ-5D dimensions, VAS and utility, and self-assessment of treatment (SATII) are presented. No formal statistical testing was performed and treatment deltas were compared with a 90% CI in this safety study.

Results: Regarding pain severity index, the mean [SD] change from baseline to end of study (EoS) was -1.9 [1.8], -2.2 [1.9] and -0.9 [1.7] with capsaicin 30 min, capsaicin 60 min and SOC, respectively; mean difference [90% CI] with capsaicin 30 min and 60 min versus SOC was -0.9 [-1.3, -0.6] and -1.2 [-1.6, -0.9], respectively. With pain interference index, the mean change from baseline to EoS was -1.9 [2.1], -2.0 [2.3] and -0.8 [1.9] with capsaicin 30 min, capsaicin 60 min and SOC, respectively; mean difference for capsaicin 30 min and 60 min versus SOC was -1.0 [-1.4, -0.6] and -1.2 [-1.6, -0.8], respectively. A greater mean improvement in EQ-5D utility index of 0.07 was observed in both capsaicin arms versus SOC from baseline to Month 12. Regarding EQ-5D items, a greater proportion of patients at EoS in both capsaicin arms versus SOC had no problems with mobility, self-care, usual activities, pain or discomfort and anxiety or depression. Regarding EQ-5D VAS, the mean change from baseline to EoS was 10.4 [18.5], 11.2 [21.4] and 5.5 [18.1] with capsaicin 30 min, capsaicin 60 min and SOC; mean difference with capsaicin 30 min and 60 min versus SOC was 4.9 [1.1, 8.6] and 5.7 [2.0, 9.4], respectively. With SATII at EoS, a greater proportion of patients in both capsaicin arms versus SOC reported improvements in pain level, activity level, quality of life, willingness to undergo treatment again, and also preferred treatment compared with their previous treatment.

Conclusion: Repeat treatment with the capsaicin 8% patch in PDPN over 52 weeks was associated with improvements in pain severity and interference, QoL and treatment satisfaction compared with SOC alone, further supporting the broad effectiveness of capsaicin treatment in PDPN.

Clinical Trial Registration Number: NCT01478607
Supported by: Astellas Pharma Europe B. V.

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Capsaicin 8% patch repeat treatment versus standard of care in painful diabetic peripheral neuropathy: a randomised, open-label, 52-week study

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Background and aims: PACE assessed the safety and efficacy of repeat treatment over 52 weeks with the capsaicin 8% patch plus standard of care (SOC) versus SOC alone in painful diabetic peripheral neuropathy (PDPN). The Norfolk QOL-DN scale was utilised to assess any patient-reported functional consequences of potential small-fibre dysfunction that may be induced by capsaicin treatment. Sensory testing, including the Utah Early Neuropathy Scale (UENS), was also performed to assess capsaicin safety.

Materials and methods: PACE was a Phase III, randomised, controlled, open-label, 52-week, safety study. Patients with painful, distal, symmetrical, sensorimotor diabetic polyneuropathy, glycosylated haemoglobin ≤9% and average daily pain ≥4, were randomised (1:1:1) to capsaicin 8% patch 30 min plus SOC, capsaicin 8% patch 60 min plus SOC, or SOC alone. Capsaicin arms received 1-7 treatments with at least 8-week intervals. The primary endpoint was percentage change from baseline to end of study (EoS) in Norfolk QOL-DN total score. Secondary endpoints were UENS score, ratings of evoked sensations, average daily pain, ≥30% pain response and patient global impression of change (PGIC). No formal statistical testing was performed and treatment deltas were compared with a 90% CI in this safety study.

Results: In total, 468 patients with mean [SD] duration of PDPN 4.4 [3.6] years, glycosylated haemoglobin 7.4% [1.0], average daily pain 5.6 [1.3], Norfolk QOL-DN total score 41.4 [18.7] and UENS total score 16.4 [6.9], were randomised to capsaicin 30 min (n=156), capsaicin 60 min (n=157) or SOC alone (n=155). A greater improvement from baseline to EoS in mean Norfolk QOL-DN total score was observed with capsaicin 30 min versus SOC (mean difference [90% CI]: -20.9% [-31.7, -10.1]) and capsaicin 60 min versus SOC (-26.1% [-36.8, -15.4]). Subscale scores for symptoms, physical functioning/large fibre, small fibre, autonomic and activities of daily living improved with capsaicin over SOC. Mean [SD] changes in UENS total score from baseline to EoS also showed improvement and were -2.1 [5.0], -3.0 [5.1] and -1.2 [4.2] in capsaicin 30 min,

capsaicin 60 min and SOC arms, respectively. A greater reduction in average daily pain score from baseline to EoS was observed with capsaicin 30 min versus SOC (-1.0 [-1.4, -0.6]) and capsaicin 60 min versus SOC (-1.2 [-1.6, -0.9]). The 30% responder rate by EoS was 67.3%, 67.5% and 40.6% in the capsaicin 30 min, capsaicin 60 min and SOC arms, respectively. With regard to PGIC at EoS, a very much improved or much improved general state of health since study start was reported by 24.2%, 24.5% and 9.5% of patients in the capsaicin 30 min, capsaicin 60 min and SOC arms, respectively.

Conclusion: Repeat treatment with capsaicin 8% patch over 52 weeks in patients with PDPN was well tolerated, not associated with any functional consequences arising from potential impaired small-fibre function, or any sensory deterioration, and pain relief was sustained and progressively improved compared with SOC.

Clinical Trial Registration Number: NCT01478607

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Capsaicin 8% patch versus standard of care in painful diabetic peripheral neuropathy: efficacy of seven consecutive treatments over 52 weeks versus SOC

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Background and aims: PACE was a Phase III, randomised, controlled, open-label, 52-week, safety study that assessed the safety and efficacy of repeat treatment with the capsaicin 8% patch plus standard of care (SOC), versus SOC alone in painful diabetic peripheral neuropathy (PDPN). Efficacy results in patients who received seven consecutive capsaicin 8% patch treatments versus SOC are presented.

Materials and methods: Patients (N=468) with painful, distal, symmetrical, sensorimotor diabetic polyneuropathy, glycosylated haemoglobin ≤9% and average daily pain ≥4, were randomised (1:1:1) to capsaicin 8% patch 30 min plus SOC, capsaicin 8% patch 60 min plus SOC, or SOC alone. Capsaicin arms received 1-7 treatments with at least 8-week intervals. Efficacy endpoints were average daily pain, Brief Pain Inventory-Diabetic Neuropathy (BPI-DN) pain severity index, BPI-DN pain interference index and patient global impression of change (PGIC). No formal statistical testing was performed and treatment deltas were compared with a 90% CI in this safety study.

Results: The SOC arm comprised 155 patients and seven treatments were received by 84/156 (53.8%) and 83/157 (52.9%) patients in the capsaicin 30 min and 60 min arms, respectively. Baseline mean [SD] daily pain was 5.6 [1.3], 5.7 [1.4] and 5.7 [1.3] in the capsaicin 30 min subset, 60 min subset and SOC arm, respectively. From baseline to end of study (EoS), the mean change in average daily pain was -2.4 [1.7], -2.6 [2.1] and -1.1 [2.0] in capsaicin 30 min subset, 60 min subset and SOC arm, respectively; mean difference [90% CI] between capsaicin 30 min and 60 min versus SOC was -1.3 [-1.8, -0.9] and -1.5 [-2.0, -1.1], respectively. Regarding pain severity index, the mean change from baseline to EoS was -2.3 [1.6], -2.4 [1.8] and -0.9 [1.7] in capsaicin 30 min subset, 60 min subset and SOC arm, respectively; mean difference between capsaicin 30 min and 60 min versus SOC was -1.4 [-1.8, -1.0] and -1.5 [-1.9, -1.1], respectively. The mean change in pain interference index from baseline to EoS was -2.2 [1.7], -2.5 [2.0] and -0.8 [1.9] in capsaicin 30 min subset, 60 min subset and SOC arm, respectively; mean difference between capsaicin 30 min and 60 min versus SOC was -1.4 [-1.8, -0.9] and -1.6 [-2.1, -1.2], respectively. With regard to PGIC at EoS, a very much improved or much improved general state of health since study start was reported by 22.6%, 27.7% and 9.5% of patients in the capsaicin 30 min subset, 60 min subset and SOC arm, respectively. Compared with observations in the total capsaicin arms who received 1-7 treatments, numerically similar or larger improvements in all efficacy endpoints versus SOC were observed following seven consecutive capsaicin treatments.

Conclusion: In patients with PDPN, seven consecutive treatments with the capsaicin 8% patch over 52 weeks were associated with sustained and progressively improved pain relief, reduced pain severity and pain interference indices, as well as an improved global impression of change compared with SOC treatment.

Clinical Trial Registration Number: NCT01478607

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Capsaicin 8% patch versus standard of care in painful diabetic peripheral neuropathy: safety of seven consecutive treatments over 52 weeks

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Background and aims: PACE was a Phase III, randomised, controlled, open-label, 52-week, safety study that assessed the safety and efficacy of repeat treatment with the capsaicin 8% patch plus standard of care (SOC), versus SOC alone in painful diabetic peripheral neuropathy (PDPN). The Norfolk QOL-DN scale was utilised to assess any patient-reported functional consequences of potential small-fibre nerve dysfunction that may be due to capsaicin treatment. Sensory testing, including the Utah Early Neuropathy Scale (UENS), was also performed to assess capsaicin safety. Safety results in patients who received seven consecutive capsaicin 8% patch treatments versus SOC are presented.

Materials and methods: Patients (N=468) with painful, distal, symmetrical, sensorimotor diabetic polyneuropathy, glycosylated haemoglobin ≤9% and average daily pain ≥4, were randomised (1:1:1) to capsaicin 8% patch 30 min plus SOC, capsaicin 8% patch 60 min plus SOC, or SOC alone. Capsaicin arms received 1-7 treatments with at least 8-week intervals. The primary endpoint was percentage change from baseline to end of study (EoS) in Norfolk QOL-DN total score. Secondary safety endpoints were UENS score and ratings of evoked sensations (warm, cold, sharp and vibration), plus assessment of deep-tendon reflexes.

Results: The SOC arm comprised 155 patients and seven treatments were received by 84/156 (53.8%) and 83/157 (52.9%) patients in the capsaicin 30 min and 60 min arms, respectively. Baseline mean [SD] daily pain was 5.6 [1.3], 5.7 [1.4], 5.7 [1.3], and Norfolk QOL-DN total score was 41.5 [20.1], 42.4 [19.8], 41.0 [18.5], in capsaicin 30 min subset, 60 min subset and SOC arm, respectively. The mean percentage reduction (improvement) from baseline to EoS in Norfolk QOL-DN total score was -31.2% [50.9], -40.5% [38.8] and -6.7% [54.1] in capsaicin 30 min subset, 60 min subset and SOC arm, respectively. Regarding UENS total score, the mean reduction (improvement) from baseline to EoS in capsaicin 30 min subset, 60 min subset and SOC arm was -2.3 [4.8], -4.1 [5.3] and -1.2 [4.2], respectively. Ratings of evoked sensation for warm, cold and sharp at EoS compared with baseline indicated that fewer patients within all three treatment arms reported no sensation and a numerically greater proportion reported slightly diminished or normal sensation by EoS. Ratings for vibration followed a similar trend from baseline to EoS within the capsaicin 30 min and 60 min subsets, but no noticeable changes were observed within the SOC arm. There were no noticeable

differences from baseline to EoS within all three treatment groups for ratings of deep-tendon reflexes. All safety observations in the capsaicin subsets were consistent with the total capsaicin arms.
Conclusion: In patients with PDPN, seven consecutive treatments with the capsaicin 8% patch over 52 weeks were well tolerated and not associated with any functional consequences from potential impaired small-fibre function, or adverse sensory effects.
Clinical Trial Registration Number: NCT01478607
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Hyperglycaemia and hypoxia induce overexpression of GLUT1 in models of diabetic retinopathy

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Background and aims: Diabetes mellitus is a group of metabolic diseases characterized by high blood glucose that leads to several complications, including diabetic retinopathy (DR). DR is a blinding disease characterized by retinal microvascular changes caused by chronic exposure to hyperglycemia leading to low tissue oxygenation and ultimately to neovascularization. It has been hypothesized that an increase in glucose transporters such as GLUT1 is responsible for increased glucose levels in retinal cells. In the retina, glucose is the only fuel source for the cells and its cellular intake occurs exclusively through GLUT1. GLUT1 expression is also controlled by HIF-1, a key player in neovascularization. To understand the role of GLUT1 in DR, we have characterized the GLUT1 expression in both in vitro and in vivo models of DR.
Materials and methods: Retinal epithelium cells (D407), subjected to normoxia and hypoxia were cultured in DMEM with different glucose concentrations, corresponding to non-diabetic (5 mM of glucose) and diabetic conditions (25 mM of glucose). GLUT1 expression and localization were analyzed by Western Blot and immunofluorescence. Analysis of GLUT1 in vivo was performed using retinas of 6 month-old Ins2Akita diabetic mice by western blot and immunofluorescence and compared with the retinas of age-matched non-diabetic mice (C57Bl6 WT).
Results: Our in vitro results show an increase in GLUT1 expression in a glucose-dependent manner, under hypoxia conditions. This increase is associated to the translocation and stabilization of GLUT1 in the cell membrane, as observed by immunofluorescence. In vivo, GLUT1 was overexpressed in the retina of Ins2Akita mice compared with the non-diabetic mice. The immunofluorescence analysis of the retina of diabetic mice shows an accumulation of GLUT1 in the cell membrane of the innermost layers of the retina, such as the photoreceptors' layer, which is avascular and known to be under hypoxic conditions in diabetic environment. As expected, this was not observed in the retina of the WT mice, corroborating our in vitro results.
Conclusion: In this study the expression of GLUT1 was analyzed in vitro in conditions simulating DR and in vivo in a mouse model of DR. The results show an overexpression of GLUT1 induced by hyperglycemia and low oxygen supply. This overexpression was associated to an increase of GLUT1 in the cell membrane of the cells, which points to a significant contribution of GLUT1 to the progression of DR.
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High glucose-induced metabolic and structural changes in mitochondria promote retinal Müller cell loss associated with diabetic retinopathy

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Background and aims: Müller cells are the principal glial cells in the retina located in the inner nuclear layer with processes extending toward the outer limiting membrane and in the opposite direction toward the inner limiting membrane. These processes come in frequent contact with retinal blood vessels allowing coupling between Müller cells and vascular cells necessary for cell survival and retinal vascular homeostasis. Müller cell loss in the diabetic retina can have a profound effect on retinal dysfunction associated with diabetic retinopathy. However, it is currently unclear how high glucose promotes Müller cell loss in diabetes. In this study, we determined whether high glucose compromises mitochondrial structure and function, and thereby promotes apoptosis in rat retinal Müller cells (rMC-1).
Materials and methods: To determine if high glucose (HG) induces changes to mitochondrial metabolic function, rat retinal Müller cells (rMC-1) were grown in normal (N, 5 mM glucose) or HG (30 mM) medium for 7 days and subjected to bioenergetic assay using the XF24 analyzer. In particular, altered mitochondrial metabolic function under HG was assessed by measuring oxygen consumption rate (OCR) and extracellular acidification rate (ECAR). In parallel, differential staining method was performed to identify cells undergoing apoptosis, and digital images of mitochondria stained with MitoTracker Red were captured through live cell imaging under confocal microscopy. Images were analyzed for mitochondrial morphology changes based on Form Factor (FF) and Aspect Ratio (AR).
Results: rMC-1 grown in HG showed a significant decrease in steady state OCR (100.5±23 vs. 155±6 pmol O2/min in normal; $p<0.05$) and maximum OCR (85.5±26 vs. 171.5±16 pmol O2/min in normal; $p<0.05$), compared to those in cells grown in N medium. Similarly, ECAR was significantly decreased in cells grown under HG condition compared to those grown in N medium (40±4% of normal; $p<0.05$). Cells grown in HG exhibited significant increase in the number of apoptotic cells (202±29% of control; $p<0.05$), and the mitochondria became fragmented and punctate compared to the long, tubular mitochondrial networks seen in cells grown in normal medium.
Conclusion: Findings from this study indicate that HG induces changes to mitochondrial metabolic function by altering OCR and ECAR while inducing mitochondrial morphology and structural changes in retinal Müller cells. Such changes are likely contributing to retinal Müller cell loss associated with diabetic retinopathy.

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Hydroquinone-damaged human retinal pigment epithelial cell proliferation by advanced glycation end-products via up-regulation of VEGF gene

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Background and aims: Age-related macular degeneration (AMD) is the important cause of irreversible blindness in elderly patients. The disease has been classified into dry and wet forms. The dry form AMD was defined as progressive destruction of retinal pigment epithelial (RPE) cells. While, exudative form AMD is characterized by choroidal neovascularization, which is led by some angiogenic cytokines such as vascular endothelial growth factor (VEGF). Although recent research showed that advanced glycation endproduct (AGE), which is accumulated by the history of diabetes mellitus, and hydroquinone (HQ), which is generated by the smoking, are strongly correlated to the pathogenesis of AMD, the mechanism how AGE and HQ induce or accelerate AMD remains elusive. In the study, we investigated effects of AGE on RPE cell proliferation and *VEGF* expression in HQ-damaged human RPE cells.
Materials and methods: ARPE-19 and h1RPE7 cells, human RPE cell lines, were treated HQ (20-40 μM), AGEs (300 μg/ml), and HQ+AGE for 12 h. After the treatment, viable cell numbers, apoptosis, and replicative DNA synthesis was measured by WST-8 cleavage, TUNEL method, and 5'-iodo-2'-deoxyuridine (IdU) incorporation, respectively. *VEGF* mRNA was measured by real-time RT-PCR and VEGF, secreted into culture medium, was measured by ELISA. Reporter plasmids containing several length of *VEGF* promoter and mutants of potential binding sites for SP1 (GC box) were constructed on pGL4.17 vector and introduced them into RPE cells. After 12 h incubation with HQ and/or AGE, cells were recovered and promoter activity was determined.
Results: The viable cell numbers of ARPE-19 and h1RPE7 were markedly reduced by HQ ($P<0.0001$). The addition of AGE to the HQ-treated cells increased cell numbers ($P<0.002$). HQ significantly increased apoptosis ($P=0.0002$). AGE alone did not change apoptosis and the addition of HQ+AGE did not prevent the HQ-induced apoptosis. Replicative DNA synthesis was increased by the addition of AGE in HQ-treated cells (No addition vs HQ+AGE, $P=0.0013$; HQ vs HQ+AGE, $P=0.0001$), indicating that AGE increases cell number by activating HQ-treated cell replication. Real-time RT-PCR revealed that *VEGF* mRNA was increased by the combined addition of HQ+AGE. The increase of VEGF in the HQ+AGE treated cell culture medium was confirmed by ELISA ($P=0.0064$ vs no addition, $P=0.0433$ vs HQ, $P=0.0063$ vs AGE). Inhibitors for VEGF/VEGF receptor (sulochrin, Ki8751, and CBO-P11) as well as *siVEGF* attenuated the HQ+AGE-induced increases in RPE cell numbers ($P<0.05$). Introduction of *siRNA for receptor of AGE (RAGE)* into RPE cells attenuated the HQ+AGE-induced increase in RPE cell numbers ($P=0.0175$). Deletion analysis of *VEGF* promoter revealed that GC boxes in the -102~-43 region were essential for the *VEGF* promoter activation. Site-directed mutaions of the GC boxes in the *VEGF* promoter and knockdown experiment using *SP1 siRNA* revealed that SP1 is an essential transcription factor for *VEGF* expression in RPE cells.
Conclusion: HQ induces RPE cell apoptosis and leads to dry AMD. AGE stimulation enhances *VEGF* transcription via the AGE-RAGE pathway in HQ-damaged cells. As a result, the secreted VEGF acts as an autocrine/paracrine growth factor for RPE and/or adjacent vascular cells, causing wet AMD.

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The role of erythropoietin in physiological angiogenesis of mouse retina

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Background and aims: Diabetic retinopathy (DR) is a prevalent microvascular complication and it is characterized by increased vascular permeability, ischemia and neovascularization. Angiogenesis is modulated by growth factors such as vascular endothelial growth factor and angiopoietin-2. Recently, it has been found that erythropoietin, besides neuroprotection, has angiogenic effects and its level is highly elevated in vitreous fluids of patients with proliferative diabetic retinopathy. However, the role of erythropoietin in physiological angiogenesis of the retina and its cooperation with angiopoietin 2 remains unclear. This study investigated the effects of erythropoietin on the physiological angiogenesis of the mouse retina.
Materials and methods: C57Bl/6 wild type and angiopoietin 2 transgenic mice were intraperitoneally injected at postnatal days P1, P3, P5, P7

and P9 with human recombinant erythropoietin at doses of 256 IU/kg. Retinae at P10 were stained with isolectin B4, NG2 and Iba1 antibodies, respectively. Angiogenic parameters of retinal vasculatures were analyzed using a Leica confocal microscope TCS SP2 system.
Results: Erythropoietin promoted the outgrowth of the deep vessels by 21% (*p<0.05) and increased pericytes recruitment in the superficial and deep layer by 23% and by 42%, respectively (*p<0.05). The microglial number was reduced in the superficial layer of retinal vessels (27%, *p< 0.05), but increased in the deep layer (60%, ***p<0.001). Microglia were located in front of tip cells and acted as a bridge to connect neighboring sprouts. Angiogenic activity was elevated in the deep layer of the retinal vascular plexus, especially with cooperation of angiopoietin 2.
Conclusion: Erythropoietin promotes angiogenesis and the maturation of the vascular plexus in the mouse retina.
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Identification of pro-apoptotic markers responsible for hypoxia and hyperglycaemia-induced pericyte apoptosis
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Background and aims: Loss of pericytes is the key-event in the pathogenesis of microvascular diabetic retinopathy. We have previously demonstrated that human retinal pericytes (HRP) are more vulnerable to intermittent than stable high glucose concentrations, with an increase in their apoptosis. Somatostatin, a well-known neuroprotective drug, may protect retinal cells, both microvascular and neuronal, from stress conditions. The aim of the present study was to investigate the effects of hypoxia combined with high glucose on HRP and explore the expression of molecules involved in the pro-apoptotic and survival pathways. This research will further clarify the mechanisms of action of these diabetic-like stress stimuli.
Materials and methods: HRP were exposed intermittently at 48 hr-intervals to high (28 mM)/physiological (5.6 mM) glucose for 8 days (intHG) and/or hypoxia for the last 48 hrs, with or without the addition of somatostatin. Cell proliferation was assessed by cell counting and BrdU incorporation, and apoptosis as DNA fragmentation (ELISA). The expression of pro-apoptotic (FasL, pro-caspase-8, active-caspase-8, Bid, t-Bid, Bax, calpain-2) and anti-apoptotic (PCNA, p-Akt) molecules were evaluated by Western blot.
Results: Hypoxia, alone and combined with intHG, is able to increase HRP apoptosis (+27 and +34%, respectively, p<0.05 vs ctrl) and decrease their proliferation (-28 and -31% vs ctrl, p<0.005). Pro-apoptotic molecules (FasL, active caspase-8, t-Bid, Bax) were significantly increased in HRP cultured in hypoxic conditions, both in physiological and intHG conditions, while no significant differences, but a trend towards decrease, were observed in the expression of survival markers (PCNA, p-Akt).
Conclusion: Diabetic-like conditions (intHG and hypoxia) are able to stimulate pericyte apoptosis through activation of pro-apoptotic molecules, thus leading to an imbalance between pro-apoptotic and survival signaling pathways. Our identification of such intermediates could help finding new therapeutic approaches for the prevention of DR.
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Decreasing diabetes-induced overexpression of lysyl oxidase prevents development of retinal vascular lesions associated with diabetic retinopathy
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Background and aims: Retinal capillary basement membrane (BM) thickening is closely associated with the development of vascular lesions in diabetic retinopathy (DR). Thickened capillary BM has been shown to compromise blood-retinal-barrier (BRB) characteristics and thereby contribute to retinal vascular permeability, a significant clinical manifestation of DR. We have previously shown that high glucose increases the expression and activity of lysyl oxidase (LOX), a cross-linking enzyme, in retinal endothelial cells. Additionally, concomitant with overexpression of LOX, increased vascular permeability was observed in streptozotocin (STZ)-induced diabetic rat retinas. However, it is unknown whether decreased LOX expression may have beneficial effects against development of retinal vascular lesions in diabetes.
Materials and methods: To examine whether reduced LOX expression has protective effects against diabetes-induced development of retinal vascular lesions characteristic of DR, four groups of mice: wild type (WT) control mice, STZ-induced diabetic mice, LOX +/- mice, and STZ-induced diabetic LOX +/- mice were used for this study. Diabetes was induced via STZ and maintained for 3 months; at the end of the study, retinas were assessed for LOX protein level by Western Blot (WB) analysis. Retinal capillary networks were isolated from the four groups of animals and retinal trypsin digest (RTD) was performed with hematoxylin/periodic acid Schiff staining. Digital images of the stained retinal capillary networks were captured to assess the number of acellular capillaries (AC) and pericyte loss (PL). In parallel, retinal vascular permeability was determined in retinal whole mounts following FITC-dextran injection.
Results: WB analysis showed significant increase in LOX expression in the diabetic retinas (135.13±23.6% of WT) compared to those of the WT control retinas, and a significant decrease in LOX expression in the diabetic LOX +/- retinas (105.29±9.8% of WT) compared to those of the diabetic retinas. RTD images exhibited a significant increase in the number of AC (197±52% of control; p<0.05) and PL (566±26% of control; p <0.05) in the retinas of diabetic mice compared to those of the WT control mice. Interestingly, the number of AC and PL was significantly decreased in the retinas of the diabetic LOX +/- mice compared to those of the diabetic mice (42%, p<0.05; 19%, p<0.05, respectively). Additionally, retinal vascular permeability was decreased in the retinas of the diabetic LOX +/- mice compared to those of the diabetic mice.
Conclusion: Findings from this study suggest that reducing diabetes-induced LOX overexpression may have beneficial effects against the development of retinal vascular lesions characteristic of DR. Therefore, LOX overexpression may be a potential therapeutic target in preventing retinal vascular cell loss and excess permeability associated with DR.
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Deficiency in nucleoside diphosphate kinase B aggravates the development of diabetic retinopathy through upregulation of angiopoietin-2 via FOXO1

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Background and aims: Nucleoside diphosphate kinase B (NDPKB) is a ubiquitous enzyme required for the synthesis of nucleoside triphosphates. It is thus involved in many cellular functions. Based on its role in endothelial cells and glucose metabolism, we hypothesize that NDPKB may play a role in diabetic retinopathy (DR).
Materials and methods: Diabetes was induced in NDPKB knockout (KO) and wild type (WT) mice with streptozotocin. Pericyte (PC) coverage and acellular capillary (AC) formation were evaluated in the retina 6 month after diabetes induction. The expression of angiopopietin-2 (Ang2) and forkhead box protein O1 (FOXO1) was assessed by western blotting and/or immunofluorescence staining in the diabetic retinas in vivo, and in NDPKB knockdown human umbilical vein endothelial cells (HUVEC) as well as in Müller cells isolated from NDPKB KO retinas and stimulated with high glucose (HG) in vitro. The knockdown of NDPKB and FOXO1 was performed by transfection with specific siRNAs.
Results: The number of PCs in the non-diabetic (NC) NDPKB KO retinas (1542/mm2 capillary area) was significantly decreased compared to WT NC retinas (1741/mm2 capillary area, $P<0.01$) and similar to diabetic (DC) WT retinas (1586/mm2 capillary area, $p<0.05$ vs. WT NC). Diabetic conditions further decreased PC number in NDPKB KO retinas (1400/mm2 capillary area) compared with KO NC and WT DC ($p<0.05$). Additionally, diabetes significantly enhanced the formation of AC in WT retinas (DC: 34/mm2 retinal area, NC: 25/mm2 retinal area, $p<0.05$). The number of AC in NDPKB KO NC retinas (28/mm2 retinal area) was similar to WT NC retinas. However, NDPKB KO DC retinas displayed a significant enhancement in AC formation (44/mm2 retinal area) compared with WT DC retinas and KO NC retinas ($p<0.05$). Concerning the essential role of Ang2 in pericyte loss in diabetic retinopathy, we determined the Ang2 levels in the NDPKB KO retinas. Compared to WT NC retinas, the expression pf Ang2 was significantly upregulated in NDPKB KO NC retinas ($p<0.05$ vs. WT NC) and to a similar extent in WT DC retinas ($p<0.05$ vs. WT NC). No difference in Ang2 expression was detected between NDPKB KO DC and NDPKB KO NC retinas. Endothelial and Müller cells are the main source of Ang2 in the retina. As shown by western blotting and immunofluorescence in HUVEC as well as in Müller cells, HG and NDPKB depletion similarly increased Ang2 expression, which was paralleled by enhanced FOXO1 levels and activity. Conversely, Ang2 expression was dramatically suppressed in the NDPKB- and FOXO1- double depleted HUVEC.
Conclusion: Our data indicate that NDPKB deficiency aggravates the development of diabetic retinopathy through an upregulation of Ang2 in endothelial and Müller cells. The NDPKB-dependent expression and activity of the transcription factor FOXO1 likely regulates Ang2 levels.
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Mechanism of action of calcium dobesilate in the early stages of diabetic retinopathy

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Background and aims: Calcium Dobesilate (CaD) has been prescribed to prevent the progression of diabetic retinopathy (DR). However, its clinical efficacy is still a matter of controversy. Recently, it has been reported that administration of the CaD in patients with DR may reduce the serum levels of endothelin-1 (ET-1). It should be noted that ET-1, a potent vasoconstrictor, is involved in the development of DR and also contributes to retinal neurodegeneration. The aim of the present study was to determine the effect of CaD in preventing retinal neurodegeneration and early microvascular abnormalities induced by diabetes in a murine model (db/db mouse). In addition, the effect of CaD on ET-1 and its receptors were also assessed.
Materials and methods: For this purpose we evaluated a total of 24 diabetic mice (db/db) aged 8 weeks that were randomly assigned to daily oral treatment with CaD (200 mg/kg/day) ($n=12$) or vehicle ($n=12$) for two weeks. Twelve non-diabetic mice (db/+) were used as control group. Retinal neurodegeneration was evaluated by measuring glial activation (GFAP immunofluorescence) and apoptosis (TUNEL, cell count in ganglion cell layer). Glutamate excitotoxicity, ET-1 pathway and VEGF expression were assessed by immunohistochemistry and Western blotting analysis. Functional retinal abnormalities were measured by focal electroretinography (fERG). Statistical analysis was performed by unpaired t-student test and results were expressed as mean±SEM.
Results: In summary, we found that CaD significantly decreases the main hallmarks of retinal neurodegeneration (glial activation and apoptosis). In addition, a significant improvement of ERG parameters was observed. CaD also reduces glutamate excitotoxicity induced by diabetes. Furthermore, CaD prevented the up-regulation of ET-1 and its cognate receptors in diabetic retinas. Finally, we have found that CaD treatment reduces the expression of VEGF and preserves the sealing function of the blood retinal barrier (BRB).
Conclusion: Our results suggest that CaD treatment could be effective in preventing neurodegeneration and microvascular abnormalities (disruption of BRB) in early DR. Better understanding of the mode of action of CaD could be important to guide physicians in targeting the treatment more appropriately.
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Prevalence and progression of diabetic retinopathy in people with type 2 diabetes dependent on the duration of diabetes

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Background and aims: At present the guideline for diabetic retinopathy of the DDG (German diabetes association) and the NVL (national guideline for diabetic eye disease) recommend annual screening of patients with type 2 diabetes and no prior retinopathy. It is because the onset of diabetes complications under good glycaemic control is a matter of years that this practice presumably leads to frequent superfluous examinations. The aim of our investigation was to examine the prevalence of retinopathy dependent on the diabetes duration in order to assess the probability of the disease progressing.
Materials and methods: In a retrospective database analysis from a university outpatient department for endocrinology and metabolic diseases we analyzed 17461 consultations of 4513 patients with type 2 diabetes (age 64,5 years, duration of diabetes 12,8 years, BMI 31,4 kg/m^2, HbA_{1c} DCCT adjusted 7,4% / 51,9 mmol/mol, blood pressure 146/82 mmHg, 50,3% women). The obtained data included clinical evidence from 1987 to 2014. 50,5% of the patients (n=2279) had at least one documented result of fundoscopy and were enclosed in the analysis (age 65,4 years, duration of diabetes 14,9 years, BMI 31,6 kg/m^2, HbA_{1c} DCCT adjusted 7,25% / 52,2 mmol/mol, blood pressure 145/80 mmHg, 47,3% women).
Results: 25,8% of the patients had retinopathy (20,2% non-proliferative, 4,7% proliferative, 0,7% were not classified, 0,1% blindness). The prevalence of retinopathy dependent on the duration of diabetes was 1,1% at diagnosis, 6,6% after 0<5 years, 12% after 5<10 years, 24% after 10<15 years, 39,9% after 15<20 years, 52,7% after 20<25 years, 58,7% after 25<30 years and 63% after ≥30 years. From 586 (25,7%) patients retinal photography of three consecutive years could be obtained and evaluated. Out of these 7,3% showed deterioration after one year and 13% after two years. On the other hand 3,4% improved after one year and 3,6% after two years. 201 (34,3%) of these patients had less than 10 years diabetes and showed an even lower tendency of deterioration (4,5% worsened after one and 9,5% after two years). Notably these patients mainly transformed from no retinopathy at all to non-proliferative retinopathy. Only four patients (2%) developed a proliferative retinopathy within this period.
Conclusion: The prevalence of the diabetic retinopathy increases with the duration of diabetes. Within the first ten years of diabetes the prevalence of retinopathy is low and the progression of the disease infrequent. Most patients have a non-proliferative form which can be reversible and requires no intervention in most cases. In patients with type 2 diabetes and no prior retinopathy as well as good glycaemic control, intervals of fundoscopy could be widened from annual to two yearly screenings within the first five years of diabetes duration given that an initial examination of the eyes has been obtained.

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Lipoprotein(a) predicts development of diabetic retinopathy in patients with type 2 diabetes mellitus

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Background and aims: We investigated factors that might influence the development of diabetic retinopathy (DR) in patients with type 2 diabetes.
Materials and methods: From January 2003 to December 2004, a total of 767 patients with type 2 diabetes without diabetic retinopathy were consecutively enrolled. The serum lipoprotein(a) [Lp(a)] concentration was measured by a one-step sandwich enzyme-linked immunoassay, and a standardized clinical examination and retinal photographs were checked annually by opthalmologist. We used a Cox proportional hazard regression analysis to test the associations between diabetic retinopathy and Lp(a).
Results: Of the 765 patients who were enrolled in this study, 483 (63.1%) completed the follow-up evaluation. The median follow-up time was 10.3 years. The mean age was 53.9±10.6 years, and the duration of diabetes was 6.4±5.6 years. During the follow-up period, 225 patients (46.6%) developed DR. The patients in the DR group had a longer duration of diabetes (p<0.001), higher baseline HbA_{1C} levels (p<0.001), received more insulin treatment (p<0.001) and a higher level of Lp(a) (12.6 [Interquartile range 7.9-24.0] vs 15.2 [9.2-29.7] mg/dl; p=0.010). A Cox hazard regression analysis revealed that the development of DR was significantly associated with the high level of serum Lp(a) level (hazard ratio 1.13; 95% CI 1.03-1.24; p=0.008) per 10 mg/dl Lp(a) increase after adjusting for sex, age, diabetic duration, mean HbA_{1c}, treatment of insulin, ACE inhibitor/angiotensin receptor blocker and lipid lowering agents.
Conclusion: The development of DR was independently associated with serum Lp(a) level in patients with type 2 diabetes.

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Cognitive function may be a predictor of retinopathy progression in patients with type 2 diabetes

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Background and aims: Cognitive function,depression and anxiety may be associated with type 2 diabetes(T2DM) and its complications. In particular diabetic retinopathy (DR) is a complication that affects people with diabetes.
In an observational prospective study of a cohort of T2DM patients followed for 8 years,we monitored a number of clinical, psychological and cognitive variables to identify possible predictors of progression of DR.
Materials and methods: Patients (n=498) with T2DM, of which 249 not treated by insulin (NIT) and 249 on insulin treatment (IT), were enrolled at baseline. The following variables were recorded: age, gender, diabetes duration, schooling, occupation, social status, smoking, self-monitoring of blood glucose, hypertension, menopausal status, fasting blood glucose, HbA1c, BMI, total and HDL cholesterol, triglyceride, retinopathy grading, presence of foot ulcers, depression and anxiety scores (Zung questionnaire) and cognitive function (Minimal Mental State Examination, MMSE). The same variables were collected again after 4 (JEI 37,79-85, 2014) and 8 years. DR assessment was by grading of digital 45° photographs of the macular and nasal areas. Grading of the worst eye was considered.

Results: Out of 477 patients for whom fundus evaluation was available at baseline, 240 (160 IT and 80 NIT) had no DR, 110 (48 NIT and 62 IT) had mild non proliferative DR and 127 (24 NIT and 103 IT) had moderate or more severe DR. After 8 years, 357 patients were available for analysis, of whom 191 had remained with no or mild DR and 166 had developed moderate or more severe DR. Patients who had moderate or more severe DR at baseline were not included in the follow-up analysis. On multivariate analysis, being on insulin treatment (OR 1.90, 95%CI 1.02;3.55, p= 0.043) and having mild vs no DR at baseline (OR 4.51, 2.44;8.31, p< 0.0001)were associated with progression to moderate/more severe DR, whereas male gender (OR 0.49, 0.26;0.92, p=0.028) and a higher MMSE score (indicative of better cognitive ability) (OR 0.90 per scoring point; 0.83;0.99, p=0.026) were protective. None of the other baseline variables was associated with progression of DR in this cohort.
Conclusion: Lower MMSE score may represent a novel risk factor for progression of DR, on top of other known determinants. Microangiopathy might develop at both brain and retinal level and manifest itself with changes in cognitive function and retinopathy.
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Evaluation of medically treatable risk factors in subjects with significant diabetic retinopathy from a local health economy area in the UK

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Background and aims: Diabetic retinopathy is the leading cause of blindness in people of working age in the western world. People with high risk retinopathy are also at risk of other diabetes and vascular complications. The aim of this study was to categorize and evaluate people with high risk diabetic retinopathy within our local health economy. We aimed to assess HbA_{1c} and four other key risk factors in this group to develop an understanding of the current provision of care.
Materials and methods: People with high-risk diabetic retinopathy (DR) were identified from our local health economy centralized electronic diabetes register and ophthalmic laser register. All known subjects with either currently active or previously treated significant diabetic retinopathy were included. People who were out of area or who had laser treatment for non-diabetic reasons were excluded. Prevalence was ascertained for each stage of DR. Data was derived from local IT systems for HbA_{1c} and four other important medically manageable risk factors- Blood pressure (BP), Creatinine, albumin/creatinine ratio (ACR) and cholesterol. These were then classified into good or poor control in accordance with internationally recognized standards such as BHS and NICE. Glycaemic control was assessed according to HbA_{1c} (%) categories as denoted: HbA_{1c}<7=A; 7.0-8.5=B; 8.5-10=C; HbA_{1c}>10=D. Number of risk factors not controlled were then assessed and analyzed for different grades of retinopathy and correlated against HbA_{1c} and other risk factors.
Results: Of 16,693 people with diabetes, 1440 (8.6%) were identified to have high-risk current or previously treated diabetic retinopathy (age range 20-94). 32 subjects were excluded from analysis due to incomplete dataset. Mean age was 63.2±14.6 and M:F ratio 1.5:1 (F=571). 1095 (78%) subjects had type 2 diabetes. 868 subjects (61.6%) were insulin treated. Breakdown of retinopathy grading: R1 940 (67%), R2 231 (16%), R3 239 (17%); M1 952 (67.6%). HbA_{1c} categories: A=529 (38%), B=317 (23%), C=330 (23%), D=234 (16%). BP of <140/80 mm Hg was achieved in 922 (65%) and Cholesterol of <4 mmol/l in 570 (40%). Diabetic Nephropathy (microalbuminuria and Creatinine>120) was seen in 392 (27%). All risk factors were controlled in 60 females (4.2%) and 64 males (4.5%). 845 (60%) subjects had 2 or more risk factors uncontrolled. For subjects with HbA_{1c} categories A,B,C positive correlation found between proportion of people with R3 and increase in number of risk factors above defined threshold. Significant positive correlation (p=<0.01) was also found between retinal score and HbA_{1c} and ACR on individual variate analysis.
Conclusion: Available retinopathy grades and risk factor data suggests that this group of high risk individuals have accessed local healthcare services within the recent past in the majority of cases (>97%). Despite this over one third of subjects with significant diabetic retinopathy had poor or very poor glucose control & suboptimal blood pressure control. Nearly two thirds of these subjects had 2 or more risk factors which were above a significant threshold. A correlation between degree of retinopathy and HbA_{1c} and ACR underlines the significance of managing microvascular risk factors in this cohort. Overall this study suggests that it is the service element that needs to be addressed in this group to target data driven care & improve outcomes.

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Multiparametric in-vivo imaging in degenerative retinopathy

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Background and aims: The transgenic polycystic-kidney-disease rat (PKD) develops neurodegeneration and subsequent vasoregression morphologically similar to diabetic retinopathy. The neurodegeneration over time has already been characterized using different parameters that are the in-vivo measurements of electroretinograms and the ex-vivo quantified retinal thickness histologically measured in retinal sections. In this study we analyzed the timecourse of the neurodegeneration with a multiparametric in-vivo imaging device to establish a method allowing us to monitor structural and functional changes over time and to identify specific sets of markers suitable to define the outcome of possible therapeutics.
Materials and methods: PKD rats were analyzed at different timepoints (1, 2, 3, 5, 7 and 12 months of age) matching our previously published ex-vivo results. Age matched Sprague Dawley (SD) rats served as controls. All measurements were performed using the RETImap multi-parametric imaging device. Retinal thickness was measured with the optical coherence tomography (OCT) module quantifying the thickness in five locations at the border of the central and peripheral retina equivalent to the area measured histologically. For the determination of the retinal function the multifocal electroretinography (mfERG) module with a seven frame focal algorithm centered on the optic nerve head was used. The groups were compared to each other and the retinal thickness was compared to our already published histological analysis.
Results: The in-vivo analysis showed a similar timecourse of disease progression as the histological data with a significantly decreased retinal thickness from one month on in PKD rats compared to SD rats (p< 0.001). Comparing the OCT-data to the histological data we achieve a Pearson Correlation Coefficient of R^2=0.97 for SD and R^2=0.87 for PKD rats (both p<0.05). For the retinal function the A-wave amplitude, reflecting the function of photoreceptors, revealed no significant difference between SD and PKD, the B-wave amplitude, reflecting the function of interneurons but also of glial cells, is decreased from two months on in PKD (p<0.01). In order to identify a functional parameter reflecting also the structural changes, we correlated them with the OCT-data showing a Pearson Correlation Coefficient for the B-wave amplitude of R^2=0.88 in SD and R^2=0.83 in PKD rats (both p<0.05).

Conclusion: In this study we demonstrate the suitability of the multiparametric in-vivo imaging device RETImap to detect and monitor retinal pathological changes affecting structure and neuroretinal function. By using this device it is possible to check the outcome of therapeutic interventions and to dramatically reduce the number of animals needed to perform this kind of studies. We also are able to show that the B-wave amplitude measured by mfERG is the most suitable parameter to determine early changes in neuroretinal function as it adequately takes the structural changes into account. This again suggests an impact of Müller cell function on the development of degenerative retinopathies.
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Genome-wide association study of severe diabetic retinopathy in type 1 diabetes

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Background and aims: The aim of the study is to find genetic risk factors for severe diabetic retinopathy (SDR) in type 1 diabetes with genome-wide association studies (GWAS) in the Finnish Diabetic Nephropathy (FinnDiane) Study. We also estimated the heritability of SDR.
Materials and methods: The patient population in the FinnDiane GWAS consisted of 1518 cases with SDR and 1622 controls without SDR. Type 1 diabetes was defined as being diagnosed <39 years of age and insulin treatment started within 1 year of diagnosis or if not available, a basal C-peptide level ≤0.2 pmol/ml. Patients were not excluded based on duration of diabetes. SDR was defined at baseline visit as presence of severe non-proliferative diabetic retinopathy or worse in ophthalmic exam/fundus photography or a history of scatter laser treatment (panretinal photocoagulation). This is equivalent to an ETDRS-score of 50 or worse in FinnDiane. Participants with mild or no retinopathy were considered as controls (ETDRS-score 45 or lower). The GWAS in FinnDiane was genotyped using Illumina 610Quad chip and SNP imputation was performed with HapMap II CEU reference population. The GWAS was performed using multiple logistic regression model with SDR status as the dependent variable and allele dosage as the independent variable (PLINK). Covariates in the analysis included age, gender, HbA_{1c} measured at baseline, diabetes duration, and quadratic transformation of duration (duration^2) all of which were significantly associated with retinopathy. The heritability estimation of SDR was calculated using GCTA (Genome-wide Complex Trait Analysis) with and without the covariates. A prevalence of 30% was used and samples with estimated relatedness of 0.025 or higher were excluded.
Results: 14 SNPs showed a p-value of $<10^{-5}$. The lowest p-value for SDR was found for a SNP on chromosome 1p13.3 with an odds ratio of 0.72 (95% confidence interval 0.707-0.819) and a p-value of 1.1×10^{-6} on *AKNAD1* gene. The results from the heritability estimation show heritability of SDR of <1% (heritability: 1×10^{-6}, standard error (S.E.) =0.130, p=0.5) without covariates. However, when including covariates (age, sex, HbA_{1c}, duration, duration^2) the heritability was estimated to 18% (S.E. =0.158, p=0.119).
Conclusion: Although no locus reached genome-wide significance, our finding show suggestive evidence of an association on chr1 to SDR in patients with type 1 diabetes. The heritability of SDR in our study is ~18% when the major covariates such as age, sex, HbA_{1c}, duration and duration^2 are considered.
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Implication of genetic loci identified in genome-wide association studies of diabetic retinopathy in Chinese patients with type 2 diabetes

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Background and aims: Diabetic retinopathy (DR), a leading cause of blindness, is the most common microvascular complication of type 2 diabetes (T2DM). Recent genome-wide association studies (GWAS) had identified novel DR-susceptibility genetic loci in various populations. If validated, these genetic markers may be utilised for risk profiling of DR in patients with diabetes and facilitate their early diagnosis. This study aimed to examine the association of the newly identified DR-associated single nucleotide polymorphisms (SNPs) with severe DR in Chinese T2DM patients.
Materials and methods: We conducted a cross-sectional case-control study on sight-threatening DR (STDR) based on Chinese subjects of the Hong Kong West Diabetes Registry. STDR cases were T2DM patients with either proliferative DR (PDR) or pre-PDR, or clinically significant macular oedema, or with photo-coagulation (with laser photocoagulation marks). Non-STDR controls were T2DM patients with no retinopathy. The grading of retinopathy was based on 2-field fundal photographs, according to the United Kingdom National Screening Committee (NSC) classification. A total of 36 SNPs showing top association signals with DR in previous GWAS were genotyped in 567 STDR cases, including 309 subjects with PDR, and 1490 non-STDR controls. Multiple logistic regression models with adjustment for confounding factors, including age, gender, duration of diabetes, and presence of hypertension, were employed.
Results: The strongest association was found at rs2115386, an intronic SNP in the insulin receptor gene *INSR* : adjusted $P=9.13\times10^{-4}$ (OR[95%CI]: 1.28[1.11-1.48]) for STDR, and adjusted $P=1.12\times10^{-4}$ (OR: 1.44[1.20-1.74]) for PDR. rs599019 located downstream of *COLEC12* (adjusted P=0.019; OR: 1.19[1.03-1.38]) and rs4462262 located at an intergenic region between *ZWINT* and *MRPS35P3* (adjusted P=0.041; OR: 1.38[1.01-1.89]) were also found to be significantly associated with STDR, but not with PDR alone. On the other hand, rs10199521 (*MYT1L-LOC729897*; adjusted P=0.022; OR: 1.25[1.03-1.51]), and rs899036 (*API5*; adjusted P=0.049; OR: 1.36[1.00-1.85]) showed significant independent associations only with PDR. Similar results were obtained when glycated hemoglobin (HbA1c) was also included in the adjustment models. The combined genetic risk score constructed of the significant SNPs showed an OR of 1.24 (adjusted $P=1.20\times10^{-5}$) and 1.35 (adjusted $P=2.00\times10^{-6}$) for each additional risk allele with STDR and PDR, respectively, in the combined genetic risk analyses.
Conclusion: We have successfully confirmed the significant independent associations of several SNPs identified in previous GWAS with severe DR (STDR and/or PDR) in our Chinese T2DM patients. Their highly significant combined genetic risk scores suggest potential applications in risk stratification for enhanced DR screening. Our findings on rs2115386 of the insulin receptor gene *INSR* are supportive of the role of insulin resistance, or the compensatory hyperinsulinaemia, in the pathogenesis of DR.
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Hyperglycaemia down-regulates levels of miR-126 in endothelial cells

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Background and aims: MicroRNAs have been implicated in the epigenetic regulation of key pathways in many diseases including type 2 diabetes (DM). miR-126, a microRNA expressed in endothelial cells, regulates expression of genes involved in neovascularization and vascular permeability, including Vascular Endothelial Growth Factor-A (VEGF-A) and hypoxia-inducible factor-1α (HIF-1α). VEGF-A has a pathologic role in microvascular diabetic complication, such as Diabetic retinopathy (DR) and diabetic macular edema (DME) which are the leading causes of blindness in the working-age population. Since levels of miR-126 are significantly lower in T2DM patients than in healthy controls, the aim of this study is to investigate whether hyperglycemia affects expression of miR-126 in a model in vitro of blood retinal barrier.
Materials and methods: We used commercially available human retinal pigment epithelial cells (ARPE-19) and endothelial cells (HECV). HECV cells were seeded in transwell inserts, let adhere for 24 hours and then co-cultured for 24 hours with ARPE-19 cells in DMEM normal glucose (5.5 mmol/Ll; CTR) or DMEM high glucose (25 mmol/L; HG) supplemented with 10% FBS. Total RNA was extracted from HECV cells and levels of miR-126 were detected using quantitative real-time PCR. Then mRNA expression of the miR-126 targets VEGF-A and HIF-1α was evaluated by quantitative real-time PCR. Another set of cells was lysed in RIPA buffer and HIF-1α protein expression was evaluated by Western blot.
Results: Levels of miR-126 were strongly decreased in HECV cells cultured with HG compare to CTR (-62.5%±10.61%, $p<0.001$ vs CTR). Lowered levels of miR-126 in HG condition are associated with increased mRNA expression of VEGF-A and HIF-1α (respectively + 30%±11.79%, $p<0.05$ vs CTR; and +33.3%±10.26%, $p<0.01$ vs CTR). In addition HIF-1α protein levels was up-regulated in HECV cells cultured under hyperglycemic condition (+51.7%±8.622%, $p<0.001$ vs CTR).
Conclusion: Here we show evidence that hyperglycemia directly lowers levels of miR-126 in endothelial cells. These results suggest that HG enhanced VEGF-A and HIF-1α mRNA expression by down-regulating miR-126. Furthermore the increased expression of VEGF-A induced by HG may be due to the synergic effect of miR-126 down-regulation and HIF-1α protein stabilization. These findings suggest that hyperglycemia may contribute to microvascular complication of diabetes by impairing levels of miR-126.
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miR-126 is downregulated in pericytes following exposure to mesenchymal stem cell-derived extracellular vesicles obtained in diabetic-like conditions

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Background and aims: Loss of pericytes in the early phases of diabetic retinopathy (DR) may disrupt their stable association with endothelial cells (EC), leading to EC proliferation and, eventually, angiogenesis. We have recently shown that extracellular vesicles (EV) derived from mesenchymal stem cells (MSC) in diabetic-like conditions may play a role in vessel destabilization by interfering with the strict EC/pericyte/extracellular matrix interactions. Thus they might contribute to angiogenesis through paracrine signalling; in particular, a role for MMP-2 has been described. MicroRNAs (miR) are short RNA sequences acting as gene modulators and playing important roles in angiogenesis and inflammation. MiR-126 is secreted mostly by the endothelium and controls vascular integrity and regeneration after injury. A down-regulation of miR-126 was observed in experimental models of DR, in diabetic retina extracts and in chorioretinal EC in hypoxic conditions, correlating with an increase in VEGF and MMP. Our aim in this study was therefore to investigate miR-126 expression in pericytes and the possible influence of EV derived by MSC cultured in diabetic-like conditions.
Materials and methods: Pericytes (HRP) were cultured in physiological (NG), stable high (HG) and intermittent high (intHG) glucose conditions for 8 days. In other experiments, EV were extracted from the supernatant of MSC cultured in hypoxic (hypo) and/or HG conditions and added to HRP cultured in NG for 6, 24 and 48 hrs. Real-Time PCR for miR-126 was performed on RNA extracts.
Results: HRP express miR-126 and this expression is down-regulated by 20% in intHG. miR-126 expression is not significantly modified by 6 and 24 hr exposure of HRP to EV. After 48 hrs, miR-126 is up-regulated by exposure to NG-EV (+62% vs ctrl). HG-EV and NG-hypo-EV do not influence significantly miR-126 expression, while EV obtained by MSC cultured in HG + hypoxia (HG-hypo) down-regulate miR-126 (-41% vs ctrl and -64% vs NG-EV, $p<0.05$).
Conclusion: We show for the first time in our knowledge that HRP express miR-126 and that its expression is down-regulated in diabetic-like conditions. Moreover, exposure of HRP to EV obtained in diabetic conditions is able to decrease miR-126 expression, consistently with previous observations of its involvement in DR and providing further insights for our findings of EV contribution to vessel destabilization.
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PS 107 Diabetic retinopathy screening

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Retinopathy in children with diabetes: When should they be screened?

