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Relationship between autonomic function and parameters of beta cell secretion across the entire spectrum of glucose homeostasis

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1. ABSTRACT

Background: type 2 diabetes is determined by a reduction of β cell mass and function besides a defect in insulin sensitivity. It was demonstrated that pancreatic islets are innervated by sympathetic nervous system (SNS) and parasympathetic nervous system (PNS) fibers and autonomic function contributes to the regulation of glucose homeostasis. An alteration in neuronal control of β cell function could be involved in the pathogenesis of the type 2 diabetes.

Aim: we focused on finding a possible association between autonomic function and the different parameters that describe β cell function in The Maastricht Study, a population-based cohort. We sought this association also in the population of Verona Newly Diagnosed Type 2 Diabetes Study (VNDS), a study of patients with newly diagnosed type 2 diabetes.

Research design and Methods: in the Maastricht study population from 24-h electrocardiogram we derived Heart Rate Variability (HRV) time and frequency domains (individual z-scores, based upon seven and six variables, respectively). From a standard 2-hour 75 g OGTT we estimated different aspects of β cell function, i.e. C-peptidogenic index t0-30, overall insulin secretion, β cell glucose sensitivity, β cell potentiation factor, and β cell rate sensitivity, using formula-based methods and mathematical modeling.

In the VNDS study cardiovascular autonomic function was assessed by a computerized system which analyzed heart rate and blood pressure variations during lying to standing (LS), deep breathing (DB), and Valsalva maneuver (VM), following the criteria presented by Ewing and Clarke. From a 5-hour 75g OGTT we estimated through mathematical modelling two main parameters of beta cell glucose sensitivity: derivative (first phase) and proportional control (second phase) of insulin secretion.

Results: in the Maastricht study we analyzed 2007 individuals with a mean±standard deviation (SD) age of 59.8 ±8.2 years, of whom 52% were men and 24% with type 2 diabetes (oversampled by design). After adjustment for age, sex, educational level and Matsuda index, time and frequency domain HRV were significantly and directly associated with C-peptidogenic index, β cell glucose sensitivity and β cell potentiation factor, but not with overall insulin secretion. Then, further adjustment for cardiovascular risk factors (model 4) did not

materially alter these associations, though only the association of HRV with C-peptidogenic remained statistically significant (standardized β [95%CI] per 1-SD increment in HRV TIME domain, for respectively C-peptidogenic index, overall insulin secretion, β cell glucose sensitivity, and β cell potentiation, 0.05 [0.00; 0.09]; 0.04 [-0.00; 0.08]; 0.04 [0.00; 0.08] ; and 0.04 [-0.00; 0.08]; standardized β [95%CI] per 1-SD increment in HRV FREQUENCY domain, for respectively C-peptidogenic index, overall insulin secretion, β cell glucose sensitivity, and β cell potentiation, 0.05 [0.00; 0.09]; 0.04 [-0.00; 0.08]; 0.04 [0.00; 0.08] ; and 0.04 [-0.00; 0.08]). HRV time and frequency domain weren't significantly associated with rate sensitivity.

Furthermore, we evaluated data of 537 patients with newly diagnosed type 2 diabetes with a mean ± SD age of 58.3 ± 9.6 of whom 66.3% were male. 91 subjects (16.9%) showed at least one abnormal test used to evaluate cardiovascular autonomic function (CAN). We found a worse derivative control of beta cell function in people with signs of cardio autonomic neuropathy as compared to the other group. This difference however did not reach statistical significance (p=0.063).

Conclusion: In summary, in the present research we analyzed a possible association between autonomic function and β cell secretion, estimated from OGTT. We found that autonomic dysregulation could contribute to β-cell dysfunction, in particular affecting the first phase of insulin secretion. This mechanism could add to the other factors that lead to the impairment of glucose homeostasis.