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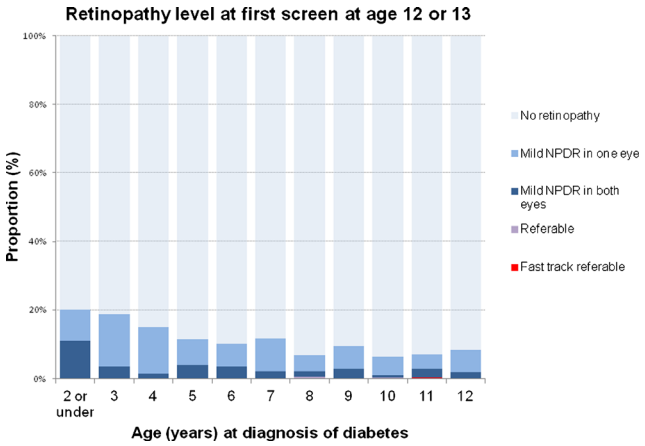
Background and aims: Current advice in the UK is to commence screening for diabetic retinopathy with digital retinal photography at twelve years of age. Here we report on the relationships between age at diagnosis of diabetes, age at diabetic eye screening and severity of Diabetic Retinopathy (DR) in children aged 12 or 13.
Materials and methods: Data were extracted from 6 UK screening programmes. Time from diagnosis of diabetes to first screening and age at diagnosis were calculated.
Results: Data were available for 2125 children screened for the first time at age 12 or 13 and diagnosed with diabetes before the age of 13. The proportion with any retinopathy decreased monotonically from 20% in those diagnosed at age of 2 years or below to 8% in those diagnosed at 12 ($p<0.0001$). The proportion with mild non-proliferative retinopathy in both eyes decreased from 11% in those diagnosed at 2 or below and 2% those diagnosed at 12. Only 3 children (aged 8, 10 and 11 respectively at time of diagnosis of diabetes) had images graded with referable retinopathy and of these 2 had non-referable DR at all subsequent screenings. Of 1703 children with subsequent images 25 were graded with referable DR over a mean follow-up of 3 years, incidence rate of 5 per 1,000 per year. Those with longer duration of diabetes at age 12 were at higher risk of progression to referable DR with hazard ratio of 1.3 per year (95% CI 1.18 to 1.50)
Conclusion: Although the rate of retinopathy in children below the age of 13 at first screening in the UK is significantly higher in those diagnosed with diabetes in early childhood, the rate is low and few children progress to referable DR. In this large cohort there is no evidence to suggest that earlier screening or more frequent screening is needed in this age group.



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Automated screening of diabetic retinopathy is safe and effective: a large-scale study on 55,074 diabetic patients

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Background and aims: Diabetic retinopathy (DR) is the leading cause of new-onset blindness in working-age adults in the industrialized world today. Fully-automated screening solutions are indispensable to screen the large and ever-increasing number of diabetic patients for preventable vision loss due to DR. EyeArt is a computerized, high diagnostic efficacy, cloud-based DR screening system that can screen thousands of patient images in just a few hours and thus enables seamless, large-scale screening deployment to aid triage of DR patients in most need of eye-care. The aim of this study is to validate EyeArt for detecting referable DR defined to be moderate non-proliferative DR (NPDR) or higher on the International Clinical Diabetic Retinopathy (ICDR) severity scale and assess its ability to reduce the workload of retina experts.
Materials and methods: A total of 440,072 color retinal fundus images of 55,074 diabetic patient visits or encounters were obtained from the EyePACS database without any patient identification data. Each of these encounters had 2-24 images including external lens-shots. These deidentified images were captured between April 2006 and December 2013 at various DR screening centers that use the EyePACS professional image grading services. The DR severity on the ICDR scale provided by human graders at EyePACS were also made available and considered to be the gold standard. The images were analyzed by EyeArt. External lens-shots and images of inadequate quality that were ungradable due to factors like poor focus or poor illumination were automatically flagged by the EyeArt system and were not further analyzed for detecting referable DR. A "refer" recommendation was provided for patients with apparent signs of moderate NPDR or higher on the ICDR scale and a "no refer" recommendation was provided for patients with no apparent signs of DR or signs of mild DR. EyeArt's referral recommendation performance was evaluated by using sensitivity, specificity, and area under the receiver operating characteristic curve (AUROC).
Results: Among the 55,074 encounters, prevalence of encounters with moderate NPDR or higher was 14.7% and prevalence of encounters with potentially treatable DR (severe NPDR or proliferative DR) was 3.5%. EyeArt's screening sensitivity was 90% (95% CI: 89.3% - 90.6%) and specificity was 47.5% (95% CI: 47.1% - 48.0%). This corresponds to 31,926 "refer" recommendations and 809 false negatives out of which 766 did not meet the general treatment criteria (i.e. had moderate NPDR). The AUROC was 0.847 (95% CI: 0.842 - 0.852). EyeArt's sensitivity for detecting potentially treatable DR was 97.8% (1878/1921 encounters). If the 23,148 encounters with "no refer" recommendations and 4,512 encounters with confident "refer" recommendations (operating point at 98% specificity) are not seen by graders, the graders need to grade only 49.8% of the total encounters with EyeArt. Therefore, in this mode of operation, EyeArt can help reduce the workload of graders by a factor of about 2.
Conclusion: EyeArt has high sensitivity and specificity for identifying patients with referable DR, making it safe and effective for integration into DR screening workflow. It can help save costs by greatly reducing the workload of image graders.
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Stratification of screening intervals for diabetic retinopathy based on one versus two consecutive negative screening events

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Background and aims: There is ongoing debate about the appropriate interval for diabetic retinopathy (DR) screening. It has been proposed that persons with diabetes without evidence of DR at two consecutive negative screening events are at low risk of developing referable DR (RDR) and can safely be screened bi or triennially. The question remains whether one screening event with no evidence of DR is adequate to similarly extend the screening interval. The aim of this study was to compare the incidence of RDR in persons without evidence of DR at either one or two prior consecutive screening events.

Materials and methods: The diabetic retinopathy screening service for Wales (DRSSW) screens annually all persons with diabetes within Wales over the age of 12 years. The service utilises standardised image capture i.e. 2× 45 degree digital retinal per eye using Canon DgI cameras and grading according to a nationally agreed protocol (DRSSW protocol). All persons included in the analysis were identified as having no DR, background DR or referable DR (RDR) i.e. pre-proliferative, proliferative DR or exudative maculopathy. Kaplan Meier survival estimates were obtained for the population undergoing screening and the incidence rates of RDR over 5 years were compared between those with one or two prior consecutive negative screening events for type 1 and type 2 diabetes separately.
Results: 1,338 persons with type 1 diabetes and 48,908 type 2 diabetes without evidence of DR at their first screening event between 2000 and 2013 were included in the analysis. There were 39 and 506 new cases of RDR over 5 years in those with type 1 and type 2 diabetes, respectively, with the 5 years cumulative incidence of RDR being 3.7% and 1.3% respectively. Of those with no DR at first screening 879 (65.7%) persons with type 1 diabetes and 37,958 (77.6%) persons with type 2 diabetes remained without DR at their second screening event which occurred at a mean of 606.7 (304.4) days and 574.8 (267.2) days respectively after the initial screening event. There were 10 and 146 new cases of RDR respectively within this group at 5 years, with the cumulative incidence of RDR being 5.1% and 1.3% respectively. The 1-5 year cumulative incidence of RDR for persons with type 1 diabetes was 0, 0.5, 0.7, 1.9 and 3.7% for those with 1 negative screening event and 0, 0.2, 1.2, 2.7 and 5.1% for those with 2 consecutive negative screening events without DR. The 1-5 year cumulative incidence of RDR for persons with type 2 diabetes was 0, 0.2, 0.5, 0.8 and 1.3% for those with 1 negative screening event without DR and 0, 0, 0.3, 0.7 and 1.3% for those with 2 negative consecutive screening events without DR.
Conclusion: There appears to be little added benefit of needing 2 consecutive annual negative screening events in order to safely extend the interval of screening to biennial in those persons considered to be at low risk as the incidence of RDR was 0% with 1 or two subsequent screening events. If screening intervals were extended to triennial then the incidence of RDR was slightly lower in those persons with 2 prior consecutive negative screening events at 0.2% compared to 0.5% for type 1 diabetes with 1 screening event and 0% compared to 0.2% respectively for type 2 diabetes.

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Diabetic retinopathy severity grading using digital image processing algorithms

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Background and aims: Diabetic retinopathy (DR) is an important cause of visual impairment. To ensure early detection and treatment, patients should undergo periodic eye examinations. In them, retinal images of the patient are usually captured using digital fundus cameras. Ophthalmologists further review these images to determine the degree of DR and establish the follow-up period accordingly. However, with the growing incidence of diabetes, the number of images to be examined can be prohibitively large. For this reason, automatic methods to detect DR severity level could be helpful to reduce the workload of ophthalmologists.
Materials and methods: We analysed 249 images from 18 diabetic patients without DR and 35 diabetic patients with different degrees of DR. Images were divided in a training set with 81 images and a test set of 168 images (96 without DR lesions and 72 with DR lesions). An ophthalmologist manually marked the location of hard exudates (EXs) and red lesions (RLs) in these images and classified the severity of DR for each patient. Besides, an automatic method for the location of EXs and RLs in the images was developed using digital image processing algorithms based on neural-networks (NNs). Three different types of NNs were analysed: multilayer perceptron (MLP), radial basis function (RBF) networks and support vector machine (SVM). The level of DR severity for a patient was subsequently established according to the number of lesions located in each image of the patient.
Results: The best results on the location of EXs and RLs were obtained with MLP. Using a lesion-based criterion, we achieved a mean sensitivity (SEl) of 60.0% and a mean positive predictive value (PPVl) of 50.0% for EXs. For RLs we obtained SEl =51.9% and PPVl =50.5%. With an image-based criterion, a mean sensitivity (SEi) of 76.4%, a mean specificity (SPi) of 55.2% and a mean accuracy (ACi) of 62.9% were obtained for EXs. For RLs we achieved SEi =71.2%, SPi =50.0% and ACi =58.5%. In a second phase, we assessed the usefulness of the proposed method for DR severity grading according to the detected lesions. Our results show that we were able to detect the correct DR severity grade in 65.7% of the patients. In 97.1% of the incorrectly classified cases, the automatic method provided the grade immediately above that given by the ophthalmologist. Besides, we reached an accuracy of 77.1% in distinguishing referable DR from non-referable DR.
Conclusion: Automatic methods for DR lesion detection and DR severity grading could be an important aid for ophthalmologists in the screening and evaluation of DR. Our results show that, in most cases, the frequency of revisions given by the automatic method would be equal or lower than that indicated by the ophthalmologist. However, further efforts should be directed to improve our results in the location of DR lesions and classification of DR severity grade, by improving the different stages of the algorithm and revising the definition of DR grades for automatic methods.

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Validation of an algorithm to predict the risk of sight threatening retinopathy in a multi-ethnic patient group treated in a Dutch hospital

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Background and aims: The majority of guidelines recommend a biennial fundoscopy to monitor retinopathy for all persons with type 2 diabetes mellitus (T2DM) free of elevated risk factors, and an annual fundoscopy for all other persons with T2DM. Only a minority of this population develops sight threatening retinopathy (STR). Recent studies indicate that it is possible to estimate STR risk based on individual patient characteristics and calculate a screening interval accordingly. In persons with a low STR risk, the screening frequency could be reduced, where it could be increased in the small high risk group. This personalized screening method would probably reduce the total number of fundoscopies, which would contribute to issues of the high and rising costs of T2DM care, the increasing demand for health care, and the limited eye care capacity. The algorithm developed in Iceland proved to predict the STR risk quit well in a mainly Caucasian population treated in a Primary Care Diabetes centre. However, it is yet unknown how the algorithm acts in a multi-ethnic patient group treated in secondary care, which was the aim of this study.
Materials and methods: Data of 888 persons with T2DM, treated in a hospital in the Netherlands were analysed. Using the Icelandic model, STR risk and an accompanying screening interval ranging from 6 to 60 months were calculated for each person based on gender, diabetes duration, HbA1c, systolic blood pressure and presence of retinopathy. In patients who developed STR during follow-up, we checked whether STR occurred before or after the model-recommended time of screening. Outcomes of omitted fundus photographs according to the model and potentially missed cases of STR were checked.

Results: The persons in this study had a Caucasian (n=403 (45.4%)), Hindustan-Surinam (n=178 (20.0%)), Negroid (n=33 (3.7%)), unknown (n=246 (27.7%), or other background. During the average follow-up duration of 40 months, 47 patients (5.3%) developed STR, of which 15 (31.9%) in the Hindustan-Surinam group. In 7 patients (15.2%) STR had developed before the recommended date of screening. Of these, 4 patients (57.1%) had a Caucasian background, 1 patient (14.3%) had a Hindustan-Surinam background and 2 patients (28.6%) had an unknown ethnic background. Using the algorithm, 50% of the fundoscopies would be omitted.

Conclusion: The model was less accurate in a multi-ethnic population of T2DM patients treated in secondary care. It remains to be determined whether this lower accuracy is caused by the multi-ethnic background of the population or the secondary care setting. Nevertheless, the number of missed cases of STR suggests that there is room for improvement of the current model for this multi-ethnic population treated in secondary care.

Ethnic background	# patients	# fundoscopies	# fundoscopies saved (%)		# STR (%)		Missed cases of STR (%)	
Negroid	33	63	30	48%	1	3,0%	0	0%
Hindustani	178	391	213	54%	15	8,4%	1	7%
Indians	16	43	27	63%	2	12,5%	0	0%
Non-Hindustani Surinams	12	27	15	56%	1	8,3%	0	0%
Unknown	246	483	237	49%	9	3,7%	2	22%
White	403	776	373	48%	19	4,7%	4	21%
TOTAL	888	1783	895	50%	47	5,3%	7	15%

Table 1. Validation results of Aspelund's algorithm in a multi-ethnic population treated in secondary care

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Screening for diabetic retinopathy: a collaborative model in Italy

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Background and aims: Systematic screening for diabetic retinopathy (DR) is not centrally organized in Italy, where screening is performed mostly on an opportunistic basis. The REaD (REtina and Diabetes) programme was launched in April 2013 to establish a network of screening centres where diabetes and eye specialists work in the same settings. This paper reports on the results obtained up to December 2014.

Materials and methods: Screening was conducted in 33 diabetes clinics by non-mydriatic digital fundus cameras (DRS, Centervue, Padua, Italy) connected to ophthalmology units within the same health structures in Italy. Two partially overlapping non-stereoscopic 45 degree fields (central and nasal) of each eye were obtained by trained operators and sent via web to partnering eye units. Images were then evaluated by an ophthalmologist to assess the presence of diabetic retinopathy (DR) and/or macular oedema (DMO) according to a severity scale with standardized referral guidelines.

Results: In total, 16,275 patients were screened, of which 53.0% were male; 51.3% were≤64 years old and 48.7% were≥65 years old. Out of 77,309 images collected, 22 centres collected approximately 2,000 each and 11 collected more than 2000. In total, 87.2% of patients could be properly assessed. The percentage of ungradable images decreased from 14% (November 2013) to 9.5% (December 2014). Most ungradable images (57% of those obtained with mydriasis and 62% without mydriasis) were observed in patients aged 61 to 80. Of the 14,185 patients with gradable images, 3,260 (23%) had diabetic retinopathy and were referred for further assessment and treatment.

Conclusion: The REaD programme is the largest screening campaign conducted in Italy in a setting where screening is mostly performed on an opportunistic basis. Non-mydriatic digital fundus cameras may be a cost-containing screening tool for DR. The number of ungradable images was a main limitation, at its maximum when the programme started, possibly due to operator-related factors, then stabilized around 10%. It appeared mainly related to patient age and non-induction of mydriasis. Systematic screening for DR with non-mydriatic digital cameras allows diabetes specialists to obtain a basic fundus evaluation directly in their units and to promptly organize referrals to ophthalmic centers in case of abnormal findings.

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Regional differences in macular thickness in the early stages of diabetic retinopathy in type 2 diabetes

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Background and aims: Following the advent of optical coherence tomography (OCT) for the measurement of macular thickness there has been interest in the relationship between macular thickness and diabetic retinopathy (DR). Research to date has focussed on fovea thickness with conflicting results. The variations in the observations may reflect different grading systems utilised to assess retinopathy status. As part of the SUMMIT consortium this study aims to examine the relationship between early stages of DR and macular thickness in all subdivision of the OCT ETDRS grid.

Materials and methods: 780 participants with type 2 diabetes (461, 162 and 157 with no, microaneurysms only or mild background DR respectively) were recruited across two centres. DR was graded from two-field retinal photography. Macular thickness (inner limiting membrane to the mid-point of the retinal pigment epithelium) was measured in both eyes using a standard OCT 512×128 scanning protocol. Participant characteristics were recorded. The eye with the worse retinopathy and its corresponding OCT data were utilised in the analysis. If retinopathy was the same in both eyes the data from the right eye was utilised. The relationship between retinal thicknesses in each ETDRS region with DR (entered as no DR, microaneurysms only and mild background DR) was investigated using regression analysis, adjusting for age, gender and centre.

Results: There was no relationship between the thickness of the fovea or inner quadrants with DR. In the outer quadrants there was a significant positive association between thickness in the temporal region and DR (unstandardised β (standard error): 1.639(0.648)μm; standardised β= 0.092, p=0.012). This association remained when further adjusted for glycaemic control (HbA1c) and duration of diabetes (unstandardised β (standard error): 1.655(0.709)μm; standardised β=0.093, p=0.020). This association was not present in the nasal, superior or inferior outer quadrants.

Conclusion: This study suggests that there are small differences in the net thickness in the outer temporal region in the early stages of DR. An attractive explanation is that in the early stages of DR there is thinning of the retinal nerve fibre layer (RNFL) accompanied by thickening of another retinal layer, resulting in no net change in thickness in the majority of the ETDRS grid. As the outer temporal region has minimal RNFL there is net thickening in this region.

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Cataract associated with diabetes and diabetic retinopathy in type 2 diabetes

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Background and aims: Cataract is the commonest cause of preventable blindness worldwide. In patients with diabetes, cataract occurs at a younger age and progresses more rapidly than senile cataract, resulting in higher rates of cataract surgery at a quite young age and poorer vision outcomes especially in operated eyes with active proliferative retinopathy and/or preexisting macular edema. The exact pathogenesis that leads to lens opacification in diabetes is still insufficiently understood and presumed to involve diabetic complications risk factors. The aim of this study was to investigate the role of diabetes duration, metabolic risk factors, diabetic retinopathy (DR) and nephropathy in cataract development in type 2 diabetes.
Materials and methods: This was a cross-sectional study including 107 type 2 diabetic patients (67 male / 40 female, mean age 66.74±8.01 years, mean diabetes duration 15.05±5.69 years). Metabolic risk factors: glycated hemoglobin (HbA1c), total cholesterol, HDL cholesterol, LDL cholesterol and triglycerides were determined using routine laboratory methods. Blood pressure was measured with a mercury sphygmomanometer after a 10-minute resting period. Serum creatinine, creatinine clearance and albumin/creatinine (A/C) ratio were used to determine the presence of nephropathy. Ophthalmologic examination included best corrected visual acuity (BCVA), biomicroscopy of the lens, binocular indirect slit lamp fundoscopy and color fundus photography of two fields (macular field, disc/nasal field) of both eyes according to the EURODIAB retinal photography methodology. Lens opacity was graded according to the Lens Opacity Classification System version III (LOCSIII).
Results: According to the 4 grading scales of the LOCSIII patients were divided into three groups: group 1 - patients with clear crystalline lens (n=16), group 2 - patients with initial cataract (NO1-NO2, NC1-NC2, C1-C2, P1-P2; n=74), and group 3 - patients with immature cataract (NO3-NO4, NC3-NC4, C3-C4, P3-P4; n=17). The three groups did not differ in age, gender, diabetes treatment, SBP and DBP, total cholesterol, HDL, LDL and triglycerides. Group 3 had significantly longer diabetes duration (17.12±6.38 years vs. 10.81±4.09 years; p<0.001) and marginally higher HbA1c (7.11±1.06 vs. 6.38±0.82; p=0.052) than group 1. DR was significantly more severe (35/29/36% vs. 82/6/12%; p=0.047) and creatinine clearance significantly lower (1.53±0.25 vs. 1.90±0.72; p=0.017) in group 3 than in group 1. Cataract was positively correlated with DBP (p=0.002), DR (p=0.009) and A/C ratio (p=0.004). DR was positively correlated with HbA1c (p=0.018), triglycerides (p=0.041) and A/C ratio (p=0.007). Multiple regression analysis showed that diabetes duration (β coefficient=0.365; p<0.001), SBP (β coefficient=0.379; p=0.003), DBP (β coefficient=0.503; p<0.001) and DR (β coefficient=0.243; p=0.013) were the main predictors of cataract development in type 2 diabetic patients.
Conclusion: This study showed that diabetes duration, poor glycemic control, blood pressure, retinopathy and nephropathy play an important role in the cataract development in type 2 diabetes. These findings point to the need for even more reducing risk factors as a means of preventing not only retinopathy but also cataract in order to improve the quality of life of diabetics and reduce the economic burden due to disability and surgery related to cataract.

PS 108 Diabetic nephropathy: pathogenesis and predictors

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Genetic exploration of a causal role of dyslipidaemia on diabetic nephropathy in type 1 diabetes

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Background and aims: Dyslipidemia has been suggested as a risk factor for diabetic nephropathy (DN), but epidemiologic studies do not reveal whether these associations are causal. Genome-wide association studies have uncovered a number of single nucleotide polymorphisms (SNPs) affecting plasma lipid fractions. In three cohorts participating in the Genetics of Nephropathy-An International Effort (GeNIE) cohort, we tested if genetic risk scores (GRS) for LDL cholesterol, HDL cholesterol, total cholesterol and triglycerides were associated with DN in 6126 adult patients with type 1 diabetes.
Materials and methods: We created weighted GRS constructed from established variants for HDL cholesterol (71 SNPs), LDL cholesterol (58 SNPs), triglycerides (40 SNPs) and total cholesterol (74 SNPs) weighting each risk allele by its published effect size, in each of 3 cohorts. DN was defined as having macroalbuminuria or end-stage renal disease (ESRD). Controls with normal albumin excretion rate were required to have a diabetes duration of more than 15 years. Association results from each cohort were combined in a meta-analyses using a fixed effect model.
Results: Genetic risk scores for lipid fractions (LDL cholesterol, HDL cholesterol, triglycerides, and total cholesterol) were not significantly associated with DN in meta-analyses. However, there was a trend towards increased odds of DN with a higher LDL cholesterol GRS (OR=1.048 per 1 SD of GRS [95% CI, 0.994 -1.105], P=0.085) and a trend towards decreased odds of DN with a higher HDL cholesterol GRS (OR=0.954 per 1 SD of GRS [95% CI, 0.904-1.006], P=0.080). We also investigated the impact of GRS lipid fractions on DN subtypes; macroalbuminuria and ESRD. We found that a higher HDL cholesterol GRS was significantly associated with decreased odds of macroalbuminuria (OR=0.936 per 1 SD of GRS [95% CI, 0.879-0.996], P=0.036) and a higher triglyceride GRS with increased odds of macroalbuminuria (OR=1.069 per 1 SD of GRS [95% CI, 1.005-1.138], P=0.034). None of the GRS lipid fractions were significantly associated with ESRD.
Conclusion: In conclusion, genetic factors associated with increased HDL and TG are associated with macroalbuminuria in patients with T1D suggesting that these lipids have a causal role on development of diabetic nephropathy. Further research is needed to verify these findings.
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Long-term high level of LDL cholesterol associated with a new onset of chronic kidney disease in patients with type 2 diabetes mellitus

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Background and aims: We investigated factors that might influence the development of chronic kidney disease (CKD) in patients with type 2 diabetes.
Materials and methods: From January 2003 to December 2004, a total of 861 patients with type 2 diabetes without CKD (estimated glomerular filtration rate [GFR]≥60 ml/min/1.73 m^2) were consecutively enrolled. The blood lipid concentrations for total cholesterol, triglycerides, high-density lipoprotein (HDL) cholesterol, low-density lipoprotein (LDL) cholesterol, and the estimated GFR were measured annually and mean LDL cholesterol level was calculated from annual LDL cholesterol data during the study. The new onset CKD was defined as estimated GFR< 60 ml/min/1.73 m^2 using a Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation. We used a Cox proportional hazard regression analysis to test the associations between new onset CKD and potential explanatory variables.
Results: Of the 861 patients enrolled in this study, 582 (67.6%) completed the follow-up evaluation. The median follow-up time was 10.1 years. The mean age was 55.0±10.0 years, and the duration of diabetes was 8.0 ±6.6 years. The baseline estimated GFR was 95.2±15.1 ml/min/1.73 m^2. The patients who developed a new onset of CKD had higher level of mean LDL level (2.67±0.56 vs 2.85±0.58 mmol/l; p=0.004) than those who did not develop CKD. During the follow-up period, 184 patients (31.6%) progressed to chronic kidney disease. The hazard ratio of CKD development was 1.61 (95% CI 1.14-2.26; p for trend=0.006) per 1.0 mmol/l mean LDL cholesterol level increase after adjusting for sex, age, diabetic duration, mean HbA_{1c}, albuminuria, treatment of insulin, ACE inhibitor/angiotensin receptor blocker and lipid lowering agents.
Conclusion: The development of CKD was independently associated with serum LDL cholesterol level in patients with type 2 diabetes. Thus, in addition to strict glycemic control, constant long term management of dyslipidemia is important to prevent development of CKD.

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Association of monocyte-to-HDL-cholesterol ratio with microalbuminuria in patients with type 2 diabetes mellitus

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Background and aims: Chronic kidney disease (CKD) due to diabetes mellitus is a growing health problem worldwide. Chronic proinflammatory state exists in adults with CKD. Previous studies have demonstrated that monocyte count to high density lipoprotein cholestrol (HDL-C) ratio (MHR) is one of the indicators of oxidative stress and systemic inflammation and showed that renal dysfunction was associated with both a reduction in serum HDL-C level and increased circulating monocyte count. Thus, we aimed to evaluate the relationship
Materials and methods: Between June 2014 and January 2015 a total of 80 patients with type 2 DM (n=34 with microalbuminuria and n=46 without microalbuminuria) who admitted to our outpatient clinic were enrolled. 24-h urine analysis for microalbuminuria, calculation of MHR and serum C-reactive protein (CRP) were performed for each patient. Patients with malignancy or acute/chronic infectious/inflammatory state were excluded.
Results: Patients with microalbuminuria were older, with higher glycosylated hemoglobin levels (HbA1c). The MHR and CRP levels were also significantly higher in patients with microalbuminuria (p=0.002 and p< 0.001, respectively). There was a positive correlation between MHR and serum CRP (r=0.462, p<0.001). In multivariate logistic regression analysis, MHR was found as independent predictor for the presence of microalbuminuria in patients with diabetes (OR: 1.44, p<0.001).
Conclusion: Higher MHR which indicates an enhanced inflammation and oxidative stress was significantly and independently associated with the presence of microalbuminuria in patients with diabetes. Besides, MHR was positively correlated with serum CRP level as a conventional marker for systemic inflammation.

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Functional polymorphisms in the promoter region of heme oxygenase-1 and susceptibility to diabetic nephropathy in type 2 diabetes

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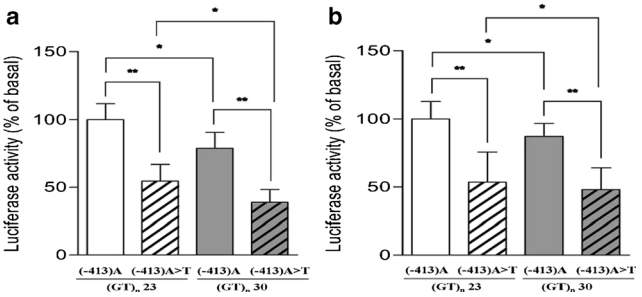
Background and aims: Heme oxygenase (HO)-1 is a key enzyme in cytoprotective mechanisms against oxidative stress in the cardiovascular-renal system. The T(-413)A single nucleotide polymorphism (SNP) and $(GT)_n$ microsatellite polymorphism in the HO-1 gene promoter modulate the HO-1 gene transcriptional activity and these polymorphisms are associated with various human diseases. We investigated the association between HO-1 promoter polymorphisms and nephropathy in type 2 diabetes and assessed the effect of SNP on promoter activity in kidney cell-lines in vitro.
Materials and methods: We sequenced the T(-413)A SNP and (GT)n repeat segments of the HO-1 gene promoter in 536 patients with type 2 diabetes. (GT)n alleles were divided into 2 groups: short (S, ≤25 GT repeats) and long (L, >25 GT repeats) alleles. Diabetic nephropathy was defined as follows: urinary albumin/creatinine ratio≥30 mg/mg or albumin excretion≥30 mg/day in a 24 h urine analysis. The regulatory effects of SNPs in the HO-1 promoter were explored in two kidney cell-lines using luciferase assay after site-specific mutagenesis of HO-1 promoter.
Results: Patients with the TT genotype in the T(-413)A SNP were significantly more susceptible to diabetic nephropathy than those carrying the A allele, with an odds ratio (OR) of 1.577 (95% confidence interval, 1.088−2.285; P=0.016). Subgroup analysis showed that patients carrying the TT genotype with long duration of diabetes (≥20 years, OR= 3.325, P<0.05), poor glycemic control (HbA_{1c}>7.4%, OR=1.780, P< 0.05), and male gender (OR=2.043, P<0.05) had higher odds ratios for the development of diabetic nephropathy. Regarding to the $(GT)_n$ repeats, the LL genotype showed a higher odds ratio for the development of diabetic nephropathy only in patients with hypertension when compared to the S allele. In vitro experiments, promoter activity of the T(-413)A SNP was higher with A allele than T allele in human embryonic kidney cells and mesangial cells.

Conclusion: In conclusion, the T(-413)A SNP in the HO-1 promoter is significantly associated with diabetic nephropathy development in type 2 diabetes patients, especially with longer duration and poor glycemic control.



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T-cadherin gene (CDH13) polymorphisms and nephropathy in subjects with type 1 diabetes

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Background and aims: T-cadherin is a receptor for the active forms of adiponectin, and variants in the T-cadherin gene (*CDH13*) have been associated with adiponectin levels. Plasma adiponectin levels are associated with diabetic nephropathy (DN). We investigated associations of *CDH13* allelic variations with kidney complications in subjects with type 1 diabetes.

Materials and methods: Three *CDH13* polymorphisms (rs11646213, rs3865188, rs4783244) were genotyped in 1297 Caucasian type 1 diabetic patients from SURGENE (n=340), GENESIS (n=501) and GENEDIAB (n=456) studies. Follow-up data were available for all SURGENE participants and for 462 GENESIS and 283 GENEDIAB participants. Duration of follow-up was 10, 9 and 5 years, respectively. Renal events were defined as new cases of DN or progression to a more severe stage of DN. We used ANCOVA to compare means of clinical and biological parameters between genotypes. Odds-ratios (OR) and hazard-ratios (HR) were estimated for the prevalence and incidence of DN using logistic regression and Cox model analyses, respectively. GENEDIAB and GENESIS data were pooled for the analyses. All analyses were adjusted for sex, age, duration of diabetes, body mass index, retinopathy stages and cohort membership (when adequate).

Results: In the SURGENE study, the variants rs11646213 (HR 1.69; CI 95% 1.01-2.71; p=0.04) and rs3865188 (HR 0.74; CI 95% 0.55-0.99; p=0.04) were associated with the incidence of renal events during follow-up. At the end of the study, these variants were associated with the prevalence of DN (OR 3.30; CI 95% 1.28-8.38; p=0.01 for rs11646213 and OR 0.54; CI 95% 0.30-0.93; p=0.03 for rs3865188). In the pooled analysis of GENEDIAB and GENESIS studies, the AA genotype of rs11646213 was associated with the prevalence of established/advanced DN (OR 1.47; CI 95% 1.18-1.85; p=0.0009), with higher urinary albumin excretion (p=0.01) and diastolic blood pressure (p=0.01). The same genotype was associated with higher incidence of end-stage renal disease (ESRD) during follow-up (HR 1.96; CI 95% 1.06-3.51; p=0.03). Furthermore, the minor alleles A and T of rs3865188 and rs4783244, respectively, were also associated with the prevalence of established/advanced DN (OR 0.71; CI 95% 0.57-0.90; p=0.004 for rs3865188 and OR 0.79; CI 95% 0.63-0.99; p=0.05 for rs4783244) and the incidence of ESRD (HR 0.47; CI 95% 0.27-0.84; p=0.01 for rs3865188 and HR 0.44; CI 95% 0.25-0.79; p=0.006 for rs4783244).

Conclusion: In subjects with type 1 diabetes, *CDH13* polymorphisms were associated with the prevalence of established and advanced diabetic nephropathy, as well as with the incidence of renal events and ESRD. The genotypes at risk have been previously associated with lower plasma adiponectin levels. Further investigations are needed to determine whether the variations in plasma adiponectin and/or t-cadherin levels can explain these associations.

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Markers of chronic kidney disease - albuminuria and glomerular filtration rate encoded by different genetic determinants in type 2 diabetes

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Background and aims: The major markers of chronic kidney disease (CKD) - albuminuria (AU) and glomerular filtration rate (GFR) have the different clinical pattern due to diabetes type: in type 1 diabetes AU is a predictor of subsequent GFR decrease, in type 2 diabetes (T2D) it might develop in parallel or after GFR decrease may be due to different genetic determinants. The aim of our study was to assess the association of candidate genes polymorphisms encoding the key mediators of kidney damage with AU and GFR in T2D patients.

Materials and methods: We enrolled 262 patients with T2D divided into groups due to the rate of AU>/<30 mg/per 24H (AU+/-, n=128/134) and GFR≥/<60 ml/min/1.73 m2 estimated by MDRD (CKD+/CKD-, n=86/176) and analyzed a complex of 6 pm in genes involved in coding of major mediators of kidney injury: endothelial dysfunction [nitric oxide synthase-3 (ecNOS4a/4b in NOS3), angiotensin I-converting enzyme (I/D in ACE)]; lipids metabolism [apolipoprotein B (I/D in APOB), apolipoprotein E (e2/e3/e4 in APOE)]; insulin secretion and sensitivity (rs5219 in KCNJ11 coding protein Kir6.2 subunits of potassium inwardly-rectifying channel, rs7903146 in TCF7L2 coding transcriptional factor 7-like 2). Statistic analysis was assessed by $\chi 2$, OR, $p<0.05$. The study was approved by local ethical committee; informed concern was obtained from all the patients.

Results: Significant association with AU showed one of the studied pm: 4a/4b in NOS3 gene: we observed excess of 4a allele and 4a4a genotype in group with AU did as risk factors [4a: OR=1.7; 95%CI 1,1-2,5, 4aa: OR=9.89; 95%CI 2,05-47,72]. Significant association with GFR decrease showed pm in 4 genes. NOS3 gene: the prevalence of 4b/4bb in CKD- group [OR=0.45/0.45; 95%CI 0,2-0,7/0,26-0,77, respectively]; while allele 4a and genotype 4a/4a did as risk factors [OR=2,2/9,9; 95%CI 1,4-3,6/2,1-47.7, respectively]. APOB gene: genotype DD protected from CKD [OR=0,2; 95%CI 0,05-0,8, p=0,04] in dominant inheritance model compared with carriers of II and ID genotypes that did as risk factors [OR=5.00; 95%CI 1,1-21, p=0,02]. KCNJ11 gene: an excess of allele T facilitate to CKD existence [OR=2,4 95%CI 1,4-4,1], allele C/genotype CC were protective [OR=0,52/0,44, 95%CI 0,3-0,8/0,2-0,8 respectively]. TCF7L2 gene: genotype TT [OR=0,12, 95%CI 0,03-0,53] had a protective role. We didn't find a variant association of pm's in ACE and APOE with CKD. CKD risk highly depended on the amount and combination of risk/protective genotypes (r=0,971, p<0,05). The absence of any risk genotypes protected from CKD [RR=0], if one of risk genotypes detected RR=0,86, if any 2 of them RR=1,7, any 3 risk genotypes increased CKD risk with RR=2,2 and RR=3,27 was the highest in carriers of 4 predisposing variants of studied pm.

Conclusion: The CKD development might have genetic susceptibility with different genetic determinants for AU and GFR in T2D patients. Risk of AU is associated with polymorphism of NOS3 gene; risk of GFR decline - with pm in NOS3, APOB, KCNJ11 and TCF7L2 genes. CKD risk was highly depended on the amount of risk genotypes with highest risk in carriers of 4 predisposing variants. The data obtained allow offering these pm as diagnostic panel for allocation of high-risk patients, which require more frequent screening and active prevention for this complication.

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Uric acid contributes to the faster progression of diabetic kidney disease and higher incidence of major cardiovascular event in type 2 diabetic patients

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Background and aims: Recent evidence suggests that increased serum uric acid (SUA) is associated with increased risk of diabetic kidney disease (DKD) development in patients with type 1 diabetes. Data on the role of SUA in the progression of DKD in type 2 diabetes (T2DM) are lacking. Polymorphisms in genes encoding for uric acid transporters in the kidney (SLC2A9, ABCG2) and gut (ABCG2) affect SUA and were associated with hyperuricaemia and gout but were not studied with respect to DKD yet. The aims of the study were to assess (i) the prognostic value of baseline SUA and (ii) variability in selected genes involved in the regulation of uric acid metabolism for DKD progression in T2DM.
Materials and methods: 422 patients with T2DM and different stage of DKD (defined as macroalbuminuria or ESRD and/or CKD III and higher) at baseline were included in the prospective study. Median follow-up was 47 [IQR 27-79] months. Hyperuricaemia was defined as initial SUA≥ 420 μmol/l in men and ≥360 μmol/l in women or as a treatment with allopurinol at the inception in the study. Following endpoints were defined: 1) DKD progression (i.e. the decline of GFR<60 ml/min per 1.73 m2 during the follow-up period for those with GFR≥60 ml/min per 1.73 m2 at baseline or achieving ESRD, or the development of overt proteinuria in normo- and microalbuminuric subjects at baseline or the progression of CKD by at least stage for those with CKD III and IV at baseline) with all-cause mortality as a competing risk and 2) major cardiovascular event (MACE - i.e. fatal or non-fatal myocardial infarction or cerebrovascular event, limb amputation, revascularization) with non-CVD death as a competing risk. Using PCR-based methodology we detected 5 SNPs in the genes formerly associated with hyperuricaemia/gout (4 in SLC2A9 and 1 in ABCG2). Statistical analysis involved survival analysis, competing risk analysis and Cox regression.
Results: Cumulative incidence of DKD progression was 54.4%, MACE 32.2% and total mortality 43.8%. Initial hyperuricaemia was confirmed as a significant risk factor for both DKD progression and MACE (P< 0.00001 and P=0.0021, log-rank test). Allopurinol treatment did not have protective effect with respect to DKD progression. Patients with and without initial hyperuricaemia did not differ in the frequency of risk genotype in any of studied SNPs. Allele T of SLC2A9 rs16890979 was marginally associated with faster DKD progression (P=0.05, Kaplan-Meier).
Conclusion: Our results suggest that hyperuricaemia significantly contributes to the progression of DKD and to the higher incidence of MACE in patients with T2DM. The effect of studied genetic variants appears to be minor.
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Uric acid associated with decline of GFR in diabetic nephropathy

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Background and aims: Diabetes is the major cause of chronic kidney disease (CKD). Evidence from epidemiological and prospective studies indicates that serum uric acid (UA) is a risk factor for development and progression of CKD and loss of kidney function. We evaluated the effect of serum UA on change in GFR in patients with type 1 diabetes (T1DM) in a previous conducted clinical trial.
Materials and methods: Post hoc analysis of a prospective, double-blinded, clinical intervention trial of the long-term renoprotective effect of AT1 receptor blockade (losartan 100 mg) on progression of diabetic nephropathy in T1DM homozygous for the I (n=26) or D (n=27) allele of the ACE/ID polymorphisms. Mean follow-up time was 3 years (range 1.5 - 3.5). Serum UA was measured at baseline. Primary end-point was GFR measured with Cr51-clearance every 6 month. Effect of UA was tested in a linear regression model with and without adjustment for known progression factors (Gender, HbA1c, systolic blood pressure, cholesterol, baseline GFR and baseline urinary albumin excretion rate (UAER)) .
Results: Mean baseline UA was 0.339 mmol/L (SD ±0.1), GFR 87 ml/min/ 1.73 m2 (SD ±23), Geometric mean of UAER 1023 mg/ 24 h (IQR, 631 - 1995). Similar association between UA and change in GFR was present for the two ACE/ID polymorphisms. In an unadjusted linear model UA was positively associated with decline in GFR (r^2=0.06, p= 0.09). After adjustment for known progression factors the association increased to a significant level (r^2=0.35, p=0.011). In the backward elimination UA remained in the model, together with baseline UAER and baseline GFR (r^2=0.26, p=0.0031).
Conclusion: Uric acid was positively associated with decline in GFR in type 1 diabetic patients with nephropathy. UA was a significant predictor together with UAER and baseline GFR. The clinical significance of UA is currently investigated in a multicenter clinical trial (PERL Study).
Supported by: DDA and AU

PS 109 Diabetic nephropathy: treatment

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The effect of sensor augmented insulin pump treatment versus multiple daily injections on albuminuria: a one year randomised controlled study

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Background and aims: The effect of tight glycaemic control on persisting albuminuria remains unclear. Insulin delivery method may play an important part by affecting HbA1c and/or glucose variability. We investigated the effect of 1 year sensor augmented insulin pump treatment (SAP) versus multiple daily injections (MDI) on albuminuria in type 1 diabetes patients with a history of elevated urinary albumin excretion.
Materials and methods: In a randomized controlled open-label parallel trial 60 type 1 diabetes patients were randomized to 1 year SAP or MDI treatment. At screening patients had urinary albumin creatinine ratio (UACR) ≥10 mg/g in 2 out 3 morning urines. All were on stable RAS inhibition 4 weeks prior to screening and throughout the study. UACR was measured in 3 morning urines at all visits. Glucose variability (CGM) and glomerular filtration rate (51Cr-EDTA GFR) was measured at beginning and end of study. Changes in UACR between treatment groups were analysed using a mixed linear model including all measurements during the study and adjustment for HbA1c.
Results: 57 patients (SAP: n=28; MDI: n=29) completed the study. At baseline 86% had UACR ≥30 mg/g. Age was (mean ± SD) 51±10 years, duration of diabetes was 33±12 years. UACR was (geometric mean, IQR) 99 (37-235) mg/g. GFR: 94±22 ml/min/1.73 m2, HbA1c: 75± 12 mmol/mol, glucose variability (given as SD): 4.0±1.0 mmol/mol and systolic blood pressure (SBP): 143±17 mmHg; no difference between groups (P≥0.08 for all). After 1 year, HbA1c decreased 14±11 vs. 6±11 mmol/mol (P=0.013), glucose variability decreased 0.9±1.1 vs. 0.3±1.0 mmol/mol (P=0.037), GFR declined 5.6±9.6 vs. 3.4± 13 ml/min/1.73 m2 (P=0.50), SBP decreased 1.6±17 vs. 3.6±17 mmHg (P=0.68) in SAP vs. MDI treatment, respectively. UACR (mean(95%CI)) decreased 13 (-39-22)% with SAP vs. 30 (-12-92)% increase on MDI (unadjusted: P=0.052; adjusted for HbA1c: P=0.042). In the subgroup of patients with UACR ≥30 mg/g at baseline (n=48) UACR decreased 18 (-44-20)% in the SAP treatment group (n=24) while MDI treated patients (n=24) had an increase of 38 (-11-71)% (unadjusted: P=0.01; adjusted for HbA1c: P=0.01).
Conclusion: In conclusion, sensor augmented insulin pump treatment tended to reduce UACR in a randomized controlled trial in type 1 diabetes patients with a history of albuminuria on stable RAS inhibition. Significance was reached after adjustment for HbA1c. In the subgroup with albuminuria at baseline, SAP treatment reduced UACR significantly. SAP treatment reduced glucose variability and HbA1c and might have a beneficial effect on diabetic nephropathy.
Clinical Trial Registration Number: NCT01454700
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Effect of different types of insulin therapy on transplant state, metabolic and haemodynamic factors control in patients with type 1 diabetes after kidney transplantation

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Background and aims: To evaluate the impact of different types of insulin therapy (continuous subcutaneous insulin infusion (CSII) using insulin pump or multiple insulin injections (MII)) on carbohydrate metabolism, the state of the transplant, cardiovascular system, calcium and phosphorus metabolism in patients with type 1 diabetes (DM1) after kidney transplantation (KT).
Materials and methods: The study included two groups of patients with DM1 after transplantation: 1) 17 patients treated with CSII 2) 10 with MII. Mean duration of diabetes in the first group was 25 years [20,5;34, 5], the second group - 24,5 years [20;30]. Posttransplantation period in both groups was comparable - 8,0 [7,0;36,0] and 7,5 [7,0:19,0] months.
Results: The mean level of glycated hemoglobin (HbA1c) in groups before the study did not differ: 9.0% [8.0;9.6] and 9.0% [8,7; 9.8], respectively. When patients were transfer into CSII - HbA1c was significantly lower in this group after 3-6-9 months -7,4% [7,4; 8,6](p<0,0005), while in the MII group - 8,1% [7,5;8,7] (p<0,026). Recurrent diabetic nephropathy at the stage of microalbuminuria was diagnosed in 2 patient (20%) in the MII group with continuing poor glycemic control (the level of albuminuria was 35-65 mg/l, reanalysis - 68-85 mg/l). All patients, treated CSII had normal albuminuria. Glomerular filtration rate (GFR) (CKD-EPI) in both groups was comparable: 67 ml/min/1,73 m2 [65,86] and 65 ml/min/1,73 m2 [60,0;75,0], respectively. Stabilization of diabetic retinopathy (proliferative stage mainly) was observed in all patients after repeated laser photocoagulation. Positive of hemoglobin, parathyroid hormone, calcium phosphorus product and blood pressure dynamics did not differ in the groups after KT.
Conclusion: The CSII using insulin pump allows to reach target values faster and more efficiently, CSII seems to be more effective than MII in reducing glycemic variability (tabl.1), and this improving the control of complications and overall prognosis in patients with DM1 after KT.