1.1 Riassunto

Il diabete di tipo 2 è determinato da una riduzione sia della funzione che della massa β cellulare in associazione ad un difetto di sensibilità all'insulina. È stato dimostrato che le isole pancreatiche sono innervate dal sistema nervoso simpatico e dal sistema nervoso parasimpatico e la funzione autonomica contribuisce alla regolazione dell'omeostasi del glucosio. Un'alterazione nel controllo neuronale della funzione delle cellule β potrebbe essere coinvolta nella patogenesi del diabete di tipo 2.

Obiettivo: in questa ricerca abbiamo studiato una possibile associazione tra la funzione autonomica e i diversi parametri che descrivono la funzione delle cellule β all'interno del Maastricht study, un ampio studio di cohorte. Abbiamo inoltre studiato tale associazione anche nella popolazione di Verona Newly Diagnosed Type 2 Diabetes Study (VNDS), uno studio composto pazienti con diabete di tipo 2 di nuova diagnosi.

Metodi: nella popolazione del Maastricht Study abbiamo ottenuto dall'elettrocardiogramma delle 24 ore i parametri di variabilità della frequenza cardiaca (Heart Rate Variability: HRV) e li abbiamo utilizzati per costruire un "dominio tempo" e un "dominio frequenza" (calcolando uno z score, basato rispettivamente su sette e sei variabili). Da un OGTT standard di 2 ore con 75 g di glucosio abbiamo stimato i diversi parametri della funzione β cellulare e cioè: indice C-peptidogenico, secrezione totale di insulina, sensibilità della β cellula al glucosio, fattore di potenziamento della β cellula e controllo derivativo della secrezione insulinica (rate sensitivity) utilizzando metodi basati su formule e un modello matematico.

Inoltre all'interno del VNDS la funzione autonomica è stata valutata da un sistema computerizzato che ha analizzato le variazioni della frequenza cardiaca e della pressione sanguigna durante la posizione eretta, la respirazione profonda e la manovra di Valsalva, secondo i criteri di Ewing e Clarke. Da un OGTT prolungato di 5 ore abbiamo stimato attraverso modello matematico due parametri della sensibilità al glucosio delle beta cellule: controllo derivativo (o di prima fase) e controllo proporzionale (o di seconda fase) della secrezione di insulina.

Risultati: abbiamo studiato 2007 individui con un'età media ± deviazione standard (DS) di 59,8 ± 8,2 anni, di cui il 52 % erano uomini e il 24% era affetto da diabete di tipo 2 (maggiormente rappresentati come da disegno dello studio). Dopo aggiustamento per età, sesso, livello di istruzione e indice di Matsuda, il dominio tempo e frequenza dell'HRV erano positivamente e significativamente

associati all'indice C-peptidogenico, alla sensibilità al glucosio delle β cellule e al fattore di potenziamento delle β cellule, ma non alla secrezione totale di insulina. Inoltre, un ulteriore aggiustamento per i fattori di rischio cardiovascolare (modello 4) non ha alterato sostanzialmente queste associazioni, sebbene solo l'associazione di HRV con l'indice C-peptidogenico sia rimasta statisticamente significativa (β standardizzato [95%IC] per incremento di 1-DS nel dominio tempo di HRV, rispettivamente per l'indice C-peptidogenico, la secrezione complessiva di insulina, la sensibilità al glucosio delle cellule β e il fattore di potenziamento delle cellule β, 0,05 [0,00; 0,09]; 0,04 [-0,00; 0,08]; 0,04 [0,00; 0,08] e 0,04 [-0,00; 0,08]; β standardizzato [IC 95%] per incremento di 1 DS nel dominio frequenza di HRV, rispettivamente per indice C-peptidogenico, secrezione complessiva di insulina, sensibilità al glucosio delle cellule β e fattore di potenziamento delle cellule β, 0,05 [0,00; 0,09]; 0,04 [- 0,00; 0,08]; 0,04 [0,00; 0,08] e 0,04 [-0,00; 0,08]). Il dominio tempo e frequenza dell'HRV non erano significativamente associati al controllo derivativo della funzione β cellulare.

Inoltre, nello studio del VNDS sono stati valutati i dati di 537 pazienti con diabete di tipo 2 di nuova diagnosi con un'età media ± DS di 58,3 ± 9,6 di cui il 66,3% erano maschi. Novantuno soggetti (16,9%) presentavano un'alterazione in almeno un test usato per valutare la neuropatia cardioautonomica. In questi soggetti abbiamo trovato una secrezione insulinica di prima fase più bassa (controllo derivato) rispetto agli altri. Questa differenza tuttavia non raggiungeva la significatività statistica (p=0,063).

Conclusioni: in sintesi, nella presente ricerca abbiamo analizzato una possibile associazione tra funzione autonomica e secrezione β cellulare. Abbiamo trovato che una disregolazione del sistema autonomo potrebbe contribuire alla disfunzione delle cellule β, in particolare impattando sulla prima fase della secrezione di insulina. Questo meccanismo potrebbe aggiungersi agli altri fattori che portano alla compromissione dell'omeostasi del glucosio.

2. INTRODUCTION

2.1 Epidemiology of type 2 diabetes

The worldwide explosion of obesity has resulted in an increasing prevalence of type 2 diabetes. Without concerted efforts to address the pathogenesis and treatment of this syndrome, the harmful macrovascular and microvascular outcomes of type 2 diabetes will remain a major burden for the next decades. Globally, about 537 million individuals between the ages of 20 and 79 are affected by diabetes, and the prevalence in this age group is 9.3%, with a slightly higher prevalence in men than in female (9.6% in men and 9.0% in women). It was demonstrated that there is a progressive increase in prevalence with increasing age: from a prevalence of 1.4% in the range of 20-24 years to a prevalence of 19.9% in the range of 75-79 years.

In the last years there was an important spread of the disease, so much that in the last 20 years the number of cases tripled: in 2000 there were about 151 million cases, in the 20-79 age range. Therefore, if the trend will be the same, in 2045 about 700 million cases will be expected (Figure 1, IDF Diabetes Atlas, 10th edn). The increase in prevalence represents a global phenomenon, which affects not only industrialized countries, but also developing countries; the latter, concomitantly with the improvement in economic conditions, seem to be the regions most affected by the increase in the number of cases (Zheng Y et al, 2018).

A serious problem is represented by undiagnosed cases: it is estimated that in 2019 one out of every two people, in the 20-79 year-old range, did not know that they had diabetes mellitus. It must be said that these percentages vary according to the regions considered, in fact in Africa the percentage of undiagnosed cases is around 60%, while it drops to 34% in North America (Unnikrishnan R. et al, 2017). Geographical constraints, such as vast rural areas, and limited resources probably make access to care more difficult, thus contributing to the increase in this percentage in African, Southeast Asian and Western Pacific countries (IDF Diabetes Atlas, 10th edn).

2.2 Etiology of Type 2 Diabetes

Genes and the environmental factors together are important determinants of the onset of type 2 diabetes. Genetic determinants exert their effect following exposure to an environment characterized by sedentary behavior and high-calorie intake. Advances in technology and analytical approaches have led to the discovery of genes linked to type 2 diabetes (Prasad RB et al, 2019). Using the candidate gene approach, PPARγ was the first gene identified (Sarhangi N. et al,2020). Since then, using largely genome-wide association studies (GWAS), over 400 genetic variants have been associated with type 2 diabetes, the vast majority linked to the β-cell (McCarthy MI, 2010). Some are related to known gene products, but for most of these genes the products have not yet been identified. Together these genes only explain 18% of the risk of type 2 diabetes (Mahajan A et al, 2018). In addition, the risk associated with individual variants is very modest (usually<1.2), with the exception of a variant in the TCF7L2 gene (Lyssenko V et al, 2007). This could be attributed to variability within the type 2 diabetes phenotype. A more precise phenotype definition could goa long way in explaining the missing heritability (Prasad RB et al, 2019).