Tabl.1 Glycemic variability in patients with DM1 after KT, using different types of insulin therapy

	MII	CSII
	MEAN	
3 month	9,7[9,4;10,2]*	7,3[6,9;7,4]
6 month	9,0[9,5;9,7]*	7,0[6,5;7,4]
9 month	8,3[8,1;9,7]*	7,5[6,9;8,2]
	MAGE	
3 month	6,7[5,5;7,3]*	3,0[2,6;3,9]
6 month	5,1[4,4;5,9]*	2,4[2,2;3,3]
9 month	5,2[4,6;5,5]*	2,6[2,1;2,9]
	Lability index	
3 month	28,6[19,7;32,5]*	5,2[2,9;7,1]
6 month	11,6[8,5;18,9]*	3,1[1,8;5,6]
9 month	15,5[11,0;17,8]*	3,1[1,5;3,9]

*p<0,05 comparison of the 1-st group with 2-nd

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The effects of vildagliptin added-on insulin therapy in well-controlled type 2 diabetic patients on markers of glomerular and tubular kidney injury

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Background and aims: Several recent experimental studies have demonstrated beneficial effects of dipeptidyl peptidase 4 inhibitor vildagliptin on certain processes associated with reduced renal function in diabetes, but its renal effects in patients with type 2 diabetes (T2D) have still remained unclear. The study aimed to assess the effects of vildagliptin added-on insulin therapy on kidney dysfunction markers in type 2 diabetic patients.
Materials and methods: The study aimed to assess the effects of vildagliptin added-on insulin therapy on kidney dysfunction markers in type 2 diabetic patients.In a single-center, unblinded, randomized comparative clinical study we investigated the dynamic of serum (estimate GFR using creatinine (eGFRcr) and cystatin C (eGFRcys)) and twice-measured urinary markers of renal dysfunction, based on a first morning urine spot (indicators of glomerular injury - albuminuria, and excretion of collagen type IV; proximal tubular (neutrophil gelatinase-associated lipocalin (NGAL)), and distal tubular (liver fatty acid-binding protein (L-FABP)) damage markers in forty three well-controlled insulin-treated patients with T2D randomized either to continue insulinotherapy (insulin-treated group (IG), n=22), or to receive 6-month vildagliptin treatment in daily dose 50 mg added-on insulin (vildagliptin/insulin-treated group (VIG),n=21). Non-inclusion criteria were severe micro- and macrovascular diabetic complication, uncontrolled hypertension, inflammatory and oncological conditions, nephrotoxic drugs using, and renal disease other than diabetic nephropathy.
Results: Studying groups were comparable on the basis of sex and age (from 54 to 67 years) , and duration of T2D ranged from 5 to 17 years. At baseline, in VIG eGFRcr was 76,7±7,8 ml/min/1.73 m^2, eGFRcys 84,2± 10,2 ml/min/1.73 m^2, albuminuria 37±11,4 mg/l, excretion of collagen type IV 3,98±1,16 μg/l, urinary NGAL 46,7±16,8 ng/ml, and urinary L-FABP was 848±87,8 ng/ml, and these parameters didn't statistically differ compared to IG. Although there were no comparable changes in eGFRcr, albuminuria, and NGAL in both studying groups, eGFRcys, and excretions of collagen type IV were significantly improved in VIG after 6-month vildagliptin treatment (89,6±8,7 ml/min/1.73 m^2, 3,46±1, 1 μg/l, (respectively, p≤0,5). Also the tendency to lowering urinary L-FABP was observed (805,6±66,8 ng/ml, P=0,052).
Conclusion: The results of the study showed that 6-month vildagliptin treatment in daily dose 50 mg added-on insulin therapy in well-controlled type 2 diabetic patients could result in amelioration of glomerular dysfunction.

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Urinary albumin excretion with sitagliptin compared to sulfonylurea as add on to metformin in patients with type 2 diabetes and albuminuria: a real-world evidence study

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Background and aims: We compared the change in urinary albumin to creatinine ratio (UACR) in patients with type 2 diabetes (T2DM) and albuminuria who initiate sitagliptin to those who initiate sulfonylurea (SU) as add-on to metformin monotherapy.
Materials and methods: A cohort of T2DM patients with albuminuria (UACR >30 mg/g), who initiated sitagliptin or SU as add-on therapy to metformin from 2008-2014, was extracted from the computerized medical records of a large managed care organization in Israel. Patients with available UACR measurements at treatment initiation and 120-365 days afterwards were included. A propensity score matching with a 1:1 ratio and 17 matching factors including demography, comorbidities, baseline levels of HbA1c, UACR, BMI, eGFR, and ACE/ARB use, was implemented. The changes in UACR were compared between the matched pairs using generalized estimating equations.
Results: A total of 282 eligible pairs were identified. During a mean follow-up of 9 months, median UACR changes were -35% (IQR=-73% to 5%) and -31% (IQR=-72% to 21%) in the sitagliptin and SU groups, respectively. Mean absolute HbA1c reductions among sitagliptin and SU groups were 0.9% and 1.0%, respectively. The magnitude of UACR reduction generally increased with greater magnitude of HbA1c reduction in both treatment groups. After controlling for HbA1c reduction and the interaction between HbA1c reduction and UACR reduction, sitagliptin users demonstrated a trend toward a greater reduction in UACR compared to SU users (odds ratio=1.20; 95% confidence interval: 0.99-1.47, P=0.063).
Conclusion: Our results suggest that both sitagliptin and SU reduce albuminuria as an add-on therapy to metformin, but sitagliptin may provide greater reductions in albuminuria per improvement in glycemic control when compared to SU. We could not rule out chance in our study, however, and a larger population study is required to explore this further.
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Glucagon-like peptide-1 receptor agonists decreases albuminuria in overweight patients with type 2 diabetes

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Background and aims: Glucagon-like peptide-1 (GLP-1) is a gut incretin hormone that stimulates insulin secretion from pancreatic β-cell in a glucose-dependent manner. In kidney, the GLP-1 receptors are expressed in glomerular capillary and vascular walls. Oxidative stress produced by chronic hyperglycemia has a central role in the development and progression of diabetic nehropathy. Activation of GLP-1 receptor stimulates the production of cAMP and subsequent activation of protein kinase A (PKA) and contributes to various physiological actions including insulin secretion and antioxidative effects that protect various tissues from oxidative injury. GLP-1 receptor agonists exenatide and liraglutide have structural similarity and bind to the GLP-1 receptor displaying a similar broad range of activities relevant to improving glycemic control and antioxidative effects. The aim of this study was to investigate the effects of GLP-1 receptor agonists exenatide and liraglutide on metabolic and renal function parameters in overweight patients with type 2 diabetes (T2DM).
Materials and methods: A total of 75 overweight T2DM with normal or mildly decreased (estimated GFR≥60 mlmin-11.73 m2) renal function were included in this study and followed for 17 months (age 58±8 years, 33 M/42 F, body mass index (BMI) 39±5 kg/m2, weight 112±20 kg, HbA1c 8.4±1.2%, duration of diabetes 12±6 years, serum creatinine 73± 20 umol/L, estimated GFR 89±16 mlmin-11.73 m2, urinary albumin excretion rate (UAE) 184.8±560.1 mg/24 h, exenatide/liraglutide group 36/39 patients). UAE was measured from at least two 24-h urine samples. Estimated GFR was calculated using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) formula. Microalbumin was measured spectrophotometrically by turbidimetric immuno-inhibition. Liraglutide was started as 0.6 mg once daily dose and increased up to 1.8 mg once daily. Exenatide was started as 5 μg twice daily dose and increased to 10 μg twice daily.

Results: Treatment with GLP-1 receptor agonists caused a significant decrease in HbA1c from 8.4±1.2 to 8.0±1.3% (p=0.03), BMI from 39 ±5 to 36±5 kg/m2 (p<0.001), and weight from 112±20 to 103±20 kg (p <0.001). However, the 17-months administration of GLP-1 receptor agonists caused a significant decrease in UAE from 184.8±560.1 to 82.8± 268.9 mg/24 h (p=0.04), while serum creatinine (from 73±20 to 73±22 umol/L (p=0.4)) and estimated GFR (from 89±16 to 89±19 mlmin-11.73 m2 (p=0.4)) did not significantly changed. The effects of GLP-1 receptor agonists on metabolic and renal function parameters were similar in both exenatide/liraglutide groups (data not shown) and were not significantly different compared to presented combination results.
Conclusion: The results of our study suggest that therapy with GLP-1 receptor agonists liraglutide and exenatide may significantly reduce UAE in overweight T2DM. It has been suggested that GLP-1 receptor agonists has a crucial role in protection against increased renal oxidative stress under chronic hyperglycemia via inhibition of NAD(P)H oxidase and PKA activation which resulted in reduced albuminuria and mesangial expansion.

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Primary prevention of albuminuria using renin-angiotensin system inhibitors in patients with type 2 diabetes: a systematic review

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Background and aims: Early prevention of diabetic nephropathy by way of blocking the renin angiotensin system (RAS) in patients with normoalbuminuria seems rational, but trials have so far shown conflicting results. The present meta-analysis was undertaken to investigate if such treatment can prevent development of microalbuminuria and to assess whether available trials can provide sufficient information for such conclusions.
Materials and methods: We searched MEDLINE, EMBASE and the Cochrane Library for double-blinded randomised controlled trials, with a population of patients with type 2 diabetes and normoalbuminuria, comparing angiotensin enzyme inhibitors (ACEi) or angiotensin receptor blockers (ARB) to placebo. At least one year of follow-up was required, and trials had to have at least 50 participants in each arm. Random effects and fixed effect models were performed as well as trial sequential analysis.
Results: Six trials (Ravid et al, BENEDICT, MICRO-HOPE, ADVANCE, DIRECT, ROADMAP) were identified and included in the analysis (n=16921). Overall risk of bias was low. In a fixed effects model analysis ACE or ARB treatment was superior to placebo in relation to development of microalbuminuria, relative risk 0.84 (95% CI 0.79, 0.88) p<0.001, I2 23%, risk difference -4% (-5%, -3%), p=0.001. Similar results were seen with the random effects model. Trial sequential analysis confirmed a 16% RRR for development of microalbuminuria suggesting sufficient trial data are available to draw a conclusion.
Conclusion: Sufficient trial data are available for the meta-analysis to conclude that in patients with type 2 diabetes and normoalbuminuria, ACEinhibitors or ARBs reduces the risk for development of microalbuminuria.

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Patiromer lowers serum potassium in patients with elevated potassium and diabetes and advanced CKD on RAAS inhibitors: Results from OPAL-HK and AMETHYST-DN

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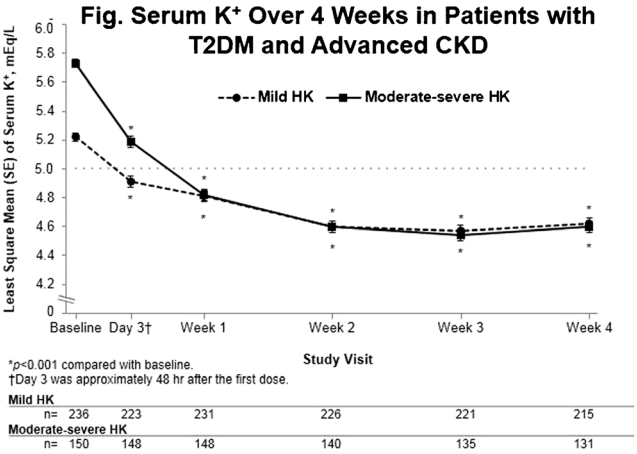
Background and aims: Albuminuria and decreased glomerular filtration rate (GFR) are associated with increased risk of end stage renal disease and cardiovascular events in patients (pts) with diabetes (DM). Guidelines recommend renin-angiotensin-aldosterone system inhibitors (RAASi) for the management of micro- and macro-vascular complications of DM and advanced chronic kidney disease (CKD), however such pts are at high risk for development of hyperkalemia, which may limit or prevent RAASi use. We evaluated the effect of patiromer, a novel investigational potassium (K^+)-binder, on serum K^+ in DM pts with advanced CKD on RAASi.
Materials and methods: OPAL-HK was a 12-week, 2-part (Part A, treatment phase; Part B, randomized withdrawal phase), single-blind study (n=243); AMETHYST-DN was a 52-week, randomized, open-label study (n=304). Eligible pts had estimated GFR (eGFR) 15-59 mL/min/1.73 m^2, were on ≥1 RAASi and, in AMETHYST-DN, had Type 2 (T2) DM. Entry serum K^+ was 5.1-<6.5 mEq/L (OPAL-HK) and >5.0-<6.0 mEq/L (AMETHYST-DN). In a post-hoc subgroup analysis, efficacy data were pooled to determine the change from baseline in serum K^+ during the first 4 weeks of patiromer treatment (primary endpoint in both studies) in pts with hyperkalemia, T2DM and eGFR <60 mL/min/1.73 m^2. Pts were stratified by baseline serum K^+: >5.0-5.5 (mild) and >5.5-<6.0 mEq/L (moderate) in AMETHYST-DN; 5.1-<5.5 (mild) and 5.5-<6.5 mEq/L (moderate-severe) in OPAL-HK.
Results: The pooled analysis includes 386 pts with T2DM and advanced CKD and hyperkalemia on stable doses of ≥1 RAASi, who then received patiromer at a starting dose of 4.2 or 8.4 g twice daily, respectively, for mild or moderate-severe hyperkalemia for 4 weeks. For pts with mild and moderate-severe hyperkalemia, respectively, mean (SD) time since T2DM diagnosis was 13±8 and 14±9 yr, mean (SD) eGFR was 38±12 and 32±12 mL/min/1.73 m^2, and mean (SE) serum K^+ was 5.2± 0.02 mEq/L and 5.7±0.03 mEq/L at baseline (Day 1). With patiromer mean serum K^+ was reduced to <5.0 mEq/L by the first post-baseline visit (Day 3, approx. 48 hr after first dose) in mild hyperkalemia pts and by Week 1 in moderate-severe hyperkalemia pts and continued to improve (Fig). By week 4, mean (95% CI) serum K^+ change from baseline was -0.58 mEq/L (-0.65,-0.51) in mild hyperkalemia and -1.12 mEq/L (-1.20, -1.04) in moderate-severe hyperkalemia pts (both p<0.001). Adverse events in these pts were predominately mild-to-moderate gastrointestinal complaints. Adverse events led to patiromer discontinuation in 10.5% of pts over 52 weeks in AMETHYST-DN and in 5.0% of pts in Part A (first 4 weeks) and 0% in Part B (weeks 5-12) in OPAL-HK.

Conclusion: Patiromer significantly reduced serum K^+ in hyperkalemic pts with T2DM and advanced CKD over 4 weeks. Patiromer may be an option for hyperkalemia treatment in pts with T2DM and advanced CKD.



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1111

Death, cardiovascular and renal events in type 2 diabetic patients with proteinuria followed by nephrologists: results of the 2-year ALICE PROTECT study

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Background and aims: Type 2 diabetes **(**T2DM) is the leading cause of chronic kidney disease (CKD) in western countries. The combination of T2DM and CKD increases the risk of end stage renal disease (ESRD), cardiovascular events and all-cause mortality. UKPDS study showed that the risk of death was higher than the risk of renal disease progression. Early control of blood pressure (BP) and proteinuria (Pu) is crucial to slow down the progression of the CKD and prevent cardiovascular events and mortality.
Materials and methods: Prospective, multicenter, observational study conducted from 2010 to 2013. Overall, 153 French nephrologists included 986 T2DM patients with Pu (≥0.5 g/day) and an eGFR>15 ml/min/1.73 m^2. Data from 729 patients were available after a 2-year follow-up. The primary objective was to assess the proportion of patients reaching the BP (<140/90 mmHg) and Pu targets (<0.5 g/day) at the end of the study. We also looked at renal (doubling of plasma creatinine and/or ESRD) and cardiovascular events.
Results: At baseline, 74% of the patients were male, mean age was 70 years. The mean T2DM duration was 17 years with a mean HbA1c of 7.4%. All were treated for hypertension and 33% had a controlled BP; 81% had dyslipidemia and LDLc was <1 g/L for 54%; 44% had retinopathy, 40% macrovascular complications and 12% heart failure. Mean Pu was 2 g/day and eGFR 40±20 mL/min/1.73 m^2, with 13%,18%, 32% and 37% of the patients in respectively stage 2, 3a, 3b and 4 CKD. After two years, 21% reached the Pu target and 39% the BP target. The mean eGFR of 40±20.3 ml/min/1.73 m^2 at baseline dropped to 36.5±20.9 by year one and to 33.9±22.6 ml/min/1.73 m^2 by year two ($p<0.001$).This corresponded to a mean annual eGFR reduction of 3.2 ml/min/1.73 m^2. 118 patients presented a renal event (16.2%): doubling of serum creatinine for 86 patients (11.8%) and start of dialysis for 72 (9.9%); 176 patients (24.1%) developed at least one cardiovascular complication during the follow-up period, and among these, 50 had also developed renal complications. Overall, 61 patients (8.4%) had a coronary event, 25 patients had a stroke (3.4%), 32 patients underwent a lower limb revascularisation procedure (4.4%), 17 patients required amputation (2.3%) and 80 patients were hospitalised with heart failure (11%). Sixty patients died, i.e. 8.2%, 26 patients from cardiovascular causes.
Conclusion: Our study highlights that achieving BP and Pu targets remains a major challenge in patients with T2DM and nephropathy, even in a nephrology environment. In contrast to older studies, renal failure is more frequent than death.
Supported by: Novartis

PS 110 Diabetic nephropathy: identification and quantification

1112

Triglyceride variation and renal function in type 2 diabetes

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Background and aims: Diabetic nephropathy progression is linked to glycemic and blood pressure control. The effect of lipid metabolism is not totally understood. Hypertriglyceridemia is thought to be a risk factor to renal failure progression. The author's objective was to investigate the association between triglyceride variation (Δ TG), renal function and metabolic control in type 2 diabetic patients (T2D).
Materials and methods: Retrospective cohort observational study of T2D patients, followed between 2005 and 2014, for at least two years. Secondary forms of diabetes, children and renal transplant patients were excluded. Δ TG was determined by calculating the mean of yearly standard deviation of triglyceride levels per patient and compared to eGFR (obtained by CKD-EPI formula), glycemic control, blood pressure and drugs. We used descriptive statistical methods, Pearson's correlation, student t-test and ANOVA for continuous and chi-square for categorical variables.
Results: There were 2322 patients (38% males) with a median follow-up time of 6.9±4.9 years. Initial median GFRe was 71 (7-138)ml/min/1, 73 m2 and the final 63 ml/min/1,73 m2. Δ TG was strongly correlation with GFRe worsening (p=0,002). Higher fasting plasma glucose, HbA1c, albuminuria and uric acid were also associated to TG variation (p<0, 001). A modest correlation was obtained between Δ TG and weight (p=0.045), but not significantly with BMI. There was not an impact of Δ TG in inflammatory markers, hepatic function and systolic blood pressure.
Conclusion: Triglyceride variation was significantly associated with renal function worsening, but also with worse glycemic control (higher fast plasma glucose and HbA1c) and hyperuricemia. Hypertriglyceridemia control may be an important factor to slow the diabetic nephropathy progression.

1113

Evolution of albuminuria depending on HbA_{1c} in diabetes mellitus type 1 patients. 20-year follow-up study

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Background and aims: To investigate the rate of albuminuria (AU) according to changes of glycated hemoglobin (HbA1c) in patients with debut of diabetes mellitus type 1 (DMT1) in their childhood during 20 year observational period.
Materials and methods: 256 patients with DMT1 (girls-143, boys-113) took part in the follow-up study from 1994 to 2014. Average age of DMT1 manifestation was 9.95±3.01 years. HbA1c was measured 4 times a year. Complications screening was done at least once a year.
Results: AU was diagnosed in 44 patients (17.2%) at the age of 15.53± 3.93 years after 5.6±2.76 years from beginning of DMT1 in accordance with categories A2 (30-300 mg/L) and A3 (>300 mg/L) with average level of HbA1c 9.57±2.32%. Correlation between changes in the rate of AU and annual fluctuation of HbA1c level was established in 37 patients (84%). All measurements of HbA1c were conditionally divided into 2 groups: 1) HbA1c<9%; 2) HbA1c≥9%. We determined annual crucial changes of HbA1c level, which were associated with manifestation of AU or its progression: in the group of HbA1c<9% average yearly fluctuation of HbA1c was -1.92±1.08÷1.59±1.09; in the group of HbA1c≥9% - -1.39±1.4÷1.41±1.3. The difference between negative average values of the HbA1c crucial interval in two groups was statistically significant (p<0.01). The safe interval of annual HbA1c level changes leading to AU reduction or even regression to the category A1 (<30 mg/L) was established: in the group of HbA1c<9% - -0.78± 0.78÷0.76±0.74; in the group of HbA1c≥9% - -0.98±0.88÷0.94±0.94. The divergence between negative average values of HbA1c changes in both groups was estimated with p=0.05.
Conclusion: In our study the correlation between AU and HbA1c fluctuation rates was established. We also determined safe HbA1c fluctuation rate interval as renal dysfunction risk factor in patients with DMT1 debut in childhood.

1114

The association between glycaemia, blood pressure, and lipidaemia control and the risk of renal function progression in type 2 diabetes patients

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Background and aims: Studies have demonstrated that the intensive control of glycemia, blood pressure, or lipidemia can slow progression of kidney outcomes. In addition to glycemia control of patients with diabetes, blood pressure and hyperlipidemia should also be managed. However, there is no study to discuss the association between simultaneous control of diabetes mellitus, hypertension, and hyperlipidemia and renal function. Thus, this study was to examine the interactive effects of the intensive control of three diseases on rapid progression of renal function.
Materials and methods: This prospective cohort study enrolled 2768 adult patients with type 2 diabetes from eight hospitals in Taiwan in October, 2008 to September, 2014. Demographic characteristics was collected using structured questionnaires. Clinical examination was obtained using medical chart reviews. Rapid progression of renal function was defined as a continued decline in eGFR of more than 5 ml/min/1.73 m −2/year. The poor control of diabetes mellitus, hypertension and hyperlipidemia was defined as if a patient had a HbA1C ≧7%, MAP >100 mmHg, and total cholesterol≧200 mg/dl and triglyceride≧150 mg/dl, respectively. Cox proportional hazard regression models were used to study the associated between interactive effects of diabetes, hypertension and hyperlipidemia controls and the rapid progression of renal function. Covariates included age, sex, stroke, CVD, anemia, CKD, BMI, baseline eGFR, BUN, smoking, alcohol, and Chinese herb drug.
Results: A total of 27.1% (751/2768) had rapid progression of renal function during a mean follow-up of 2.4 years (SD: ±1.16). The mean age of the cohort was 62.9±11.5 years, and male gender accounted for 53.6%.
When considered the single effect of the control of diabetes, hypertension and hyperlipidemia, the poor control of glycemia had the highest risk of the increase risk of rapid progression of renal function, with an adjusted HR of 1.41 (95% CI: 1.22-1.64). The poor control of hypertension and hyperlipidemia was also associated the increase risk of rapid progression of renal function, with an adjusted HR of 1.27 (95% CI: 1.03-1.56) and 1.25 (95% CI: 1.01-1.56), respectively. When considered the interactive effect of the poor control of all three diseases, we treated the patients with an intensive control of all three disease as the reference group. Compared

to the reference group, the poor control of all three diseases had the highest risk of rapid progression of renal function, with an adjusted HR of 2.84 (95% CI, 1.80-4.47). Even if the patients with an intensive control of glycemia, the result showed the poor control of both hypertension and hyperlipidemia was associated with the increased risk of rapid progression of renal function, with adjusted HR of 1.92 (95% CI of 1.11-3.29) than the reference group.

Conclusion: A poor control of glycemia, blood pressure, and lipidemia were found to be associated with the increased risk of declining renal function.

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1115

Decline in renal function was not associated with steatosis evaluated by proton-spectroscopy in a population of people with type 2 diabetes

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Background and aims: Data regarding the link between hepatic steatosis and renal function in people with type 2 diabetes are controversial. Liver fat content was associated with the development of diabetic nephropathy in cross-sectional studies but not in one longitudinal study. We aimed to determine whether liver fat content was associated with a decline in renal function in patients with type 2 diabetes.

Materials and methods: Two hundred and twenty-three patients with type 2 diabetes were included in the study. Liver fat content was measured by proton spectroscopy at inclusion. Steatosis was defined by liver fat content greater than 5.5%. The decline in renal function was assessed by changes in the estimated glomerular filtration rate (eGFR), (MDRD formula) over time. Diabetic nephropathy at inclusion was defined by the presence of microalbuminuria or macroproteinuria.

Results: One hundred and thirty-four (60.1%) patients had steatosis. Those with steatosis had higher BMI (p=0.0003), lower diabetes duration (p=0.005) and a lower frequency of diabetic nephropathy at inclusion (12.6% vs 24.7%, p=0.03). Age, HbA1c and eGFR at inclusion were not statistically different between groups. The median follow up was 4.4 years. Hepatic steatosis was not associated with the rate of decline in renal function calculated by year, but the difference was borderline significant (Patients with steatosis had a lower annual renal decline than those without steatosis (+0.48 vs. -0.98 ml/min per year, p=0.06). In multivariate analysis, steatosis was associated with BMI, but not with age, diabetes duration, diabetic nephropathy at inclusion, eGFR at inclusion, renal decline per year or gender.

Conclusion: When we used an accurate method to evaluate liver fat content, the presence of steatosis was not associated with the decline in renal function in our cohort of patients with type 2 diabetes. Our study suggests that early identification of hepatic steatosis in people with type 2 diabetes may not be useful to identify people at the highest risk of renal decline.

1116

The risk of incident chronic kidney disease in a Korean metabolically healthy obese population

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Background and aims: Obesity is considered to be an important risk factor for chronic kidney disease (CKD).The metabolically healthy obese (MHO) phenotype refers to obese individuals with a favorable metabolic profile. Its prognostic value remains controversial and may depend on the health outcome being investigated. We examined the risk of MHO phenotype with incident CKD in a Korean population.

Materials and methods: The study population comprised 40,579 Koreans without CKD. Participants were stratified by body mass index (BMI) (cut-off value, 25.0 kg/m^2) and metabolic health state (assessed using Adult Treatment Panel-III criteria). Incident CKD was assessed by the Modification of Diet in Renal Disease (MDRD) study equation and defined by a glomerular filtration rate (GFR) less than 60 ml/min/1.73 m^2 during the follow-up period.

Results: Over the median follow-up period of 39.0 months (range, 4.8-83.8 months), 315 of the 40,579 individuals (0.8%) developed incident CKD. Compared with the metabolically healthy nonobese (MHNO) group, the MHO group showed increased risk of incident CKD with a multivariate-adjusted hazard ratio (HR) of 1.52 (95% confidence interval [CI], 1.10-2.11). Nonobese but metabolically unhealthy individuals were at an increased risk of incident CKD (multivariate-adjusted HR, 1.44 [95% CI, 1.01-2.00]) than the MHNO group.

Conclusion: Metabolically unhealthy obese individuals were at the highest risk of incident CKD. A healthy metabolic profile does not protect obese adults from incident CKD. Thus, it is important to consider metabolic health and obesity when evaluating CKD risk.

1117

The prevalence of diabetic nephropathy in patients with type 2 diabetes mellitus in Slovakia: the NEFRITI Study

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Background and aims: To assess: 1) the prevalence of diabetic nephropathy (DNeF), 2) proportion of patients with complications and accompanying diseases, according to the stage of chronic kidney disease (CKD) and albuminuria, and 3) the proportion of patients with GFR<60 ml/min/1,73 m2 with normal creatinine values.

Materials and methods: Randomized, multicentric, epidemiological survey in cohort of 1831 patients with type 2 diabetes mellitus. Urinary albumin was determined by turbidimetric method, GFR was calculated by CKD-EPI formula.

Results: Male 46,5%, age 63,85±9,48 years (y), duration of diabetes 9,29±7,21 y, HbA1c 7,5±1,6 median 7,2% (DCCT), BMI 31,5±5,6 kg.m-2, hypertension 82,1%, dyslipidemia 76,8%, serum creatinine 80,3±44,0 umol/l, GFR 87,2±27,7 ml/min/1,73 m2, UACR 8,1±36,0 median 1,41 mg/mmol. Proportions of patients with normoalbuminuria (nA) resp. microalbuminuria (miA) resp. macroalbuminuria (maA) were 67,7% resp. 25,2% resp. 5,01%. Proportion of patients with CKD stage 1 (CKD1) 42,9% CKD2 42,8%, CKD3a 9,4% CKD3b 3,3%, CKD4 1,2% CKD5 0,39%. The prevalence of DNeF based on a presence of maA or miA, eGFR>30 ml/min/1,73 m2 and diabetic retinopathy was 12,7%. The prevalence of DNeF was increasing with duration of diabetes:

less than 5y / 5-10y / 10-15y / 15y and more was 4,8 / 9,9 / 15,9 / 26,8%. Chronic renal failure (CKD5 and/or dialysis) was found in 0,45%. The proportion of patients with complications of diabetes and accompanying diseases were rapidly increasing with both increasing stage of CKD and albuminuria (Tab. 1).

Conclusion: The prevalence of DNeF in patients with Type 2 diabetes in Slovakia was 12,3%. Both, increase in stage of CKD and albuminuria were associated with increase in all complications of diabetes and accompanying diseases. Normal serum creatinine values were found in 35,4% of patients with eGFR<60 ml/min/1,73 m2. Namely in elderly women.The study has been reviewed by the Local Ethics Committee and therefore it been been performed in accordance with the ethical standards laid down in the Helsinki Declaration.

Table 1. The proportion of patients with complications of diabetes and accompanying diseases according to the stage of CKD and albuminuria

	NeuP	NPDR	PDR	DF	Amp1	Amp2	CAD	ACS	HF	Stroke	Canc
CKD1 (n=763)	37,33	15,99	2,6	1,11	0,99	0	23,73	5,07	2,23	4,21	4,82
CKD2 (n=762)	39,58	18,49	2,78	1,71	1,19	0,53	33,38	4,76	3,96	1,59	8,3
CKD3 (n=226)	51,11	25,22	4,87	2,66	1,33	0,44	48,67	9,73	7,96	10,62	9,73
CKD4+5 (n=29)	89,66	48,15	39,29	14,29	7,14	3,6	60,71	3,57	28,57	27,58	10,7
	NeuP	**NPDR**	**PDR**	**DF**	**Amp1**	**Amp2**	**CAD**	**ACS**	**HF**	**Stroke**	**Canc**
Normoalbuminuria (n=948)	35,7	16,77	1,92	0,74	0,42	0,106	28,56	4,56	3,62	2,98	7,30
Microalbuminuria (n=383)	47,52	21,52	5,25	4,2	3,14	0,52	37,86	7,05	5,74	4,43	6,28
Macroalbuminuria (n=71)	67,61	43,48	14,08	9,86	7,14	1,43	56,34	14,08	15,5	7,04	7,04

CKD – chronic kidney disease, NeuP – diabetic neuropathy, NPDR – non-proliferative diabetic retinopathy, PDR- proliferative diabetic retinopathy, DF – diabetic foot with ulcer, Amp1- amputation under ankle, Amp2 – amputation over ankle, CAD –coronary artery disease, ACS – acute coronary syndrome, HF- heart failure, Stroke – stroke, CANC – Cancer

1118

Frailty and the relationship between albuminuria and renal function in elderly patients with type 2 diabetes (ZODIAC)

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Background and aims: In elderly patients with type 2 diabetes mellitus (T2DM), a moderate reduced renal function (eGFR between 45 and 60 ml/min) is not related to mortality. Other cardiovascular risk factors, such as blood pressure, also have different consequences in old age. Previous studies showed that frailty influences the relationship between several risk factors, e.g. blood pressure, and mortality in old age. We hypothesized that the relationship between renal function and mortality may also be influenced by the level of frailty.

Materials and methods: Patients with T2DM participating in a prospective observational cohort study (ZODIAC) in the Netherlands were included. Patients aged 60 years and older were selected from this cohort of primary care treated patients. Frailty was defined as a score less than 80 on the subscale 'physical functioning' of the RAND-36 questionnaire. After median follow-up for 14 years, multivariate Cox regression analyses were performed to evaluate the association between albuminuria/renal function and (cardiovascular) mortality. Analyses were performed in strata according to the frailty level ('physical functioning' score 80). We adjusted for age, gender, BMI, duration of diabetes, systolic blood pressure, serum creatinine level (for model with albuminuria), total cholesterol-HDL ratio, HbA1c, macrovascular complications, albuminuria (for model with eGFR), lipid lowering drugs and the use of antihypertensive drugs. Renal function was estimated by the Modification of Diet in Renal Disease equation (MDRD).

Results: Approximately 60% of our study population was female. Median age (interquartile range) was 72 (67-77) years and median diabetes duration was 6 (3-12) years. Frailty was highly prevalent in our study population; 629 out of 858 patients (73%) fulfilled the criterion. Albuminuria was related to increased all-cause and cardiovascular mortality in both frail and non-frail patients (table 1). Every 10 ml/min decrease in eGFR was related to a 41% (95% confidence interval (CI): 12-76%) and 18% (95% CI: 8-27%) higher cardiovascular mortality risk in non-frail and frail patients, respectively. No significant associations were observed between eGFR and all-cause mortality.

Conclusion: The presence of albuminuria and decreased eGFR were related to cardiovascular mortality in both frail and non-frail elderly patients with T2DM. However, the associations were the strongest for non-frail patients. Therefore, we conclude that frailty seems to attenuate the relationship between renal function and cardiovascular mortality, but not for all-cause mortality.

	All-cause mortality	CVD mortality
Non-frail subjects		
Albuminuria	1.50 (1.00-2.25)	1.98 (1.01-3.86)
eGFR (per 10ml/min decrease)	1.14 (0.99-1.31)	1.41 (1.12-1.76)
Frail subjects		
Albuminuria	1.55 (1.26-1.90)	1.50 (1.09-2.06)
eGFR (per 10ml/min decrease)	1.03 (0.98-1.08)	1.18 (1.08-1.27)

Table 1. Hazard ratios and their 95% confidence intervals of albuminuria and eGFR for all-cause and cardiovascular disease (CVD) mortality. Analyses were stratified according to frailty.

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Albumin- and haemoglobin- corrected HbA_{1c} detects uncontrolled diabetic patients on haemodialysis better than glycated serum protein: a continuous glucose monitoring study

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Background and aims: It is reported that HbA1c is unreliable in hemodialysis patients, underestimating the level of glycemic control. Anemia and administration of erythropoietin are the primary factors that influence its validity. Alternatively the glycated serum protein (GSP) has been proposed as a more reliable marker of glycemic control in these patients. The aim of the study was to compare these two markers of glucose control estimation in hemodialysis patients with diabetes.

Materials and methods: We studied 38 hemodialysis patients with diabetes (36 type 2), sex: 22 male, age: 62.8 (16.2) years, BMI: 27.0 (3.9) Kg/m2, serum albumin (Alb): 4.0 (0.3) g/dl, hemoglobin (Hb): 10.9 (1.4) g/dl, HbA1c: 6.5 (1.3)% and GSP: 622.8 (184.3) μmol/L. A 7-day continuous glucose monitoring system was used (iPro2 CGM) in order to measure the average glucose (mAG: 162.5±35.3 mg/dl) and the standard deviation of mAG (mSDG: 49.1±17,0 mg/dl). The predicted HbA1c (preHbA1c) according to mAG was calculated, based on equation: preHbA1c=mAG+46.7 / 28.7. Moreover we estimated the AG according to HbA1c, based on equation for patients in hemodialysis: eAG=104.8+ (29.7 X HbA1c) - (18.4 X Alb) - (4.7 X Hb) and thereafter we estimated the corrected HbA1c (corHbA1c=eAG+46.7 / 28.7). ROC analysis was performed to determine which of the three markers (HbA1c, corHbA1c and GSP) could predict better mAG≥184 mg/dl, which in patients with normal renal function corresponds to HbA1c≥8% and detects the uncontrolled diabetic patients in hemodialysis. Statistical analysis was performed with SPSS 18.0. P value<0.05 was considered statistically significant.

Results: preHbA1c and corHbA1c were 7.3 (1.2)% and 7.6 (1.4)% respectively. There was a significant difference between HbA1c, preHbA1c and corHbA1c (p=0.001). HbA1c was statistically significantly lower compared to preHbA1c (p=0.033) and to corHbA1c (p=0.001). There was no statistically significant difference between preHbA1c and corHbA1c (p=0.509). The mAG and the mSDAG were significantly correlated with HbA1c, corHbA1c and GSP (p>0.001). The area under receiver-operating characteristic curve (AUC) for HbA1c, corHbA1c and GSP to detect mAG≥184 mg/dl was 0.786 (0.623-0.902), 0.771 (0.606-0.891) and 0.694 (0.523-0.832) respectively. There was no significant statistical difference between the AUCs. The sensitivity of HbA1c≥8%, corHbA1c≥8% and GSP≥650.0 μmol/L to detect mAG≥184 mg/dl was 36.4%, 72.7% and 81,8% respectively. The specificity of HbA1c≥8%, corHbA1c≥8% and GSP≥650.0 μmol/L to detect mAG≥184 mg/dl was 85.2%, 85.2% and 63.0% respectively. In total, the false negative and

false positive values of HbA1c≥8%, corHbA1c≥8% and GSP≥ 650.0 μmol/L to detect mAG≥184 mg/dl were 11 (28.9%), 7 (18.4%) and 12 (31.5%) respectively.
Conclusion: In hemodialysis diabetic patients, HbA1c should be corrected according to serum albumin and hemoglobin. The albumin- and hemoglobin- corrected HbA1c seems to detect uncontrolled diabetic patients on hemodialysis better than glycated serum protein.

PS 111 Diabetic nephropathy: mechanisms

1120

High glucose induces membrane-bound transcription factor peptidase site1 via carbohydrate response element binding protein to modulate ER stress in mesangial cells

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Background and aims: High glucose (HG) evokes a variety of gene expressions in mesangial cells (MC) that alter cellular functions responsible for the development of diabetic glomerulopathy. We previously reported that HG activates hypoxia-inducible factor-1α and its target genes expression in MC, leading to an extracellular matrix expansion in diabetic glomeruli. A glucose responsive transcription factor, carbohydrate response element binding protein (ChREBP), plays a pivotal role in such derangement of gene regulation. To provide more insight into glucose-mediated gene regulation at genome-wide level, we performed chromatin immunoprecipitation with anti-ChREBP antibodies followed by DNA microarray analysis (ChIP-chip) and identified membrane-bound transcription factor peptidase site1 (MBTPS1) as a novel target gene of ChREBP in MC. MBTPS1 proteolytically activates a class of transmembrane transcription factors at the endoplasmic reticulum (ER) and participates in the regulation of cellular events such as response to ER stress; however the role of MBTPS1 in MC under diabetic circumstances is largely unknown. In the present study, we examined the mechanism by which MBTPS1 is induced by HG in MC, and the role of MBTPS1 in the regulation of cellular function.
Materials and methods: Cultured human MC were incubated in HG (25 mM glucose) and normal glucose (NG; 5.6 mM glucose), and the gene and protein expression were analyzed. Gene expressions in the kidney of diabetic model mice were determined by real time PCR.
Results: ChIP-chip assay revealed binding of ChREBP to MBTPS1 gene at 2.5 kb in 3'-flanking region. In validation analyses, exposure to HG for 24 h enhanced MBTPS1 mRNA expression in cultured MC (2.4±0.43 fold, compared to NG). Knock-down of ChREBP abrogated this induction response. MBTPS1 is known for the proteolytic activation of activating transcription factor-6 (ATF6), which is involved in the adoptive response to ER stress. In support of these issues, HG induced the activation of ATF6 in MC and the inhibitor of MBTPS1 cancelled this activation. Interestingly, the target genes of ATF6 were not universally induced by HG; C/EBP homologous protein, a critical determinant of ER stress-induced apoptosis, was induced by 4.8±1.1 fold ($p<0.05$), whereas X-box-binding protein 1 was induced by 1.8±0.2 fold ($p<0.05$) of and glucose-regulated protein-78 was not induced, indicating that MBTPS1 may participate in the control of the fate of MC exposed to HG. Moreover, the expression of MBTPS-1 mRNA was upregulated in the kidney of streptozotocin-induced diabetic model mice compared to control mice (1.36 fold).
Conclusion: HG-mediated induction of MBTPS1 via ChREBP operates in response to ER stress in MC, which may provide novel insights into the pathogenesis and therapeutic intervention of diabetic nephropathy.
Supported by: JSPS KAKENHI

1121

Advanced Glycation End products (AGEs) increase human renal tubular foam cell formation by endoplasmic reticulum stress

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Background and aims: Diabetic nephropathy caused by advanced glycation end products (AGEs) is associated with lipid accumulation in renal tubule. This study was designed to investigate whether Nε-(carboxymethyl) lysine (CML) (one of AGEs family) increase lipid accumulation in human renal tubular epithelial cell line (HK-2) via increasing cholesterol synthesis and uptake, reducing cholesterol efflux though endoplasmic reticulum stress (ERS).
Materials and methods: HK-2 were incubated with CML. Cell viability was evaluated through the CCK-8 assay, and cell apoptosis was evaluated by Annexin V-FITC Kit. Intracellular cholesterol content was assessed by Oil Red O staining and cholesterol enzymatic assay. Expression of mRNA and protein of molecules controlling cholesterol homeostasis in the treated cells was examined by real-time quantitative PCR and western blotting, respectively.
Results: CML reduced the cell viability and increased the cell apoptosis of HK-2 cells, which could be inhibited by anti-RAGE (RAGE, receptor of AGEs). CML increased cholesterol accumulation in HK-2 cells, however anti-RAGE reduced the lipid content. Exposure to CML increased expression of 78-kDa glucose-regulated protein (GRP78) and C/EBP homologous protein (CHOP), which up-regulated transcription factor SREBP-2 to the Golgi for activation by cleavage enhanced gene transcription of HMGCoA reductase and LDL receptor, down-regulated adenosine triphosphate-binding cassette transporter A1 (ABCA1) and Liver X receptor (LXR), which were reversed by anti-RAGE. The effects of CML were blocked by 4-PBA(4-PBA was used to inhibit ERS).
Conclusion: CML increased HMG-CoAR-mediated cholesterol synthesis and LDLr-mediated cholesterol uptake, reduced ABCA1-mediated cholesterol efflux though ERS, ultimately, caused lipid accumulation in HK-2 cells. These data imply that inhibiting ERS might have a potential renal protective role in prevention of renal tubular foam cell formation.