Besides the obvious increases in caloric intake and decreased energy expenditure, other environmental factors seem to be important. Nutrient composition, specifically increased consumption of dietary fat and saturated fat are important in determining the development of obesity, insulin resistance, β-cell dysfunction and glucose intolerance (Rice Bradley BH et al, 2018). Furthermore, with increasing age, the aging of β cells determines the reduction in the β-cell's responsiveness to carbohydrate. The in utero environment, determined in part by the mother's body habitus, may well produce epigenetic and gene expression changes that determine the risk of the offspring to development of obesity and type 2 diabetes (Yajnik CS, 2009). Recent studies have also focused on the role of environmental chemicals in the obesity and diabetes epidemics (Thayer KA et al 2012).

2.3 Pathophysiology of type 2 diabetes

Abnormally high glucose levels in blood result from a malfunctioning of the feedback loops between insulin action and insulin secretion (Kahan et al 1993). In the case of β -cell dysfunction, insulin secretion is reduced, limiting the body's capacity to maintain physiological glucose levels. On the other hand, insulin

resistance contributes to increased glucose production in the liver and decreased glucose uptake both in the muscle, liver and adipose tissue (Weyer et al, 1999). Even if both processes take place early in the pathogenesis and contribute to the development of the disease, β-cell dysfunction is usually more severe than IR. However, when both β-cell dysfunction and insulin resistance are present, hyperglycaemia is amplified leading to the progression of type 2 diabetes. Beside the reduction of insulin secretion and insulin sensitivity there are other factors that contribute to the onset of type 2 diabetes that all together constitute the known 'ominous octet' (R.A. DeFronzo, 2009).

Insulin secretion

It is known that the progression from normal glucose tolerance to prediabetes and type 2 diabetes is a continuum in which besides the presence of insulin resistance the continuous decline in insulin secretion from β-cells determines the progressive increase in glucose, even across the normal range (Kahan, 2009).

The different mechanisms leading to the gradual deterioration of β-cell insulin secretion are matter of intense investigations. β-cell dysfunction has been traditionally associated with β-cell death (Christensen, A.A et al, 2019). However, recent evidence suggests that the dysfunction of β-cells in T2DM might be due to a more complex network of interactions between the environment and different molecular pathways implicated in cell biology (Halban, P.A., 2014). In an excessive nutritional state hyperglycemia and hyperlipidemia are often present, favoring insulin resistance and chronic inflammation. Under these circumstances, β-cells, due to differences in their genetic susceptibility, are subject to toxic pressures including inflammation, inflammatory stress, endothelial reticulum (ER) stress, metabolic/oxidative stress, amyloid stress, with the potential of ultimately leading to a loss of islet integrity (Christensen A.A et al, 2019). An excess of Free Fatty Acids (FFAs) and hyperglycemia determine β cell dysfunction by inducing ER stress through the activation of the apoptotic unfolded protein response (UPR) pathways (Yamamoto, W.R et al 2019). In fact, lipotoxicity, glucotoxicity and glucolipotoxicity occurring in obesity, cause metabolic and oxidative stress that leads to β-cell damage (Meyer JJ and Bonadonna RC, 2013). Stress derived from high levels of saturated FFAs can activate the UPR pathway by several mechanisms including inhibition of the sarco/endoplasmic reticulum Ca^{2+} ATPase (SERCA) responsible for ER Ca^{2+} mobilization; activation of IP3 receptors or direct impairment of ER homeostasis. In addition, sustained high