1122

Effects of high free fatty acids on human kidney epithelial cells

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Background and aims: Diabetes is an epidemic metabolic syndrome, becoming one of the most common causes of kidney diseases leading to the end stage renal failure and dialysis requirement. Several factors including hyperglycemia and dyslipidemia have been identified as risk factors for diabetic nephropathy. Also, an elevation of free fatty acids (FFA) level is commonly observed in diabetic patients. This study examined potential adverse effects of high FFA on kidney epithelial cells and investigated relevant signaling pathways.
Materials and methods: RC124 cells (a human kidney epithelial cell line) were treated with high concentrations (0.5 and 1 mM) of the saturated palmitic acid (PA) alone, or a mixture of equal amount of PA and the monounsaturated oleic acid (OA), for 12, 24 or 48 hours, followed by assessments of cell viability and various signaling pathways using immuno-blotting and assay kits run on Muse Cell Analyzer.
Results: Morphological changes occurred after kidney epithelial cells were exposure to high FFA, displaying coarse surface and membrane blebbing. When OA was present, cell confluence was higher than that by high PA treatment alone. High PA treatment reduced (up to by 80%) viability of kidney epithelial cells in both time- and dose- dependent manner. By contrast, co-treatment with equal amount of OA was able to block such adverse effects by PA to a large degree. The underlying mechanisms for high FFA effects on renal cell viability were studied by examining related signaling events. High PA treatment activated caspase-3 and induced apoptotic cell death at 24 h. In addition, PA treatment evoked endoplasmic reticulum (ER) stress (as indicated by increased phosphorylation of eIF2α) and induction of autophagy (assessed by detection of LC3-II) in renal cells. Both high PA-induced ER stress and autophagy occurred at 12 h, a time point prior to apoptosis occurrence, suggesting their possible causative relationship in the course of injuring cells. On the other hand, co-treatment with OA abolished such PA-induced ER stress, autophagy and apoptosis of renal cells.
Conclusion: High PA may exert lipotoxicity effects on kidney epithelial cells via induction of ER stress, autophagy and apoptosis, which may contribute to the development of renal diseases in diabetes. OA appears capable of antagonizing high PA's adverse action and potential for development of targeting drugs.
Supported by: Singapore National Kidney Foundation

1123

The function of ELMO1 in renal development under diabetic conditions within zebrafish and in human diabetic nephropathy

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Background and aims: Although the engulfment and cell motility 1 (ELMO1) protein functions as a guanine exchange factor for Rac1, regulating cell migration and early vascular development within the zebrafish (ZF); recently, it was found to protect endothelial cells from apoptosis via activation of the Rac1/PAK/AKT signalling cascade in vitro and in vivo. GWAS data suggests that polymorphisms in different regions of human ELMO1 acts as a potential contributing factor for the development of diabetic nephropathy (DN). However, the function and altered expression of ELMO1 remains unclear with respect to the development of DN and this study thus aims to illustrate the role played by ELMO1 in renal development in ZF, under diabetic conditions, and DN patients.
Materials and methods: ELMO1 expression within the Wt1B:GFP transgenic zebrafish line was knocked out using the CRISPR:Cas9 technology. The renal system in Wt1B:GFP fish line is tagged with GFP and hence was used to observe developmental changes within the pronephron. ZF embryos were rendered diabetic via the knockdown of PDX1 using specific splice blocking morpholinos. Nephron functional assays were carried out with the help of TexasRed labelled Dextran to observe leakage via the pronephron. TUNEL assay was used to measure increase in apoptosis within the ZF embryos. Lastly, human kidney paraffin sections from controls and DN human patients suffering from Type 1 and Type 2 DM were stained to observe and compare ELMO1 expression within the glomeruli.
Results: It was observed that a knockout of ELMO1 within ZF embryos leads to pathophysiological changes within the pronephron, adversely affecting pronephric structure and appropriate ultrafilteration. Subsequently, renal structure and function is restored in rendered diabetic ZF embryos upon over-expression of ELMO1. ZF embryos upon loss of ELMO1 showed a significant increase of apoptosis within the ZF pronephron, which consequently lead to the renal pathophysiological effects. Lastly, expression of ELMO1 within kidney samples of human patients suffering from Type 1 and Type 2 DM was greatly reduced as compared to control samples.
Conclusion: Collectively, the results indicate that ELMO1 is extremely important for glomerular protection and renal cell survival via decreasing apoptosis, especially within the diabetic diseased renal conditions.
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1124

Defining the role of pyroptosis and apoptosis in diabetic nephropathy

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Background and aims: Cell death and inflammation are both associated with the progression of diabetic nephropathy (dNP). Glomerular cell apoptosis is thought to contribute to diabetic nephropathy. However, direct experimental evidence supporting a causative role of apoptosis is lacking. Of note, common cell death assays like TUNEL fail to distinguish apoptosis from other cell death forms, leaving the question open, whether apoptosis has a prevailing role for establishing dNP. To evaluate the role of apoptosis for experimental diabetic nephropathy we used various pharmacological and genetic approaches.
Materials and methods: Murine models of type 2 (db/db mice) and type 1 (Streptozotocin [STZ] -uninephrectomized mice) diabetes were analyzed. dNP was quantified based on albuminuria and histological changes. Expression levels of cleaved caspase-1 (Casp1), Casp3, and IL1β were determined by immunoblotting and immunohistochemistry. A subset of mice was injected with M-920 (a pan caspases inhibitor targeting Casp1, 3, 4, 5, 6, 7) and CIX (an inhibitor of Casp3, 6, 7, 8, 10). Furthermore, Casp3 and Casp1 deficient mice were used to distinguish the role of apoptosis and pyroptosis.
Results: Of the two pharmacological approaches used in db/db mice only one (M-920, targeting also Casp1) efficiently ameliorated diabetic nephropathy. Furthermore, Casp1 inhibitor, but not CIX, prevented glucose induced Nlpr3 expression as well as IL-1β and caspase-1 cleavage in podocyte in vitro and in vivo. Importantly, while Casp1 deficient mice with STZ induced persistent hyperglycemia were protected from diabetic nephropathy, Casp3 deficient mice were not. Hence, manifestation of dNP is independent of caspase-3, but requires caspase-1 in mice.
Conclusion: Taken together, these studies question the role of Casp3 dependent cell death while emphasizing the role of inflammasome and Casp1 activation and potentially of pyroptosis for diabetic nephropathy. Therefore, we propose that inflammasome inhibition is a more feasible therapeutic approach to successfully target dNP than apoptosis inhibition.
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1125

The antifibrotic microRNA crosstalk in the action of N-acetyl-seryl-aspartyl-lysyl-proline on kidney fibrosis in diabetes

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Background and aims: N-acetyl-seryl-aspartyl-lysyl-proline (AcSDKP) is an endogenous peptide and provides renal-protective effects by inhibiting renal fibrosis.
Materials and methods: We found that the suppression of AcSDKP and concomitant induction of dipeptidyl peptidase-4 (DPP-4) were connected with insufficient levels of antifibrotic microRNAs in the kidney of streptozotocin (STZ)-induced diabetic CD-1 mice, considering the possible collaborations among these molecular defects in the onset of kidney fibrosis of diabetes.
Results: By the comparative studies using streptozotocin (STZ)-induced diabetic non-fibrotic (129Sv) kidneys, we found that diabetic CD-1 mice with fibrotic kidney was differentiated from non-fibrotic diabetic 129SV mice by the suppression of AcSDKP, prominent induction of DPP-4 protein expression/activity, endothelial to mesenchymal transition (EndMT), and the suppression of anti-fibrotic microRNAs (miRs) such as miR-29 s and miR-let-7 s. These alterations in diabetic CD-1 mice were all reversed by AcSDKP treatment. Transfection studies in human microvascular endothelial cells revealed that miR-29 s and miR-let-7 s are bidirectionally regulated each other against EndMT program, and such regulation of miR-29 s and miR-let-7 s played an essential role in maintaining the antifibrotic program in AcSDKP action. The suppressed level of AcSDKP in diabetic CD-1 mice also induced inflammatory responses by enhancing both IFN-γ and TGF-β signaling, resulting in inhibition of antifibrotic miRs crosstalk.
Conclusion: In conclusion, the present study provides new insight into the physiologically relevant antifibrotic actions of AcSDKP via restoration of antifibrotic miRs, and such anti-fibrotic programs mediated by AcSDKP would have potential utility in combating diabetic nephropathy.
Supported by: Japan Society for the Promotion of Science to DK and KK

PS 112 Diabetic nephropathy: biomarkers

1126

High levels of fasting plasma pro-enkephalin predict deterioration of kidney function and incidence of chronic kidney disease

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Background and aims: Recently, high levels of pro-enkephalin A (p-ENK) have been associated with decreased estimated glomerular filtration rate (eGFR) in an acute setting. Here we examined, if plasma levels of p-ENK may predict chronic kidney disease (CKD), a decline of eGFR or increase of cystatin C, and analyzed the genetic determinants of p-ENK using a genome-wide association study (GWAS).
Materials and methods: The study included 2568 individuals, aged 46-68 years, without CKD (eGFR≥60 ml/min/m2), and with measured fasting plasma p-ENK at baseline (1991-1994). Incidence of CKD was defined as≤eGFR 60 ml/min/m2 at follow-up re-examination (2007-2012). Logistic regression was used to calculate the odds ratio (OR) of CKD according to tertiles of fasting plasma concentrations of p-ENK adjusting for sex, age, eGFR, fasting glucose, systolic blood pressure, anti-hypertensive medication, BMI at baseline and follow-up time. Further, we conducted a GWAS for p-ENK levels in 4150 participants and tested if the genetic markers identified genome wide significantly associated with p-ENK may prospectively predict incidence of CKD from baseline to follow-up re-examination.
Results: During a mean follow-up time of 16.6 years we observed a CKD incidence rate of 32%. Among participants in the highest tertile of p-ENK concentration at baseline the OR for CKD was increased to 1.51 (95% CI 1.18-1.94) compared to participants within the lowest p-ENK tertile. The yearly mean decline of eGFR was significantly higher (P=0.0002) among participants with high levels of p-ENK and likewise did cystatin C (P=0.005) and creatinine (P=0.000003) increase in individuals with high p-ENK concentrations. In GWAS we identified 19 SNPs up- and 5 SNPs down-stream of the PENK locus that altered p-ENK concentration on a GWAS significant level. Per copy of the p-ENK increasing minor alleles of the lead SNP rs1012178 (P: 4.666-21) the risk for incidence of CKD was significantly increased (OR: 1.19; 95% CI 1.02-1.38). The mean decline of eGFR per year was significantly higher (P=0.0007) and creatinine (P=0.002) and cystatin C (P=0.031) levels increased more compared to homozygote major allele carriers.
Conclusion: Our large prospective study suggest circulating p-ENK as a potential biomarker to predict incidence of CKD. GWAS followed by analyses for the lead SNP provided evidence for that increased risk for CKD observed in participants with high p-ENK concentrations may be partially genetically determined.

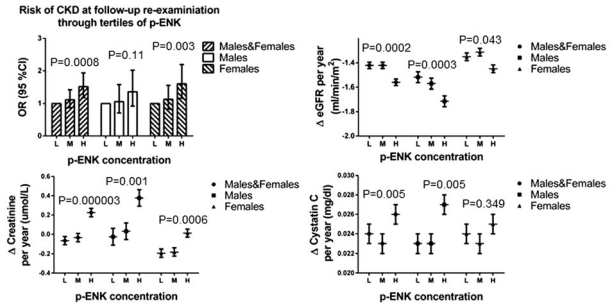

Figure 1: a) Risk for CKD at follow-up re-examination in relation to baseline p-ENK concentration in 2568 participants of MDC-CC. The logistic regression model was adjusted for sex, age, eGFR, fasting glucose, systolic blood pressure, anti-hypertensive medication, BMI at baseline and follow-up time. Changes per year in b) eGFR (estimated Glomerular Filtration Rate CKD-EPI 2012 based on Inker et al.), c) creatinine and d) Cystatin C through levels of fasting plasma p-ENK in a linear model adjusted for age, (sex), and baseline levels. P= P-trend.

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1127

Increased copeptin in plasma predicts mortality and renal outcome in patients with type 1 diabetes

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Background and aims: Copeptin is a surrogate marker for vasopressin in plasma and associated with cardiovascular disease (CVD) and renal disease in patients with type 2 diabetes. Here, we examined the association between copeptin concentrations and cardiorenal disease in patients with type 1 diabetes.
Materials and methods: Baseline complications were defined as albuminuria (n=358) (urinary albumin excretion rate (UAER) ≥30 mg/24 h) and CVD (n=143) (myocardial infarction, revascularisation, stroke or peripheral arterial disease). Adjustments in the regression model included gender, age, diabetes duration, eGFR, UAER, HbA_{1c}, cholesterol, systolic blood pressure, insulin dose, sodium excretion, BMI, smoking and antihypertensive treatment. During follow up we assessed 1) the development of a combined endpoint including end stage renal disease (ESRD) (dialysis, renal transplantation), doubling of baseline creatinine and all-cause mortality, and 2) annual change in eGFR.
Results: In 665 patients with type 1 diabetes (age (mean ± SD) 55± 13 years, 301(45%) females), the median (interquartile range) copeptin concentration was 5.5 (3.3-10.9) pmol/L. In multivariate analysis, copeptin concentrations were higher in males and correlated positively with UAER and HbA_{1c} and, inversely with eGFR and systolic blood pressure (p≤0.008). Moreover, copeptin concentrations were higher in patients with albuminuria than in patients without (8.0 (3.8-16.0) *vs.* 4.4 (2.8-6.8) pmol/L; adjusted p=0.035). During a follow up of median [interquartile range] 3.9 [0.16-5.0] years, the mean ± SD, annual eGFR decline was 0.30±4.1 ml/min/1.73 m^2. Increased copeptin concentrations predicted the combined endpoint (n=47) with a hazard ratio of 3.5 (1.6-7.7) (per doubling in ln-copeptin; p= 0.002) adjusted for gender, age, systolic blood pressure, eGFR and previous CVD, and was associated with a larger annual decline in eGFR (adjusted p=0.02).**Conclusion:**

Increased copeptin concentrations were associated with impaired renal function and larger decline in eGFR. Moreover, increased copeptin concentration independently predicted the combined endpoint. Copeptin may thus be a useful cardiorenal risk marker in patients with type 1 diabetes. *Clinical Trial Registration Number: NCT01171248*

1128

Serum adiponectin and glomerular filtration rate in patients with type 2 diabetes

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Background and aims: High serum adiponectin has been associated with decreased renal function in the general population. Only sparse and conflicting results, all derived from small studies mainly carried out in non-European populations, have been reported in patients with type 2 diabetes (T2D), a subgroup of individuals who are at high risk of renal dysfunction. The aim of this study was to fill up this gap of knowledge by investigating such association among diabetic patients of European ancestry.
Materials and methods: The association between serum adiponectin levels and estimated glomerular filtration rate (eGFR by Modification of Diet in Renal Disease equation) was investigated in 1,212 patients with T2D from two different Italian samples: 847 patients from San Giovanni Rotondo (SGR) and 365 patients from Foggia.
Results: Serum adiponectin was inversely associated with eGFR in SGR [β (SE) for 1 SD of adiponectin=-2.53 (0.69), p=2.0*10-4] and in Foggia [β (SE)=-5.63 (1.66), p=0.001] samples, as well as in the two studies combined [β (SE)=-3.46 (0.69), p=6.0*10-7]. The association was somehow reduced but still significant after adjusting for sex, smoking habits, BMI, diabetes duration, HbA1c, anti-diabetic, anti-hypertensive and anti-dyslipidemic treatments [β (SE)=-1.79 (0.73), p=0.015]. For each adiponectin SD increment, the odds of having eGFR<60 ml/min/1.73 m2 increased by 46% (OR =1.46; 95% CI 1.24-1.71; p=3.2*10-6) in SGR sample, 52% (OR=1.52; 95% CI 1.19-1.94; p=0.001) in Foggia sample, and 47% (OR=1.47; 95% CI 1.29-1.68; p=8.7*10-9) in the two studies considered together. Further adjustment for the above mentioned covariates did not change the observed association (OR=1.36; 95% CI 1.16-1.59; p=9.6*10-5).
Conclusion: This is the first report of an association between high serum adiponectin and low eGFR in a large study of patients with T2D of European ancestry. Such counterintuitive, paradoxical association deserve further attempts to be deeper understood.

1129

Associations of serum N-glycans with glycaemic control and albuminuria in type 1 diabetes

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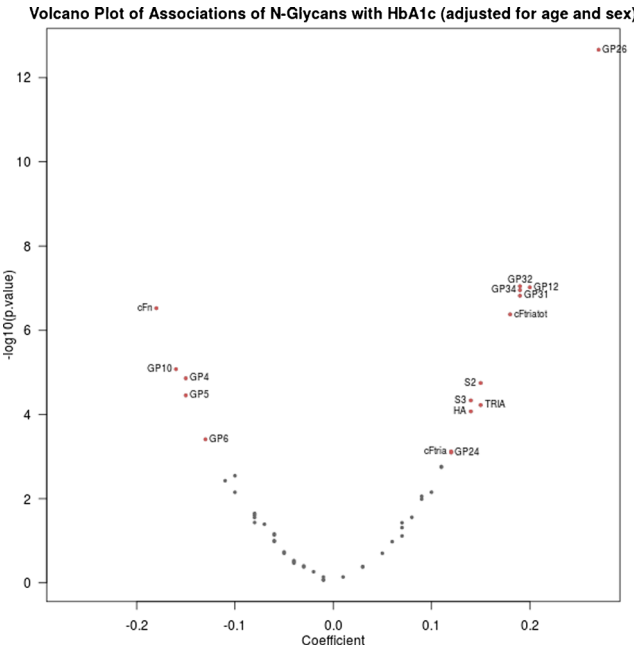
Background and aims: Higher glycaemia levels leading to altered flux through the hexosamine pathway is considered to be a pivotal pathway in diabetic complications, as this produces UDP-N-acetylglucosamine (UDP-GlcNAc), the substrate for N-linked glycosylation. The N-glycan profile reflects oligosaccharide modifications on secreted proteins resulting from N-linked glycosylation. We tested whether in people with type 1 diabetes the N-glycans profile in serum differs by glycaemic control and albuminuria status.

Materials and methods: Study participants comprised 744 people with T1D recruited from diabetes clinics and primary care into the Scottish Diabetes Research Network Type 1 Bioresource. The N-glycan profile in non-fasting serum samples were analysed using hydrophilic interaction ultra performance liquid chromatography (HILIC-UPLC) and divided into 39 glycan groups (GP1-GP39). We investigated associations with HbA1c adjusted for age and sex, through linear regression where the relative quantity of each N-glycan was evaluated independently and then further adjusted for diabetes duration. We also examined associations with 14 summary variables reflecting the ratios of different branching patterns and levels of sialylation and galactosylation. We used a Bonferroni adjusted p-value to consider significance of associations. We used a cross-validated forward selection to define a set of N-glycans associated with HbA1c and used it to compute an N-glycan-based score for glycaemia and tested it for association with albuminuria status adjusted for age, sex, diabetes, duration and HbA1c.
Results: At baseline median (inter-quartile range) for age was 43.6 (31.4, 54.8) years, duration 20.6 (11.0, 30.2) years, HbA1c 75.7 (63.0, 85.9) mmol/mol, eGFR 108.1 (66.6, 135.6) ml/min/1.73 m2, 23.7% of the subjects had micro- or macroalbuminuria (13.2% and 10.5% respectively). Of the 39 N-glycans and 14 summary measures, 16 showed strong associations in either direction with HbA1c at p<0.0001 (Fig 1). The proportion of N-glycans that were tri-antennary glycans was higher with poorer glycaemic control and the level of non-sialylated glycans was decreased. Further adjustment for diabetes duration did not alter these associations. The N-glycan-based score was associated with albuminuria status adjusted for age, sex, diabetes duration and HbA1c (regression coefficient=0.06, 95% CI: (0.02, 3.52), p=0.0005).
Conclusion: In type 1 diabetes the serum N-glycan profile shows strong associations with glycaemic control in a pattern consistent with increased hexosamine flux. Further studies are needed to evaluate whether the cross sectional association with albuminuria is found in prospective studies.



Supported by: Chief Scientist Office, Diabetes UK, JDRF

1130

Arterial stiffness is an independent predictor of renal function decline and stroke in patients with type 2 diabetes with preserved renal function

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Background and aims: Aortic pulse wave velocity (Ao-PWV) the gold standard measure of arterial stiffness is an independent predictor of cardiovascular disease (CVD) in T2DM patients with advanced renal disease. The role of Ao-PWV as a predictor of glomerular filtration rate (GFR) decline in T2DM patients with relatively preserved renal function is unclear.
Materials and methods: In a prospective, observational study of 119 T2DM consecutive patients (81 males) attending a diabetes clinic in a university hospital, we evaluated if Ao-PWV measured (by applanation tonometry (AtCor Medical, Sydney, NSW, Australia) at baseline, was a predictor of renal function decline, CVD or cerebrovascular events (CVA). Patients were followed at least annually with standardized clinical and laboratory assessments. CVD and CVA events were obtained from hospital records.
Results: Median duration of follow up was 9 yrs (range 3-11). At baseline mean age was 60 yrs (range 34 to 80), duration of diabetes 9 yrs (range 4-28), mean ± SD estimated GFR (Modification of Diet in Renal Disease equation) 85±24 ml/min, Ao-PWV 10.1±2.6 m/s, urine albumin to creatinine ratio (median 1.3 mg/mmol, interquartile range 0.6-4.7) and HbA1c 7.9±1.5%. Patients were divided into two groups above (n=72) or below (n=47) 60 yrs of age. Patients below 60 yrs reaching an eGFR value below the median for this group at the end of the observation period had a higher Ao-PWV value at baseline (9.2 vs. 7.9 m/s, mean difference 1.3 m/s 95% CI 0.2-2.4 p=0.02) and a higher rate of GFR decline (-3.3 ml/min/yr, 95% CI -5.0 to -1.5 p=0.001). Ao-PWV was an independent risk factor for decline of renal function after adjustment for age, pulse pressure, gender, baseline eGFR, and albuminuria. In those above 60 yrs baseline Ao-PWV was similar (11.6 vs. 11.1 m/s) in patients above or below final median eGFR for this group. During the follow up there were 4 (3.3%) CVA events and 17(14%) CVD events. Ao-PWV was an independent predictor of CVA events (p=0.015, odds ratio=2.5) but not CVD after adjustment for conventional risk factors.
Conclusion: In 'younger' T2DM subjects with preserved renal function Ao-PWV is an independent predictor of renal function decline. In the whole cohort, Ao-PWV predicted CVA independent of traditional risk factors. Treatments that target Ao-PWV reduction may delay the progression of renal function decline in T2DM.

1131

Microalbuminuria, but not mildly reduced eGFR, predicts cardiovascular outcomes in patients with type 2 diabetes

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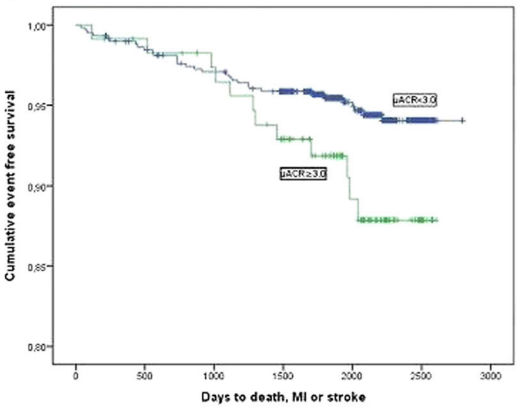
Background and aims: A growing body of evidence emphasizes the importance of albuminuria as a predictor for cardiovascular disease and mortality in patients with type 2 diabetes. However, it is still a matter of controversy if mild renal impairment is an independent risk factor for macrovascular complications. Thus, the aim of this study was to explore the impact from microalbuminuria and mild renal impairment on cardiovascular events in patients with type 2 diabetes.
Materials and methods: Data were analyzed from 706 patients from baseline in the Cardiovascular Risk factors in Patients with Diabetes - a Prospective study in Primary care (CARDIPP). The participants were consecutively recruited 2005-2008, at an age of 55-65 years from 22 primary health care centers in Sweden. The mean age of the patients was 60.7 years. At baseline, urine and blood samples for standardized laboratory analyses were taken in the morning following at least 10 hours fasting. Presence of microalbuminuria was defined as a u-albumin/creatinine ratio (uACR) ≥3.0 mg/mmol. Estimated Glomerular Filtration Rate (eGFR) was calculated by use of the MDRD formula and reduced renal function was defined as level of eGFR<60 ml/min/1.73 m2. Cardiovascular disease and death data were retrieved from the hospital discharge register and the cause of death register in Sweden until 31st December 2012. The primary endpoint was a composite endpoint of the first non-fatal or fatal event of hospitalization for acute myocardial infarction or stroke or cardiovascular mortality diagnosed according to ICD-10, I00-99. The relations of microalbuminuria and renal impairment to risk of the outcomes were investigated using Cox proportional hazard models.
Results: When uACR was used as a continuous variable, covariates in the final model were age, sex, smoking status, previous MI, LDL-cholesterol, triglycerides, HbA1c, mean 24-h systolic blood pressure and use of ACE inhibitors, ARB, beta-blockers and statins in Cox proportional hazard models. There was a strong association between uACR and the endpoint, P=0.001 HR: 1.03; CI 1.01-1.04. When uACR was replaced by eGFR in the analysis there were no significant association between eGFR and the primary endpoint crude or in any model of adjustments of covariates. The association between uACR and eGFR and the primary endpoint are further illustrated in the Figure by Kaplan-Meier curves with uACR dichotomized at ≥3.0 mg/mmol and eGFR<60 ml/min/1.73 m2 .
Conclusion: Our study confirms that microalbuminuria defined as uACR, but not renal impairment defined by eGFR, predicts cardiovascular complications in middle-aged patients with type 2 diabetes.

Figure



Kaplan-Meier estimates above shows significance for uACR, p=0.035 (log-rank test).
For comparison of survival with eGFR higher or lower than 60 ml/min/1.73m^2 there was no statistical significance, p=0.326.

Clinical Trial Registration Number: Dnr M26-05
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1132

Albuminuria is positively associated with elevated numbers of circulating endothelial pre- and mature cells, but inversely associated with circulating fibrocytes

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Background and aims: Diabetic nephropathy is characterized as a microvascular disease with enhanced vascular leakiness in the diabetic

kidney together with aberrant tissue remodelling. Abnormal number and function of endothelial cells, stem cells and activation of leukocytes is considered as contributing mechanisms to the "kidney-micro"-vascular leaky syndrome. We aim to determine if circulating endothelial pre- and mature cells, fibrocytes or monocyte sub-populations are abnormally regulated in type 2 diabetic patients with albuminuria.

Materials and methods: Cross-sectional study of 37 type 2 diabetic patients, 18 with normoalbuminuria (<30 mg/24 h) and 19 with albuminuria (≥30 mg/24 h) randomly selected from our outpatient clinic. 8-color flow cytometry (FACS analysis) of peripheral blood was performed. Analysis of covariance compared -expression of cell markers as well as absolute number of specific cell populations in patients with normoalbuminuria *vs*. albuminuria. Adjustment included age, sex, body mass index, total cholesterol, 24 h systolic blood pressure, smoking, Hba1c and eGFR.

Results: The cohort included 30% women, mean ± SD age was 62.4± 9.4 years, and median (interquartile range) UACR was 61.5 (10-234) mg/24 h. Expression of VEGFR2 was significantly enhanced in patients with albuminuria compared to normoalbuminuria ($p=0.009$). Also, the total number of circulating mature endothelial cells (CEC) was significantly enhanced in patients with albuminuria ($p<0.001$). Moreover, the circulating fibrocyte number and collagen-1 expression was inversely associated with presence of albuminuria ($p\leq0.037$ for both). The TGFbeta stabilizing and M2-associated galectin-3 expression on M1 and M0-M2 macrophages was positively associated with presence of albuminuria ($p\leq 0.029$ for all). In contrast, the expression of the M1-like marker CD11c ($p=0.042$) was inversely associated with presence of albuminuria on all macrophages and particularly on the M0-like macrophages ($p=0.014$).

Conclusion: The enhanced number of CEC together with elevated expression of VEGFR2 may indicate an aberrant function of the CEC with reduced capacity to heal the kidney microvascular disease. The imbalanced M2-polarization of macrophages may favour improper repair leading to excessive tissue fibrosis. Hence therapeutic approaches addressing migratory pattern of CEC, providing adjuvant activating signals and restoring immune balance might provide novel individualized treatment regimes.

1133

Systemic inflammation and glucose variability in type 2 diabetic patients with and without chronic kidney disease

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Background and aims: A growing body of evidence indicates that chronic macrophage-mediated inflammation is involved in the pathogenesis of diabetic kidney disease. Furthermore, it was demonstrated that increased glucose variability (GV) can enhance the systemic inflammatory response in diabetes. The aim of our study was to assess the relationship between acute-phase and macrophage-related inflammatory markers and GV parameters in type 2 diabetic patients with and without chronic kidney disease (CKD).

Materials and methods: We observed 211 insulin-treated patients, 47 M/164 F, 43-70 years of age, HbA1c 8.4, 7.2-9.6% (median, 25th-75th percentile). Sixty four patients had estimated glomerular filtration rate (eGFR) <60 mL/min/1.73 m^2. The serum levels of high-sensitivity C-reactive protein (hsCRP), alpha1-acid glycoprotein (AGP), macrophage colony stimulating factor (M-CSF), macrophage inflammatory protein 1alpha (MIP-1alpha), macrophage migration inhibiting factor (MIF), as well as urinary albumin and type IV collagen were determined by ELISA and compared to control (30 healthy subjects). The parameters of GV were derived from the blinded continuous glucose monitoring (CGM) recordings. The values of nine GV indices were estimated, including lability index, M-value, J-index, continuous overlapping net glycemic action, mean absolute glucose, high blood glucose index, low blood glucose index, mean amplitude of glucose excursions and average daily risk range.

Results: The increased serum levels of hsCRP, AGP, M-CSF, MIP-1alpha and MIF were found in observed diabetic patients as compared to control ($p<0.0001$, $p<0.0001$, $p<0.0001$, $p=0.03$ and $p=0.0006$ respectively). The concentration of hsCRP correlated positively with AGP and M-CSF ($r=0.44$ and $r=0.37$, both $p<0.01$), AGP demonstrated correlations with M-CSF and MIP-1alpha ($r=0.51$ and $r=0.4$, both $p<0.01$). Concentrations of hsCRP, M-CSF and MIP-1alpha, as well as urinary type IV collagen, were higher in patients with reduced eGFR as compared to those without ($p=0.04$, $p=0.02$, $p=0.02$ and $p=0.01$ respectively). Serum AGP and M-CSF correlated positively with urinary albumin/creatinine ratio ($r=0.39$ and $r=0.37$ respectively, $p<0.0001$), meanwhile hsCRP, M-CSF and MIP-1alpha demonstrated weak relationships with type IV collagen excretion ($r=0.31$, $r=0.37$, $r=0.4$, all $p\leq0.01$). Subjects with reduced GFR did not differ from those without by mean monitored glucose and all investigated GV parameters. No relationships were found between concentrations of inflammatory markers and GV indices.

Conclusion: The serum levels of acute-phase and macrophage-related inflammatory proteins are associated with CKD in type 2 diabetic patients. The parameters of GV seem to be not related to inflammatory markers in these patients.

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PS 113 Hypertension

1134

Type 2 diabetes is associated with greater carotid stiffness and greater pressure-dependency of carotid stiffness: The Maastricht Study

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Background and aims: Arterial remodeling is considered an adaptive process aimed at maintaining mechanical arterial integrity under altered arterial environmental conditions, e.g. hypertension and hyperglycemia. This process has been shown operative in both impaired glucose metabolism and type 2 diabetes, but eventually may even become maladaptive leading to excessive arterial stiffening. A specific marker of arterial remodeling, hypothesized to be closely linked to structural alterations of the arterial wall itself, we propose is the pressure-dependency of arterial stiffness. The pressure-dependency of arterial stiffness is quantified as the systolic-diastolic difference in pulse wave velocity (δPWV), with greater δPWV signifying greater pressure dependency.Thus far, the relationship between arterial stiffness pressure dependency with deteriorating glucose metabolism status (GMS) has not been investigated. Therefore, we investigated in The Maastricht Study the associations of carotid arterial stiffness (cPWV) and the pressure-dependency of carotid arterial stiffness (δPWV) with deteriorating GMS. Additionally, we investigated the interdependency of cPWV and δPWV to evaluate whether arterial remodeling may act differentially upon cPWV and δPWV.
Materials and methods: The study population consisted of 746 individuals (415 normal glucose metabolism [NGM], 126 impaired glucose metabolism [IGM] and 205 type 2 diabetes [T2D]). Each participant underwent carotid ultrasonography and tonometry to determine cPWV and δPWV. Linear regression analyses were used to investigate the associations of cPWV and δPWV with deteriorating GMS (NGM as reference category). Models were adjusted for age, sex, mean arterial pressure (MAP), anti-hypertensive medication, prior cardiovascular disease, eGFR, BMI and smoking and δPWV or cPWV as appropriate.
Results: After adjustment for age, sex and MAP, T2D was associated with greater cPWV (β (95% CI; 0.377 (0.124; 0.629) and δPWV (0.311 (0.027;0.595)). Further adjustments did not change these associations materially. After additional adjustment for δPWV or cPWV the associations of T2D with cPWV and δPWV were attenuated (0.292 (0.051; 0.534) and 0.182 (-0.090; 0.454), respectively). IGM was not associated with either cPWV or δPWV.
Conclusion: In T2D, but not in IGM, both cPWV and δPWV were increased. These associations were only partially interdependent which suggests that the process of arterial remodeling may act differentially on carotid arterial stiffness and the pressure dependency of carotid arterial stiffness. This study hereby provides a pathophysiological framework to understand how arterial remodeling may shift from being adaptive to maladaptive with deteriorating GMS.
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1135

Hypertension among urban and rural type 2 diabetic subjects in Bangladesh

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Background and aims: Hypertension is known to be more common among people with type 2 diabetes mellitus (T2DM), but the prevalence and risk factors of the comorbidity vary from population to population. The present study was undertaken to estimate the proportion of hypertension among urban and rural Bangladeshi Type 2 diabetic subjects.
Materials and methods: An analytical cross-sectional study was conducted among 640 urban (M/F, 244/396; age in years, 52.23±11.8; BMI, 25.95±3.9 Kg/m2) and 560 rural (M/F, 244/396; age in years, 50.89±11.9; BMI, 24.13±4.6) subjects, selected purposively from various health care facilities in Dhaka and Northern Districts of Bangladesh. The World Bank criteria were followed for the selection of the urban and rural areas. Diagnosis of T2DM and hypertension was done by WHO Study Group Criteria (following a 2-sample OGTT) and WHO/ISH Guideline respectively.Data were collected by face-to-face interview and by physical examinations through interviewer administered pretested semi-structured questionnaire and check-list. Data were analyzed by univariate, bivariate and multivariate statistics as appropriate.
Results: Hypertension was present among 45.5% urban (95% CI,41.6-49.4) and 43.6% rural (CI,39.5-47.7) subjects. There was no significant difference in the proportion of hypertension between the two demographic locations. Males and females did not differ in the proportion of hypertension either in the urban or in the rural region. The trend in the proportion did not vary when the data were analyzed for systolic and diastolic hypertension separately. Urban hypertensive subjects had higher age compared to their normotensive counterparts (age in years, 53.85±10.5 vs 50.88±12.7; p<0.001). The rural hypertensive subjects showed similar difference in age (54.69±11.4 vs 47.95±11.5; p<0.001). The BMI of the hypertensive subjects (25.56±4.1 Kg/m2) was higher compared to normotensive (24.73±4.4 Kg/m2) and the difference was significant (p< 0.001). The waist circumference (WC) of the hypertensive subjects (90.11±13.1 cm) was similarly higher compared to normotensive subjects (86.30±14.2 cm)and difference was significant (p<0.001). Physical activity was significantly less in hypertensive compared to the normotensive subjects (p=0.032). On Pearson correlation analysis hypertension correlated positively with WC (r=0.092, p<0.001), and duration of DM (r =0.122, p<0.001). On multiple regression analysis higher age (p< 0.001, OR=1.036, CI,1.025-1.048), greater WC (p<0.001, OR=1.014, CI,1.004-1.025) and less physical activity (p<0.001, OR=1.396, CI, 1.098-1.775) were found as the main predictors of hypertension.
Conclusion: A large proportion of Type 2 diabetic subjects in Bangladesh, both males and females, irrespective of urban or rural origin, suffer from hypertension. Higher age, central obesity and reduced physical activity are the major predictors of hypertension in this population.Only an appropriate lifestyle intervention can minimize the risk of hypertension among T2DM subjects.

1136

Diastolic but not systolic orthostatic hypotension is associated with organ damage and with increased risk for cardiovascular complications in type 2 diabetes

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Background and aims: Orthostatic hypotension (OH) is a marker for increased cardiovascular risk, and is common in patients with diabetes. It is likely that the diastolic and systolic components of OH represent two different clinical entities, but their respective impacts on cardiovascular prognosis have not been compared in patients with diabetes. The aim of this study was to determine the association between diastolic and systolic OH, respectively, with markers of macrovascular atherosclerosis and with subsequent cardiovascular prognosis, in a cohort of patients with type 2 diabetes.
Materials and methods: Office blood pressures were measured in the sitting and in the standing position in 749 patients with type 2 diabetes, as part of the baseline examination of the observational cohort study

CARDIPP (Cardiovascular Risk factors in Patients with Diabetes - a Prospective study in Primary care). Systolic OH was defined as a systolic blood pressure drop≥20 mmHg, and diastolic OH was defined as a diastolic blood pressure drop≥10 mmHg when rising from sitting to standing. Aortic pulse wave velocity (PWV) and carotid intima media thickness (IMT) measurements were performed at baseline. Patients were treated in usual care at their primary health care centers, and were followed until any of the primary endpoint events (fatal or non-fatal myocardial infarction or stroke, or cardiovascular death) occurred or until 31 December 2012.

Results: Among the 749 study participants, 18 had systolic OH only, 25 had diastolic OH only, and 6 had combined systolic and diastolic OH. Patients with diastolic OH had significantly higher PWV than patients without diastolic OH (11.3 m/s vs. 10.3 m/s, P=0.030), but there was no significant difference between patients with systolic OH and patients without systolic OH (11.0 m/s vs. 10.4 m/s, P=0.145). Likewise, carotid IMT was significantly higher in patients with diastolic OH than in patients without diastolic OH (0.83 mm vs. 0.73 mm; P=0.002), but did not differ significantly between patients with systolic OH and patients without systolic OH (0.74 mm vs. 0.79 mm, P=0.138). During a median follow-up time of 5.9 years, there were 48 primary endpoint events. The presence of diastolic OH was a significant predictor of cardiovascular complications (crude hazard ratio: 3.321; 95% CI: 1.412 - 7.815; P=0.006), but the presence of systolic OH was not (crude hazard ratio: 0.636; 95% CI: 0.088 - 4.609, P=0.654). When treated as a continuous variable, diastolic blood pressure drop from sitting to standing was significantly associated with increased risk for cardiovascular complications (crude hazard ratio per 1 mmHg: 1.048; 95% CI: 1.007 - 1.091; P=0.020), but systolic blood pressure drop was not (crude hazard ratio per 1 mmHg: 1.002; 95% CI: 0.976 - 1.028; P=0.894).

Conclusion: In patients with type 2 diabetes, diastolic OH is associated with more severe atherosclerosis, and with an increased risk for macrovascular diabetes complications. On the contrary, we found no significant associations between systolic OH and degree of atherosclerosis or prognosis. Diastolic OH is easily diagnosed in the usual clinical setting, and our data suggest that it is a useful marker to detect patients at higher risk for macrovascular diabetes complications.

Clinical Trial Registration Number: NCT01049737

1137

Baroreceptor stimulation enhanced vessel sensitivity to nitric oxide-mediated vasodilation, implications for treatment of diabetes and the metabolic syndrome

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Background and aims: Increasing evidence suggests endothelial nitric oxide (NO) deficit and baroreflex dysfunction to be characteristic for diabetic and cardiovascular conditions ranging from arterial hypertension to stroke and coronary heart disease with deleterious impact on morbidity and mortality. To test the hypothesis that arterial baroreflex has a modulatory effect on NO-dependent vasodilation the magnitude of the cutaneous vasodilator response to sodium nitroprusside (SNP), a spontaneous NO donor, was determined in relation to increase in baroreflex sensitivity (BRS) generated by carotid baroreceptor magnetic activation and potential implementation in NO deficiency states.

Materials and methods: Mean femoral artery blood pressure (MAP), heart rate (HR) and ear lobe skin microcirculatory blood flow, measured by microphotoelectric plethysmogram (MPPG), were simultaneously recorded in conscious rabbits before and after 40-min sinocarotid baroreceptor exposure to 350 mT static magnetic field (SMF), generated by Nd-Fe-B alloy (n=8) or sham magnets (n=8, controls). Arterial baroreflex sensitivity (BRS) was measured by changes in HR and MAP (ΔHR / ΔMAP) after intravenous bolus injections of SNP and phenylephrine.

Results: SMF sinocarotid baroreceptor exposure significantly increased BRS, HR variability and decreased phenylephrine MAP ramps. The vasodilatory effect of SNP was markedly enhanced after SMF exposure (MPPGbeforeSMF: 2.57±0.81 V vs. MPPGafterSMF: 7.82±1.61 V, p< 0.0001), Figure 1, and positively correlated with increase in BRS (r= 0.51, p=0.01).

Conclusion: The important and novel findings demonstrated in this study are that arterial baroreflex modulates NO-mediated vasodilation and that sinocarotid baroreceptor magnetic stimulation improved vessel sensitivity to NO vasodilatory effect. These may synergistically reinforce the reciprocal cross-talk relationship between insulin resistance and NO deficit arguing for baroreceptor stimulation in cardiometabolic conditions such as diabetes and the metabolic syndrome where unresponsiveness of vascular smooth muscle cells to NO amplifies deleterious consequences of the reduced endothelial NO availability importantly contributing to autonomic dysfunction, arterial hypertension and the non-lipid residual cardiovascular risk.

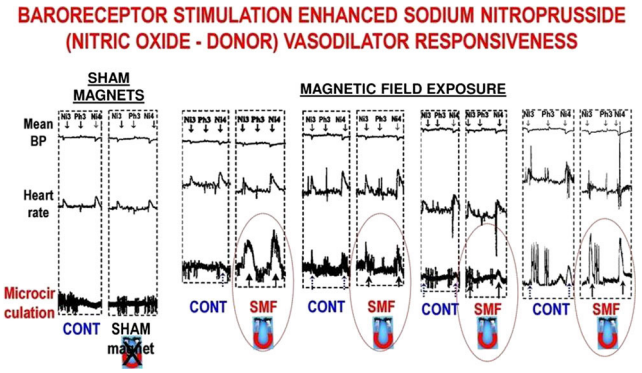

Figure 1. Segments of five experimental recordings after local exposure of sham magnets (SHAM, control experiment) or static magnetic field (SMF) to sinocarotid baroreceptors. (Ni3, 10.0; Ni4, 30.0) and (Ph3, 3.0), doses of sodium nitroprusside and phenylephrine (μg/kg), respectively, given by intravenous bolus injections for BRS testing. CONT, initial readings.
A notable increase of the vasodilation in response to same dose sodium nitroprusside after sinocarotid baroreceptor magnetic stimulation is obvious.

1138

LEADER-4: blood pressure control in patients with type 2 diabetes and high cardiovascular risk: baseline data from the trial

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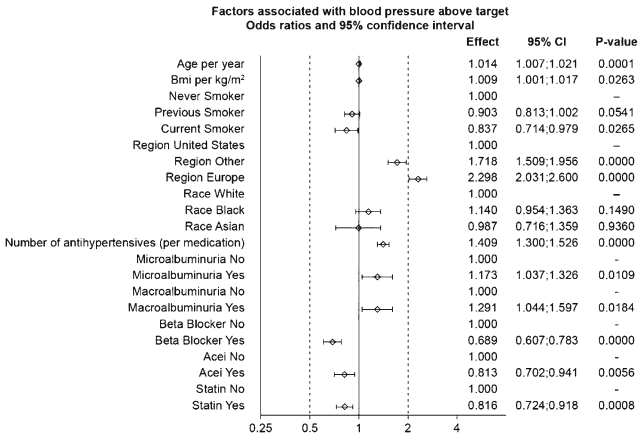
Background and aims: Blood pressure (BP) control is a key strategy for reducing cardiovascular (CV) and renal complications in patients with type 2 diabetes mellitus (T2DM). As a result, treatment guidelines include disease-specific targets but these can be difficult to achieve even with multiple antihypertensive agents. For patients with T2DM who are also obese, there is limited knowledge about the percentage whose BP reaches target and the factors associated with non-achievement of target.

Materials and methods: We collected data on BP control at baseline (2010-2012) and demographic/anthropometric characteristics from the Liraglutide Effect and Action in Diabetes; Evaluation in Cardiovascular outcomes Results (LEADER) trial. LEADER is an ongoing Phase 3B randomized, double-blind, placebo-controlled CV outcome trial examining the safety and efficacy of the GLP-1 receptor agonist liraglutide in 9340 people with T2DM from 32 countries (including USA, Europe, Asia and other countries). Mean (±SD) age was 64±7.2 years; BMI 32.5±6.3 kg/m^2; duration of diabetes 12.7±8.0 years; 81% had suffered

a previous CV event. We analysed factors associated with BP control using multiple logistic regression models in order to assess the association between dichotomized 'off-target' hypertension endpoints and predefined explanatory factors.

Results: 91% of participants had a history of hypertension but only 51% were treated to a target BP of <140/85 mmHg [European Society of Hypertension (ESH)/ European Society of Cardiology (ESC) 2013] and only 26% to the predefined BP target of <130/80 mmHg (ESH/ ESC guideline 2007). This was despite prescription of multiple antihypertensive agents: 61% of patients were on two or more agents and 19% on three or more. Those with a previous CV event had lower BP than those without (137±18.8/78±10.6 mmHg vs. 140±17.7/80±9.9 mmHg; p< 0.001) and were prescribed more antihypertensive agents (p<0.001). BP control was better in the US than Europe with a higher proportion achieving a BP target of 140/85 mmHg (63% vs. 42%, p<0.0001) and a BP target of <130/80 mmHg (37% vs. 20%, p<0.0001); this was not readily explained by baseline demographics or the intensity of antihypertensive therapy. In multifactorially adjusted logistic regression analyses, principal characteristics associated with poorer BP control were: region of residence, smoking status, and number of antihypertensive agents (Figure).

Conclusion: These data confirm that even with modern antihypertensive agents, BP is frequently poorly controlled in individuals with long-standing T2DM, including individuals who are also obese. The LEADER trial will provide important information on the effects of the GLP-1 receptor agonist liraglutide on BP and other CV risk factors in relation to CV safety and outcomes in this patient population.



Clinical Trial Registration Number: NCT01179048
Supported by: Novo Nordisk

1139

Remodelling of the aorta and kidney in L-NAME-induced hypertension in rats: comparison of the protective effect of ivabradine with captopril and melatonin

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Background and aims: Ivabradine was recently proven to reduce morbidity and mortality in patients with heart failure. It is supposed that its benefit is achieved through the reduction of heart rate via blockade of the If-channel in sinoatrial node. Besides the heart rate reduction, ivabradine seems to reduce afterload potentially through its antiproliferative effect on the aorta. The aim of our experiment was to show the effect of ivabradine on morphometry of the aorta and kidney and to compare ivabradine with well established protectives - agiotensin converting enzyme (ACE) inhibitor captopril and melatonin.

Materials and methods: 72 male Wistar rats were divided into 6 groups: controls (C), ivabradine (10 mg/kg/day) (Iv), Nω-Nitro-L-arginine methyl ester (40 mg/kg/day) (L-NAME), L-NAME+ivabradine (L-NAME+Iv), L-NAME+captopril (100 mg/kg/day) (L-NAME+Cap), L-NAME+melatonin (10 mg/kg/day) (L-NAME+Mel). Drugs were administered in drinking water for 4 weeks. Systolic blood pressure (SBP) and heart rate (HR) were measured (by tail-cuff method) once a week. After 4 weeks of the therapy rats were killed and organs (heart, aorta, kidney) were removed and processed for morphometric and biochemical investigation. One-way analysis of variance (ANOVA) and the Bonferroni test were used for statistical analysis. Differences were considered significant if the p value <0.05.

Results: Ivabradine significantly (p<0,05) reduced SBP in Iv (by 21%) and in L-NAME (by 19%) and markedly reduced HR in both Iv and L-NAME (by 26% and 21%, respectively). Ivabradine did not affect the wall thickness (WT) and cross-sectional area (CSA) of the aorta in neither controls nor in L-NAME. Captopril reduced SBP and WT in L-NAME but HR and CSA remained unchanged. Melatonin reduced both SBP (by 14%) and HR (by 10%) without any effects on WT or CSA. Enhancement of glomerular tuft area (AG) in L-NAME was decreased by ivabradine (by 8%) and captopril (by 7%). Density of glomeruli was decreased by ivabradine (16%), captopril (19%) and melatonin (14%).

Conclusion: Ivabradine reduced HR, SBP and modified kidney remodelling in L-NAME-induced hypertension, without influencing the remodelling of the aorta.

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1140

Sitagliptin and risk of hypertension in patients with type 2 diabetes mellitus: meta-analysis of randomised trials

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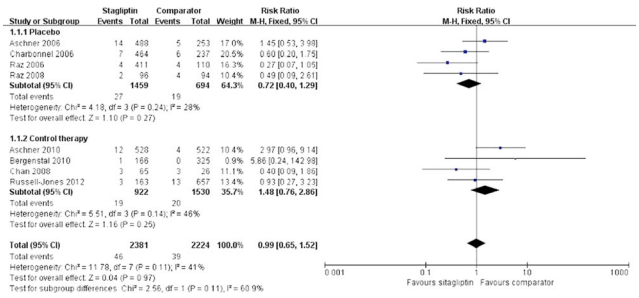
Background and aims: Recent reports of sitagliptin use have raised questions about whether the sitagliptin have beneficial effect on blood pressure of type 2 diabetes mellitus (T2DM) patients. The aim of this meta-analysis was to evaluate the effect of sitagliptin on hypertension.

Materials and methods: We searched PubMed, MEDLINE, EMBASE, the Cochrane Central Register of Controlled Trials, and product information sheets for randomized controlled trials, systematic reviews, and meta-analyses published through December 2012. Studies were included if they were randomized controlled trials of sitagliptin vs placebo or active comparator for T2DM patients also monitored hypertension. Relative risks of hypertension events were estimated using a fixed-effects meta-analysis.

Results: We analyzed 8 randomized controlled trials. Compared with placebo therapy and control therapy, sitagliptin had no significant increase in risk of hypertension (n=46/2381 vs 39/2224; RR 0.99; 95% CI, 0.65 - 1.52; P=0.97). The risk of hypertension was slightly lower in the sitagliptin group than in the placebo group, but the difference was not statistically significant (n=27/1459 vs 19/694; RR 0.72; 95% CI, 0.40 - 1.29; P=0.27). Conversely, the risk of hypertension was slightly higher in the sitagliptin group than in the control therapy group, but the difference was not statistically significant (n=19/922 vs 20/1530; RR 1.48; 95% CI, 0.76 - 2.86; P=0.25). There was no evidence of substantial heterogeneity among the trials (I2=41%, I2=28%, and I2=46%, respectively).

Conclusion: Sitagliptin has no beneficial or harmful effect on hypertension in the treatment of T2DM.



PS 114 Diabetic complications: beyond the vasculature

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The changes of the mitochondria in the lung parenchyma in streptozotocin-induced diabetes

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Background and aims: There is growing body of evidence that lungs represent another target for diabetic complications. However, the pathogenetic mechanisms of lung damage in diabetes mellitus remain not fully understood. The damage of mitochondria can be one of the underlying mechanisms of the development of this complication. Therefore, the aim of this study was to investigate ultrastructural features of mitochondria in pulmonary tissue in the streptozotocin (STZ)-induced diabetes.
Materials and methods: The study was performed on 47 STZ-induced diabetic and 43 control male Wistar rats. Diabetes was induced by single intraperitoneal injection of STZ 60 mg/kg in 0.1 M citrate buffer, pH-4.5. The tissue for the further evaluation was collected after 18 weeks of diabetes. The diabetic rats were not treated with insulin. The morphometric assessment was performed using the electron microscope PEM-125 K and the data were analyzed using the software Image Tool Version 3 (USA).
Results: We found swelling and clarification of the mitochondrial matrix, the intercrystaline intervals enlargement without change of the space between the internal and external membranes which corresponded to a reduced generation of adenosine triphosphate, fragmentation and decondensation of the mitochondrial membrane in the lung tissue in STZ-induced diabetic rats compared to non-diabetic rats. The total number of mitochondra in the lung tissue was not significantly different in STZ-induced diabetic rats compared to control group - 10,1±0,4 U/μm2 and 9,6±0,3 U/μm2, respectively, $p>0.05$. However, it was found an increased number of structurally damaged mitochondria per μm2 of the lung parenchyma - 15,3±0,8% and 4,6±0,4%, in STZ-induced diabetic rats and control group respectively, $p<0,001$. There were no signs of morphogenesis activation. Also, it was an increase of the average diameter of the of mitochondria in the diabetic rats compared to controls - 0,64 ±0,05 μm and 0,39±0,04 μm, respectively, $p<0,01$.
Conclusion: Experimental STZ-induces diabetes mellitus leads to the morphological changes of mitochondria in the lung parenchyma. The revealed mitochondrial dysfunction could be one of the possible mechanisms of the lung damage observed in diabetes mellitus.

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Carbon monoxide in exhaled air and the development of vascular complications in type 2 diabetes

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Background and aims: Decompensated diabetes leads to high levels of glycosylated hemoglobin (HbA1c), which forms close bond with oxygen and cause tissue hypoxia. On the other hand the catabolism of heme-containing compounds and proteins, lipid peroxidation, cetogenesis and other metabolic transformations lead to formation of endogen carbon monoxide (CO) that form carboxyhemoglobin and exacerbates tissue hypoxia and development of angiopathy in diabetes. The aim of our research is to study the level of CO in exhaled air and the development of vascular complications in patients with type 2 diabetes.

Materials and methods: We examined 53 patients with type 2 diabetes (age 57,5±1,18 years, 39 women and 14 men, diabetes duration 6,1±2, 1 years) and 26 patients without diabetes (age 59,5±2,18 years, 18 women and 8 men) who did not smoke and have no respiratory or blood diseases. All patients underwent clinical and laboratory examination: HbA1c levels, lipid profile, C-reactive protein, urinary albumin/creatinine ratio. CO in exhaled air was performed using Smokelyzer Bedfont Scientific Micro +. Doppler of carotid arteries was done to register diabetes complications. Statistical analysis - using Excell 2007, SPSS.

Results: The patients with type 2 diabetes had significantly higher fasting glucose (9,4±0,5 mmol/l, p<0,01) and HbA1c (7,6±0,4%, p<0.05) in contrast to comparison group (glucose=5,3±0,4 mmol/l, HbA1c=5,5±0, 1%). Violation of lipid metabolism was also confirmed by significant differences in triglyceride levels (1,08±0,07 mmol/l) and levels of very low-density lipoproteins (VLDL=0,5±0,03 mmol/l) in patients with diabetes, in contrast to comparison group (triglyceride=0,8±0,1 mmol/l, VLDL=0,4±0,03 mmol/l, p<0,01). The level of urinary albumin/creatinine ratio was significantly higher in diabetes patients (2,9±0, 6 mg/mmol),in contrast to comparison group (1,1±0,2 mg/mmol, p<0, 05) that shows endothelial dysfunction, the initial glomerular and vascular changes, and is known marker of atherosclerosis and cardiovascular events. In this situation, patients with diabetes had significantly higher CO levels in exhaled air (1,9±0,2 ppm, p<0,05), in contrast to comparison group (1,5±0,1 ppm), which had significantly direct correlation with urinary albumin/creatinine ratio (p<0,01), HbA1c (p<0,05) and confirms direct relationship of hyperglycemia, endothelial dysfunction and CO levels in exhaled air. Significant thickening of intima/media in patients with type 2 diabetes was determined by Doppler of carotid artery, which has direct correlation with total cholesterol (p<0,05), LDL (p<0,05), HbA1c (p<0,05) and was inversely related to HDL level (p<0,01); atherosclerotic plaques with restriction of blood flow were identified in 21% of diabetes patients. Our data confirm the significant relationship between glucose metabolism, endothelial dysfunction, microvascular and macrovascular complications, atherosclerosis and level of CO in exhaled air.

Conclusion: Defined in patients with type 2 diabetes significantly higher glucose levels, HbA1c level, urinary albumin/creatinine ratio, which correlated with significantly higher CO levels in exhaled air, suggests a direct relationship between hyperglycemia, endothelial dysfunction and CO levels in exhaled air. Determination of CO in exhaled air is composed of non-invasive method and can be used to analyze the development angiopathy and cardiovascular complications in general practice.

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Prevalence, associates and prognostic significance of abnormal pulmonary function in type 2 diabetes: the Fremantle Diabetes Study Phase II

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Background and aims: We found previously that type 2 diabetes (T2DM) was associated with reduced pulmonary function in the community-based observational Fremantle Diabetes Study Phase I (FDS1) in patients recruited between 1993 and 1996. Since clinical management, complications and methods of spirometric data assessment have changed over the last two decades, we re-examined the prevalence, associates and prognostic significance of abnormal pulmonary function in a larger, contemporary sample of community-based patients with T2DM from the same geographical area as FDS1.

Materials and methods: We used baseline data including spirometry from the FDS Phase II (FDS2) collected between 2008 and 2011. Mortality data were available through validated health service linkage to end-June 2013. The highest forced expiratory volume in the first second (FEV1) and forced vital capacity (FVC) from three recordings/patient were used in analyses. Predicted values and lower limits of normal (LLN; z-score<-1.96) were determined using Quanjer Global Lungs Initiative 2012 reference equations which are based on sex, age, height and ethnicity. Multivariable linear regression was used to assess independent associates of lung function measures. Cox proportional hazard modelling was used to determine whether FEV1 and FVC predicted mortality.

Results: Of 1,551 participants, 1,393 (89.8%) performed acceptable lung function tests. Of these patients, 109 (7.8%) died during a mean ± SD of 3.7±1.0 years (5,106 patient-years) of follow-up. The mean ± SD FEV1 and FVC as percentage predicted were 82.6±18.9% and 85.7±17.4%, respectively, and 294 (21.1%) and 238 (17.1%), respectively, had FEV1 and FVC values below the LLN. After adjusting for other variables including a history of respiratory disease, body mass index and smoking, the diabetes-related independent inverse predictors of both FEV1 and FVC were diabetes duration, HbA1c, ln(urinary albumin:creatinine), self-reported ischaemic heart disease, and peripheral arterial disease (ankle:brachial index ≤0.90) (P≤0.037 in each case). Although FEV1 and FVC as percentages of predicted were bivariately associated with mortality (P≤0.001), neither variable was an independent predictor of death in a fully adjusted model.

Conclusion: These spirometric data, derived from contemporary validated predictive equations, confirm that T2DM is associated with a restrictive lung defect, especially in those patients with long duration of disease, poor glycaemic control, and both micro- and macrovascular complications of diabetes. Abnormal pulmonary function is not an independent predictor of short-term mortality in T2DM.

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Metformin protects against homocysteine-induced apoptosis of osteocytic MLO-Y4 cells by activation of AMP-activated protein kinase

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Background and aims: Homocysteine (Hcy) is a sulfur-containing amino acid formed by the demethylation of methionine. Hcy is reported to be significantly increased in patients with diabetes mellitus, and it is an emerging risk factor for diabetic complications such as diabetic nephropathy and cardiovascular disease. On the other hand, accumulating evidence has shown that the risk of osteoporotic fractures is increased in type 2 diabetes mellitus. Previous studies have shown that elevated plasma Hcy level is associated with the risk of osteoporotic fracture. Osteocytes are the most abundant cells in the bone, and they play important roles in coordinating the functions of osteoblasts and osteoclasts. However, to our best knowledge, no study has described the effects of Hcy on osteocytes. While Hcy increases oxidative stress, AMP-activated protein kinase (AMPK) activation ameliorates it, suggesting that an AMPK activator, metformin, may rescue the adverse effects of Hcy on osteocytes. This study aimed to investigate whether Hcy induces apoptosis of osteocytes through regulating expressions of oxidant and anti-oxidant enzymes and determine the effects of AMPK activation by metformin and 5-aminoimidazole-4-carboxamide-1-β-D-ribofuranoside (AICAR) on the Hcy-induced apoptosis of the cells.

Materials and methods: MLO-Y4, a murine long bone-derived osteocytic cell line, was used. DNA fragment ELISA and TUNEL staining assays were performed to detect the apoptosis of the cells. Real-time PCR and western blot analysis were performed to examine the expression of NADPH (Nox) oxidase and superoxide dismutase (SOD).

Results: DNA fragment ELISA and TUNEL staining assays showed that Hcy treatments (0.1-5.0 mM) induced apoptosis of MLO-Y4 cells in a

dose-dependent manner. The detrimental effect of Hcy was partly but significantly reversed by an antioxidant (N-acetylcysteine) and NADPH oxidase (Nox) inhibitors (apocynin and diphenyleneiodonium). In addition, metformin (10-100 μM) and treatment with AICAR (0.05-0.1 mM) ameliorated Hcy-induced apoptosis of the cells. The favorable effect of metformin on Hcy-induced apoptosis was completely cancelled by an AMPK inhibitor Ara-A. Hcy increased the expression levels of Nox1 and Nox2, while it had no effects on the expressions of Nox4 or the anti-oxidant enzymes, SOD1 and SOD2. Hcy-induced increases in the expressions of Nox1 and Nox2 decreased significantly by treatments with AICAR.

Conclusion: These findings suggest that Hcy induces apoptosis of osteocytes by increasing the expression of Nox1 and Nox2, and metformin effectively prevents the detrimental reactions via AMPK activation.

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Wnt/b-catenin pathway downregulation, indicated by increased dickkopf-1 in type 1 diabetes, is probably associated with lower bone mineral density

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Background and aims: Diabetes Mellitus disrupts many bone metabolic pathways, predisposing to reduced bone mass. Increased fracture risk and elevated Dickkopf-1 and sclerostin levels, which are inhibitors of Wnt/β-catenin pathway, have been documented in adult patients with T2DM; however no relevant data exist on childhood T1DM. We aimed to study plasma Dickkopf-1 and sclerostin concentrations in children and adolescents with T1DM and controls and to correlate them with metabolic bone markers and bone mineral density(BMD).

Materials and methods: We evaluated 40 children and adolescents with T1DM (mean ± SD age:13.04±3.53 years, T1DM duration:5.15± 3.33 years), along with 40 healthy matched controls (age 12.99± 3.3 years). Dickkopf-1, Sclerostin, Receptor Activator of Nuclear factor-KappaB Ligand(s-RANKL), Osteoprotegerin, Osteocalcin, C-telopeptide crosslinks-CTX, electrolytes, PTH, total 25(OH) D were measured and lumbar spine and total body BMD were evaluated.

Results: BMD was found lower and Dickkopf-1 levels were found higher (13.56±5.34 vs 11.35±3.76 pmol/L, p=0.0194) in T1DM patients than controls. A trend for lower values was found in girls (13.36±4.04 vs 11.72±5.14 pmol/L, p=0.06) and in pubertal children (13.61±4.87 vs 11.83±4.56 pmol/L, p=0.054). Dickkopf-1 correlated with Sclerostin and L1-L4 BMD z-score only in controls and with Osteoprotegerin and i-Phosphorus only in patients, while in both groups a significant correlation with log(CTX) and √ALP was documented. A significant correlation of Dickkopf-1 with IGF-1 and insulin dose was also found in patients.

Conclusion: Higher levels of Dickkopf-1 were found in T1DM children and adolescents, indicating a downregulated Wnt signaling system and possible lower osteoblast activation that could be associated with T1DM osteopathy.

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FGF-23 in type 1 diabetes: the potential role of mineral metabolism in arterial stiffness

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Background and aims: Existing evidence suggests that an abnormal mineral metabolism, and particularly vitamin D deficiency, may promote atherosclerosis. However, the discovery of fibroblast growth factor 23 (FGF-23) has introduced a new perspective linking vitamin D metabolism and cardiovascular (CV) disease. FGF-23 is an endocrine peptide that regulates mineral metabolism and is directly associated with adverse CV outcomes. Our aim was to investigate the likely relationship between FGF-23 and vitamin D with arterial stiffness (AS), as an early sign of atherosclerosis, in a group of subjects with type 1 diabetes (T1DM) without previous clinical cardiovascular disease.

Materials and methods: 68 T1DM patients and 68 age- and sex-matched controls were evaluated for: 1) age, sex, diabetes duration, physical activity, smoking, alcohol intake, BMI, BP, fasting plasma glucose, HbA_{1c}, estimated glomerular filtration rate (eGFR) and lipid profile, 2) microvascular complications, 3) concentrations of FGF-23 and mineral metabolism parameters (calcium, phosphate, parathyroid hormone (PTH) and 25-hydroxy-vitamin D (25(OH)D)), 4) AS, assessed as aortic pulse wave velocity (aPWV), and 5) low-grade inflammation (hsCRP, IL-6, sTNFαR1, sTNFαR2) and endothelial dysfunction (ED) markers (ICAM-1, VCAM-1, E-Selectin).

Results: Subjects with T1DM had higher aPWV compared with healthy controls (6.8(6.0-7.9)m/s vs. 6.1(5.5-6.7)m/s; p<0.001), but they did not present differences in 25(OH)D (28.3(23.9-33.3)ng/mL vs. 28.1(20.2-34.5)ng/mL; p=0.462) and in FGF-23 plasma concentrations (70.1(38.4-151.9)RU/ml vs. 77.6(51.8-113.9)RU/ml; p=0.329). In patients with T1DM, higher concentrations of FGF-23 were positively associated with aPWV after adjusting for eGFR and classical cardiovascular risk factors (model 1: ß=0.202, p=0.026), other mineral metabolism parameters (model 2: ß=0.214, p=0.015), microvascular complications, low-grade inflammation and ED markers (model 3: ß=0.170, p=0.045). Lower 25(OH)D concentrations were also associated with higher aPWV after adjusting for FGF-23 and all the above-mentioned factors (model 3: ß=-0.241, p=0.015).

Conclusion: We conclude that both FGF-23 plasma concentrations (positively) and 25(OH)D serum concentrations (negatively) are associated with AS in subjects with T1DM and no previous clinical cardiovascular disease.

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Impaired balance ability, particularly one-leg stand evaluation, is associated with diabetic complications even in younger adults with type 2 diabetes

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Background and aims: Elderly individuals with diabetes were at greater falls risk, but probability for falls among younger adults with diabetes is unknown. Several clinical balance tests have been reported to be a useful predictor of falls among older adults. Our aims of this study are 1) to characterize the balance ability of subjects aged 30 and older with type 2 diabetes (T2D) compared with non-diabetic control subjects, 2) to investigate the relevance between the balance ability and diabetic complications among subjects with T2D.
Materials and methods: Two static balance tests, one-leg stand (OLS) and outer area of postural sway while standing (OAPS), and two dynamic balance tests, timed up and go test (TUG) and functional reach test (FR), were measured in 115 T2D subjects and 51 non-diabetic subjects. All subjects were interviewed for a history of falls, and were divided into six groups with age: younger (60 years) T2D (n=55), or older control (n=15). In comparison of balance ability among the different groups, Mann-Whitney test or Kruskal-Wallis test were used.
Results: T2D subjects had significantly lower balance ability compared with non-diabetic subjects in all four balance measures (OLS; 44.8 vs 60.0 sec, OAPS; 1.83 vs 1.25 cm^2, TUG: 7.3 vs 5.8 sec, FR 0.189 vs 0.230, all $P<0.01$). In subjects with T2D, the balance ability assessed by measuring OLS and OAPS decreased gradually with retinopathy progression (OLS; 52.9, 42.8, 10.3 sec, $P<0.01$, OAPS; 1.67, 1.94, 3.08 cm^2, $P<0.01$ in no retinopathy, simple retinopathy, or either preoproliferative or proliferative retinopathy, respectively). Subjects with diabetic neuropathy showed a lower balance ability assessed by OLS (30.0 vs 57.5 sec, $P<0.01$), OAPS (1.65 vs 2.11 cm^2, $P<0.05$) and TUG (6.7 vs 7.5 sec, $P<0.05$) compared with those without diabetic neuropathy. Furthermore, T2D subjects with a falls history (n=9) had a longer duration of diabetes (18.3 vs 9.7 years, $P<0.05$), a shorter time of OLS (6.1 vs 46.6 sec, $P<0.01$) and a longer time of TUG (9.7 vs 7.2 sec, $P<0.01$) compared with those without a falls history. Even in younger and middle-aged T2D subjects, the OLS, TUG and FR evaluation showed lower balance ability, compared with those of age-matched non-diabetic subjects (in younger OLS; 52.7 vs 60.0 sec, TUG; 6.8 vs 5.3 sec, FR; 0.197 vs 0.240, in middle-aged OLS; 57.5 vs 58.1 sec, TUG; 6.9 vs 5.8 sec, FR; 0.192 vs 0.238, all $P<0.01$, respectively).
Conclusion: Subjects with T2D, even in younger adults, had impaired balance ability. OLS was closely associated with the severity of diabetic neuropathy and retinopathy in every aged group, and the previous falls history. Therefore OLS may be the most suitable clinical measurement for evaluating balance ability and falls risks among subjects with T2D.
Clinical Trial Registration Number: UMIN 000008698
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Severe vertebral fracture and low osteocalcin level increase the mortality in postmenopausal women with type 2 diabetes mellitus

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Background and aims: Accumulating evidence has shown that patients with type 2 diabetes mellitus (T2DM) have an increased risk of osteoporotic fracture independently of bone mineral density. Although osteoporotic fractures such as hip fracture and vertebral fracture (VF) are known to increase mortality in general population, there are no studies investigating whether the fractures influence the mortality of patients with T2DM. On the other hand, previous studies have shown that osteocalcin, which is secreted from osteoblasts, has a hormonal function improving glucose tolerance by increasing insulin secretion and sensitivity. Because serum osteocalcin levels are decreased in diabetic patients, we hypothesized that serum osteocalcin may be associated with the mortality. Therefore, the aim of this study was to examine whether the presence of VF and bone turnover markers including osteocalcin were associated with the mortality in postmenopausal women with T2DM.
Materials and methods: This is a historical cohort study with the endpoint of all-cause mortality in postmenopausal women with type 2 diabetes. We recruited 190 postmenopausal women with T2DM who previously underwent lateral X-ray examination of thoracic and lumbar spine to check VF from 1993 to 2009 at Shimane University Hospital. A VF was diagnosed if at least one of three height measurements along the length of the same vertebrae had decreased by >20% compared to the height of the nearest uncompressed vertebral body. VFs were classified as follows; grade 1 (G1), a reduction of 20-25%; G2, 25-40%; G3, more than 40%. The participants were observed up to 7 years, and the association between VF and bone turnover markers versus all-cause mortality was explored using Kaplan-Meier method, logrank test, and Cox regression analysis.
Results: At the entry of this study, mean age and duration of diabetes were 67.5 and 12.3 years, respectively. Mean serum osteocalcin level was 6.9 ng/mL, and 54 patients (28.4%) had vertebral fractures. Of 190 subjects, 19 patients died during the observation period. Logrank tests showed that lower serum osteocalcin (<6.5 ng/mL), multiple and G3 VFs were associated with higher mortality compared to higher osteocalcin (≥6.5 ng/mL) or non VFs, respectively (at least $p<0.01$). Moreover, age-adjusted Cox regression analyses showed that G3 VF was significantly associated with the mortality [relative risk (RR)=3.7, 95% confidence interval (CI)=1.1-13.0, p=0.04] and multiple VFs were marginally with it (RR=2.8, 95%CI=0.9-8.7, p=0.08) compared to non VFs, and that serum osteocalcin was significantly associated with age-adjusted mortality (Hazard ratio (HR)=0.36, 95%CI=0.16-0.80 per SD increase, p=0.012). Moreover, the association of osteocalcin and mortality was still significant even after additional adjustment for duration of diabetes mellitus, body mass index, HbA1c, and serum creatinine (HR=0.30, 95%CI=0.11-0.80 per SD increase, p=0.02).
Conclusion: The present study for the first time showed that severe VF and low serum osteocalcin were associated with all-cause mortality in postmenopausal women with T2DM.

PS 115 Diabetes and the liver: pathogenetic insights

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Relation of 25OHD3 availability to VDR expression and NF-κB-dependent proinflammatory signalling pathway in diabetes-induced liver failure

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Background and aims: Diabetes is known to be characterized with upregulation of pro-inflammatory factors in various tissues. It is known that nuclear factor kappa B (NF-κB) is a key regulator of inflammatory process and its activation leads to increased expression of inducible nitric oxide synthase (iNOS) and vascular permeability factor (VPF) involved in inflammation. Vitamin D3 (D3) is currently recognized as a potent immunomodulator affecting various inflammatory and autoimmune diseases. Importantly, hormonally active form of vitamin D3 (1,25(OH)2D3) binds to vitamin D3 receptors (VDR) functioning as transcription factor to modulate gene expression. However, precise mechanisms of vitamin D3 influence on immune regulation has not been clearly established. We therefore investigated the hepatoprotective role of vitamin D3 in VDR-mediated regulation of pro-inflammatory factors (p65 NF-κB, VPF, iNOS) expression in diabetic liver.
Materials and methods: Type 1 diabetes was induced in male C57BL/J6 mice (24.0±1.6 g) by i.p. injection of high-dose STZ (150 mg/kg b.w.). After 2 weeks of STZ-induced diabetes mice were treated with or without vitamin D3 (15 IU/mouse per os, for 8 weeks). Serum 25OHD3 was assessed by ELISA. The expression of phosphorylated NF-κB/p65 (Rel A), VPF, iNOS and VDR mRNA were measured by RT-PCR and Western-blot analysis. Histological features were assessed using H&E staining.
Results: Serum level of 25OHD3 was shown to be reduced to 27.4±2.0 in diabetes vs. 41.6±2.9 nmol/L in control, indicative of diabetes-induced D3 deficiency ($p<0.05$). These changes were accompanied by 1.3-fold overexpression of VDR mRNA, that can be a compensatory mechanism in response to D3 deficiency. It should be noted, that diabetes was associated with 2.4-fold augmentation of RelA expression in hepatic tissue of diabetic mice. NF-κB overexpression was accomponied by 1.53- and 1.28-fold increase in VPF and iNOS mRNA levels in liver. The results of RT-PCR correlated with results of Western blot analysis that showed a 1.21- , 2.04- , 1.62- and 1.32-fold elevation of liver tissue protein contents of VDR, NF-κB p65, VPF and iNOS respectively. Diabetes also caused structural lesions in liver, which were characterized by altered blood microcirculation (dilated microvessels), degenerative changes in cytological structure of hepatocytes and their mitotic activity. Full restoration of 25OHD3 content and partial normalization of liver tissue structure were achieved by D3 treatment. Vitamin D3 administration caused a partial normalization of mRNA expression and protein contents of VDR, pNF-κB/p65, VPF and iNOS in liver tissue. It can be suggested that D3 prevents overexpression of pNF-κB/p65 and nuclear translocation of phosphorylated p65 in hepatocytes and its effect is mediated through 1, 25(OH)2D3 and VDR.
Conclusion: The study confirmed that diabetes-induced liver abnormalities are associated with upregulation of VDR and inflammatory markers NF-κB/p65, VPF and iNOS expression that correlated with insufficient vitamin D3 availability. Our results can explain protective VDR-mediated effects of vitamin D3 action against diabetes-induced liver cells injury.
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Metformin attenuates hepatitis C virus-induced hepatic fibrosis through up-regulation of AMPK and orphan nuclear receptor SHP

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Background and aims: Metformin, a commonly prescribed oral hypoglycemic agent, has pleiotropic benefits including lowering cancer development and cancer mortality in patients with type 2 diabetes. Metformin was also independently associated with a reduction in hepatitis C virus (HCV)-induced cirrhosis in patients with type 2 diabetes. Mounting evidence suggests that the potential beneficial effects of metformin are associated with AMPK and SHP activation. However, little is known about the protective effects exerted by metformin induced AMPK and SHP activation against HCV-induced hepatic fibrosis.
Materials and methods: Levels of SHP, p-AMPK and fibrotic markers in HCV-infected human liver and in Huh-7.5 cells infected with HCV genotype 2a (JFH-1) were investigated. The effect of adenovirus-mediated overexpression of SHP (Ad-SHP) and AMPK activation by metformin on fibrotic gene expression was evaluated in HCV-infected cells. Finally, we examined the effect of Ad-SHP and metformin on invasion and activation of LX2 human hepatic stellate cells induced by conditioned media (CM) from HCV-infected hepatocyte.
Results: In human livers infected with HCV and Huh-7.5 cells infected with JFH-1, SHP mRNA and protein levels were diminished compared with control livers and Huh-7.5 cells, respectively, whereas profibrotic factors, including TGF-β, were increased. In addition, levels of phosphorylated p38 mitogen-activated protein kinase, c-Jun N-terminal kinase, and Smad3 were also increased. Metformin-induced AMPK activation recovered HCV-suppressed SHP expression, and Ad-SHP inhibited mRNA and protein levels of profibrotic factors and fibrotic genes. SHP overexpression also inhibited HCV-stimulated nuclear factor-kappa B activation, which led to decreased TGF-β production. CytoSelect invasion assay revealed that increased activity and invasiveness of HSCs induced by CM from HCV-infected Huh-7.5 cells were attenuated by treatment with metformin or Ad-SHP.
Conclusion: These results demonstrate that metformin-induced activation of AMPK and SHP reverses profibrogenic features of HCV-infected cells by overcoming HCV-induced diminution of AMPK and SHP expression, and concomitantly decreasing TGF-β and fibrotic gene expression. These findings provide a rationale for metformin as a possible therapeutic agent against HCV-induced hepatic fibrosis, especially when accompanied by type 2 diabetes.

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Non-alcoholic fatty liver disease is associated with vascular inflammation: analysis by 18 F-fluorodeoxyglucose positron emission tomography

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Background and aims: Growing evidence suggests that non-alcoholic fatty liver disease (NAFLD) is associated with cardiovascular disease as well as metabolic syndrome. FDG-PET is a novel imaging technique that detects vascular inflammation, which may reflect rupture-prone vulnerable atherosclerotic plaques.

Materials and methods: Vascular inflammation was measured as the maximum target-to-background ratio (maxTBR), along with various cardiometabolic risk factors in 51 subjects with NAFLD, and compared them with 100 age- and gender-matched subjects without NAFLD. The liver attenuation index (LAI), which was measured using computed tomography, was used as a parameter for the diagnosis of NAFLD.
Results: After adjusting for age and sex, both maxTBR and LAI values were associated with several cardiometabolic risk parameters. Furthermore, there was a significant inter-relationship between LAI and maxTBR values (r=-0.227, P=0.005). Individuals with NAFLD had higher maxTBR values than those without NAFLD (P=0.026), although their carotid intima-media thickness values did not differ. The proportion of subjects with NAFLD showed a step-wise increment following the tertiles of maxTBR values (P for trend=0.015). In multiple logistic regression analysis, maxTBR tertiles were independently associated with NAFLD after adjusting for age, gender, systolic blood pressure, triglycerides, HDL-cholesterol, glucose, BUN, creatinine and homeostasis model assessment of insulin resistance (HOMA-IR) (P=0.030). However, their relationship was attenuated after further adjustment for waist circumference, LDL-cholesterol or high sensitive C-reactive protein.
Conclusion: The present study first shows that patients with NAFLD have an increased risk for vascular inflammation as measured via FDG-PET/CT.
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The hepatic protective effect of hepassocin on hyperglycaemic crisis
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Background and aims: Hyperglycemic crisis is a metabolic emergency associated with uncontrolled diabetes that may result in morbidity. Although it is known that reactive oxygen species (ROS) production by hyperglycemia leads to hepatic dysfunction, only a mild increase of aspartate aminotransferase (AST) and/or alanine aminotransferase (ALT) level is observed in patients with hyperglycemic crisis, and the exact mechanisms were still undefined. Hepassocin is a hepatokine that plays as a mitogen for hepatocytes and exerts a hepatic protection action against chemical-induced liver injury. However, the effect of hepassocin in hyperglycemia-induced hepatic dysfunction remains obscure. Thus, the aim of this study is to investigate the role of hepassocin in hyperglycemia-induced hepatic dysfunction.
Materials and methods: Thirty patients with hyperglycemic crisis, including diabetic ketoacidosis and hyperosmolar hyperglycemic state, without obvious infection were enrolled at our university hospital. Standard treatment of hyperglycemic crisis including insulin, hydration, and correction of electrolyte imbalance were given. After resolution of hyperglycemia, the blood samples were collected to examine hepassocin concentrations by enzyme-linked immunosorbent assay. In addition, streptozotocin-induced hyperglycemic mice and HepG2 cell models were used to evaluate the role of hepassocin in hyperglycemic crisis.
Results: The serum concentrations of hepassocin were significantly increased in patients with hyperglycemic crisis. After treatment to relieve hyperglycemic status, hepassocin levels were significantly reduced ($P<0.01$). In addition, we found a dose-dependent increase in the hepassocin expression of HepG2 cells exposed to high glucose condition. In streptozotocin-induced hyperglycemic mice, treatment with hepassocin significantly decreased the levels of AST and ALT without affecting the concentrations of blood glucose and insulin in circulation ($P<0.001$). In addition, the hepatic SOD1 expression was increased after the hepassocin treatment. Furthermore, we found that hepassocin increased the activity of ERK1/2, Nf-E2 related factor-2 (NRF2) translocation to nuclear, as well as SOD1 expression. Blockade of ERK1/2 by U0126 inhibited NRF2 translocation and SOD1 expression, indicating hepassocin increased SOD1 through an ERK1/2-NRF2-SOD1 dependent pathway. As a result of increased SOD1 expression, HepG2 hepatocytes were protected by hepassocin from ketone body (acetoacetate)-induced ROS production and cell death.
Conclusion: Hepassocin plays a protective role in hyperglycemia-induced hepatic dysfunction by regulating ERK1/2 activity. Hepassocin induced SOD1 expression through an ERK1/2-NRF2-SOD1 dependent pathway, and this effect further decreased ROS production and protects hepatocytes from hyperglycemia-induced hepatocyte injury.
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Liver proteome and microRNAs in insulin receptor knockout mice reveal novel molecules involved in the diabetes pathophysiology
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Background and aims: Type 2 Diabetes Mellitus (T2DM) is a disease characterized by alteration of insulin signaling in specific target tissues, such as skeletal muscle, adipose tissue, and liver. Dysfunction and later failure of insulin-producing pancreatic beta cells (β-cells) and peripheral insulin resistance induce hyperglycemia in pre-diabetes conditions. Fundamental is discovering early biomarkers to delay or prevent onset of T2DM. Recently, some studies are seeking for biomarkers through proteomic and microRNA (miRNA) approaches, but none identified specific hepatic proteins which trigger early stages of T2DM. Therefore we are reaching the goal to reveal new proteins involved in the onset of diabetes and/or its relative complications.
Materials and methods: miRNAs were extracted from ten mice for each genotype (IR+/+, IR+/-, and IR-/- its integrity assayed on Nano Chip and Small Chip Agilent, and then hybridised with miRNA Microarray System with miRNA Complete Labeling and Hyb kit (Agilent) for expression profiling. Expression levels of specific miRNAs were validated by qRT PCR using TaqMan assay. We used miR-Walk algorithm to determine miRNA targets and mRNAs expression were validated by qRT PCR. Different protein profiles of IR+/+, IR+/-, and IR-/- were analyzed by 2D PAGE and nLC MS/MS analysis. HMGB1 acetylation and SIRT1 expression were analyzed by Immunoprecipitation and Western Blot, while HMGB1 secretion was studied by ELISA kit (Shino Test), and immunofluorence microscopy.
Results: We analyzed protein and miRNA patterns by proteomic and miRNA arrays in insulin receptor knockout (IR-/-) and heterozygous (IR+/-) mice as a murine model of liver metabolic dysfunction associated with diabetic ketoacidosis and insulin resistance. We evaluated protein expressions by using protein 2-DE MALDI-TOF/TOF and peptic nLC-MS/MS shotgun profiling. Twenty-eight proteins identified by 2-DE analysis and 24 identified by nLC-MS/MS shotgun, were differentially expressed among the 3 genotypes. Bioinformatic analysis revealed a central role of High Mobility Group Box 1/2 and huntigtin (HTT) never reported to be associated with metabolic and related liver disease. MiRNAs array identified only 4 miRNA differently expressed between IR+/+, IR+/- and IR-/- miR-376b, miR-154, miR-543, and miR-199b. Quantitative Real time polymerase reaction (qRTPCR) confirmed these results, and bioinformatic analysis reveals interesting mRNA targets involved in metabolic pathways linked with proteomic analysis. Among these targets, the most interesting is SIRT1, a deacytetilase involved in

HMGB1 translocation, and then, in the activation of inflammatory state mediated by HMGB1.
Conclusion: These results provide new insight into pathophysiology of T2DM and non alcoholic fatty liver disease, and should be useful to be confirmed as novel biomarkers to predict risk for diabetes and its complications.

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Tissue-specific effects on mitochondrial dysfunction in type 1 and 2 diabetic mouse models

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Background and aims: During diabetes, increased hyperglycemic flux is considered to raise the production of reactive oxygen species (ROS). However, it remains under debate whether this increase is due to or causing functional changes of the mitochondria. Several studies have been published to determine the effect of diabetes on the function of the mitochondria. Yet, the results are contradictory. Here, we present a screen analyzing the effects of diabetes on the mitochondria of heart, kidney and liver using low-dose streptozotocin (STZ)-induced wild-type mice as a model for type 1 diabetes (T1D) and db/db mice as a model for type 2 diabetes (T2D).
Materials and methods: Mitochondria were isolated by differential centrifugation and purity was determined using FACS. Functionality of the mitochondria was analyzed using fluorescence-photometric and spectrophotometric assays. Gene expression profiles were analyzed using quantitative PCR. Data was expressed as fold change to control (mean +/- SD) and statistical significance was calculated using students t-test.
Results: After 3 months of diabetes (3 M) oxygen consumption was decreased (0.11 +/- 0.09, P-value<0.001) and ATP production showed a tendency to decrease in heart-derived mitochondria of T1D mice. In T2D oxygen consumption (0.68 +/- 0.07, P-value<0.01), ATP (0.71 +/- 0.20, P-value=0.03) and superoxide (0.78 +/- 0.01, P-value=0.02) production were decreased in cardiac mitochondria. In the mitochondrial fraction of T1D kidneys increased ATP production (1.25 +/- 0.15, P-value<0.01) and a tendency of increased oxygen consumption was measured. In T2D oxygen consumption (0.31 +/- 0.23, P-value<0.01) was decreased whereas ATP and superoxide production remained unchanged. In T1D increased superoxide production (1.31 +/- 0.28, P-value=0.04) and a tendency of increased oxygen consumption and ATP production was measured in the liver. In T2D superoxide production (1.48 +/- 0.17, P-value<0.01) was increased in the same extent as in T1D. However, ATP and oxygen consumption (10.29 +/- 5.40, P-value=0.02) increased to a higher extent in T2D. Besides the comparison of T1D and T2D, mitochondrial changes were also analyzed during the progression of diabetes using the T1D model. 6 months after the induction of diabetes (6 M) ATP and superoxide production were unchanged compared to 3 M. In the heart- and liver-derived mitochondria oxygen consumption remained unchanged as well. However, oxygen consumption in the kidney increased in 6 M compared to 3 M. Gene expression analysis showed a decrease in mitochondrial complexes and antioxidative enzymes in the diabetic kidney in 3 M and 6 M. In the heart and liver changes in the expression were first detected in 6 M and less pronounced than in the kidney.
Conclusion: In this study it was found that the effect of diabetes on mitochondrial dysfunction depends on the tissue and model studied and that results obtained from one organ and model cannot be applied to another. Furthermore, the kidney was found to be the organ which was affected first and most severe in T1D, suggesting that tissues which are highly susceptible to chronic hyperglycaemia are likely to be the most affected with respect to mitochondrial properties.
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PS 116 Getting to the heart of the matter: cardiovascular complications in diabetes

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Long-term clinical outcomes in patients with acute coronary syndrome and dysglycaemia

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Background and aims: Diabetes and impaired glucose tolerance (IGT) are major risk factors for atherosclerosis including coronary artery disease (CAD). The aim of the present study was to investigate the importance of glucose tolerance for long term clinical outcome in patients with acute coronary syndrome (ACS).
Materials and methods: 1062 consecutive patients, 781 men and 281 women, aged 32-80 years, admitted to the coronary care unit at a University Hospital, for ACS from year 2006 to 2008. At discharge, the patients were categorized according to an OGTT as having normal glucose tolerance (NGT); n=295 (28%), impaired glucose tolerance (IFG) and impaired glucose tolerance (IGT); n=299 (28%), diabetes discovered by OGTT; n=156 (15%) or known diabetes at admission to the hospital; n=312 (29%). Mortality and reinfarction rates were studied during a mean follow-up time of 4.0 (±0.8) years. Clinical outcome data were obtained from the Swedish Coronary Angiography and Angioplasty Registry and the Swedish National Registry.
Results: Patients with known diabetes at admission had compared to patients with dysglycaemia discovered by OGTT and NGT a significant increase (p<0.001) in death within 14 days (2.9% vs 0.2% and 0%), 30 days (4.8% vs 0.2% and 0%), 6 month (9% vs 1% and 0%), 1 year (12% vs 2% and 0%) and 3 years (25% vs 5% and 3%). During the follow-up, reinfarction was significantly higher (p<0.001) in patients with known diabetes compared to patients with dysglycaemia discovered by OGTT and NGT (28% vs 17% and 12%). The composite endpoint of mortality and reinfarction was significantly higher (p<0.001) in patients with known diabetes compared to patients with dysglycaemia discovered by OGTT and NGT (44% vs 22% and 15%). Furthermore, it was also a significant difference (p<0.001) between patients with dysglycaemia discovered by OGTT as compared to patients with NGT.

Conclusion: A majority of patients admitted for ACS have disturbed glucose metabolism including diabetes with high prevalence of previously undiagnosed dysglycaemia. Both known diabetes and dysglycaemia discovered by OGTT are associated with poor clinical prognosis in these patients.

Figure 1: Kaplan-Meier curves showing cumulative death within 3 years

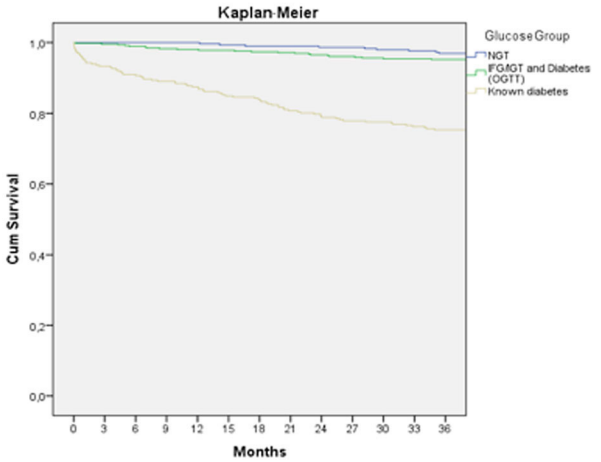

Figure 1. Kaplan-Meier curves showing cumulative death within 3 years. Blue line represents patients with NGT, green line patients with IFG+IGT and diabetes discovered by OGTT and beige line patients with known diabetes at admission.

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HbA_{1c} and risk of stroke in 33,414 type 1 diabetes patients compared to 166,097 controls from the general population

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Background and aims: Earlier studies have found patients with type 1 diabetes at increased risk of stroke compared to the general population. In this study we estimated the risk of stroke among individuals with type 1 diabetes according to HbA1C level, and compared it to the risk in the general population.
Materials and methods: We included 33,414 individuals with type 1 diabetes, aged 18 years or older (mean 35.5 years), registered in the Swedish National Diabetes Register 1998 to 2011 and 166,097 age, sex and county matched controls from the general population. The endpoints all stroke, ischemic stroke and hemorragisk stroke were captured using ICD-10 codes I61, I62.9, I63, I64 and I67.9 from the Swedish Inpatient and Cause of Death Registers. The risk of stroke for patients with type 1 diabetes were estimated in 5 different HbA1C categories by Cox hazard regression analysis adjusted for age, sex, diabetes duration, born in Sweden, highest education level and baseline comorbidities with controls from the general population as reference.
Results: Medium follow-up was 8.6 years and 9.0 years for patients with type 1 diabetes and controls respectively. Among patients with type 1 diabetes 759 (2.3%) received a non-fatal or fatal stroke diagnosis compared to 1425 (0.9%) among the controls. Unadjusted incidence values of all stroke expressed as cases per 1000 person years were 2.86 (2.66 - 3.08) for patients with type 1 diabetes and 1.04 (0.99 - 1.10) for controls. The risk for all and ischemic stroke was significantly increased for patients with type 1 diabetes in all HbA1C categories. The risk rose gradually by each HbA1C category from a HR of 1.56 (95% CI 1.21 - 2.01) for all stroke in the lowest category (≤6.9% (≤52 mmol/mol)) to a more than 7 times increased risk for patients with type 1 diabetes in the highest HbA1c category (HR for all stroke 7.52 (95% CI 6.10 - 9.26) in HbA1c category ≥9.7% (≥83 mmol/mol)). For hemorrhagic stroke the risk for patients with type 1 diabetes was increased in all but the lowest HbA1C category compared to the controls. After a gradual increase in risk over the HbA1c categories, patients with type 1 diabetes in the highest HbA1C category ≥9.7% (≥83 mmol/mol) had a HR of 7.74 (4.74 - 12.62) for hemorrhagic stroke.
Conclusion: Individuals with type 1 diabetes experience a considerably higher risk for stroke compared to the general population. The risk increases gradually with worse glycemic control from a 50% higher risk in the lowest HbA1C category to more than 700% higher risk in the highest HbA1c category.
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Blood pressure and stroke risk in 408,076 type 2 diabetes patients compared to 1,913,507 controls from the general population

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Background and aims: Patients with type 2 diabetes have an elevated risk of stroke. In this study we estimated the risk of stroke among patients with type 2 diabetes at different blood pressure levels compared to the risk in the general population in Sweden.
Materials and methods: This case-control study was based on 408,076 individuals with type 2 diabetes, 18 years or older, registered in the Swedish National Diabetes Register 1998-2011 and 1,913,507 age- and sex matched controls from the general population. Stroke diagnoses were retrieved by ICD-10 codes I61, I62.9, I63, I64 and I67.9 from the Swedish Inpatient and Cause of Death Registers. Cox hazard ratios (HRs) and 95% confidence intervals (CIs) adjusted for age, sex, diabetes duration, born in Sweden, highest education level and baseline comorbidities were estimated at 6 different blood pressure levels (<110/<65, 110-119/65-69, 120-129/70-79, 130-139/80-89, 140-159/90-99, >160/>100 mm Hg)
Results: During a median follow up of 4.0 years for patients with type 2 diabetes and 4.1 years for controls, 19,548 (4.8%) patients with type 2 diabetes and 61,690 (3.2%) controls were diagnosed with non-fatal or fatal stroke. Corresponding unadjusted incidence values for any stroke as cases per 1000 person years were 10.63 (10.48-10.78) for patients with type 2 diabetes and 6.80 (6.75-6.86) for controls. Multivariable adjusted Cox regression analysis, using controls as reference, yielded a significantly increased risk of any stroke for patients with type 2 diabetes in blood pressure categories above 130-139/80-89 mm Hg (hazard ratio HR of 1.11 (95% CI 1.07-1.15)), with gradually increasing HR to 1.80 (95% CI 1.73-1.86) in the highest blood pressure category (>160/>100 mm Hg). A similar pattern was observed for ischemic stroke. The association was more complex for hemorrhagic stroke with a significant reduced risk for patients with type 2 diabetes compared to controls in lower blood pressure categories and significantly increased only in blood pressure category >160/>100 mmHg (HR 1.49 (95% CI 1.33-1.68)).
Conclusion: Individuals with type 2 diabetes and blood pressure below 130/80 mm Hg have a risk of all stroke and ischemic stroke comparable to that of the general population.
Supported by: Swedish Scientific Council, Swedish Heart and Lung Foundation, Diabetes Wellness

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Remnant cholesterol predicts cardiovascular event risk in patients with type 2 diabetes independently from the baseline coronary artery disease state

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Background and aims: Remnant cholesterol, which is calculated as total cholesterol minus LDL cholesterol minus HDL cholesterol recently has attracted interest as a marker of cardiovascular event risk. We aimed at investigating the power of remnant cholesterol to predict cardiovascular events in patients with type 2 diabetes (T2DM) as well as in non-diabetic patients in whom the baseline coronary artery disease (CAD) state was verified angiographically.
Materials and methods: We enrolled 1774 consecutive patients undergoing coronary angiography for the evaluation of established or suspected stable CAD. Prospectively, cardiovascular events were recorded over a mean follow-up period of 7.5±2.9 years. Diabetes was diagnosed according to ADA criteria.
Results: During follow-up, 32.5% of our patients suffered cardiovascular events; the event rate was significantly higher in patients with T2DM (n=513) than in nodiabetic subjects (40.5 vs. 29.3%; $p<0.001$). Remnant cholesterol significantly predicted cardiovascular events in the total study population, among patients with T2DM, as well as among non-diabetic subjects both univariately (HR 1.18 [1.10-1.27], $p<0.001$, 1.20 [1.05-1.38], $p=0.008$ and 1.19 [1.09-1.30], $p<0.001$, respectively) and after multivariate adjustment including presence as well as extent of baseline CAD (HR 1.15 [1.07-1.24], $p<0.001$, 1.21 [1.05-1.39], $p=0.009$ and 1.15 [1.05-1.25], $p=0.002$, respectively).
Conclusion: From our data we conclude that remnant cholesterol predicts cardiovascular event risk in patients with type 2 diabetes as well as in non-diabetic patients independently from the baseline CAD state.

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Contrasting eight cardiovascular risk equations for use in type 2 diabetes cohorts using the CORE Diabetes Model

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Background and aims: Within health economic evaluations concern persists regarding the generalizability of cardiovascular (CV) risk equations (REs) and their impact upon cost-effectiveness predictions. The IMS CORE Diabetes Model (CDM) is a widely published and validated decision support tool that has been recently updated to include eight sets of CV risk equations derived from European, American, Asian and Global cohorts. The objective of this study was to compare and contrast cardiovascular incidence and predicted quality adjusted life expectancy (QALE) across these REs.
Materials and methods: Lifetime analyses comparing outcomes for metformin+ sulphonylurea (M+S) versus metformin + DPP-4 (M+D) were undertaken using the CDM with basal insulin rescue therapy applied to both arms at an HbA1c threshold level of 7.5%. Baseline characteristics and treatments effects from the published EDGE study were utilised: mean age 57.8 years, duration diabetes 5.5 years, 54.8% male, HbA1c 8.2% and BMI 29 kg/m2. HbA1c reductions applied were -0.99% and -1.19% for M+S and M+D respectively with a -0.3 kg and -1.6 kg change in body weight for M+S and M+D respectively. A comparison of cumulative incidence (CI) of myocardial infarction (MI) and stroke, QALE and incremental cost effectiveness ratio (ICER) was made for the following risk equations Swedish-National Diabetes Registry (S-NDR); UKPDS 68 (UK); UKPDS 82 (UK); ADVANCE (Global); FREMANTLE (Australian); ARIC (US); HONG KONG (Asia) and PROCAM (European). UK 2013 costs were applied; costs and health benefits were discounted at 3.5%.
Results: Across all REs, overall mean (standard error [SE]) predicted CI of MI and stroke were 23.9% (4.1) and 35.7% (4.6) respectively. Averaged across treatment arms, the Swedish-NDR RE predicted highest CI of stroke (58.3%) and ARIC the lowest (4.3%); PROCAM the highest CI of MI (69.7%) and ARIC lowest (9.8%). Mean (SE) predicted QALE across all RE was 9.2 (0.2) with the highest (10.4) from ARIC and lowest (7.8) from PROCAM. The overall mean (SE) ICER across all RE was £8,569 (£280) with the lowest (£6,980) from UKPDS 68 and highest (£10,964) from PROCAM (range £3,984).
Conclusion: There was a noteworthy difference in predicted CI of CV events across the eight RE and therefore economic assessments should utilise RE from the most representative population. Importantly, estimated QALE and ICERs were relatively stable. Nevertheless, choice of RE may have a significant impact on cost-effectiveness evaluations, particularly where results lie close to the willingness-to-pay threshold.

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Testosterone in patients with acute myocardial infarction and glucose abnormalities and in matched controls: a report from the GAMI study

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Background and aims: Low levels of testosterone are prevalent in men with diabetes and have been associated with impaired cardiovascular health. A potential relationship between testosterone, diabetes and cardiovascular disease needs to be further elucidated. The present aims were to investigate the prevalence of low testosterone (<350 ng/dl) and the dynamic of levels over time in patients with acute myocardial infarction (AMI) with or without newly detected glucose abnormalities and in controls. Further, the study explored the relation between testosterone and cardiovascular prognosis.
Materials and methods: The material comprises male patients with AMI without known diabetes (n=123) and age-matched controls without AMI (n=124). Testosterone levels were measured at baseline for controls and at the first morning after admission, at discharge, and 3 and 12 months post-discharge for patients. Study participants were categorized as having normal or abnormal glucose tolerance (NGT or AGT=diabetes or impaired glucose tolerance) by means of an oral glucose tolerance test. The prognostic impact of testosterone was studied by Cox regression hazard analyses (by +1 SD) with major cardiovascular events (cardiovascular death, non-fatal MI, non-fatal stroke or severe heart failure) as primary endpoint during 11 years follow-up.
Results: At the first morning after admission, 76% of the patients had low testosterone (vs. 43% in controls) and their median testosterone levels were significantly lower than among controls (Table 1). Testosterone was lower in patients with AGT than NGT during hospitalization. The prevalence of low testosterone was 83% in AGT vs. 65% in NGT patients, and 57% in AGT vs. 34% in NGT controls. Testosterone did not predict cardiovascular events in patients (events=50) or controls (events=27). Subgroups analyses were not performed due to limited number of events.