glucose levels increase proinsulin biosynthesis and islet amyloid polypeptides (IAAP) inside the β-cells, leading to the accumulation of misfolded insulin and IAAP and increasing the production of oxidative protein folding-mediated reactive oxygen species (ROS) (Yamamoto W.R et al, 2019). These effects alter physiological ER Ca^{2+} mobilization and favor proapoptotic signals, proinsulin mRNA degradation and induce interleukin (IL)-1 β release that recruits macrophages and enhances local islet inflammation (Christensen A.A et al, 2019) As previously mentioned, insulin secretion has to be finely regulated to precisely meet metabolic demand. Indeed, proper islet integrity must be conserved in order to allow β-cells to respond to metabolic needs. Under pathogenic conditions, the mechanism described above can ultimately lead to disruption of islet integrity, impairing optimal cell-to-cell communication within pancreatic islets, contributing to alter the regulation of insulin and glucagon release and ultimately exacerbating the hyperglycemia. Defects in the synthesis of any insulin precursors, or insulin itself, as well as disruption of the secretion mechanism, can lead to insulin secretory dysfunction, the primary driver of β-cell failure that conduce to the onset of type 2 diabetes (Meyer JJ and Bonadonna RC, 2013).

Insulin resistance

Insulin resistance can be defined as the inability of insulin to produce its usual biological actions at circulating concentrations that are effective in normal subjects (Dali-Youcef N et al, 2013). Insulin resistance in the context of glucose metabolism leads to impaired suppression of endogenous glucose production, under basal conditions as well as after eating, when the physiological rise in insulin in response to glucose entry from the gut normally shuts down glucose production by the liver, and to reduced peripheral uptake of glucose. These alterations result in hyperglycaemia and a compensatory increase in insulin secretion (Czech, M.P. 2017). Resistance to the ability of insulin to suppress very-low-density lipoprotein (VLDL) production from the liver enhances circulating serum triglycerides, which, in turn, leads to a decrease in high-density lipoprotein (HDL) cholesterol and formation of atherogenic, small, dense, low-density lipoprotein (LDL) particles. Resistance in adipose tissue increases the flux of non-esterified fatty acids (NEFA) both to the liver and skeletal muscle, and reduces the action of insulin on glucose metabolism in these tissues (Choi S.H. et al, 2011).

Other factors that lead to the onset of type 2 diabetes

Fasting plasma glucagon levels are inappropriate high in the context of hyperglycaemia and hyperinsulinaemia in type 2 diabetes, and contribute to the increased rate of hepatic glucose output characteristic of type 2 diabetes (Dunning BE et al, 2007). Normal suppression of glucagon following a carbohydrate or mixed meal is blunted in patients with type 2 diabetes. Dysregulated α-cell release of glucagon, manifests as increased fasting glucagon concentrations and a failure to adequately suppress glucagon secretion following meal ingestion, contributes to the development of hyperglycaemia.

The gastrointestinal tract produces a variety of peptides, not all of which directly modulating nutrient absorption. Glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP), known as the "incretins", act on the pancreatic islet. GLP-1 acts on both β- and α-cells to enhance insulin and suppress glucagon secretion, respectively (Drucker DJ et al, 2006). Plasma GLP-1 levels are not in general different in individuals with normal glucose tolerance, impaired glucose tolerance or type 2 diabetes. Therefore, the problem is that the β-cell response to GLP-1 following meal ingestion is deficient, as shown following intravenous administration of GLP-1 under controlled conditions (Nauck MA et al, 2011). This defect is not a primary cause of type 2 diabetes since it can be partly restored by improved glycemic control and is only observed in the diabetic twin of identical twins discordant for type 2 diabetes (Holst JJ et al, 2009). Therapeutic approaches for enhancing incretin action include degradation resistant GLP-1 agonists (incretin mimetics) and inhibitors of dipeptidyl peptidase 4 (DPP4) activity (DPP4 inhibitors).

The intestinal microbiome also appears to be important in the pathophysiology of type 2 diabetes. The microbiome contains about 100 times the genetic information of the human genome and together these comprise the human metagenome. Many products of the microbiome provide functions beyond that of the host genome, thereby serving an important role in human physiology. These gut communities are thought to play an important role in a number of conditions including obesity and type 2 diabetes, although which bacterial species may be involved in altering human metabolism remains to be determined (Diamant M et al, 2011).