Conclusion: Low testosterone levels are common in patients with AMI, especially in combination with AGT. Over time, the difference in testosterone levels between patients with or without glucose abnormalities diminished. The results raise questions on the proposed causal relationship between testosterone and glucose abnormalities. Also, it indicates that testosterone substitution should not be instituted based on samples taken during hospitalization for AMI.

Table 1. Testosterone levels (ng/dl) in patients and controls presented as median and interquartile range (IQR) for the total cohort and with regards to glucose categorization.

	Patients				Controls
	Day after admission (n=123)	Discharge (n=115)	3 months (n=101)	12 months (n=87)	Baseline (n=124)
Total	243 (184-346)	310 (204-426)	345 (265-429)	357 (270-485)	380 (276-476)
NGT	308 (199-387) (n=43)*	286 (112-429)	299 (44-417)	308 (121-445)	386 (315-481) (n=77)*
AGT	230 (178-301) (n=75)*	201 (20-360)	285 (26-394)	264 (30-382)	341 (266-441) (n=47)*
$p^{\#}$	0.04	0.03	0.55	0.14	0.15

*Individuals with glucose categorization available.
$^{\#}p$-value for group wise comparison of AGT vs. NGT.

Supported by: Bayer AG

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Myo-inoisitol and d-chiro-inositol prevent palmitate-induced autophagy and senescence in human cardiac progenitor cells

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Background and aims: Supplementation with myo-inositol and D-chiro-inositol has been shown to induce insulin-mimetic effects in humans and to improve cardiac performance in experimental heart failure. The aim of this study was to evaluate the effects of myo-inositol and D-chiro-inositol on lipotoxicity-induced autophagy and senescence in human cardiac progenitor cells (CPC).
Materials and methods: Human CPC were isolated from right auricle biopsies of patients undergoing elective heart surgery and exposed to palmitate (0.25 mM up to 16 h). Autophagy was evidenced by monodansylcadaverine and autophagolysosome labeling, and by immunoblotting of microtubule-associated protein 1 light chain 3 (LC3)-II and beclin1. Cellular senescence was detected by evaluation of senescence-associated β-galactosidase activity, p21(WAF1/Cip1) and p16(Ink4a) gene and protein expression. Protein expression and phosphorylation were detected by immunoblotting.
Results: Incubation of CPC with 0.25 mmol/Lpalmitate for 16 h increased cell autophagy, evidenced by monodansylcadaverine staining and confirmed by increased levels of LC3-II and beclin-1, respectively ($p<0.05$). Palmitate also induced cellular senescence, demonstrated by increased senescence-associated β-galactosidase activity. Cellular senescence was confirmed by the detection of increased p21(WAF1/Cip1) gene and protein expression ($p<0.05$), whereas the p16(Ink4a) pathway appeared to be unaffected. Chemical inhibition of autophagy with 3-methyladenine resulted in reduced β-galactosidase staining, suggesting that increased autophagy mediates palmitate-induced cellular senescence in human CPC. When cells were pretreated with myo-inositol or D-chiro-inositol (1-1000 micromol/L), both the palmitate-induced autophagosome formation and increase in LC3-II were abrogated ($p<0.05$), and so was the increase in p21 protein levels ($p<0.05$). Increased Akt and p42/p44 MAPK phosphorylation ($p<0.05$) was observed in CPC exposed to myo-inositol or D-chiro-inositol for up to 30 min.
Conclusion: Palmitate increases both autophagy and senescence in human CPC. Both myo-inositol and D-chiro-inositol activate survival kinases and counteract the effects of palmitate. Inositols may thus protect the myocardium from lipotoxic damage by preserving the CPC pool in type 2 diabetic and/or obese subjects with elevated free fatty acid levels.

PS 117 Adverse consequences of glycation

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ApoA-I glycation; linking diabetes and CVD

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Background and aims: Diabetic patients have an increased risk of suffering from atherosclerosis. Apolipoprotein A-I (ApoA-I) function and metabolism in high-density lipoprotein (HDL) is closely linked to diabetes. Recognized positive qualities of ApoA-I in HDL include removal of cholesterol from macrophage-derived foam cells at the vascular wall in the reverse cholesterol transport pathway, as well as anti-inflammatory and anti-thrombotic functions, all leading to reduced development of atherosclerosis and cardiovascular disease. Importantly, novel features of this lipoprotein have recently emerged describing ApoA-I/HDL as regulator of glucose homeostasis (insulin secretion and glucose disposal in target tissues), thus suggesting dysfunctional ApoA-I/HDL as a contributing factor to the development of type 2 diabetes. In support of this, our recent *in vivo* data show massive impact (increase) on glucose disposal capacity following acute ApoA-I injections. Intensive glycemic control management reduces CVD events with the most significant reduction related to HbA1c. Hyperglycemia results in increased non-enzymatic reaction of sugars with proteins to form advanced glycation end products (AGEs). Taking all this information into account we hypothesize that the glycation of ApoA-I structurally and functionally modifies the protein and it has an important impact in the impairment of its ability to control glucose homeostasis and cholesterol efflux thus leading to the increase of diabetes and CVD complications. The aim of this study is to elucidate the role of hyperglycemia/glycation of ApoA-I/HDL in its loss of functionality, especially related to its impact on glucose homeostasis.
Materials and methods: Recombinant ApoA-I is *in vitro* glycated with glycolaldehyde (GA) or methylglyoxal (MG) and modifications of the protein are determined by mass spectrometry analysis. Functional modification of the protein are tested by acute injections of ApoA-I (modified and non-modified) in diet-induced obese (DIO) and insulin resistant mice followed by a glucose tolerance test (GTT) and insulin concentration determination (ELISA), lipid clearance assay (LCA), HDL formation assay and finally cholesterol efflux experiments.
Results: Glucose clearance in a glucose tolerance test is markedly improved by the injection of non-modified ApoA-I. When the protein is glycated by MG, a less efficient glucose clearance can be observed, especially in the first 15 minutes of the test ($n=15$, $p<0,05$). The capacity of ApoA-I to form HDL particles is clearly reduced when it is modified as seen by the LCA and also, cholesterol efflux from macrophages is reduced. Mass spectrometry studies revel a different modification pattern for each of the reactive carbonyls used (GA or MG).
Conclusion: Non-enzymatic glycation of ApoA-I partially impairs the capacity of the protein to form HDL particles leading to reduced glucose clearance in DIO mice. Different reactive carbonyls lead to different profiles in glucose clearance suggesting that there are specific residues in the protein implicated in the glucose homeostasis functions.
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Glycation as an atherogenic modification of lipoproteins, and the contribution of copper

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Background and aims: Cardiovascular disease is the leading cause of death and disability in diabetes. Low-density lipoprotein (LDL) is the permissive factor in the development of atherosclerosis. LDL must undergo oxidation and / or glycation to participate in atherogenesis. Glycated LDL is present in the circulation under physiological conditions and at higher concentrations in diabetes and in those destined to experience myocardial infarction, but LDL glycation *in vitro* requires supraphysiological glucose concentrations. Protection against glycation is a recognised function of high-density lipoprotein (HDL). The role of copper in promoting *in vitro* glycation requires further investigation as it may be possible to manipulate this process therapeutically with a copper chelating agent to protect lipoproteins against glycation.
Materials and methods: LDL (1.019-1.063 g/ml) and HDL (1.063-1.21 g/ml) were isolated from human serum using sequential preparative ultracentrifugation before incubation of LDL at a concentration of 1 mg/ml with glucose and copper at physiological concentrations (5-25 mMol/l and 1.25-5 umol/l respectively) for 3 days. The ability of HDL and the divalent copper-selective chelator triethylenetetramine to protect against glycation were also assessed. Glycated lipoproteins were separated from non-glycated lipoproteins using *m*-aminophenylboronate affinity chromatography. Apolipoprotein B (apoB) was measured immunoturbidimetrically and glycated apoB by a validated in-house high-sensitivity ELISA. Lipid hydroperoxide (LPO) formed during glycation was measured by a cholesterol oxidase colorimetric assay. The cholesterol efflux capacity of HDL *in vitro* was determined by a previously validated assay.
Results: *In vitro* glycation occurs more readily in the presence of physiological concentrations of copper. Incubation with 10mMol/l glucose and 1.25umol/l and 2.5umol/l copper sulphate for 3 days significantly increased the proportion of glycated apoB ($P=0.003$ and 0.01 respectively). Similar effects were observed in the presence of copper chloride ($P=0.05$) but not copper-histidine complex ($P=0.28$) or ferrous sulphate ($P=0.18$). Addition of HDL prevented glycation of apoB ($P=0.02$). We also for the first time report a potential therapeutic role for copper-selective chelation in the protection of lipoproteins against glycation. Glycation also appears to impair the capacity of HDL to promote cholesterol efflux ($P=0.36$), with a further reduction in the presence of 1.25umol/l copper sulphate ($P=0.21$).
Conclusion: This work offers insight into HDL functionality in diabetes and supporting evidence for the emerging link between copper dysregulation and the accumulation of advanced glycation end-products, and a potential role for copper in lipoprotein glycation.
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Unravelling the methylglyoxal effect on the angiogenic process

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Background and aims: Much of the morbidity and mortality associated with diabetes mellitus (DM) reflects its deleterious effects on micro and macrocirculation. DM impairs physiological angiogenesis by molecular mechanisms that are not fully understood. Generation of Advanced Glycation End-products (AGEs) has an important role in the development of hyperglycaemia-induced endothelial damage. The highly reactive dicarbonyl methylglyoxal (MG) is the most reactive AGE precursor in endothelial cells. It is a potent dicarbonyl glycating agent formed from glycolysis intermediates of in cells and degraded by the glyoxalase system (Glo). Glo1 activity prevents the accumulation of MG, thereby suppressing dicarbonyl-mediated glycation reactions and cellular damage. The concentration of MG is increased 2 to 5-fold in plasma of diabetic patients. Recent experimental evidence indicate that MG is implicated in the pathogenesis of diabetic complications, however, the mechanisms by which MG accumulation leads to endothelial dysfunction and possibly to the vascular complications associated with DM need to be clarified. Homeobox (Hox) genes and miRNAs-mediated epigenetic regulation have a crucial role in the neovascularization both in physiological and pathological conditions. This work aims at elucidating the MG effect on the angiogenic ability of endothelial cells and the molecular mechanisms involved.
Materials and methods: Proliferation and migration were evaluated by cell-growth curves, scratch and transwell migration assays on mouse aortic endothelial cells isolated from heterozygous mice for glo1 deletion (Glo-/+ MAEC) and their wild type littermates (WT MAEC). Glo1, HoxA5 and miRNAs expression levels were measured by Real-time PCR and MG intracellular concentrations by HPLC.
Results: Glo1-/+ MAEC show a 50% reduction of Glo1 mRNA levels and a 5-fold increase of MG intracellular concentrations compared to WT MAEC ($p=0.0001$). Cell-growth curves reveal that Glo1-/+ MAEC grow slower than WT MAEC (Glo1-/+ MAEC 106833±15610 vs WT MAEC 202733±62699 cells at 24 h, $p=0.002$). Preliminary data indicate that Glo1-/+ MAEC have a delayed wound healing upon VEGF stimulation even when the proliferation is inhibited by mitomycin C (Glo1-/+ MAEC 55.1±13% vs WT MAEC 100% of wound closure at 12 h) and an impaired migration capacity (Glo1-/+ MAEC 0.062±0.008 vs WT MAEC 0.09±0.024 OD) compared to WT MAEC. To investigate the molecular mechanisms by which angiogenic function may be compromised by MG in MAEC, HoxA5 and vascular function-related miRNAs expression has been evaluated. mRNA levels of the anti-angiogenic gene HoxA5 are 2-fold increased in Glo1-/+ MAEC compared to WT MAEC (Glo1-/+ MAEC 1.98±0.48 vs WT MAEC 1 REU, $p=0.01$). Moreover, miRNA126 and miRNA503 levels are 0.5-fold reduced and 2-fold increased, respectively, in Glo1-/+ MAEC compared to WT MAEC.
Conclusion: These results suggest that MG accumulation impairs the angiogenic ability of Glo1-/+ MAEC. This effect is associated to the overexpression of the anti-angiogenic HoxA5 and the altered levels of miRNA-126 and miRNA-503, found to be modified in vascular dysfunction. A better understanding of the molecular mechanisms by which MG alters the angiogenic process may allow to identify novel approaches in the prevention and treatment of diabetes vascular disease.
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Fructose-derived advanced glycation end-products induce a negative crosstalk between lipogenesis and muscle reprogramming in mice through SREBP-1c

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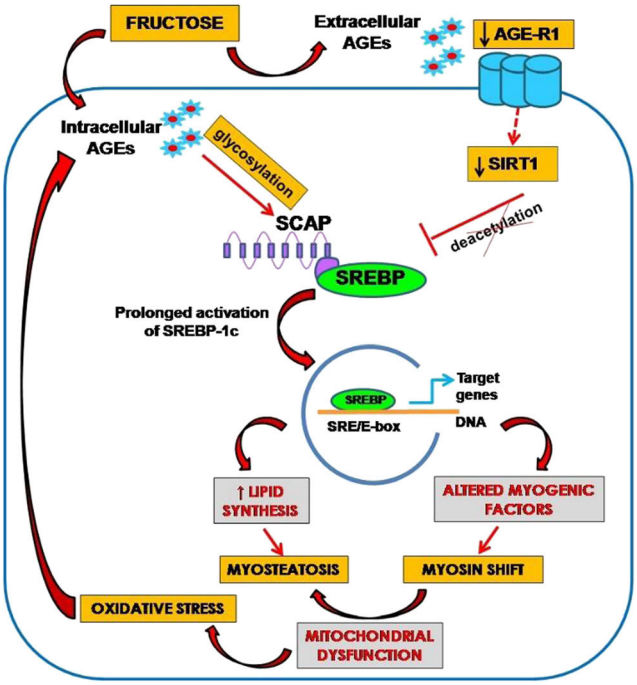
Background and aims: Advanced Glycation End-Products (AGEs) have been recently linked to the onset of obesity, adiposity, and insulin-resistance. AGEs can endogenously be formed by dietary sugars, in particular fructose, which is widely used as added sweetener in foods and drinks. We previously reported that chronic fructose exposure generates AGEs in mice plasma and liver at higher level than glucose. Recently we documented that AGEs accumulate in gastrocnemius muscle in obese mice and that this is associated to activation of the SCAP/SREBP-1c lipogenetic pathway. We have now investigated whether mice skeletal muscle structure and metabolism can be affected by chronic exposure to a high-fructose diet and we have better characterized the causal role of fructose-derived AGEs by using the glycation inhibitor pyridoxamine.
Materials and methods: C57Bl/6J mice were fed a standard diet (SD) or a 60% fructose diet (HFRT) for 12 weeks. Two subgroups of SD and HFRT mice received the anti-glycative compound pyridoxamine (120 mg/kg/day) in the drinking water.
Results: At the end of protocol HFRT mice showed increased plasma glucose ($P<0.05$ vs SD), insulin ($P<0.05$ vs SD), triglycerides ($P<0.05$ vs SD) and cholesterol ($P<0.001$ vs SD), and impaired OGTT curve. Very high levels of AGEs were detected both in plasma and gastrocnemius muscle ($P<0.001$ vs SD). We found that AGEs enhanced the activation of SREBP-1c through either downregulation of the SREBP-inihibiting enzyme SIRT-1 and increased glycation of the SREBP-activating protein SCAP. Interestingly, the AGEs-induced SREBP-1c activation also had dramatic repercussions on myogenic regulatory factors and myosin heavy chain isoforms expression, paralleled by depletion of muscle strength ($P<0.05$ vs SD) and increased fatigability ($P<0.05$ vs SD). This could be related to the impaired individual oxidative activity of myofibers, and oxidative stress increase. The use of pyridoxamine almost completely counteracted not only AGEs generation, but all the fructose-induced muscle alterations.

Conclusion: The present data indicate for the first time that fructose-derived AGEs exert a multiple interference on SREBP-1c activity which in turn enhances fatty acids synthesis and impairs muscle proteins expression, leading to a negative crosstalk between lipogenesis and muscle reprogramming. Thus, these findings revealed an unsuspected involvement of AGEs in muscle derangements that could contribute to further progression of insulin resistance and obesity.



Supported by: ex-60% University of Turin

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Lens autofluorescence relates to skin autofluorescence but poorly to glycated haemoglobin in patients with diabetes

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Background and aims: Advanced glycation endproducts (AGEs) play an essential role in the pathophysiology of diabetes and its complications. Their accumulation in the lens reflects the intensity of glycation process and it is demonstrated by specific lens autofluorescence. Recently, a new confocal biomicroscope ClearPath DS-120 easily measuring lens autofluorescence (LAF) has been developed. The aim of this study was to compare lens autofluorescence (LAF) in diabetic and non-diabetic subjects with their skin autofluorescence (SAF) and parameters of glucose control.
Materials and methods: LAF was measured in the left eye in 126 diabetic patients (55 Type 1 /T1DM/ aged 42±14 yrs, 71 Type 2 /T2DM/ aged 61±10 yrs) and 59 healthy controls (aged 47±13 yrs) by ClearPath DS-120 (Freedom Meditech, US). SAF was measured on forearm by AGE-Reader (Diagnoptics BV, Netherlands) in subgroup with 116 subjects (32 T1DM, 35 T2DM and 49 controls). Results were expressed in arbitrary units (AU) and compared with age, anthropometrical data, current glycated hemoglobin HbA1c (in IFCC units) and mean HbA1c in the last 2 years, renal function (using MDRD formula) and albuminuria.
Results: In both T1DM and T2DM LAF was significantly higher as compared to healthy controls (0.26±0.09, 0.23±0.06 vs 0.17±0.04 AU, $p<0.0001$). Similar results were found in SAF (2.01±0.45, 2.28±0.57 vs 1.78±0.33 AU, $p<0.0001$). Significantly higher mean current HbA1c found in T1DM and T2DM as compared to controls (65±16; 58±18 vs 34±5 mmol/mol, $p<0.0001$) was related to LAF only in T2DM (T1DM: $r=0.19$, NS; T2DM: $r=0.37$, $p<0.01$; controls: $r=0.28$, NS). The same was true for the two-year HbA1c. LAF was inversely related to glomerular filtration in T1DM ($r=-0.34$, $p<0.05$) and controls ($r=-0.29$, $p<0.05$), but not in T2DM ($r=-0.10$, NS). No significant difference of LAF was observed in diabetic patients with or without positive albuminuria (0.27±0.08 vs 0.24±0.07 AU, NS). Significant relationship of LAF and SAF was found in all groups (T1DM: $r=0.53$, $p<0.005$, T2DM: $r=0.36$, $p<0.05$; controls: $r=0.30$, $p<0.05$).
Conclusion: Our study demonstrates significant association of lens and skin autofluorescence in subjects with or without diabetes. It confirms similar effect of non-enzymatic glycation in two different tissues. LAF is elevated in diabetes, but is not entirely related to current or average two-year HbA1c, since LAF is likely a very-long-term glycation marker independent on HbA1c variability. On the other hand, LAF does not reflect more advanced changes as it was not significantly different in patients with or without albuminuria or with normal or decreased MDRD. A follow-up study will be necessary to evaluate the usefulness of LAF in clinical practice.

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Synergistic effects of transplanted endothelial progenitor cells and RWJ67657 in diabetic ischaemic stroke models

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Background and aims: An immature vascular phenotype in diabetes may cause more severe vascular damage and poorer functional outcomes after stroke, and it would be feasible to repair damaged functional vessels using endothelial progenitor cell (EPC) transplantation. However, high glucose induces p38 mitogen-activated protein kinase (MAPK) activation, which can accelerate the senescence and apoptosis of EPCs. The aim of this study was to investigate the combined effects of EPC transplantation and p38 MAPK inhibitor administration on diabetic stroke outcomes.
Materials and methods: Bone marrow-derived EPCs, labeled with a multi-functional nanoprobe modified with paramagnetic chelators and fluorophores, were injected intra-arterially into wild-type and db/db mice after ischemic stroke induction. RWJ 67657 (RWJ), a p38 MAPK inhibitor, was administered orally into db/db mice for 7 consecutive days, with the first dose given 30 min before stroke induction.
Results: The signal on magnetic resonance (MR) and optical imaging of diabetic stroke models was significantly lower than that of wild-type controls ($P<0.001$, $n=4$ to 6 per group). However, the signal intensity of diabetic stroke models significantly increased after oral administration of RWJ ($P<0.05$, $n=4$ or 5 per group), indicating that more transplanted EPCs migrated to the ischemic brain. Neither EPC transplantation nor RWJ administration alone significantly improved diabetic stroke outcome, although RWJ displayed a potent anti-inflammatory effect. By both improving the functioning of EPCs and reducing inflammation, EPC transplantation plus RWJ administration in vivo synergistically promoted angiogenesis ($P<0.05$, $n=4$ per group) and neurogenesis ($P<0.05$, $n=6$ or 7 per group) after diabetic stroke . In addition, fractional anisotropy value and number of fibers in the ispileasional internal capsule derived from MR diffusion tensor imaging (MR-DTI), together with myelin basic protein-positive cells were significantly increased in the combination group, indicating more white matter remodeling. Furthermore, behavioral scores, infarct volume reduction, and expressions of VEGF and BDNF were significantly increased in diabetic mice treated with both EPCs and RWJ.
Conclusion: The dual-modal imaging strategy suggested that diabetes reduced the number of transplanted EPCs homing to the ischemic area, while consecutive intragastric administration of RWJ could protect EPC from being damaged in diabetic mice. The combination of EPC

transplantation and RWJ administration accelerated recovery from diabetic stroke, which might have been caused by increased levels of pro-angiogenic and neurotrophic factors.
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PS 118 Speculations on inflammation

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A novel role for the Krüppel-like factor 14, klf14, on macrophage inflammatory response and atherosclerosis development

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Background and aims: Genome-wide association studies have shown that Krüppel-like factor 14(KLF14) is associated with both Type 2 diabetes mellitus (T2DM) and lipid metabolism. However, its role in chronic inflammatory responses and the pathogenesis of atherosclerosis remains unknown. The present study was designed to investigate in both vivo and vitro the impact of KLF14 on chronic inflammatory responses and atherosclerosis.
Materials and methods: In vivo, KLF14-knockdown ApoE KO mice by a adenovirus-mediated RNAi was used to assessed the effects of KLF14 knockdown on metabolic parameters and the formation of atherosclerosis on a high fat diet. Atherosclerotic lesions in the aorta were analyzed by en face analysis of the spread total aorta and analysis of cross sections of the aortic sinus with oil red O staining. The circulating levels of pro-inflammatory cytokine in ApoE KO mice were detected by ELISA. In vitro, Both the effects of up-regulation and down-regulation of KLF14 on the ac-LDL-induced cholesterol accumulation in macrophage were evaluated. Effects of KLF14 on the expressions of cytokines and the activities of mitogen-activated protein kinases (MAPKs) were detected by real time-PCR and western blot, respectively. The specify inhibitors of MAPKs were used to analyze the mechanisms by which KLF14 regulates the accumulation of cholesterol and cholesterol ester.
Results: Here, we have shown that in ApoE KO mice, a well established animal model of atherosclerosis, fed with both high fat diet (HFD) and standard chow diet (SCD), expression of KLF14 in aortas tissues was higher than in C57BL/6J mice. Consistent with this, adenovirus-mediated KLF14 knockdown markedly reduced the circulating levels of pro- inflammatory cytokine and the formation of atherosclerotic lesions in HFD-fed ApoE KO mice. In RAW264.7 macrophages, KLF14 overexpression significantly increased ac-LDL induced expressions of inflammatory cytokine and further strengthened ac-LDL-induced increase in total cholesterol (TC), cholesteryl ester (CE) and the ratios of CE to TC. Conversely, KLF14 knockdown in these cells remarkably attenuated ac-LDL-induced increase in TC, CE and the ratios of CE to TC and the expressions of inflammatory cytokine. KLF14 overexpression remarkably elevated the phosphorylation levels of p38 MAPK and ERK1/2 in ac-LDL- stimulated RAW264.7 macrophages. Importantly, treatment with a p38 MAPK (PD098059) and ERK1/2 inhibitors (SB203580) significantly nullified the ability of KLF14 to promote inflammatory cytokine expressions in these cells.
Conclusion: These data demonstrate an important role for KLF14 expression in atherosclerotic lesion formation. Thus, KLF14 may be a potential target for therapy of atherosclerosis.
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Increased infiltration of inflammatory immune-cells in sciatic nerves but not in dorsal root ganglions in late diabetic neuropathy in the murine STZ model of diabetes

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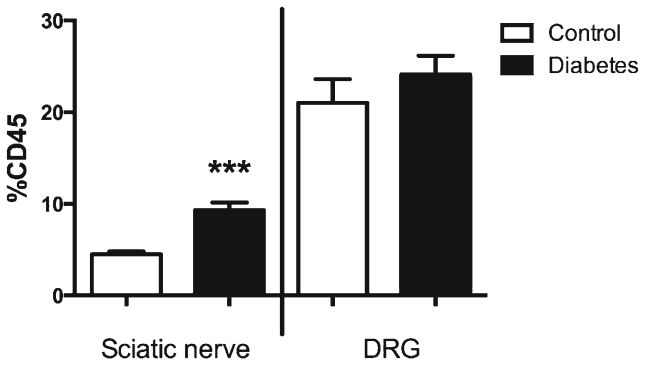
Background and aims: The diabetic condition is associated with a pro-inflammatory phenotype of immune-cells infiltrating into different organs

where inflammatory macrophages contribute to the pathogenesis of the disease and impair tissue re-generation. Infiltrating immune-cells into the peripheral nervous system during DN, in absence of an injury, has not yet been demonstrated in the streptozotocin (STZ) mouse model of diabetes. The aim of this study is to investigate changes to immune-cell population in sciatic nerve (SN) and dorsal root ganglion (DRG) in late diabetic neuropathy.

Materials and methods: Diabetes was induced in WT C56BL/6, mice or in mice lacking T-cells (RAG2-/-) by STZ (50 mg/kg/day i.p. for 5 days). At 6 months, thermal hypoalgesia was assessed by hotplate and Hargreave's and neuronal blood flow of SN by laser-doppler. Peritoneal cavity lavage was collected and SN and DRGs were digested and stained with antibodies against CD45, CD68 and CD206. The cells were analyzed by flow cytometry. Statistical significance was tested with two-tailed student's T-test.

Results: At 6 months of STZ-induced diabetes, WT mice showed significant hypoalgesia, as compared to the age-matched WT controls; hotplate response times were increased by 6.5 secs on average ($p < 0.002$) and 2.8 secs by Hargreave's ($p < 0.0001$). SN blood-flow was significantly reduced in WT diabetic mice (74% as compared to controls, $p < 0.01$, $n = 5$). In contrast, the diabetic RAG2-/- mice showed no significant differences either in measurements of thermal hypoalgesia or SN-blood flow. In the SN of the WT diabetic mice, a 2-fold increase of infiltrating CD45+ leukocytes was observed by flow cytometry (from 4,5% of all nucleated cells to 9,3%, $p < 0.001$, $n = 11$), but no significant increase in infiltration was observed in the DRGs. These findings were confirmed by immunohistochemistry stainings of SN and DRGs cryosections. No increase in infiltrating leukocytes was observed in the SN of the RAG2-/- diabetic mice, as compared to the control RAG2-/- mice. In SN of both the control and diabetic WT mice, 50-70% of the infiltrating CD45+ cells expressed the macrophage marker CD68. However, fewer of the SN CD68+ macrophages in the WT diabetic mice expressed the M2 marker, CD206 (reduced from 70% of macrophages in control to 58% in diabetic mice, $p < 0.01$). The proportion of macrophages in peritoneal cavity lavage expressing CD206 was reduced from 10% in WT control mice to 4% in WT diabetic mice ($p < 0.05$).

Conclusion: Late diabetic neuropathy is associated with increased infiltration of leukocytes in SN but not DRG of STZ-induced diabetic mice. Leukocyte infiltration into SN and neuropathic symptoms, such as thermal hypoalgesia and reduced SN blood-flow, were reduced in mice lacking T and B cells. We conclude that late diabetic neuropathy is associated with changes of macrophage populations in peripheral nerves, which require the adaptive immune system.



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New insights for an old marker: TGF-beta, diabetic nephropathy and metabolic memory

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Background and aims: Metabolic memory has been pointed out as an important phenomenon to explain the increasing incidence of diabetic nephropathy (DN) and other diabetes complications even by patients under intensive glycemic control. TGF-β is thought to play a key role in mediating major kidney alterations due to diabetes, such as hypertrophy and fibrosis. Although the mechanisms by which TGF-β causes renal fibrosis are well characterized, the relationship between TGF-β and metabolic memory remains unexplored, which is the purpose of this study.

Materials and methods: Male Wistar rats were made diabetic by a single injection of streptozotocin. After 4 or 12 weeks of diabetes induction, animals were kept under intensive glycemic control for the same period, until 8 or 24 weeks, respectively. Treatments were performed with insulin alone or combined with metformin (100 mg/kg). For the 24 weeks study, a group treated with insulin plus N-acetylcysteine (NAC, 750 mg/kg) was included. Control groups consisted of non-diabetic and diabetic animals after 8, 12 and 24 weeks. Body weight and blood glucose were monitored weekly, while HbA1c and renal function were assessed at different time points along the study. Renal expression of TGF-β was analyzed by western blotting, and intracellular metabolites related to glycolytic pathway and tricarboxylic acid cycle were quantified in kidney tissue by reverse phase ion pair HPLC-ESI-MS/MS.

Results: High levels of HbA1c were detected after 4 weeks of diabetes, but the decline of renal function started just after 8 weeks, as evidenced by increased proteinuria, albuminuria and kidney/body weight ratio. This impairment on renal function occurred in parallel to the raise of TGF-β expression ($p = 0.0018$), ATP ($p = 0.0039$), and fumarate ($p = 0.0103$) levels, compared to non-diabetic animals. Treatments with either insulin alone or combined with metformin recovered all of these early alterations. After 12 weeks of diabetes, the decline of renal function was more pronounced, including tubular injury, assessed by KIM-1 quantification in urine ($p = 0.0003$). TGF-β expression remained elevated ($p = 0.0040$), as well as glutamine ($p = 0.0024$) and glutamate ($p = 0.0043$), in comparison to non-diabetic rats. At the end of 24 weeks, when diabetic animals presented a clear impairment of glomerular and tubular function, renal expression of TGF-β was even higher ($p = 0.0198$) and none of the treatment strategies were able to normalize it. Concomitantly, greater levels of pyruvate ($p = 0.001$), lactate ($p = 0.0002$), malate ($p = 0.0464$), succinate ($p = 0.0166$), glutamine ($p = 0.0051$), and glutamate ($p = 0.0211$) were detected in diabetic kidneys, suggesting the occurrence of tricarboxylic acid cycle changes.

Conclusion: Increased TGF-β expression is an early alteration related to DN, which is just normalized by early, but not late, glycemic control, even though renal function is re-established. This uncontrolled TGF-β expression may lead to mitochondrial damage and mediate other long-term modifications related to the occurrence of metabolic memory, which deserves more investigation.

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Impaired cytokine responses from circulating monocytes in type 2 diabetes mellitus

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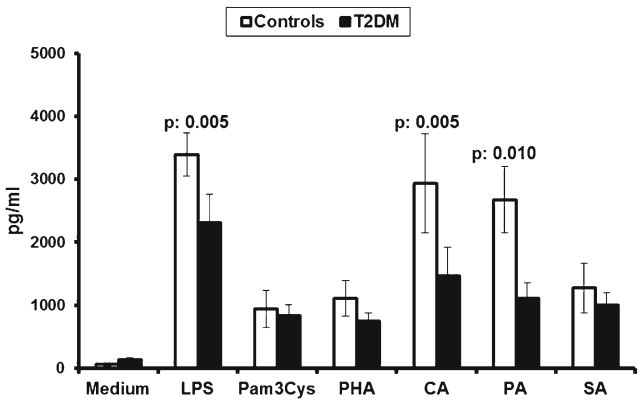
Background and aims: Although type 2 diabetes mellitus (T2DM) is associated with predisposition to infection, the mechanism is poorly studied. We explored the impairment of innate immune responses to microbial antigens as part of the mechanism underlying infection predisposition.
Materials and methods: Peripheral blood mononuclear cells were isolated from 20 healthy controls and 34 treatment-naïve patients (HbA1c 9.64±1.48%) and stimulated with each of the following six stimuli: a) the purified Toll-like receptor (TLR)- 4 ligand lipopolysaccharide (LPS) of Escherichia coli O55:B5; b) the TLR2 ligand Pam3Cys; c) the lymphocyte mitogen phytohemmaglutin (PHA); and d) heat-killed isolates of Pseudomonas aeruginosa (PA), Staphylococcus aureus (SA) and Candida albicans (CA). TNFα and IL-1β were measured in cell supernatants. Stimulations were repeated after 3 and 6 months of treatment and related with the level of glycemic control. Any less than 5-fold increase of cytokine production to at least four stimuli compared to medium-treated cells was considered appropriate response. HbA1c below 7% was considered proper glycemic control.
Results: All healthy controls (100%) had appropriate cytokine responses compared to only 18 (52.9%) of T2DM patients ($p<0.0001$). This was predominantly shown for IL-1β after stimulation with LPS, PA and CA (Figure). In treatment month 3, IL-1β produced after LPS stimulation of cells of patients with proper glycemic control was 5277.6±1262.0 pg/ml compared with 1235.8±488.8 pg/ml of cells of patients with improper glycemic control ($p<0.0001$).
Conclusion: T2DM is characterized by impaired cytokine production from circulating monocytes. This is pronounced for LPS that is a ligand of the cell wall of Gram-negative bacteria and it is partly restored after proper glycemic control. Results provide insight into the mechanism of predisposition for Gram-negative infections in T2DM and of the importance of proper glycemic control for prevention.



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IL-1beta, a novel predictive factor of cardiovascular disease in patient with newly diagnosed, drug-naïve type 2 diabetes mellitus

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Background and aims: Cardiovascular disease (CVD) is a major cause of death in patients with type 2 diabetes mellitus (T2DM). So development of markers to detect earlier and prevent CVD in patients with T2DM is very important. Chronic activation of innate immune system through the nucleotide binding and oligomerization domain-like receptor family pyrin domain-containing 3 (NLRP3) inflammasome leads to the maturation and secretion of proinflammatory cytokines such as IL-1beta and 18 and plays an essential role in the pathogenic mechanism of T2DM and CVD. In our previous study, NLRP3 inflammasome activation is elevated in myeloid cells from drug-naïve, newly diagnosed type 2 diabetic patients and antidiabetic treatment with metformin contributes to modulation of inflammasome activation. However, the study whether the NLRP3 inflammasome activation and its products in diabetic patients can predict the risk of CVD has not been reported. Therefore, we examined the association of CVD risk using the Framingham risk equation with the NLRP3 inflammasome activation in two group; patents with newly diagnosed, drug-naïve T2DM (New-DM) vs with patients taking anti-diabetic drug over one year (Old-DM).
Materials and methods: We used the serum, cultured monocyte-derived macrophages (MDMs) and their supernatants for measuring the mRNA expression and secreted maturation form of IL-1beta in New-DM (n=44) and Old-DM (n=16). Statistical analyses were completed using unpaired t-test, Pearson correlation, and one-way ANOVA.
Results: Although the mean values of Framingham 10-year CVD risk score were not significantly different between two groups (8.9±7.1 vs 9.3 ±7.4, p=0.863), the serum value of IL-1beta showed the positive linear correlation with Framingham 10-year CVD risk score only in New-DM (r=0.314, p=0.038), not in Old-DM (r=-0.083, p=0.760). To evaluate whether IL-1beta and NLRP3 inflammasome activation were different between the low risk group (10-year CVD risk score<5%) and the intermediate to high risk group (10-year CVD risk score≥10%), we compared the values of mean ± SD between two groups in New-DM and Old-DM. The serum value of IL-1beta showed the significantly increase in the intermediate to high risk group compared to the low risk group only in New-DM (18.03±15.36 vs 28.41±10.65, p=0.046), not in Old-DM (23.25±13.43 vs 21.47±8.99, p=0.960) as expected. In addition, the intermediate to high risk group compared to the low risk group had significantly elevated levels of IL-1beta in LPS-primed MDMs after treatment with different "danger signal" molecules, such as ATP (349.35± 141.32 vs 383.17±157.60, p=0.017), monosodium uric acid crystals (MUS) (262.94±69.98 vs 283.30±82.68, p=0.003) and free fatty acids (FFAs) (384.41±127.11 vs 392.55±141.60, p=0.004) only in New-DM, not in Old-DM.
Conclusion: In conclusion, serum IL-1beta was positively correlated with CVD risk score using Framingham risk equation only in New-DM. The serum value of IL-1beta also increased significantly in the intermediate to high risk group compared to the low risk group only in New-DM. Our study suggests that serum IL-1beta might be used as a marker for prediction of CVD in newly diagnosed type 2 diabetic patients.

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Glycaemic variability correlates with 1.5-AHG but not with markers of oxidation, inflammation and endothelial function: baseline results of the CAROLINA® CGM substudy

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Background and aims: DPP4-inhibitors enhance glucose-induced insulin secretion, decreases glucagon secretion and might effectively reduce blood glucose variability. This is investigated in the CGM substudy of CAROLINA®,an ongoing, cardiovascular outcome trial assessing potential differing impact of linagliptin or glimepiride predominantly when given as second line therapy in patients with relatively early type 2 diabetes (T2D).
Materials and methods: To explore potential relationships between glycemic variability (assessed with CGM, Dexcom SEVEN PLUS) and circulating markers of glucose variability (1.5 - anhydroglucitol [1.5-AHG]), oxidative stress (8 isoprostane prostaglandin $F_{2\alpha}$) endothelial function (asymmetric dimethylarginine and Endothelin-1) and systemic inflammation (interleukin-6) we analyzed the baseline glucose excursion patterns and assessed whether glycemic variability was correlated with five vascular markers. Specifically, we characterized the baseline ambulatory glucose profiles (AGP) with glucose exposure (by total area under the curve), glucose variability (by inter-quartile range) and glucose stability (by the average hourly absolute change) of all CGM readings over a 2-week period. We also calculated the mean amplitude of glycemic excursions (MAGE).
Results: Mean ± SD age of the 44 patients (91% males) with T2D was 66 ±9 years and HbA1c 7.0±0.4%. Median (min, max) diabetes duration was 11.0 (4.0, 29.0) years and 43% had a history of vascular complications. 79.5% were treated with metformin (66% in monotherapy), 34.1% received a sulfonylurea (34% as monotherapy)and 11.4% did not receive and treatment. As indicated in the table, a modest, significant negative correlation between glycemic variability and 1.5 AHG was observed, but no significant correlations between the vascular markers and glucose variability were seen.
Conclusion: A modest, significant negative correlation between glycemic variability and 1.5 AHG was observed indicating a potential applicability of this marker to identify patients with excess glucose pertubations. We found no significant correlations between glycaemic variability and the vascular markers (Table). Future analysis of the prospective CAROLINA® CGM substudy will determine if glimepiride and linagliptin have differing impact on glycemic excursions patterns.

Table. CGM and biomarker characteristics of the CAROLINA® CGM-substudy population

Markers of glycemic variability			
Glucose variability (mean±SD)	44.2±12.6 mg/dL		
Glucose stability (mean±SD)	8.9±3.3 mg/dL/hr		
Glucose exposure (mean±SD)	3506.1±491.1 mg/dL*24hr		
MAGE*	72.5±26.9 mg/dL		
Biomarkers*		**Correlations between glucose variability and biomarkers**	
		Spearman's correlation coefficient	p-value
1.5 AHG (mean±SD)	12.8±6.2 µg/ml	-0.366	0.0186
IL-6 (median, min, max)	3.0 (1.4, 22.2) pg/ml	0.034	0.8329
TNF-α (median, min, max)¤	2.9 (2.2, 5.6) pg/ml	0.191	0.2963
8-iso $PGF_{2\alpha}$ (median, min, max)	23.4 (17.2, 60.2) pg/ml	0.104	0.5161
ADMA (median, min, max)	0.5 (0.4, 0.7) µmol/l	0.256	0.1063
Endothelin-1 (median, min, max)	1.9 (0.7, 19.9) fmol/ml	-0.08	0.6223

*: n=41, ¤: n=32 Abbreviations: BP – blood pressure; CRP – c-reactive protein; MAGE –mean amplitude of glycemic excursions; AHG – anhydroglucitol; TNF-α – tumor necrosis factor α; 8-iso $PGF_{2\alpha}$ -8 isoprostane prostaglandin $F_{2\alpha}$; ADMA - asymmetric dimethylarginine.

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Role of SGK-1 in regulating eNOS activity in human coronary artery endothelial cells

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Background and aims: Cardiovascular diseases (CVD) are the leading cause of death globally and especially in patients with diabetes. CVD is a class of diseases that involve the blood vessels, in fact the initial step in development of CVD is the impairment in endothelial homeostasis, which is modulated by nitric oxide (NO) production. Important studies performed in endothelial cells recognized some signaling, such as the activation of PI3K-AKT-1 pathway, that improves endothelial nitric oxide synthase (eNOS) activation as a result of insulin stimulation. While it is known that Akt-1 directly phosphorylates human eNOS at Ser1177 resulting in an increase of eNOS activity, on the contrary the role of Serum and glucocorticoid-inducible kinase (SGK)-1 in modulation of eNOS activity is still sparse. In this study, we analyzed the role of SGK-1 in regulating eNOS activation in a cell model of human coronary artery endothelial cells (HCAEC).
Materials and methods: A retrovirus system was used to infect HCAEC with different constructs such as SGK-1wt, SGK-1Δ60 (lacking of the N-60 amino acids, not ubiquitinated and more active construct), and SGK-1Δ60KD (kinase dead constructs). eNOS phosphorylation at Ser1177 was measured in cells infected with different SGK-1 constructs in diverse experimental conditions: 1. insulin stimulation alone (10-7 M for 1 h); 2. SGK1 inhibitor (GSK650394, 103 nM, added 30 min before insulin); 3. AKT-1 inhibitor (iAKT, 10 µM, added 18 h before insulin) in presence or absence of insulin.
Results: AKT-1 inhibitor completely inhibited phosphorylation and activation of AKT-1 in Ser473 in all constructs, but inhibited only partially eNOS phosphorylation in Ser1177. In a similar way, SGK-1 inhibitor, inhibited only partially eNOS activity and phosphorylation but this inhibition was significantly less ($p<0.05$) in SGK-1Δ60 cells than other constructs, probably due to an increased activity of SGK-1 in this cells.
Conclusion: These results demonstrated that SGK-1 and AKT-1 may have a synergistic and compensatory effect in inducing eNOS phosphorylation in Ser1177 modulating NO production in HCAEC cells. Further studies in different cellular systems and in vivo are needed to better define the role of SGK-1 in diabetic cardiovascular diseases.

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Previously unknown glucose abnormalities are common in individuals with periodontitis, especially in those with a previous myocardial infarction

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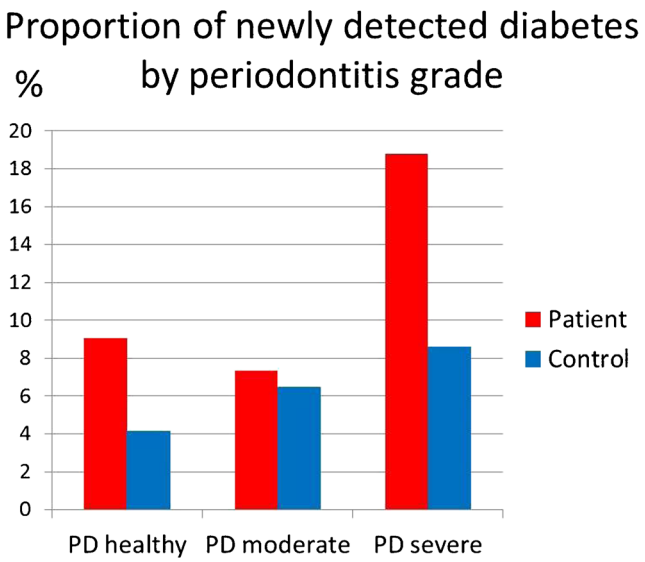
Background and aims: Periodontal disease (PD) is more common in diabetes (DM) and is suggested as a risk factor for myocardial infarction (MI) as well as for dysglycaemia. The prevalence of unknown abnormal glucose tolerance (AGT) in people with PD and if this is more common in MI patients with PD has not been investigated. We studied the prevalence of AGT in different grades of PD in patients with a first MI and controls.
Materials and methods: Patients (n=805) <75 years with a first MI in Sweden 2010-2014 and age and gender matched controls (n=805) were examined for risk factors, including an oral glucose tolerance test (OGTT). Dental bone loss was examined by panoramic radiographs

and PD classified as healthy (≥80% remaining bone), mild to moderate (79-66%) or severe (<66%). Known diabetes was present if reported by the participant. AGT was based on OGTT according to WHO (DM=F-glucose: ≥7.0 mmol/l and or F-glucose<7.0 and 2-h above 11.0 mmol/l, impaired glucose tolerance (IGT); F-glucose <7.0 and 2-h glucose ≥7.8 - 11.0 mmol/l).

Results: Mean age was 62±8 years (81% males). Previously known DM was present in 10% of patients and 8% of controls, newly detected DM in 9 and 5% (p=0.002) and new IGT in 20% and 12% (p<0.0001) respectively. AGT (new DM +IGT) was present in 29% of patients and 17% of controls (p<0.0001). Among study participants with signs of PD (moderate + severe) 62% of patients and 37% of controls had unknown AGT. Newly detected DM (Figure) was more common in patients with severe PD than in those without PD (18% vs. 9%) and more common than in controls (9% vs. 4%).

Conclusion: Unknown AGT is more common among patients with a first MI than in controls and especially apparent in the presence of PD. Newly detected DM is particularly apparent in patients with severe PD. This indicates that PD may induce a chronic inflammatory condition and provoke glucose abnormalities as well as MI supporting the assumption of a two-way relationship between dysglycaemia and PD.



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The relationship between LDL-c reduction during statin therapy and the risk of new-onset diabetes: a meta-analysis

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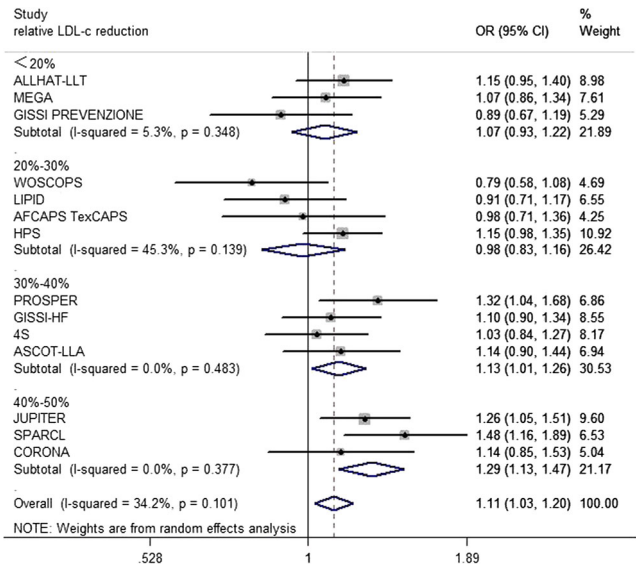
Background and aims: Recent studies demonstrated that lower target LDL-c level of statin use is associated with excess risk of diabetes. This study is to investigate under what circumstances we should focus on statin-induced diabetes according to the new ACC-AHA cardiovascular prevention guidelines.

Materials and methods: We searched Pubmed, Embase, and the Cochrane Central Register of Controlled Trials, with no time constraints, for randomized placebo and standard care-controlled endpoint trials of statins. According to the new ACC-AHA cardiovascular prevention guidelines, the included trials were stratified by the relative LDL-c reduction of 10% to 20%; 20% to 30%; 30% to 40%; or 40% to 50%. We calculated an overall odds ratio (OR) with a random-effect model.

Results: Totally, 4559 patients developed diabetes during duration in 14 trials. The risks of incident diabetes elevate by 13% and 29% when relative LDL-c reduction were 30-40% and 40-50%, respectively. However, incident diabetes did not increase when relative LDL-c reduction were <20% and between 20-30%. In absolute terms, one additional case of diabetes is diagnosed per 137 patients and per 108 patients whose relative LDL-c reduction were 30-40% and within 40%-50% when taking statin therapy for 4 years.

Conclusion: LDL-c reduction is positive related with new-onset diabetes risk. We should pay close attention to incident diabetes among the "major statin benefit groups", especially when LDL-c reduction more than 30% during the process of lipid lowering.



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Effects of lipid drugs on glycaemic control in type 2 diabetes

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Background and aims: Several studies showed that statins increase the risk of incident diabetes; however, the relationship between statins and glycemic control in subjects with established diabetes has not been well characterized. Aim of this study was to evaluate the effect of lipid drugs, statins, ezetimibe and fenofibrate, on glycemic control in patients with type 2 diabetes (DM2).
Materials and methods: In a retrospective study, a consecutive series of 423 (210 F, 213 M) DM2 clinic outpatients, aged 67.7±8.8 years, were consecutively examined for HbA1c levels before starting lipid drugs and after 6, 12 and 18 months with lipid therapies. All patients studied remained with unchanged diabetes therapies for all the study duration. Subjects with current corticosteroids drugs and chronic kidney disease were excluded. Statins were subdivided by pharmacological potency, based on their low density lipoprotein-cholesterol reduction below 40% (low-potency statins: atorvastatin 10 mg, simvastatin 10 mg, 20 mg and 40 mg, fluvastatin, pravastatin and lovastatin at all doses) and upper 40% (high-potency statins: rosuvastatin all doses, atorvastatin 20 mg and 40 mg and simvastatin+ezetimibe all doses). We compared HbA1c levels modifications during the 18 months of follow-up in subjects with or without lipid drugs.
Results: The patients studied showed HbA1c 7.0±1.1% (53±12 mmol/mol) with 13.8±7.1 years duration of diabetes. 90.6% patients assumed oral hypoglycemic agents, 9.4% insulin, and 88.0% lipid drugs. Among these, 96.0% were treated with statins and 4.0% with fenofibrate. Among subjects with statins therapy, 50.8% patients assumed high-potency statins and 49.2% low-potency statins. No statistical difference was found in HbA1c baseline levels between subjects with or without lipid therapies. During the follow-up, we observed a significant increase of HbA1c levels in patients treated with lipid drugs compared to subjects without any lipid therapy: 6.9±0.9% vs 6.5±0.8% (p=0.004) at 6 months, 6.88±0.8% vs 6.46±0.7% (p=0.003) at 12 months and 6.9±0.8% vs 6.4±0.8% (p=0.002) at 18 months. Considering the effects of statins potency on HbA1c variation, we observed an increase of 0.5% at 6 months (p=0.022), 0.45% at 12 months (p=0.003) and 0.53% (p=0.001) at 18 months in HbA1c by high-potency statins. Low-potency statins were associated to an increment of HbA1c levels 0.31% (p=0.025) at 6 months, 0.36% (p=0.03) at 1 year and 0.48% (p=0.001) at 18 months. Fenofibrate showed an increase of HbA1c 0.5% (p=0.021) at 6 months, 0.76% (p=0.002) at 1 year and 0.78% (p=0.001) at 18 months.
Conclusion: In DM2 patients, lipid therapies are associated with a modest, but significant decrease in glycemic control. This fact should not be relevant in clinical setting in the choice of lipid drugs considering their positive effects on cardiovascular protection.

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High adherence to dietary recommendations associates with lower risk of developing high triglycerides and LDL during 16 years of follow-up

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Background and aims: A high diet quality according to the Swedish nutrition recommendations associates with reduced risk of mortality and cardiovascular disease in the Malmö Diet and Cancer cohort. To further clarify this protective association we examined its relation to change in standard blood lipids during 16 years of follow-up. In addition, because genetic variation could influence the response to certain diets we examined if genetic risk scores composed of 80 validated lipid-associated genetic variants modify this association.
Materials and methods: We included 3,145 individuals from the population-based Malmö Diet and Cancer cohort study that had standard lipid concentrations (triglycerides, HDL and LDL) measured both at baseline examination (1992-1994) and after an average of 16 years of follow-up. Dietary intakes at baseline were measured using a modified diet history method, and a diet quality index was constructed based on adherence to recommended intakes for saturated fat, polyunsaturated fat, sucrose, fiber, fruit and vegetables, and fish.
Results: A high compared with a low diet quality index was associated with higher HDL concentrations at baseline and lower risk of developing high triglycerides and high LDL during follow-up. We found an inverse association between diet quality and HDL change during follow-up only among those with lower genetic risk for low HDL but not among those with higher genetic risk (p-interaction=0.04). Among those with low genetic risk for low HDL, low diet quality was associated with decreased HDL during follow-up.
Conclusion: Individuals with high adherence to the Swedish nutrition recommendation had lower risk of developing high triglycerides and LDL during 16 years of follow-up. We found no strong evidence that genetic risk for dyslipidemia modified the association between diet quality index and change in standard blood lipids.
Supported by: SMRC, SSMR, SH-L Foundation, SUH, Albert Påhlsson RF, Crafoord Foundation

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Rosuvastatin 5 mg improved carotid atherosclerotic changes regardless of LDL cholesterol level in Japanese diabetic patients: a 2-year prospective study

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Background and aims: A new AHA guideline on the treatment of blood cholesterol recommends moderate-intensity statin therapy (MIST) for diabetic patients regardless of LDL-C levels. In Japan, we set the targeted values for lipid level in detail based on medical conditions. However, the dosage of strong statin is generally less than western countries due to size of physique. Therefore, no enough evidences using MIST exist for Asian people. In this study, we had assessed the effectiveness of rosuvastatin 5 mg as MIST on carotid atherosclerotic changes for Japanese diabetic patients.
Materials and methods: Ninety-nine diabetic outpatients (59.6% male; mean age=55.8±8.0 years; average diabetic duration=9.4±6.5 years; mean HbA1c=7.26±1.3%; 33% statin-naive) were enrolled who also meet the following requirements; Age≦65 years, with hyper lipidemia, found max carotid intima media thickness (CIMT)≧1 mm or plaques in carotid ultra sound (US). 33 patients had already received statin therapy before the study. Daily oral administration of 5 mg rosuvastatin was started or switched from other statins and the fasting blood samples were collected (including TC, HDL-C, triglyceride and LDL-C was estimated by Friedwald method) every 1 to 3 months, then carotid US had been performed 1 to 2 years later. We had assessed the mean CIMT(mm) including no plaques, max CIMT(mm) and stenosis(%) using ECST measurement method and the course of primary lesion area of max CIMT and stenosis was followed. In addition, we assessed the change of US findings depending on reduced LDL-C levels.
Results: Mean levels of LDL-C were reduced from 100.4±28.4(mg/dl) to 71.5±23.7 (after 1-2 years). There were no significant changes in HbA1c levels for 2 years (mean HbA1c: 7.26→7.26→7.27%). The significant improvement was not accepted after 1 year, but accepted after

2 years through the observation of carotid US findings: mean CIMT 0.76 ±0.16→0.72±0.16 (p=0.02), max CIMT 2.0±0.9→1.8±0.8 (p=0.0002), stenosis 22.9±9.2→20.2±8.3 (p<0.0001) (by using Student's t test). Based on the levels of LDL-C after 1-2 years of administration, we divided participants into 2 groups by referring to the ESC/EAS dyslipidemia guideline recommendation for diabetic patients [LDL-C<70 mg/dl=group A (n=51; mean age=56.5 years; 63% male; mean LDL-C=55.1 mg/dl; mean HbA1c=7.2%); 70≦LDL-C=group B (n=48; mean age=55.0 years; 56% male; mean LDL-C=89.6 mg/dl; mean HbA1c=7.4%)]. Both groups had significant amelioration of max CIMT (A: p=0.016; B: p=0.001) and stenosis (A: p<0.0001; B: p=0.002) but mean IMT was improved only in group A (p=0.01) (by using Wilcoxon signed-rank test). There were no significant differences within 2 groups in the result of 2 years changing rate of each US findings (by using Mann-Whitney U test) with no significant differences in age, sex, diabetic duration and HbA1c. Mean changing rates of US findings in each group were as follows: mean CIMT [-5.6 vs -1.1(p=0.14)]; max CIMT [-9.31 vs -3.22(p=0.81)]; stenosis [-6.9 vs -12.9(p=0.95)] (%).

Conclusion: This result shows that rosuvastatin 5 mg (as MIST) administration for Japanese diabetic patients for 2 years improves carotid atherosclerotic change regardless of maintained LDL-C levels without deterioration in HbA1c.

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Does diagnosis of diabetes mellitus among patients surviving an ACS event affect lipid control measures? Results from the Dyslipidaemia International Study (DYSIS) II ACS

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Background and aims: Patients suffering an acute coronary syndrome (ACS) event with concomitant diabetes mellitus (DM) remain at very high risk for future cardiovascular complications. Current ESC guidelines recommend an LDL-C target of <70 mg/dl for very high risk patients. We aimed to determine LDL-C target achievement rates among post-ACS patients with DM and how this differs from patients without DM. We further evaluated lipid management and in those not reaching target, the distance to LDL-C <70 mg/dl.

Materials and methods: DYSIS II ACS, a multicountry observational cross sectional study, enrolled ACS patients from May 2013-October 2014 in 18 countries from Asia-Pacific, Europe, and Middle East/Africa regions. Eligible adult patients were hospitalized for an ACS event, had documented DM (previously diagnosed, fasting plasma glucose≥126 mg/dl, or currently receiving treatment for DM), full lipid profile available within 24 hours of hospital admission, on lipid lowering therapy (LLT)≥3 months or not at all, alive at discharge, and not participating in clinical trials involving medication. Patient follow-up interviews were conducted 4 months after ACS hospital admission date to capture details on recurrent events, current symptoms, laboratory values, LLT, and quality of life.

Results: Among 3855 enrolled ACS patients (mean age 62.3±12.1 years; 76.4% male), 36.9% had DM, with 23.8% of DM patients having an LDL-C <70 mg/dl versus 16.0% of patients without DM (p <0.0001). Median distance to LDL-C target at enrollment was 38.0 (16.0,66.0) mg/dl among DM patients and 48.0 (24.0,77.0) mg/dl among patients without DM (p <0.0001). Seventy-seven percent of DM patients and 58.5% of patients without DM were currently receiving LLT when admitted to the hospital. Approximately ninety-one percent of ACS patients (n=3496) had 4 month follow-up data available, with 37.5% having DM. Lipid results available for 1040 patients at follow-up showed that 38.8% of DM patients versus 28.1% of patients without DM (p <0.001) achieved an LDL-C <70 mg/dl. Median distance to LDL-C target was 21.5 (9.0, 42.0) mg/dl for DM patients and 21.0 (6.0,37.0) mg/dl for patients without DM (p=0.16). Differences in LLT management 4 months after an ACS event are shown in Table 1.

Conclusion: LLT was used the same way in both patient groups, with concomitant DM having no influence on the physicians' prescribing behavior. LDL-C was lower in patients with DM indicating towards a different lipid profile containing smaller lipoprotein particles. Additional effective lipid lowering strategies are needed among these very high cardiovascular risk patients in order to further reduce CV events.

Table 1: Lipid-Lowering Therapies 4 months after ACS Event

	DM Patients n=1231	Patients without DM n=2071
Atorvastatin equivalent dose (mg/day)[a]	33 ± 20	32 ± 22
Statin monotherapy[b]	85.5%	87.2%
Statin + ezetimibe[b]	5.0%	5.0%
Statin + other non-statin[b]	4.2%	3.2%
Non-statin monotherapy[b]	0.5%	0.4%
No statin or non-statin treatment[b]	4.8%	4.2%

[a] p <0.05
[b] Results were not statistically significant

Supported by: Merck & Co., Inc.

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Target value attainment among treated ACS and CHD patients with concomitant type 2 diabetes mellitus in 21 countries: the Dyslipidaemia International Study (DYSIS) II results

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Background and aims: ESC guidelines set target LDL-C values for patients suffering from diabetes or CHD at <70 mg/dl. The condition of concomitant CHD and diabetes puts patients at very high risk for cardiovascular events, specifically recurrent infarctions. We aimed to determine target value attainment in 2 cohorts: stable CHD patients and post-acute coronary syndrome (ACS) patients respectively, both with concomitant type 2 diabetes mellitus (T2DM). Further, we evaluated lipid management and in those not reaching the target, the distance to LDL-C <70 mg/dl.

Materials and methods: DYSIS II, a multicountry observational cross sectional study enrolled stable CHD and ACS patients in 21 countries from Asia-Pacific, Europe, and Middle East/Africa regions. Eligible adult patients were hospitalized for an ACS event or had a documented history of CHD, documented T2DM (previously diagnosed, fasting plasma glucose≥126 mg/dl, or currently receiving treatment for diabetes), full lipid profile available within 24 hours of hospital admission for ACS patients and 0-12 months prior to enrollment for CHD patients, on lipid lowering therapy (LLT)≥3 months or not at all, and not participating in clinical trials involving medication.

Results: Overall, 1346 patients with ACS and T2DM were enrolled, with 77.4% (n=1042) currently on LLT when admitted to the hospital. Only 28.3% of patients on LLT had an LDL-C <70 mg/dl (mean LDL-C 94.2±40.2 mg/dl), with median distance to LDL-C target being 32.0 (14.0, 57.0) mg/dl. Mean atorvastatin equivalent dose was 23±16 mg/day, with the majority of patients on statin monotherapy (91.4%), followed by 3.6% statin + other non-statin (fibrates or omega 3 fatty acids), 3.2% statin + ezetimibe, and 1.8% non-statin monotherapy when admitted to the hospital. Among 2620 enrolled stable CHD patients with T2DM, 94.6% (n=2479) were currently on LLT, with 36.3% achieving an LDL-C <70 mg/dl

(mean LDL-C 82.4±41.0 mg/dl). Median distance to target LDL-C was 21.0 (9.0, 38.0) mg/dl. Mean atorvastatin equivalent dose was 25±18 mg/day, with 81.9% currently on statin monotherapy followed by 10.4% statin + ezetimibe, 6.5% statin + other non-statin (fibrates, nicotinic acid, or omega 3 fatty acids), and 1.2% non-statin monotherapy.

Conclusion: Approximately two-thirds of treated ACS and stable CHD patients with concomitant T2DM were not at the recommended LDL-C target while receiving primarily statin monotherapy. DYSIS II confirms that patients at highest CV risk are treated mainly with moderate statin doses. Switching to higher statin doses, and the addition of ezetimibe, will further reduce the risk of future cardiovascular events.

Table 1: Patient characteristics among treated ACS and CHD patients with concomitant T2DM

	ACS patients n=1042	CHD patients n=2479
Mean age (years)	64.6 ± 10.7	65.9 ± 9.9
Male	69.4 %	74.5%
Hypercholesterolemia	74.0%	77.6%
Hypertension	82.5%	80.6%
Body mass index >30 kg/m²	31.7 %	32.8%
History of previous myocardial infarction	31.1%	52.5%
Family history of CHD	24.6%	31.1%
Current smoker	18.1%	9.3%

Supported by: Merck & Co., Inc.

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The abruptness of terminating nicotinic acid delivery has a profound effect on free fatty acid and insulin rebound in rats

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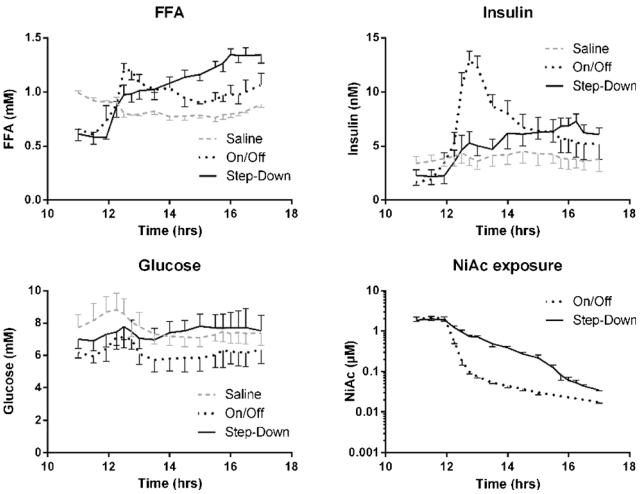
Background and aims: A major factor thought to drive insulin resistance is ectopic lipid accumulation. Reducing circulating lipids including free fatty acids (FFA) may offer an approach to ameliorate insulin resistance. Acute nicotinic acid (NiAc) infusion results in rapid reduction of plasma FFA concentrations. However, this tends to be offset following infusion cessation, by a FFA rebound, which overshoots pre-treatment levels. One possible explanation is that this is a consequence of the very short plasma NiAc half life (~3 min in the rat). The aim of this study was to determine whether a slow step-down NiAc infusion protocol (Step-Down group) *vs.* simply turning the infusion off (On/Off group) could attenuate the FFA rebound development and maximize NiAc induced FFA lowering.

Materials and methods: FFA lowering was assessed in obese Zucker rats following a 12 hr constant rate NiAc infusion with corresponding saline infused controls.

Results: It was found that, following a 12 hr constant rate NiAc infusion the On/Off group exhibited a FFA rebound and a remarkable insulin rebound. To our surprise the Step-Down group did not succeed in improving FFA control. In fact the area under the FFA curve during the 5 hr step-down phase was greater than for the corresponding period in the On/Off group (5.70 vs. 4.96 mM hr, $P<0.05$) despite substantially higher plasma levels of NiAc in the Step-Down group. The higher FFA levels were associated with, and may have been caused by, a markedly blunted insulin rebound in the Step/Down versus On/Off group possibly due to NiAc-induced inhibition of insulin secretion.

Conclusion: In conclusion, these results show that the time dependence of the infusion profile of the anti-lipolytic agent NiAc has a very significant impact on control of plasma FFA levels. Specifically, gradual step-down reduction of NiAc infusion actually degraded the anti-lipolytic effectiveness of NiAc compared to abrupt withdrawal possibly due to a NiAc induced blunting of insulin secretion.



PS 120 Non-glycaemic effects of incretin system

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Down-regulation of vascular GLP-1 receptor expression in subjects with obesity

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Background and aims: It is known that incretin signaling improve vascular relaxation response through eNOS expression and/or activity in endothelial cells. In addition, it has been shown that incretin has anti-atherosclerotic effects in vascular smooth muscle cells. On the other hand, although it has been reported that GLP-1 receptor expression in pancreatic β-cells is reduced under diabetic conditions, it remains unknown whether vascular GLP-1 receptor expression is altered under some conditions and which factor could influence vascular GLP-1 receptor expression. The aim of this study was to reveal the alteration of vascular GLP-1 receptor expression and the factors affecting its expression in human artery.
Materials and methods: We examined the subjects having artery surgery. Excised diseased artery was mechanically separated into the intima and tunica. Gene expression of various factors in the intima and tunica was analyzed by real time RT-PCR. Primer pairs encoding genes associated with GLP-1 receptor and factors related to arteriosclerosis were prepared, and real-time RT-PCR with Sybr Green was performed. Each gene expression was semi-quantified by the comparative Ct method with each result in β-actin as a control. Subjects were divided into obesity (BMI≥25 kg/m2) and non-obesity group (BMI<25 kg/m2) and comparison of the clinical parameters and various gene expression levels in the intima and tunica was performed. The results were expressed as mean± SE. A Wilcoxon test was used to test the difference between obesity and non-obesity group with $p<0.05$ regarded as significant.
Results: Subjects were 36 patients (male / female=26 / 10, abdominal or thoracic aortic aneurysm / aortic dissection / arteriosclerosis obliterans / other=11 / 17 / 6 / 2, diabetic / non-diabetic=11 / 23, age 67.3±2.3 years old, BMI 23.7±0.8 kg / m2, HbA1C 6.3±0.3%). BMI in obese and non-obese group was 28.6±0.7 kg/m2 and 20.2±0.6 kg/m2, respectively ($p<0.05$). Between the obesity and non-obesity group, there was no significant difference in FBG (121.2±8.9 mg/dl vs 119.6±6.3 mg/dl). Plasma insulin level in obesity group was higher compared to that in non-obesity group (29.2±11.0 μU/ml vs 6.5±1.3 μU/ml) ($p<0.05$). And there was no significant difference in HbA1c (5.9±0.2% vs 6.6±0.5%), triglyceride, HDL-cholesterol, LDL-cholesterol between the 2 groups. GLP-1 receptor gene expression in the intima and tunica was significantly lower in obesity group compared to that in non-obesity group ($p<0.05$). In spearman's rank correlation coefficient test, there was significantly negative correlation between GLP-1 receptor gene expression and BMI, LDL-cholesterol in the intima and tunica.
Conclusion: GLP-1 receptor gene expression in human artery was down-regulated in subjects with obesity. The present results suggest that obesity and hyperinsulinemia down-regulate GLP-1 receptor gene expression in human artery.
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1184

Resistance to the GLP-1 effects on platelets in type 2 diabetes mellitus

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Background and aims: Glucagon-like peptide 1 (GLP-1) shows pleiotropic benefits on cardiovascular system: its influence on platelets - deeply involved in the pathogenesis of diabetic vascular diseases - is poorly investigated. The GLP-1 active form is GLP-1 (7-36), rapidly degraded to GLP-1 (9-36).We previously showed that, in platelets, both GLP-1 (7-36) and GLP-1 (9-36) and the analogue Liraglutide similarly increase the sensitivity to the antiaggregating nitric oxide (NO) and decrease the agonist-induced activation of the signalling pathways PI-3Kinase (PI-3 K) and MAP-Kinase (MAPK) and reactive oxygen species (ROS) production. The GLP-1 influence on platelet response in Type 2 Diabetes Mellitus (T2DM) is not known. Aim of this study was to verify whether the GLP-1 effects observed in healthy subjects were preserved in T2DM.
Materials and methods: In platelets from 20 T2DM patients (M/F: 9/11; age 56.1±1.9 years; BMI:32.6±1.1 kg/m2; HbA1C:8.0±0.2%) and 12 healthy control subjects (M/F:6/6; age:48.2±3.4 years; BMI: 24.0± 0.7 kg/m2) we evaluated the ability of GLP-1(7-36), GLP-1(9-36) and Liraglutide (100 nmol/l) to influence: the antiaggregating effects of the nitric oxide donor Na-nitroprusside (SNP) (Born's method), the phosphorylation levels of AKT (pAKT) and ERK-1/2 (pERK-1/2)(Western Blot) and ROS synthesis (DCF-DA fluorescence assay) in the presence of the agonists ADP (10 micromol/l), Collagen (4 mg/L) and Na-Arachidonate (NaA) (100 micromol/l).
Results: The effects of GLP-1 (7-36), GLP-1 (9-36) and Liraglutide, were lower in T2DM compared with healthy subjects. In particular, in the presence of GLP-1 (7-36): the percent (%) increases of the antiaggregating effects of SNP were 4.0±1.8 vs 28.2±4.8 ($p<0.0001$) with ADP, and 17.0±5.2 vs 36.8±5.1 ($p<0.02$) with Collagen; the% reductions of pAKT were 3.2±4.2 vs 43.1±3.5 ($p<0.0001$) with Collagen, and 5.4±5.2 vs 32.1±6.8 ($p<0.004$) with NaA; the % reductions of pERK-2 were 3.5±2.2 vs 25±8.2 ($p<0.004$) with Collagen, and 10.5± 8.6 vs 40.2±9.5 ($p<0.03$) with NaA; the % reductions of ROS production were 15.1±5.2 vs 41.2±7.5 ($p<0.006$) with NaA. In the presence of GLP-1 (9-36): the % increases of the antiaggregating effects of SNP were 6.1±1.9 vs 25.6±3.0 ($p<0.0001$) with ADP, and 18.5±3.8 vs 32.7±4.1 ($p<0.02$) with Collagen; the % reductions of pAKT were 4.8±3.1 vs 45.3 ±4.6 ($p<0.0001$) with Collagen, and 6.1±3.3 vs 35.9±7.3 ($p<0.0001$) with NaA; the % reductions of pERK-2 were 4.9±2.0 vs 28±9.1 ($p<0.004$) with Collagen, and 12.6±8.1 vs 44.0±9.8 ($p<0.02$) with NaA; the % reductions of ROS production were 17.1±8.3 vs 45.9±7.9 ($p<0.03$) with NaA. In the presence of Liraglutide: the % increases of the antiaggregating effects of SNP were 5.7±2.5 vs 30.1±7.8 ($p<0.001$) with ADP, and 15.5±3.5 vs 33.5±4.4 ($p<0.003$) with Collagen; the % reductions of pAKT were 15.9±5.7 vs 39.1±9.0 ($p<0.03$) with Collagen, and 20.1±5.7 vs 36.2±5.8 (ns) with NaA; the % reductions of pERK-2 were 4.0±2.5 vs 27.9±8.8 ($p<0.003$) with Collagen, and 24.3±7.7 vs 39.2± 6.8 (ns) with NaA; the % reductions of ROS production were 26.1±5.4 vs 56.8±6.3 ($p<0.001$) with NaA.
Conclusion: T2DM is characterized by a resistance to the effects of the native GLP-1 (7-36), truncated GLP-1 (9-36) and GLP-1 analogue Liraglutide to increase the antiaggregating effects of NO, to reduce platelet activation of signalling pathways PI-3 K and MAPK and oxidative stress stimulated by platelet agonists.
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The DPP-4 enzyme correlates with plasma triglycerides in non-obese subjects and mediates palmitate-induced apoptosis in human cardiac progenitor cells

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Background and aims: Hypertriglyceridemia is an independent risk factor for cardiovascular disease, and chronic exposure to elevated saturated fatty acids (SFA) leads to cardiomyocyte dysfunction and death, both in vivo and in vitro. Circulating levels of dipeptidyl peptidase-4 (DPP-4) have been shown to correlate with cardiovascular disease in humans. Thus, we investigated the potential link between plasma DPP-4 levels, triglyceridemia and the activation of apoptotic pathways in human cardiac progenitor cells (CPC).
Materials and methods: Plasma DPP-4 levels were determined with an ELISA assay. DPP-4 activity was measured by a colorimetric assay. Human CPC isolated from right auricle biopsies were exposed to 0.25 mM palmitate up to 24 h. Cell apoptosis was detected by caspase-3 cleavage and cytosolic release of oligosomes. Expression and phosphorylation levels of the proteins under investigation were evaluated by immunoblotting techniques.
Results: In 20 non-diabetic subjects (M/F 9/11, age 47±11 y, BMI 33±9, triglycerides 135±99 mg/dL), plasma DPP-4 levels were found to positively correlate with BMI ($r=0.485$, $p<0.05$). In addition, DPP-4 levels were significantly and positively associated with plasma triglycerides in non-obese ($r=0.847$, $p<0.05$) but not in obese ($r=0.02$) individuals. Then, primary cultures of CPC were obtained from right auricle biopsies of patients undergoing elective heart surgery. When CPC were exposed to the SFA palmitate (0.25 mmol/L, up to 16 h), DPP-4 levels and activity in the culture medium were found to be increased 2- to 4-fold ($p<0.05$), and this was associated with increased phosphorylation of the stress kinase JNK and the JNK substrate c-Jun ($p<0.05$). In addition, exogenous DPP-4 (up to 500 ng/ml) activated the JNK pathway, inducing a 2.5 fold increase in c-Jun phosphorylation ($p<0.05$). Finally, palmitate augmented CPC apoptosis, evaluated by measuring cytoplasmic oligosomes and Caspase-3 cleavage, ($p<0,05$), but DPP-4 silencing with a specific siRNA prevented CPC apoptosis, as well as c-Jun phosphorylation ($p<0.05$).
Conclusion: The DPP-4 enzyme correlates with plasma triglycerides in vivo in non-obese subjects, is released from CPCs in response to palmitate in vitro, and contributes to SFA-triggered stress signals leading to cardiac cell dysfunction and death.

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Correlation of SDF-1a levels and left ventricular diastolic dysfunction in type 2 diabetes patients according to treatment with or without DPP4-inhibitors

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Background and aims: Recent studies have shown that stromal derived factor-1α (SDF-1α) is a substrate of dipeptidyl-peptidase-4 (DPP-4) inhibitors. SDF-1α is a small cytokine that belongs to the chemokine family and can stimulate endothelial progenitor cells. It has, also, been shown that SDF-1α shares antiapoptotic as well as nephroprotective properties and it has a beneficial effect in the cardiovascular system. Therefore, the aim of the present study was to estimate the effect of the treatment with DDP-4 inhibitors to SDF-1α levels according to the presence of diastolic dysfunction of left ventricle (DDLV) in subjects with type 2 diabetes (T2D)
Materials and methods: 32 participants (16 males) with T2D, mean age (±SD) 67.2±8.3 years, HbA1c 6.4±0.5%, body-mass index (BMI) 29.1±4.9 Kg/m2, duration of diabetes 8.5±4.0 years receiving antidiabetic treatment with metformin only (17 participants) or metformin plus DPP-4 inhibitors (14 participants) without known cardiovascular disease were enrolled into the study. All study participants underwent fully clinical examination and ultrasound examination of the heart while a blood sample was taken at fasting state for the estimation of SDF-1α levels.
Results: Study participants receiving metformin plus DPP-4 inhibitors had higher plasma levels of SDF-1α compared to participants on metformin therapy only (19.6±4.1 versus 6.9±1.3 pg/ml, respectively, $p=0.01$). 14 participants had DDLV, while SDF-1α levels did not differ according to the presence or not of DDLV (12.0±1.8 versus 9.1±0.1 pg/ml, respectively, $p=0.65$). 61.3% of participants had arterial hypertension, 77.4% dyslipidemia while 12.9% were current smokers. Multivariate regression analysis (backward), after controlling for age, sex, BMI, smoking, history of arterial hypertension and dyslipidemia, DDLV, white blood cells count, C-reactive protein, creatinine clearance, uric acid, LDL-cholesterol and triglycerides, showed that SDF-1α levels were positive related with treatment with DPP-4 inhibitors (beta=0.91, $p=0.001$), and negative with duration of T2D (beta=-0.42, $p=0.05$), HDL-cholesterol levels (beta=-0.46, $p=0.02$) and HbA1c (beta=-0.41, $p=0.05$).
Conclusion: The results of the present study showed that treatment with DDP-4 inhibitors has a favorable effect to SDF-1α levels. On the contrary, no statistical significant relation was found between SDF-1α levels and DDLV. Finally, long duration of diabetes is related with lower levels of SDF-1α, confirming the detrimental effects of diabetes per se.

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LEADER-5: prevalence and cardiometabolic impact of obesity in cardiovascular high-risk patients with type 2 diabetes: baseline data from the trial

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Background and aims: The association between anthropometric measures of adiposity and cardiovascular (CV) risk in patients with established type 2 diabetes mellitus (T2DM) and high CV risk has not been well studied. Some longitudinal cohort studies have shown reduced mortality with higher body mass index (BMI) in patients with T2DM and coronary artery disease, suggesting an "obesity paradox". However, this is not well-established and additional data in this patient group are needed.
Materials and methods: We used baseline data from the Liraglutide Effect and Action in Diabetes: Evaluation of cardiovascular outcome Results (LEADER) trial, which enrolled 9340 patients with T2DM, at high-risk for CVD, across 32 countries. We used cross-sectional data to investigate (1) the prevalence of overweight and obesity; (2) the association of cardiometabolic risk factors with measures of adiposity (BMI and waist circumference (WC)); and (3) the cardiometabolic risk factors affecting treatment intensity. Lipids and blood pressure measurements were considered on target according to ADA 2014 criteria. Descriptive statistics and multivariable logistic regression analyses were used.
Results: Mean BMI was 32.5±6.3 kg/m^2, with only 9.1% of patients having normal weight. The prevalence of normal WC was also low (6.4% according to harmonized criteria). The percentage of patients with LDL-cholesterol <2.6 mmol/L (100 mg/dl), triglycerides (TG) <1.7 mmol/L (150 mg/dl), and blood pressure (<140/80 mmHg) at target was generally lower for individuals with a BMI >40 kg/m^2 compared to individuals with a BMI <25 kg/m^2 (37.6% vs. 36.9%, 63.1% vs. 45.9%, 44.9% vs. 38.2%, respectively). A similar trend was observed for WC. No differences were detected for HbA1c and LDL-cholesterol. Insulin, statin, and antihypertensive agent use was more frequent at higher BMI and

WC. Multivariable adjusted logistic regression analyses yielded that obesity was mainly associated with female gender ($p<0.0001$), with not smoking ($p<0.0001$), race and region of living ($p<0.0001$ for both) (Table), as well as triglyceride level and hypertension (data not shown).

Conclusion: Overweight and obesity are extremely prevalent in high-risk patients with T2DM. BMI and WC are associated with cardiometabolic risk factors and treatment intensity is higher in patients who are overweight or obese; however, a high percentage of these patients were not at target for HbA1c, BP, and lipids at baseline. The LEADER trial will provide important information on the effects of the GLP-1 receptor agonist liraglutide on CV outcomes in this patient population.

		BMI>=30 kg/m2			WC-ATPIII off target			WC-IISHMS off target		
factor	level	Odds Ratio	(95% C.I. for OR)	p_value	Odds Ratio	(95% C.I. for OR)	p_value	Odds Ratio	(95% C.I. for OR)	p_value
Age	per year	0.956	(0.949 - 0.964)	<0.0001	0.998	(0.988 - 1.007)	0.6461	1.005	(0.990 - 1.021)	0.5158
Gender	Male	0.597	(0.525 - 0.678)	<0.0001	0.136	(0.112 - 0.164)	<0.0001	0.190	(0.137 - 0.259)	<0.0001
	Female	1.000	(1.000 - 1.000)	.	1.000	(1.000 - 1.000)	.	1.000	(1.000 - 1.000)	.
Smoking Status	Current Smoker	0.612	(0.517 - 0.724)	<0.0001	0.627	(0.516 - 0.764)	<0.0001	0.604	(0.453 - 0.813)	0.0007
	Never Smoked	0.927	(0.821 - 1.046)	0.2189	0.797	(0.689 - 0.922)	0.0022	0.911	(0.724 - 1.148)	0.4295
	Previous Smoker	1.000	(1.000 - 1.000)	.	1.000	(1.000 - 1.000)	.	1.000	(1.000 - 1.000)	.
Region	Asia	0.207	(0.137 - 0.314)	<0.0001	0.361	(0.240 - 0.543)	<0.0001	0.358	(0.199 - 0.625)	0.0004
	Europe	0.500	(0.430 - 0.579)	<0.0001	0.685	(0.569 - 0.823)	0.0001	0.901	(0.659 - 1.227)	0.5106
	Other	0.495	(0.426 - 0.576)	<0.0001	0.646	(0.536 - 0.777)	<0.0001	0.683	(0.504 - 0.925)	0.0140
	United States	1.000	(1.000 - 1.000)	.	1.000	(1.000 - 1.000)	.	1.000	(1.000 - 1.000)	.
Race	Asian	0.226	(0.159 - 0.317)	<0.0001	0.240	(0.168 - 0.341)	<0.0001	0.473	(0.290 - 0.803)	0.0039
	Black	0.663	(0.545 - 0.808)	<0.0001	0.628	(0.493 - 0.804)	0.0002	0.690	(0.474 - 1.020)	0.0565
	Other	0.598	(0.465 - 0.771)	0.0001	0.699	(0.521 - 0.944)	0.0180	0.782	(0.482 - 1.324)	0.3568
	White	1.000	(1.000 - 1.000)	.	1.000	(1.000 - 1.000)	.	1.000	(1.000 - 1.000)	.
Ethnicity	Hispanic or latino	0.528	(0.446 - 0.625)	<0.0001	0.545	(0.445 - 0.668)	<0.0001	1.121	(0.790 - 1.620)	0.5311
	Not hispanic or latino	1.000	(1.000 - 1.000)	.	1.000	(1.000 - 1.000)	.	1.000	(1.000 - 1.000)	.
Diabetes Duration	per year	0.983	(0.977 - 0.990)	<0.0001	0.981	(0.973 - 0.989)	<0.0001	0.982	(0.970 - 0.995)	0.0051

Table. Association between baseline characteristics, obesity and increased waist circumference (WC-ATPIII, WC-IISHMS) off-target.

ATPIII: National Cholesterol Education Program (Adult Treatment Panel III)

IISHMS: International Joint Interim Statement for the Harmonization of the Metabolic Syndrome criteria

Clinical Trial Registration Number: NCT01179048
Supported by: Novo Nordisk

1188

Health-related quality-of-life (HRQoL) implications of cardiovascular and hypoglycaemic events in type 2 diabetes mellitus

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Background and aims: The Saxagliptin Assessment of Vascular Outcomes Recorded in Patients with Diabetes Mellitus (SAVOR) -TIMI 53 trial was a multicenter randomized controlled phase IV trial of clinical and HRQoL outcomes in 16,492 T2DM patients with a previous cardiovascular event, or multiple risk factors for vascular disease. The analysis quantified the impact of major non-fatal clinical events on HRQoL (as measured by EQ5D utilities); and compared utilities over time according to whether patients experienced clinical events of interest.

Materials and methods: During the trial, EQ5D utility values were elicited annually and following a non-fatal clinical event, including hospitalizations for hypoglycemia, heart failure (hHF) and major cardiovascular events (myocardial infarction and stroke). Utilities were analyzed using linear mixed effects regression, adjusting for baseline characteristics (including EQ5D utility) and a time-dependent variable describing prior cardiovascular events.

Results: Most patients completed the EQ5D survey at baseline, 12 months, and closeout; 1,973 also completed the EQ5D after a clinical event. The mean EQ5D utility of the overall sample was 0.78 at baseline and all subsequent time points and did not vary by treatment arm. Despite no observed difference in mean HRQoL utility between treatment arms at planned observation points, an impact of cardiovascular events was observed: mean baseline, month 12, and closeout utilities among those with a cardiovascular event were 0.75, 0.72, and 0.71, respectively. After a cardiovascular event, mean utilities were 0.69, 0.69, and 0.72 (within 3 months, 3-6 months, and 6-12 months after the event, respectively). In the regression analysis adjusting for age, sex, treatment arm, and baseline HRQoL, utility decrements associated with cardiovascular events were -0.059, -0.045, and -0.037 over the same time periods; all coefficients were statistically significant. Utility decrements for specific cardiovascular events (MI, stroke and hHF) will be presented. Among 96 individuals who experienced a hospitalized hypoglycemic event, mean time between the event and next utility elicitation was 175 days; the adjusted utility decrement following the hypoglycemic event was 0.026, although this difference was not statistically significant.

Conclusion: In a large clinical trial T2DM population, no significant impact was observed by treatment arm for HRQoL, consistent with results reported elsewhere for clinical outcomes. However, cardiovascular events were associated with a significant decrease in HRQoL, most substantial in the initial post-event period. These data also suggest a potential relationship between hypoglycemic events and HRQoL; investigating this association would be best addressed by a study designed to include a sufficient number of hypoglycemic events. The temporal relationship observed between major cardiovascular events and utility trajectories highlights the need to elicit utilities proximal to occurrence of events of interest, in order to accurately and comprehensively quantify HRQoL burden.

Clinical Trial Registration Number: NCT01107886

PS 121 Metabolic consequences of obesity

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Insulin-like factor 5: a novel orexigenic hormone in humans is dysregulated in obesity

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Background and aims: Insulin-like factor 5 (INSL5), a member of the insulin superfamily, is expressed in the colorectum and hypothalamus. INSL5 deficient mice display impaired fertility and dysregulated glucose homeostasis. Based on the observation that INSL5 was elevated by prolonged calorie restriction and declined with feeding, it was suggested that INSL5 might be an orexigenic hormone. Our aim was to explore the relationship between circulating levels of INSL5 and different metabolic parameters in lean and obese subjects and to identify possible links between INSL5 and the development of metabolic disorders such as obesity and diabetes.
Materials and methods: INSL5 was measured in serum samples by ELISA (Cloud clone corp.). 20 lean females and males and 20 obese females and males were included. Furthermore 15 morbidly obese patients were tested before and six months after they underwent bariatric surgery. In addition we measured INSL5 levels in 10 lean and obese individuals after an overnight fasting, after a meal and during an oral glucose tolerance test (OGTT). For all groups, correlations between INSL5 concentrations and anthropometric, metabolic and hormonal measures were investigated.
Results: We found gender specific differences in INSL5 after fasting. INSL5 was significantly higher (by 25.5%, $p<0.05$) in healthy lean females than males but this gender specific difference was not observed in obese subjects. Basal INSL5 was significantly lower in the obese group (by 28.6%, $p<0.05$). Correlation analysis revealed that INSL5 was negatively correlated to testosterone in both lean and obese males ($p=0.001$, $r=-0.58$; $p=0.02$, $r=-0.52$) and to insulin in both genders. Six months after bariatric surgery 15 males lost 25% of their body weight that was associated with significant (by 97%) increase in serum testosterone and marked (by 17.4%) decline in serum INSL5. Obese subjects with type 2 diabetes (T2DM) had lower INSL5 before and after surgery compared to obese persons without T2DM. In patients without T2DM INSL5 levels decreased more (20.7%) compared to T2DM patients (14.8%). After a meal INSL5 decreased by 21.8% and 20.8% in lean females and males, respectively, while interestingly this effect was not observed in obese individuals of both genders. During an OGTT, after a pure sugar load, there was no significant effect in the lean group but a decrease in INSL5 in the obese subjects with insulin resistance.
Conclusion: Altogether, our data indicate that the gender-related difference in INSL5 was observed only in healthy lean but not in obese individuals. Thus suggesting that the regulation of INSL5 is dependent on the metabolic state in humans. Negative influence of insulin resistance and T2DM on serum levels of INSL5 in obese individuals may indicate a link between beta cell function and INSL5 regulation. Our finding that INSL5 levels were restored after weight loss let us to suggest a negative effect of fat tissue on the biosynthesis of this hormone in obesity. Therefore, INSL5 may become an interesting target for the development of new therapeutic agents to treat metabolic disorders in humans.
Supported by: ESPE Research Fellowship, IFB Adiposity Diseases

1190

Secretory products from epicardial adipose tissue from patients with type 2 diabetes impair mitochondrial respiration in cardiac myocytes via upregulation of miR-208a

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Background and aims: Alterations in cardiac energy substrate metabolism contribute to the development of diabetic cardiomyopathy. We have recently found that secretory products from epicardial adipose tissue (EAT) from patients with type 2 diabetes impair cardiac myocyte function as illustrated by the induction of insulin resistance, contractile dysfunction, and changes in microRNA expression, including miR-208a, a key regulator of energy metabolism. The present study examines whether EAT affects mitochondrial respiration in cardiac myocytes and whether this can be ascribed to the induction of miR-208a by angiotensin II.
Materials and methods: Biopsies from EAT from were collected from patients with and without type 2 diabetes and used to generate conditioned media (CM). Levels of angiotensin II in CM were determined by enzyme-linked immunosorbent assay. Mitochondrial respiration was measured in primary adult rat cardiac myocytes exposed to CM using a Seahorse XF96e analyzer. Gene and miRNA-expression as well as mtDNA copy number were measured by qRT-PCR. The cardiac mouse cell line HL-1 was used for transfection with precursor-miRNAs to investigate the impact of miRNAs on mitochondrial respiration and fatty acid oxidation. Protein expression and phosphorylation was measured by Western blot analysis.
Results: CM from EAT and from patients with type 2 diabetes (CM-EAT-T2D) increased the levels of miR-208a in both primary adult rat (1.8-fold, $p<0.05$) and primary human cardiac myocytes (1.6-fold, $p<0.05$) as compared to cells exposed to CM from patients without type 2 diabetes (CM-EAT-ND). The effects of CM-EAT-T2D on miR-208a induction were abolished by the angiotensin II receptor antagonist, losartan. Higher levels of angiotensin II were identified in CM-EAT-T2D compared to CM-EAT-ND (2-fold, $p<0.05$). Furthermore, CM-EAT-T2D reduced maximal mitochondrial respiration by 20% and spare respiratory capacity by 40% in primary adult rat cardiac myocytes. Accordingly, expression of the precursor for miR-208a reduced maximal mitochondrial respiration by 19% and spare respiratory capacity by 25% in HL-1 cardiac myocytes. Moreover, miR-208a reduced palmitate-induced maximal respiration by 22%. This decrease in mitochondrial function associated with a reduced phosphorylation of the regulatory beta subunit of AMP-activated protein kinase, and its substrate acetyl-CoA carboxylase. Finally, higher levels of miR-208a reduced mtDNA copy number and the expression of carnitine palmitoyltransferase 1, a regulator of mitochondrial β-oxidation.
Conclusion: Secretory products from epicardial adipose tissue from patients with type 2 diabetes impair mitochondrial respiration in cardiac myocytes via upregulation of miR-208a. This miRNA inhibits myocardial energy metabolism via the down-regulation of mitochondrial β-oxidation. Finally, the induction of miR-208a is prevented by the angiotensin II receptor antagonist losartan.
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The effect of a fat-rich mixed meal on metabolic and hormonal parameters, postprandial lipaemia as well as endothelial function in women with polycystic ovary syndrome

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Background and aims: Polycystic ovary syndrome (PCOS) is characterized by hyperandrogenism, chronic anovulation, and metabolic disorders like insulin resistance, glucose intolerance, dyslipidaemia and impaired endothelial function. No studies have yet investigated the metabolic response of these patients in the postprandial state. The aim of this study was to investigate the effect of consumption of a fat-rich mixed meal on metabolic and hormonal parameters as well as endothelial function in women with PCOS and to compare them with healthy controls.
Materials and methods: We studied 55 women with PCOS (age 26.0± 6.1 years; BMI 27.6±6.9 kg/m2), and 20 age and BMI matched healthy controls (age 26.5±4.3 years; BMI 25.15±7.76 kg/m2) who consumed a fat-rich mixed meal (783 kcal; 27.5% carbohydrates 52.5% fat, 20% protein). Sex hormones at times 0' and 6 h after the meal as well as glucose, insulin, total cholesterol, HDL, LDL and triglycerides at times 0', 2 h, 4 h and 6 h were measured. Additionally, all patients underwent an oGTT in a separate session, for the detection of insulin resistance using the HOMA, QUICKI, and MATSUDA indices. A subset of women (nPCOS=46 and nControls=12) underwent an endothelial function test using flow-mediated dilation and assessment of skin microcirculation using laser Doppler flowmetry.
Results: In the postprandial state, PCOS patients showed a significant reduction of Δ4-androstenedione ($p<0.001$) and a trend for reduction of free testosterone levels ($p=0.052$), a significant increase of dehydroepiandrosterone sulfate ($p=0.016$) and a trend for increase of sex hormone-binding globulin levels ($p=0.086$). We also found a trend for the triglyceride concentrations expressed as area under the curve (AUC) to be higher (464.79±158.55 vs. 587.72±287.81, $p=0.073$) and the AUC of insulin to be significantly higher (97.06±62.24 vs. 147.08±94.85, $p=0.014$) in women with PCOS compared to controls. Furthermore, patients with PCOS had worse endothelial function (brachial artery dilation of 8.52±5.80% vs. 4.78±6.42%, $p=0.036$) while skin microcirculation and insulin resistance indices were not different between the two groups.
Conclusion: In the postprandial state women with PCOS exhibited significant changes in their hormonal profile. In addition, PCOS compared to controls had a trend for higher postprandial lipaemia and worse endothelial function. These findings suggest that young women with PCOS have detrimental hormonal, metabolic and endothelial alterations. Therefore, they seem to be more susceptible to cardiovascular disease.