Finally, an adaptive response by the kidney to conserve glucose, which is essential to meet the energy demands of the body, can be altered in the diabetic patient. Instead of dumping glucose in the urine to correct the hyperglycemia, the kidney tends to hold on to the glucose. Even worse, the ability of the diabetic kidney to reabsorb glucose appears to be augmented by an absolute increase in the renal reabsorptive capacity for glucose (De Fronzo RA et al, 2008).

2.4 Autonomic activity on glucose regulation

The ability of sensing glucose is of great importance for maintaining an optimal glucose homeostasis. Therefore, it is not surprising that the brain, which is the most important center of control of our body, uses glucose sensing information as a major signal for integrating the neural activity and regulating the whole body metabolism (Levin et al. 1999; Thorens B, 2014). Evidence of this important glucose regulation mechanism dates back to the work of the physiologist Claude Bernard, who for the first time showed a causal relationship between brain stimulation of the fourth ventricle in the hindbrain and an increase in plasma glucose levels (Bernard C, 1865).

Glucose sensing cells can be found in the mouth, the hepatoportal vein area, the brainstem and the hypothalamus and are linked to each other by nervous connections. These neurons can be either glucose excited or glucose inhibited neurons that increase and decrease, respectively, their action potential frequency as extracellular glucose levels vary throughout the physiological range (Wang et al. 2004). They are thought to act activating or inhibiting either the parasympathetic and sympathetic nervous system that innervate pancreatic α and β cells, regulating their hormonal secretion and cell number (Alonge KM et al, 2021). (Figure2)

The activity of sympathetic nervous system (SNS) or parasympathetic nervous system (PNS) fibers influences secretion of both insulin and glucagon in ways that can potently impact blood glucose levels. Whereas nutrient-mediated secretion of insulin during a meal is augmented by an associated increase of PNS outflow to the pancreas (Güemes and Georgiou 2018), the hypoglycaemia-induced increase in SNS outflow to the islet (Havel PJ et al 1988) stimulates glucagon secretion and potently inhibits glucose stimulated insulin secretion (GSIS) (Faber CF et al, 2020).

SNS fibers supplying the liver are also activated as part of the counter regulatory' responses (CRR) and, together with increased plasma adrenaline (arising from activation of the adrenal medulla) and glucagon, these responses drive increased hepatic glucose production in an effort to restore normoglycaemia. Suppression of insulin secretion in this setting involves activation of α-adrenergic receptors on β cells resulting from either increased SNS outflow directly to the islet or increased circulating adrenaline levels (or both) (Halter JB et al, 1984).

It was proved that humans who carry genetic variants causing overexpression of the α2-adrenergic receptor have reduced insulin secretion and are at increased risk of developing type 2 diabetes (Rosengren AH et al, 2010). Furthermore, it was demonstrated a sexual dimorphism of the adrenergic activity in pancreatic β cells in mice; indeed its activity of inhibition of insulin secretion was less pronounced in diabetic male IRS 2 null mice than in females (Garcia-Barrado et al, 2011).

Neural control of islet function also plays a physiological role in cephalic-phase insulin release (insulin secretion in response to feeding cues but before nutrient absorption or increase in blood glucose) (Teff KL, 2011). The cephalic phase is mediated by vagal cholinergic signals, is amenable to behavioural entrainment and contributes to glucose tolerance (Thorens B, 2014). While cephalic insulin release has become the defining feature of neural regulation of insulin secretion, neural factors also contribute to the postprandial insulin response. Meal consumption triggers PNS outflow to the islet, and pharmacological blockade of these signals reduces prandial insulin in humans and animal models (D Alessio DA et al, 2001).