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Microvesicles from activated endothelial cells are decreased by weight loss and correlate with dyslipidaemia and cardiovascular risk in severe obesity

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Background and aims: Obesity, elevated triglycerides (TG) and low HDL-cholesterol are all factors related to the metabolic syndrome (MetSy), a state that includes increased cardiovascular risk. Endothelial CD36 is involved in TG clearance from TG-rich lipoproteins and up-regulated by oxidative stress, both potentially activating endothelial cells. Microvesicles (MVs) may be markers and mediators of endothelial dysfunction having impact on the cardiovascular state during obesity. The aim of this study was to evaluate the effect of weight loss on levels of total MV number, activated endothelial MVs (EMVs), and CD36 positive EMVs, and their correlation to HDL, TG, and MetSy.
Materials and methods: Twenty obese individuals (BMI of 43.0± 5.4 kg/m^2) were included. Informed consent was obtained and the study was approved by the local ethical committee. Fasting blood samples were collected at baseline and three months after weight loss. Plasma MV profile was analyzed in accordance to a published flow cytometric method by this group that includes the whole MV size range of 100-1000 nm. MVs were stained with Lactadherin-FITC, CD62e-PE, and CD36-APC antibodies. Statistical analysis included the Wilcoxon signed rank test and Spearman's Correlation test.
Results: Gastric bypass reduced BMI with 20% ($p<0.001$). Total MV number decreased by 39% ($p=0.025$), total EMV number by 47% ($p=0.002$), and CD36 positive EMVs by 73% ($p=0.004$). At baseline, total MV count correlated with TG (rho =0.5, $p=0.015$) and TG/HDL ratio (rho=0.6, $p=0.009$). Total EMVs correlated with HDL (rho=-0.6, $p=0.005$), TG (rho=0.5, $p=0.02$) and TG/HDL ratio (rho =0.8, 0.001). When data from baseline and after weight loss were pooled, total MV number was increased by 1.7 ($p<0.012$), total EMV by 1.74 ($p<0.067$), and CD36+EMV by 3.45 fold ($p<0.01$) in participants with MetSy.
Conclusion: Significant weight loss in extremely obese individuals leads to a decrease in total MV number. The decrease in EMVs and CD36+ EMVs may reflect reduced endothelial dysfunction and lower cardiovascular risk.
Supported by: The Novo Nordisk Foundation

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Cardiovascular safety of liraglutide: meta-analysis of major adverse cardiovascular events across weight management and type 2 diabetes development programmes

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Background and aims: The cardiovascular safety of liraglutide among overweight/obese individuals and those with type 2 diabetes (T2D) is unknown. We therefore performed a meta-analysis of all available individuals from five phase 2/3 liraglutide weight management trials, including follow-up through a 120-Day Safety Update. A meta-analysis of 21 trials from T2D development programmes, in which liraglutide was used as a treatment, provided further supportive information. The maximum dose of liraglutide investigated in the weight management programme was 3.0 mg, whereas the maximum dose was 1.8 mg in the T2D trials.
Materials and methods: The primary endpoint was first occurrence of adjudicated major adverse cardiovascular events (MACE) (non-fatal myocardial infarction, non-fatal stroke or CV death) on liraglutide (any dose) or pooled comparator (placebo, active) and was analysed using a Cox proportional hazards model stratified by trial. Prospective adjudication (blinded, independent) was implemented in three of the weight management trials; post-hoc adjudication was conducted for all other trials. Individuals who did not experience an event during the treatment period or within 30 days after last dose were censored at last treatment date plus 30 days. Multiple sensitivity analyses were conducted to confirm the robustness of the primary analysis. Across weight management trials

(liraglutide: n=3,872; comparator: n=2,036), baseline characteristics were: 71% women; history of CV disease, 9%; mean age, 47 years; mean BMI, 38 kg/m^2. Across T2D trials (liraglutide: n=5,511; comparator: n=2,748): 43% women; history of CV disease, 13%; mean age, 56 years; mean BMI, 30 kg/m^2.

Results: In the weight management trials, the overall number of adjudicated MACE was low and numerically lower with liraglutide (any dose: 10 events, frequency 0.2%, 0.2 events/100 patient-years of exposure [PYE]; liraglutide 3.0 mg: 7 events, 0.2%, 0.2 events/100 PYE) than with comparator (total comparator: 10 events, 0.5%, 0.4 events/100 PYE; placebo: 10 events, 0.5%, 0.4 events/100 PYE). Hazard ratios (HR) and 95% confidence intervals (CI)] for liraglutide (any dose) vs. total comparator: 0.40 [0.16; 1.01]; liraglutide 3.0 mg vs. placebo: 0.33 [0.12; 0.90]. As expected, higher event rates were observed across T2D trials: (liraglutide [any dose]: 26 events, 0.5%, 0.6 events/100 PYE vs. total comparator: 23 events, 0.8%, 1.3 events/100 PYE vs. total comparator): HR [95% CI]: 0.6 [0.35, 1.15].

Conclusion: Based on these data, there was no indication of an increased risk of MACE with liraglutide up to doses of 3.0 mg once-daily in overweight/obese individuals, or in those with T2D.

Supported by: Novo Nordisk

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Discrepancies in the relationship of BMI and traditional cardiovascular risk factors in subjects with different levels of obesity

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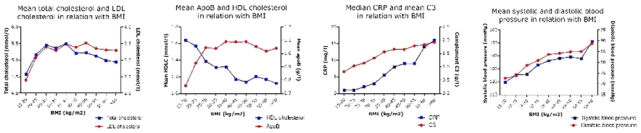
Background and aims: Obesity is related to increased cardiovascular risk. This relationship is based on the negative effects of body weight on several traditional risk factors. It remains unknown whether this association also holds at higher levels of obesity. The purpose of this study was to investigate this relation in a cohort with wide ranges of BMI.

Materials and methods: Lean subjects participating in observational studies in our out-patient clinic and obese subjects scheduled for bariatric surgery were included. Baseline characteristics and laboratory values were collected according to a standard protocol. Both, the group of lean subjects (i.e. BMI<30) and the group of obese subjects (i.e. BMI≥30) were categorized in quintiles according to BMI. The relation between BMI and cardiovascular risk factors were analyzed using ANOVA.

Results: The cohort consisted of 953 subjects (591 women, 362 men; mean age±SD, 49.9±14.8 years). The lean group consisted of 377 subjects, with a mean BMI of 25.0±2.81 kg/m2. 576 Subjects were included in the obese group, with a mean BMI of 43.8±7.58 kg/m2. The obese group included significantly more women, more T2DM, more HT and more smokers than the lean group. Mean values of LDLC, apolipoprotein B, systolic en diastolic blood pressure were significantly higher in the obese group and HDLC was significantly lower in the obese group. Diastolic blood pressure, apolipoprotein B and LDLC showed a significant dose-response relationship with increasing BMI in the lean group (p=0.002, p=0.005 and p=0.034, respectively), but this association was not observed in the obese group. In the lean group, triglycerides increased with increasing BMI (p=0.001) but decreased in the obese group (p=0.009). HDLC decreased with higher BMI in both groups, but the association was weaker in the obese. Inflammatory markers like CRP, leukocyte count and C3 increased with increasing BMI in the obese group (p<0.001 for all markers). In the lean subjects this association with BMI was also observed for C3 (p<0.001), but not for CRP and leukocytes.

Conclusion: Lipid associated risk factors showed a significant dose-response relationship with increasing BMI especially in lean subjects, but this association was weaker in obese subjects. The association with inflammatory markers and BMI was strongest in obese subjects. The relationship between cardiovascular risk factors and BMI is lost in higher levels of obesity. In that case, inflammation seems to be clinically more prevalent.

Figure 1: the relation of cardiovascular risk factors with BMI



PS 122 Cardiovascular complications in type 1 diabetes

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Type 1 diabetes as a coronary disease equivalent: an analysis of 33, 886 individuals with type 1 diabetes and 169,223 controls from the Swedish National Diabetes Registry

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Background and aims: Few studies have been performed on the contemporary incidence of myocardial infarction (MI) among patients with type 1 diabetes (T1D) in comparison to the general population. Whether diabetes represents a coronary artery disease equivalent remains controversial, and has not been specifically examined in patients with T1D.
Materials and methods: Individuals with T1D registered in the Swedish National Diabetes Register (NDR) after January 1st, 1998 (n=33,886) were included. For each patient registered in the NDR, 5 controls matched according to age, gender, and county were randomly selected from the general population (n=169,223). T1D individuals without history of MI were compared to controls with and without history of MI. Endpoints included MI, cardiovascular death and all-cause mortality. T1D individuals and controls were studied until occurrence of an endpoint or December 31st, 2011, through the Swedish In-hospital and Cause of death Registries. Analyses were performed with Cox regression adjusting for age and gender.
Results: The mean age of individuals with T1D and no history of MI (n= 33142) was 35.3 years and 35.6 years in corresponding controls (n= 168257). The mean age of controls with history of MI (n=966) was 62.5 years. The mean follow up period in T1D individuals without history of MI was 7.9 years. Mean follow up was 8.2 years and 6.8 years, respectively, in controls with and without history of MI. Hazard ratios (HRs) for MI and cardiovascular death for individuals with T1D and no history of MI versus corresponding controls were 4.6 (95% CI 4.31 to 4.95) and 3.09 (95% CI 2.78 to 3.42), respectively. The HR for all-cause mortality was 2.70 (95% CI 2.56 to 2.86). The corresponding age-adjusted HRs for T1D individuals without history of MI versus controls with history of MI were 1.20 (95% CI 1.02 to 1.42), 0.93 (95% CI 0.75 to 1.14) and 1.29 (95% CI 1.12 to 1.50).
Conclusion: Individuals with T1D without history of MI had greater risk of future MI and all-cause mortality but had similar risk for cardiovascular death compared with controls with history of MI. These results support the notion that T1D is a coronary disease equivalent.

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Decreased eGFR as a risk factor for heart failure in 13,781 patients with type 1 diabetes

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Background and aims: Heart failure (HF) is a disease with poor long time prognosis and is more prevalent among patients with type 1 diabetes. Decreased kidney function is a well-known risk factor in the general population but little is known of its importance in type 1 diabetes. This study investigates the association of eGFR (estimated GFR) and hospitalisation for heart failure in patients with type 1 diabetes
Materials and methods: Data were obtained from the Swedish National Diabetes Registry (NDR), a nationwide quality-assurance instrument for diabetes care, in conjunction with outcomes data from the Swedish hospital discharge and cause-specific death registries. A cohort of patients 18 years or older who had type 1 diabetes mellitus and no known HF and registred with at least one creatine value in the NDR between January 1998 and December 2003 was identified (N=13,718). Three equations were used to calculate eGFR and proportional hazards regression models were constructed to evaluate the association between eGFR and hospitalization for HF.
Results: Among 13,781 patients (mean age 41.1 (SD 13.3) years at baseline), 330 (2.4%) were hospitalised for HF over median follow-up of 7.0 years. Renal function was normal (eGFR >90 mL/min/1.73 m2) in 67% of patients according to the Cockcroft-Gault formula, compared to 51% and 41% according to the Chronic Kidney Disease Epidemiology (CKD-EPI) and Modification of Diet in Renal Disease (MDRD) formulas. For eGFR 45-60 ml/min/1.73 m2, hazard ratios (HRs) for hospitalisation (reference >90 mL/min/1.73 m2) were 3.18(95% CI 2.17;4.65), 2.12 (1.16;3.08), and 2.44 (1.69;3.55) using the Cockcroft-Gault, MDRD, and CKD-EPI formulas. In the group with even worse kidney function (eGFR <30 mL/min/1.73 m2) the HRs (95% CI) were respectively 3.78(2.15;5.91), 3.44(2.14;5.51) and 3.51(2.21;5.58)
Conclusion: In patients with type 1 diabetes, risk of hospitalisation for heart failure was over two times greater at eGFR 45-60 ml/min/1.73 m2 and more than 3 times greater at <30 ml/min/1.73 m2 when compared to normal eGFR
Supported by: ALF-agreement

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Incidence of atrial fibrillation in 33442 individuals with type 1 diabetes from the National Diabetes Registry compared to 167930 matched controls in Sweden from 2001-2011

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Background and aims: Atrial fibrillation (AF) is a condition that substantially increases the risk of stroke. Diabetes is a risk factor for many cardiovascular conditions, and studies of type 2 diabetes have shown that diabetes is associated with higher prevalence and incidence of AF. However the relation between type 1 diabetes (T1D) and AF has not been studied. The aim of this study was, therefore, to evaluate the relation between type 1 diabetes and atrial fibrillation.
Materials and methods: Individuals with T1D registered in the Swedish National Diabetes Registry (NDR) after January 1, 1998 were included in the study. For each individual with T1D, 5 controls matched on age, sex and county were randomly selected from the general population at time of registration in NDR. Because the diagnosis of AF was only available in the Outpatient Registry from 2001, evaluations were performed from January 1, 2001, and onwards. At baseline 253 (0.8%) individuals with T1D and 998 (0.6%) controls were excluded from further analysis due to previous AF diagnosis. After these exclusions, 33442 individuals with T1D and 167930 controls were followed using the Inpatient Registry and the Outpatient Registry with regards to AF until December 31, 2011. Mortality was assessed using the Cause of Death Registry.

Results: Mean age at baseline was 36.3 in individuals with T1D and 36.4 in controls, and 45.3% and 45.2% were women, respectively. During an average follow up of 7.37 years in the T1D group and 7.61 years in the control group, 578 (1.7%) individuals with T1D and 2353 (1.4%) controls were diagnosed with AF. The incidence rate per 1000 years was 2.35 and 1.84 in individuals with T1D and controls, respectively. The overall Hazard Ratio (HR) for individuals with T1D versus controls after adjusting for age and sex was 1.43 (95% CI, 1.31 to 1.57). When additionally adjusting for myocardial infarction (MI) and hospitalization for heart failure (HF) at baseline, the HR decreased to 1.29 (95% CI, 1.18 to 1.42). The HRs for individuals with T1D versus controls when adjusting for age was 2.66 (95% CI, 1.94 to 3.65) in women ≤50 years old, 1.56 (95% CI, 1.32 to 1.85) in women >50 years old, 1.64 (95% CI, 1.35 to 1.99) in men ≤50 years old and 1.13 (95% CI, 0.98 to 1.31) in men >50 years old. Notably, men older than 50 years had similar risk as controls, with no significant difference. The corresponding HRs with respect to age groups and sex when additionally adjusting for MI and HF was 2.66 (95% CI, 1.94 to 3.65), 1.40 (95% CI, 1.18 to 1.68), 1.61 (95% CI, 1.33 to 1.96) and 1.04 (95% CI, 0.90 to 1.20), respectively.
Conclusion: Type 1 diabetes is associated with a higher risk of AF in women all ages and in men 50 years or younger, but not in men older than 50 years. Further research is needed to confirm and explore reasons for this increased risk.
Supported by: Swedish Research Council, Swedish State, Swedish Society of Medicine + more

1198

Features of coronary artery disease in 2776 type 1 diabetes patients undergoing coronary angiography
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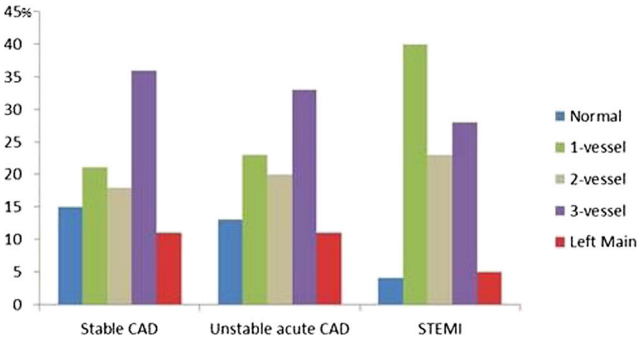
Background and aims: Individuals with diabetes have more widespread coronary artery disease (CAD) than those without which partly can explain their increased risk for cardiovascular death. However few studies have addressed type 1 diabetes in this context. The aim of this study was to assess features of coronary artery disease in type 1 diabetes undergoing coronary angiography.
Materials and methods: All patients undergoing coronary angiography during the years 2001-2012 included in the Swedish Coronary Angiography and Angioplasty Registry (SCAAR) as well in the Swedish National Diabetes Registry (NDR) with type 1 diabetes were included and followed for mortality until 31 December 2012. Type 1 diabetes was defined both with an epidemiological definition (onset age before 30 years and insulin treatment alone) and using the clinicians' diagnosis of type 1 diabetes and onset age before 50 years. The coronary angiogram was visually judged by the local coronary interventionist and divided into normal (atheromatosis/stenosis<50%), one-, two-, three- and left main-vessel disease.
Results: Of 2776 individuals with type 1 diabetes (58% male) and complete data on coronary angiogram, mean age was 57 years (SD 11), mean diabetes duration 35 years (SD 14, range 0-76) and mean HbA1c 67 mmol/mol (SD 14). The indications for coronary angiography were stable CAD (31%), unstable acute CAD (38%) and ST-elevation myocardial infarction (STEMI; 10%), heart failure (4%), atypical chest pain (5%), silent ischemia (3%) and other rare reasons. Coronary angiography revealed 21% without significant stenosis, 23% had one-vessel, 18% had two-vessel and 29% had three-vessel disease. Left main stem disease was present in 9%. Among those with stable CAD 15% had a normal angiography and 21% had one-vessel disease. The corresponding figures for unstable acute CAD were 13% and 23% (Figure).
Conclusion: In patients with type 1 diabetes the coronary angiogram was normal more often than expected or with only one-vessel affected despite a long diabetes duration. These findings might be encouraging for those who are involved in type 1 diabetes care.
Figure: Percentage of coronary artery disease by indication for the coronary angiography



Supported by: Swedish Heart-Lung Fdn., Dep. of Research and Development, Region Kronoberg

1199

Accelerated decline in brachial distensibility in women with type 1 diabetes: a cross sectional study
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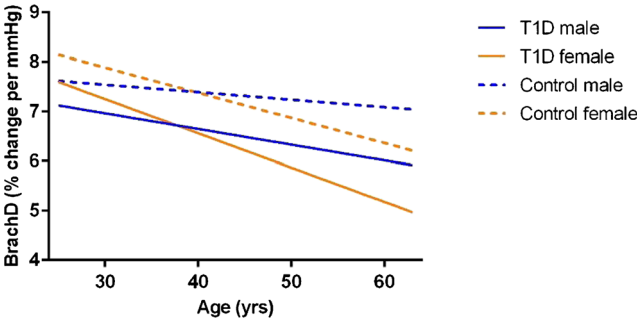
Background and aims: In patients with type 1 diabetes mellitus (T1D), cardiovascular disease (CVD) events are more common and occur earlier in life than in non-diabetics. Reduced brachial artery distensibility (BrachD), a non-invasive measure of vascular stiffness and early atherosclerosis, is an independent risk factor for development of CVD. A lower BrachD indicates increased arterial stiffness. Our aim was to determine if adults with T1D have lower BrachD compared to adults without diabetes and also to determine how age and gender affect the relationship of BrachD with T1D status. We also investigated the association of BrachD with coronary artery calcium (CAC), a marker of subclinical CVD.
Materials and methods: BrachD was measured using the Dynapulse instrument (PulseMetric, San Diego, CA) in 829 participants (352 with T1D, diabetes duration 29.4±8.8 years, age 43±9 years, range 25-63 years; 477 non-diabetics age 47±9, range 26-62 years) as part of the Coronary Artery Calcification in Type 1 Diabetes (CACTI) Study. CAC was measured twice within 5 minutes and averaged, and CAC volume was square root transformed (CVS) for linear regression analysis. An ANCOVA model was used to test the association of BrachD with age, sex, and T1D, and the significance of an age*sex*T1DM interaction. The correlation of BrachD with CAC was investigated with Spearman correlation coefficients, linear models, and logistic regression.
Results: Mean BrachD was lower in T1D patients vs. controls (6.43±1.46 vs. 7.16±1.48% change per mmHg, p<0.0001), indicating higher arterial stiffness. In a model adjusted for age, T1D, and sex, the interaction of age*T1D*sex was significant (p=0.0045). Younger women both with and without T1D had higher BrachD than men with and without T1D, but older women had lower BrachD compared to older men. Women with T1D had a steeper decline than nondiabetic women (Figure 1). BrachD was correlated with CAC score (r=-0.17, p<0.0001), but after adjusting for age and sex, neither CAC score nor CVS were associated with BrachD (p=0.95 and p=0.23, respectively).

Conclusion: BrachD is lower in T1D patients indicating increased vascular stiffness. Younger females have higher BrachD than males but the decline with age in BrachD is steeper for women and even greater in women with T1D. This greater change in BrachD with age seen in women with T1D is consistent with the increase in other CVD risk factors in women with T1D. BrachD may be an inexpensive, non-invasive method to ascertain increased CVD risk in this population. CAC was not associated with BrachD after adjusting for age and sex, suggesting that these markers of subclinical atherosclerosis may have different pathophysiologic pathways.



Supported by: NHLBI, JDRF

1200

Use of statins and risk of cardiovascular disease and death in type 1 diabetes: a report from Swedish NDR

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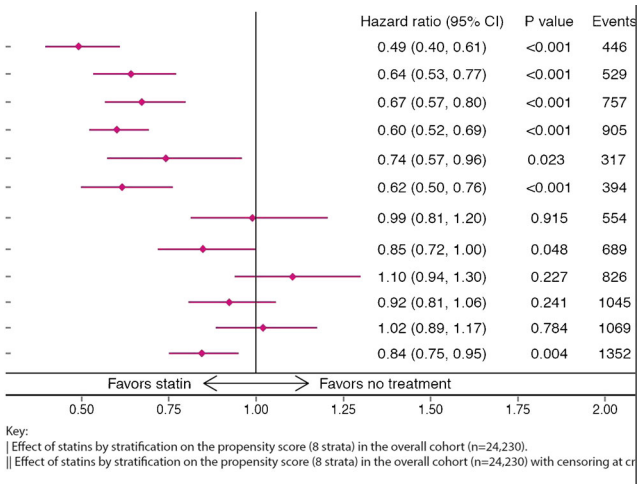
Background and aims: Individuals with type 1 diabetes (T1D) are at higher risk of cardiovascular disease (CVD) and death than the general population. Risk factor control is crucial and statin treatment is advocated by guidelines. Recommendations for persons with T1D proceed largely from extrapolation from other patient populations. The effect of statins in primary prevention among persons with T1D without previous CVD is unknown. Our aim was to examine the effect of statins on major cardiovascular events and death in T1D.
Materials and methods: We used the Swedish National Diabetes Register (NDR) to perform a propensity score based study. A total of 24,230 individuals (included during 2006-2008) with T1D without history of CVD were followed until 31/12/2012; 18,843 (53% male) were untreated and 5387 (57% male) treated with statins. Mean follow-up was 6.0 years with 146,553 person-years of follow-up. A propensity score for treatment with statins was estimated using 32 baseline variables, including socioeconomic factors, and used to balance the covariates in the two groups. Cox regression analysis was performed by stratifying (eight strata) on the propensity score, with and without censoring at cross-over (i.e. switching treatment group by starting or ending medication with statins).
Results: Unadjusted differences were marked at baseline between the groups but the propensity score allowed for balancing of all 32 covariates; there were no differences between treated and untreated after accounting for the propensity score. Figure 1 compares numbers of events and adjusted hazard ratios (HR) with 95% confidence intervals (CI) for CVD death, total death, for fatal/non-fatal acute myocardial infarction (AMI), coronary heart disease (CHD), stroke and CVD in patients with and without statin treatment in the overall cohort and in the cohort with censoring at cross-over.
Conclusion: This large observational study shows that treatment with statins reduces the risk of CVD death and total death as well as stroke in individuals with T1D without history of CVD.



1201

Mortality by affected coronary artery vessels in 2776 patients with type 1 diabetes undergoing coronary angiography

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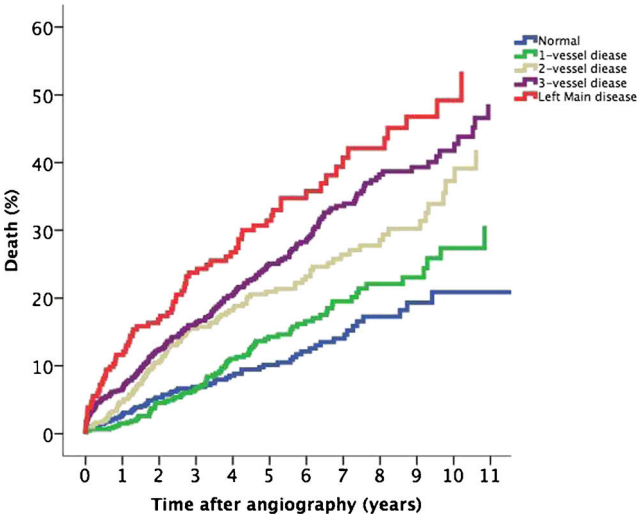
Background and aims: Individuals with diabetes mellitus have more widespread coronary artery disease (CAD) than those without which partly can explain their increased risk for cardiovascular death. However few studies have addressed type of diabetes in this context and rarely type 1 diabetes. The aim of this study was to assess long-term mortality by affected coronary vessels in type 1 diabetes undergoing coronary angiography.
Materials and methods: All patients undergoing coronary angiography during the years 2001-2012 included in the Swedish Coronary Angiography and Angioplasty Registry (SCAAR) as well as in the Swedish National Diabetes Registry (NDR) with type 1 diabetes were included and followed for mortality until 31 December 2012. Type 1 diabetes was defined both with an epidemiological definition (onset age before 30 years and insulin treatment alone) and using the clinicians' diagnosis of type 1 diabetes and onset age before 50 years. The coronary angiogram was visually judged by the local coronary interventionist and divided into normal (atheromatosis/stenosis<50%), one-, two-, three- and left main-vessel disease.
Results: Of 2776 individuals with type 1 diabetes (58% male); mean age was 57 years (SD 11), mean diabetes duration 35 years (SD 14, range 0-76) and mean HbA1c 67 mmol/mol (SD 14). Mean follow-up time was 7.2 years (SD 2.2). The most common indications for coronary angiography were stable CAD (31%), unstable acute CAD (38%) and ST-elevation myocardial infarction (STEMI) (10%). Patients with three- compared to one-vessel disease had longer diabetes duration (39 vs. 33 years) and lower onset age of diabetes (21 vs. 23 years) while actual HbA1c did not differ (67.2 vs. 66.8 mmol/mol). Mortality (Figure) was similar in those with normal and one-vessel diseases while those with two-vessel almost had comparable mortality rate to those with three-vessel disease.
Conclusion: In patients with type 1 diabetes admitted for coronary angiography mortality is increased by numbers of affected coronary vessels. Duration of diabetes seems more important than actual HbA1c for

numbers of affected coronary arteries. Figure: Time to mortality by affected coronary vessels after coronary angiography. Log-rank p=<0.0001.



Supported by: Swedish Heart-Lung Fdn., Dep. of Research and Development Region Kronoberg

1202

Cumulative smoking and the risk of acute myocardial infarction and stroke in patients with type 1 diabetes

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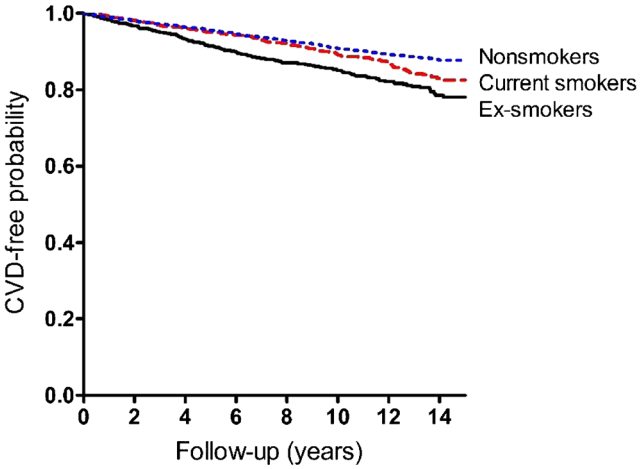
Background and aims: Previous studies addressing the association between smoking and cardio vascular disease (CVD) in patients with diabetes have mainly been focused on the CVD mortality and only few of them have included patients with type 1 diabetes. Our aim was to study the effect of cumulative smoking in pack-years on the risk of acute myocardial infarction (AMI) and stroke in patients with type 1 diabetes.
Materials and methods: The study included 3,545 patients with type 1 diabetes participating the FinnDiane (Finnish Diabetic Nephropathy) study. The smoking status was based on baseline questionnaires and patients were considered as current, ex- or nonsmokers and the amount of cumulative smoking was calculated in pack-years. The follow-up data for the development of AMI or stroke was based on The Care Register for Health Care and Causes of Death Register until the end of year 2012. The overall follow-up time was 39,481 person years and the median follow-up time was 12.0 (9.2-13.9) years. Cox-regression analyses providing HRs were used to estimate the risk for the development of AMI or stroke per each pack-year as continuous variable. Sex, duration of diabetes, systolic blood pressure, HbA1c and HDL cholesterol were used as covariates. The 14-year cumulative risk of combined AMI and stroke in current, ex- and nonsmokers was estimated with Kaplan-Meier analyses and log-rank test was used for comparison between the groups.
Results: One pack-year increased the risk of incident AMI with a HR of 1.027 (95% CI 1.016-1.037, p<0.0001) in current smokers and 1.032 (1.023-1.041, p<0.0001) in ex-smokers, compared with nonsmokers. In the multivariate model the HR of AMI was 1.011 (0.001-1.022, p<0.05) for current smokers and 1.009 (0.996-1.021, p=NS) for ex-smokers. The results were similar in stroke and the HRs were 1.029 (1.017-1.042, p<0.0001) in current smokers and 1.035 (1.025-1.045, p<0.0001) in ex-smokers compared with nonsmokers in the unadjusted model. In the adjusted model the HRs were 1.015 (1.001-1.028, p<0.05) and 1.019 (1.005-1.032, p<0.01) respectively. The combined 14-year cumulative risk of AMI and stroke was 17.1% (14.7-19.3, p=0.011) in current smokers and 21.4% (18.8-23.9, p<0.0001) in ex-smokers compared with 12.3% (10.9-13.7) in nonsmokers.
Conclusion: Smoking is a significant risk factor for CVD in patients with type 1 diabetes and the risk of developing CVD depends on the cumulative amount of smoked cigarettes. After the adjustments for other CVD riskfactors the risk for AMI in ex-smokers approaches the risk seen in nonsmokers. However, the risk for stroke is the highest in ex-smokers also after the adjustments.



Supported by: Folkhälsan RF, Stockmann F, Diabetes RF

PS 123 What can we learn from obese animals?

1203

Adipose-specific Dipeptidyl Peptidase 4 (DPP4) knockout mice display improved fasting insulin and smaller adipocytes on High Fat Diet (HFD)

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Background and aims: DPP4 is an important therapeutic target for type 2 diabetes mellitus. Our group has recently characterized this serine protease as a novel adipokine potentially linking obesity to the metabolic syndrome. By using a unique adipose-tissue (AT) specific DPP4 KO mouse model, we aimed to elucidate the role of adipose DPP4 in high fat diet-induced obesity.
Materials and methods: The AT-specific DPP4 KO mouse was generated using a Cre-lox strategy under control of the aP2 promoter on the C57BL/6J background. Metabolic phenotyping of mice on both chow and HFD (60% kcal fat) over 24 weeks was performed using Phenomaster metabolic cages, MRT, glucose and insulin tolerance tests (ipGTT, ipITT), as well as euglycemic-hyperinsulinemic clamps. DPP4 concentration and DPP4 activity in serum, DPP4 expression in mature adipocytes and stroma-vascular fraction (SVF) as well as plasma levels of glucose, insulin, triglycerides and cholesterol were measured. Adipocyte size was quantified by immunohistochemistry in subcutaneous (sWAT) and epididymal white adipose tissue (eWAT). Furthermore, gene expression was assessed in both depots by qRT-PCR.
Results: DPP4 expression in mature adipocytes from KO mice was reduced up to 65% ($p<0.01$) with unchanged expression in the SVF. Serum DPP4 was significantly lower in KO animals on both diets. KO mice gained significantly more weight, fat and lean mass on HFD. However, body fat percentage and length remained similar. The genotype exerted no effect on energy expenditure, respiratory quotient and spontaneous physical activity. ipGTT, ipITT and clamps were affected by HFD but not by genotype. On the other hand, fasting insulin and HOMA-IR were significantly lower in KO mice on HFD despite unchanged insulin sensitivity. Cholesterol was reduced in both KO mice on chow and on HFD compared to WT but triglycerides were similar. Within the HFD group, the KO mice showed a marked shift in the adipocyte size distribution towards smaller adipocytes. In both fat depots, differentiation markers remained similar whereas the M2 macrophage markers macrophage mannose receptor 1 and interleukin (IL)-10 were significantly upregulated in KO animals compared to WT under HFD. In eWAT, IL-6 and monocyte chemotactic protein 1 were significantly increased in KO mice. Serum DPP4 correlated significantly with adipocyte size in both subcutaneous and epigonadal AT while negatively with serum adiponectin.
Conclusion: This model proves that AT is an important source of DPP4 in mice. Although KO animals gained more weight and fat mass under HFD challenge, this is not followed by decreased glucose tolerance . Taken into account that KO animals display smaller adipocytes under HFD, these findings point towards a beneficial role for DPP4 deletion in adipose tissue remodeling during HFD. Nevertheless, how DPP4 deletion in adipose tissue translates into impaired metabolic performance in this mouse model must be further clarified.
Supported by: EFSD/MSD and FP7-EU-Marie Curie-IEF (ADDIO-PIEF-2012-328793)

1204

Spontaneous high fat diet overeating and body weight gain in selectively bred mice for diet-induced glucose intolerance: possible role of leptin

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Background and aims: Selective breeding is a powerful approach for studying the polygenic etiology of lifestyle-related diseases, such as type 2 diabetes and obesity. To investigate the pathogenesis of type 2 diabetes under high fat diet (HFD) conditions, we recently established 2 lines of mice with distinctively different susceptibilities to HFD-induced glucose intolerance by selective breeding; designated *s*electively bred *d*iet-induced *g*lucose intolerance-*p*rone (SDG-P) and -*r*esistant (SDG-R). In addition to the obvious glucose intolerance, SDG-P mice showed greater body weight gain, adiposity, and insulin resistance after HFD feeding as compared to SDG-R mice. Here, we investigated the differences in feeding behavior between the 2 lines of mice and further explored the mechanism underlying the different feeding behavior.
Materials and methods: Male SDG-P and SDG-R mice fed a HFD (32% energy fat) for 5 weeks (5-10 weeks of age). In a pair-feeding experiment, daily food intake of some SDG-P mice (pair-fed SDG-P) was kept the same amount of SDG-R mice during the HFD feeding period. Glucose tolerance was assessed before and after the HFD feeding by OGTT. Plasma leptin concentration was measured by ELISA. Leptin gene expression in epididymal adipose tissue was assessed by quantitative real-time PCR. Data are expressed as mean±SEM. Values of $p<0.05$ were considered statistically different between groups by Student's *t* test or ANOVA with Tukey's multiple comparisons.
Results: At 5 weeks of age (before the HFD feeding), no significant differences were shown in body weight, adipose tissue mass, locomotor activity, and oxygen consumption between SDG-P and SDG-R. In *ad libitum* feeding condition, SDG-P mice showed higher HFD intake and greater body weight gain than SDG-R [SDG-P (n=14) vs SDG-R (n=15): food intake, 4.3±0.4 vs 3.4±0.2 g/day ($p<0.001$); body weight after the 5-week HFD feeding, 36.8±4.3 vs 27.3±2.5 g ($p<0.001$)]. In the pair-feeding experiment, body weight gain of pair-fed SDG-P mice was completely suppressed to the level of SDG-R [27.1±1.5 g (n=14, $p=0.991$ vs SDG-R)]. Furthermore, HFD-induced deterioration of glucose intolerance in SDG-P mice was largely abrogated by the pair feeding. Despite the no significant differences in body weight and adipose tissue mass at 5 weeks of age, plasma leptin concentration of SDG-P mice was significantly lower than that of SDG-R mice [SDG-P (n=12) vs SDG-R (n=11): 1.7±0.7 vs 2.7±1.4 ng/ml ($p=0.039$)]. Leptin gene expression level was also lower in SDG-P mice [~50% of SDG-R (normalized to *Gapdh*), $p=0.016$]. In addition, exogenous leptin injections (50 μg/mouse twice daily for 3 days, i.p.) effectively reduced HFD intake in both lines of mice and the body weight gain of SDG-P mice was suppressed to the same level of SDG-R mice by the injections.
Conclusion: The present results demonstrate that the HFD-induced deterioration of glucose intolerance in SDG-P mice is the result of spontaneous overeating. The lower leptin levels before HFD feeding in SDG-P mice suggest a possible role of leptin as a determinant of later overeating, obesity, and the development of type 2 diabetes under HFD conditions.
Supported by: JSPS KAKENHI

1205

Methylglyoxal impairs high-fat diet-induced adipose tissue expansion causing systemic dysmetabolism

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Background and aims: Adipose tissue dysfunction is known to trigger systemic dysmetabolism in obesity and type 2 diabetes. The use of obese models consistently has shown hypoxia in adipose tissue, but previous studies did not fully explored the mechanisms involved. Many of them suggested adipocyte hypertrophy as the cause for hypoxia and dysmetabolism, due to lower oxygen diffusion. However, recent data supported the idea that instead of simple adipocyte hypertrophy, microvascular dysfunction could be the trigger for hypoxia and dysmetabolism. We previously demonstrated that methylglyoxal (MG), a byproduct of glucose metabolism, is able to induce glycation, microvascular dysfunction and inflammation, as well as impaired adaptation to decreased blood supply. This study investigates the role of MG-induced microvascular dysfunction in a context of high-fat diet-induced adipocyte hypertrophy and tissue expansion.
Materials and methods: We used animal models fed a high-fat diet (HFD), with MG-induced glycation (MG) or both (HFDMG) during 4 months, separating both variables in the experimental design, as well as a group of control (Ct) and diabetic Goto-Kakizaki (GK) rats fed a standard diet (n=6/ group). Animals were submitted to functional magnetic resonance imaging (fMRI) in order to assess tissue irrigation. Subsequently, animals were injected i.p. with the hypoxia probe pimonidazole (60 mg/Kg) and epididymal adipose tissue was collected for biochemical analyses.
Results: Rats submitted to MG administration (MG and HFDMG) showed higher accumulation of the MG-derived AGE N(epsilon)-(carboxyethyl) lysine (CEL) in adipose tissue, similarly to diabetic rats. Using fMRI imaging, lower accumulation of the contrast product was observed in high-fat diet-fed (HFD) and GK rats, but mostly in rats treated with MG alone (MG) and in HFD rats supplemented with MG (HFDMG). Moreover, pimonidazole adducts were significantly increased in the epididymal adipose tissue of HFDMG and GK rats, but not HFD or MG. In addition, decreased Perilipin A, a marker of adipocyte dysfunction, was observed in HFDMG rats. Regarding insulin signaling, despite no differences were observed in phosphorylated Akt, the HFD fed rats with MG supplementation (HFDMG) and GK rats, but not the HFD nor the MG groups, showed a significant decrease of the active form of insulin receptor (Tyr1163). These data were in accordance with the i.p. glucose tolerance test (IPGTT), where higher glucose intolerance (higher area under the curve) and hyperinsulinemia were observed in HFDMG rats.
Conclusion: Altogether, our results show that glycation induced by methylglyoxal conduces to microvascular dysfunction that impairs adipose tissue expansibility. In a context of diet-induced hypertrophy and adipose tissue expansion, such events lead to hypoxia, adipocyte dysfunction and insulin resistance, that have systemic repercussions in the glucose metabolism.
Supported by: FCT (Pest-C/SAU/UI3282/2011). Project DoIT – Diamarker (QREN- COMPETE)

1206

Glycation and high-fat diet trigger distinct functional consequences in the heart: an MRI study

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Background and aims: Cardiovascular diseases are one of the main consequences of obesity and type 2 diabetes. Among other factors, alterations of lipid metabolism and glucotoxicity are known to be involved in heart disease and decreased ability to adapt to an ischemic event. In the past, we showed that glucose-derived methylglyoxal (MG) is able to decrease the activation of survival pathways during heart ischemia, similarly to diabetic rats. More, we observed that such effects were specifically inhibited by the dicarbonyl scavenger drug pyridoxamine. Moreover, we described that diabetic rats fed a high-fat diet showed impaired response to ischemia. Thus, our goal was to investigate the mechanisms of heart dysfunction in diabetes and obesity, using a magnetic resonance imaging device specially designed for laboratory animals.
Materials and methods: Rats were maintained during 4 months with MG supplementation (100 mg/Kg/day) (MG), a high-fat diet rich in triglycerides (HFD) or both (HFDMG) and compared with controls feeding a standard diet (n=6/ group). The effects of MG-induced glycation and a high-fat diet regimen in several cardiac functional parameters were assessed using magnetic resonance imaging (MRI).
Results: High-fat diet-fed rats (HFD group) showed no significant alterations in heart function parameters, despite a trend to increased end-diastolic volume and end-systolic volume and heart weight was observed. On the other hand, accumulation of glycated products induced by MG supplementation (MG group) resulted in decreased stroke volume and cardiac output, as well as decreased peak filling rate. Interestingly, MG supplementation to high-fat diet-fed rats (HFDMG group) produced many of the functional consequences of MG and further decreased the peak ejection rate.
Conclusion: Using magnetic resonance imaging, our results suggest that moderate ventricular hypertrophy caused by the consumption of a high-fat diet is not associated with impaired heart functional parameters. On the other hand, the accumulation of MG-derived advanced glycation end products impairs filling and ejection rates, conducing to decreased cardiac output and stroke volume. This study sheds light on the mechanisms governing heart failure in obesity and type 2 diabetes, which may conduce to better therapeutic approaches in the future.

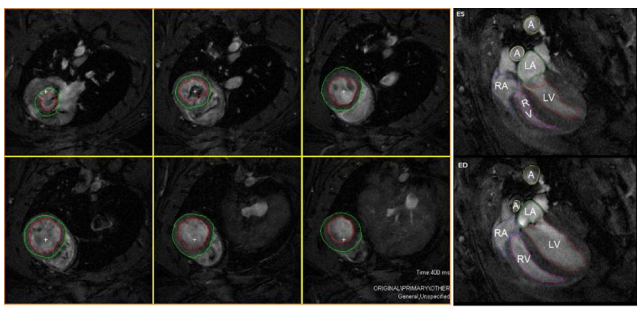

Figure1. Example of cardiac segmentation of the left ventricle (left) and 4 cameras (right).

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1207

PAR2 is upregulated by adipocyte-derived factors and high fat diet in the vascular wall

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Background and aims: Obesity is associated with impaired vascular function. However, the underlying mechanisms linking obesity and cardiovascular diseases are not fully understood. Protease activated receptor-2 (PAR2) is a G protein coupled receptor that can be activated by proteases as well as other activating peptides. Under physiological conditions, PAR2 exerts multiple functions such as controlling vascular tone and coagulation. In pathological conditions, PAR2 is upregulated in atherosclerotic lesions and promotes vasoconstriction. However, little is known about the impact of obesity on PAR2 in the vasculature. Our group has recently proposed that the adipokine soluble DPP4 (sDPP4) acts as an agonist for PAR2 leading to pro-atherogenic effects in human vascular smooth muscle cells (SMC). Thus, we aimed to investigate the impact of adipocyte-derived factors on the expression and function of PAR2 in the context of obesity-related vascular dysfunction.
Materials and methods: SMC were treated with conditioned medium (CM) from in vitro differentiated primary human adipocytes, IL-1β (10 ng/ml) or YKL-40 (100 ng/ml) for 24 h. Additionally, a time course with CM treatment was performed in SMC. In parallel, human coronary artery endothelial cells (HCAEC) were incubated with CM for 4 h. Protein and mRNA expression were assessed by Western Blot and qRT-PCR, respectively. BrdU incorporation was analyzed to determine SMC proliferation. Furthermore, C57BL/6 mice were fed with either a chow or a 60% high fat diet (HFD) for 24 weeks prior to isolation and subsequent mRNA extraction from aortas. DPP4 activity of serum samples from mice were analyzed with a DPP4 activity kit.
Results: We observed that CM treatment significantly enhanced PAR2 protein expression in both SMC and HCAEC (1.4-, 1.5- fold, respectively). Interestingly, PAR2 mRNA level in SMC showed a peak (1.7 fold) after 1 h of CM exposure. PAR2 protein level was increased in SMC by the adipokines IL-1β (1.8 fold) or YKL-40 (1.4 fold). Moreover, CM exerted a pro-atherogenic action on SMC since it promoted proliferation at 24 h. This observation was a PAR2-specific effect abrogated in the presence of the PAR2 antagonist GB83 (10 μM). In vivo, aortas of HFD-fed mice expressed significantly more PAR2 mRNA (1.5 fold) compared to aortas from lean mice. PAR2 upregulation in aortas was in parallel with an increase in the enzymatic activity of DPP4 in the serum of the animals treated with HFD (1.8 fold).
Conclusion: We showed that PAR2 is upregulated in two different vascular cell types in response to CM and in response to specific adipocyte-derived factors, leading to a pro-atherogenic effect. PAR2 expression levels were also elevated by HFD in murine aortas. In conclusion, under obesogenic conditions, where circulating levels of adipokines, such as IL-1β, YKL40 or sDPP4, are elevated, PAR2 arises as an important player linking obesity-related adipose tissue inflammation with atherogenesis.
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1208

Irisin improves endothelial function in obese mice through the AMPK-eNOS pathway

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Background and aims: Irisin is a novel hormone secreted by myocytes and has been proposed to mediate the beneficial effects of exercise on metabolism. Some studies reported that circulating irisin levels were positively associated with endothelium-dependent vasodilation in obese subjects and irisin may be involved in the regulation of endothelial function in obesity. Therefore, the objectives of this study were to explore whether irisin could improve endothelial function in obesity.
Materials and methods: C57BL/6 mice were given chow or high fat diet with or without treatment with irisin. Aortic endothelial function was determined by measuring endothelium-dependent vasodilatation (EDV). Nitric oxide (NO) in the aorta was determined. The effect of irisin on the level of phosphorylation of AMP kinase (AMPK), Akt and endothelial nitric-oxide synthase (eNOS) in endothelial cells were determined. Human umbilical vein endothelial cells (HUVECs) were used to study the role of irisin on AMPK-eNOS pathway.
Results: Treatment of obese mice with irisin improved glucose and lipids metabolism (glucose, 88.2±5.4 mg/dl vs 99.0±5.4 mg/dl; triglycerides, 83.54±12.60 mg/dl vs 124.54±16.83 mg/dl; NEFA 0.73±0.26 mmol/L vs 1.09±0.22 mmol/L; $P<0.01$), reduced serum levels of TNF-α (20.34 ±4.24 pg/ml vs 32.45±5.84 pg/ml, $P<0.01$), high sensitivity C-reactive protein (1.47±0.55 vs 2.84±0.83 mg/L, $P<0.01$), malondialdehyde (3.16 ±0.45 umol/L vs 5.33±0.72 umol/L, $P<0.01$) and increased serum adiponectin levels (16.65±1.13 ug/ml vs 14.35±1.16 ug/ml, $P<0.01$). Acetylcholine-stimulated EDV was significantly lower in obese mice compared with control mice (maximum vasodilatation rate, 39.01%±7.96% vs 82.31%±5.18%, $P<0.05$). Treatment of obese mice with irisin significantly enhanced EDV and improved endothelial function (63.23%±7.97% vs 39.01%±7.96%, $P<0.05$). This beneficial effect of irisin was partly attenuated in the presence of compound C (AMPK inhibitor), API-2/triciribine (Akt inhibitor) and L-NAME (eNOS inhibitor). Treatment of obese mice with irisin enhanced NO production (19.40±3.20 umol/L vs 10.24±2.30 umol/mg, $P<0.01$), phosphorylation of AMPK, Akt and eNOS in endothelial cells ($P<0.05$). Incubation of the HUVECs with irisin induced NO production in a dose- dependent and time-dependent manner ($P<0.05$) and also enhanced phosphorylation of AMPK, Akt and eNOS ($P<0.05$). Treatment with L-NAME abolished irisin-induced NO production ($P<0.05$). Suppression of AMPK expression by siRNA blocked irisin-induced Akt and eNOS phosphorylation and abolished irisin-induced NO production (92.40±12.30 umol/L vs 162.3±13.20 umol/L, $P<0.01$).
Conclusion: We have provided the first evidence that irisin improved endothelial function in aortas of high-fat diet induced obese mice. The mechanism for this protective effect is related to the activation of the AMPK-eNOS signaling pathway.
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Olfactory-bulbar prokineticin-2 is involved in the regulation of food intake and energy homeostasis, and proceeds through a GLP-1R dependent mechanism

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Background and aims: Food intake is influenced by olfactory cues and metabolic status can affect the olfactory function of animals and regulate feeding behavior. Prokineticin-2 (PK2) is a hypothalamic anorectic neuropeptide that is also highly expressed in the olfactory bulb (OB) as well as GLP-1 receptor (GLP-1R). Our study was aimed to assess the role of olfactory-bulbar PK2 in regulation of food intake and energetic homeostasis, in relation with GLP-1R signaling pathway.
Materials and methods: To that end, expression of both PK2 and GLP-1R mRNA was measured in fed or fasted mice either under control diet (CD) or high fat diet (HFD). In another serial, spontaneous food intake was measured in fasted mice injected with recombinant PK2 (rPK2) +/- Exendin9 (Ex9) in the OB.Energetic homeostasis was assessed after 5 weeks of down-regulation of OB-PK2 using a specific shRNA.

Results: We first evidenced that PK2 mRNA levels in the OB of C57Bl/6j mice depend on both the nutritional (fed vs fasted) and the metabolic (lean vs obese) status of the animal. Indeed, fasting resulted in a decrease of PK2 mRNA in lean mice and a significant increase of PK2 mRNA in obese mice. Moreover, we confirmed that GLP-1R mRNA was present within the OB and increased by fasting. Injection of rPK2 in the OB significantly decreased food intake during 24 h post-surgery compared to control animals, in both mice fed with CD or HFD. Inhibitory effect of rPK2 injected in the OB was lost when Ex9 was co-infused. Additionally, we injected rPK2 in the OB of GLP-1R-KO mice and could not detect any anorectic effect. Moreover, a chronic down-regulation of OB-PK2 led to variations of GLP-1R mRNAs in both the OB and the HT, and also altered energy homeostasis of animals.
Conclusion: We conclude that olfactory-bulbar PK2 reduces food intake through a GLP-1R dependent mechanism.
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