The SNS is well recognized as being implicit in cardiovascular and metabolic control and sustained sympathoexcitation results in parasympathetic withdrawal and exerts substantial metabolic effects. There is clear evidence that cardiometabolic disorders such as diabetes and hypertension are associated with chronic activation of sympathetic tone (Thorp AA and Schlaich MP, 2017). However, due to the complex interaction between the SNS, hyperglycaemia, hyperinsulinaemia and insulin resistance, the exact mechanism linking T2DM and sympathetic activation and whether sympathoexcitation is a cause or consequence of type 2 diabetes remains to be determined (Carnagarin R et al, 2018). So far, only few clinical studies investigated the influence of cardioautonomic function on glicometabolic profile. One large cohort study on more than 3500 subjects without diabetes of the Withehall II study showed that

a higher, i.e. less favorable, resting heart rate, which is also controlled by the autonomous nervous system, was associated with increases in both fasting and 2-h insulin levels, during a 5-year follow-up period (Hansen CS et al, 2019). Differently, another study on 450 patients with recently diagnosed type 2 diabetes did not find any association between HRV indices and insulin secretion measured by glucagon-stimulated incremental C-peptide (ΔC-peptide) (Ziegler D. et al, 2017). In light of these conflicting data a better understanding of the role of the nervous system in type 2 diabetes pathogenesis is required.

3. AIM OF THE STUDY

In this work we focused on finding a possible association between autonomic activity and β cell function in the Maastricht Study, a population-based cohort. We sought this association also in the population of Verona Newly Diagnosed Type 2 Diabetes Study (VNDS), a study of patients with type 2 diabetes of new onset.

We assumed that a sympathetic overdrive which characterizes many cardiometabolic disorders can have a negative impact on the different parameters of β cell function.

4. METHODS

For the purpose of this research we have analyzed two populations: The Maastricht Study and the Verona Newly Diagnosed Type 2 Diabetes Study (VNDS).

4.1 THE MAASTRICHT STUDY

Study population and design

A prospectively designed, population-based observational cohort study which started in November 2010. Eligible for participation are all individuals aged between 40 and 75 years and living in the southern part of the Netherlands (municipalities Maastricht, Margraten-Eijsden, Meersen and Valkenburg; Maastricht and Heuvelland in the province of Limburg). Participants are recruited through mass media campaigns and from the municipal registries and the regional Diabetes Patient Registry via mailings. Recruitment is stratified according to known type 2 diabetes status, with an oversampling of individuals with type 2 diabetes, for reasons of efficiency (Schram MT et al, 2014). Figure 3.

The present report includes cross-sectional data of 3,451 participants who completed the baseline survey between November 2010 and September 2013. The examinations of each participant were performed within a time window of three months. The study has been approved by the institutional medical ethics committee (NL31329.068.10) and the Minister of Health, Welfare and Sports of the Netherlands (Permit 131088-105234-PG). All participants gave written informed consent (Schram MT et al, 2014).

Social, clinical and anthropometric examination

As described previously (Schram MT et al, 2014), participants underwent assessment of educational level (low, intermediate, high), socio-economic status (income level and occupational status) (Qi Y et al, 2019), smoking status (never, former, current), alcohol use (none, low, high), and history of cardiovascular disease through questionnaire. Furthermore, the population under study underwent assessment of total energy intake through a food frequency

questionnaire (Van Dongen MC et al, 2019); of lipid-modifying, antihypertensive, and glucose-lowering medication use as part of a medication interview.

Weight, height, and waist circumference was measured during a physical examination; body-mass index (BMI) was calculated based on body weight and height; office and 24-hour ambulatory blood pressure was collected; total daily physical activity (hours/day) was obtained through an accelerometer (Van der Berg JD et al, 2016); lipid profile was measured in fasting venous plasma samples; the Matsuda Index, an index of insulin sensitivity was estimated from an OGTT (Xiang AH et al, 2014) and the estimated glomerular filtration rate (eGFR) was calculated based on serum creatinine and cystatin C (Inker LA et al, 2014).

Assessment of heart rate variability

Heart rate is the number of heart beats per minute. Heart rate variability (HRV) is the fluctuation in the time intervals between adjacent heartbeats. HRV indexes neurocardiac function and is generated by heart-brain interactions and dynamic non-linear autonomic nervous system processes. HRV is an emergent property of interdependent regulatory systems which operate on different time scales to help us adapt to environmental and psychological challenges (Shaffer F and Ginsberg JP,2017). A healthy heart is not a metronome. The oscillations of a healthy heart are complex and nonlinear. HRV can simply be obtained using one lead chest ECG trace from which R to R intervals are measured in milliseconds and plotted in sequence. Thus, HRV is a measure of electrical activity and not mechanical activity as the name might suggest.

We can describe 24 h HRV using time-domain and frequency-domain. Time-domain indices of HRV quantify the amount of variability in measurements of the interbeat interval (IBI), which is the time period between successive heartbeats (Table 1). Essentially, all these indices explore the parasympathetic activity. Frequency-domain measurements estimate the distribution of absolute or relative power into four frequency bands (table2) (Malik M et al, 1996). The components of the HRV obtained by spectral analysis provide information about both the sympathetic and parasympathetic influences on the heart (Bernardi et al, 2011).

In the Maastricht study, as published previously, all ECGs were recorded by use of a 12-lead Holter system (Fysiologic ECG Services, Amsterdam, the Netherlands) over a 24-h period (Coopmans C et al, 2020). During recording

time, participants were asked to follow their normal daily activities, except that they were asked not to take a shower or a bath. Recordings were analyzed with proprietary Holter Analysis Software at Fysiologic ECG Services (Fysiologic ECG Services, Amsterdam, the Netherlands) with an algorithm that excluded non-sinus cardiac cycles (e.g., artifacts and premature/ectopic beats), validated by manual inspection afterward. The software from Fysiologic ECG Services provided the intervals between the individual R waves of sinus beats (i.e. interbeat intervals, in milliseconds). From the obtained interbeat intervals, HRV was calculated by use of the publicly available free GNU Octave software (Eaton et al, 2019), according to the standard time and frequency domain measures defined by the (recently updated) recommendations of the Task Force document on HRV (Malik M et al, 1996 & Sassi R. et al 2015). After exclusion of non-sinus cardiac cycles, the minimum duration of the recording for HRV analysis was 18 hour. As summarized in Table 1, we calculated the following time domain measures: the standard deviation (SD) of all normal-to-normal (NN) intervals (SDNN, in milliseconds [ms]); the SD of the averages of NN intervals in all 5-min segments of the entire recording (SDANN, in ms); the square root of the mean of the sum of squares of differences between adjacent NN intervals (RMSSD, in ms); the mean of the SDs of all NN intervals for all 5-min segments of the entire recording (SDNN index, in ms); the number of pairs of adjacent NN intervals differing by >50 ms in the entire recording (NN50 count) divided by the total number of all NN intervals (pNN50, expressed as percentage). Then, as summarized in table 2, we calculated the following frequency domain measures using the Fast Fourier Transform: the variance of all NN intervals ≤0.4 Hz (total power [TP], in ms squared); power in the ultralow-frequency range (ULF, in ms squared; ≤0.003 Hz); power in the very-low-frequency range (VLF, in ms squared) (0.003–0.04 Hz); power in the low-frequency range (LF, in ms squared; 0.04–0.15 Hz); and the power in the high-frequency range (HF, in ms squared; 0.15–0.4 Hz). Individual *z*-scores were calculated for the time and frequency domain measures and combined in an overall time domain variable ([SDNN + RMSSD + SDANN + SDNN index + pNN50]/5) and an overall frequency domain variable ([TP + ULF + VLF + LF + HF]/5).

Beta cell function assessment

After an overnight fast, all participants (except those who used insulin or had a fasting plasma glucose concentration above 11.0 mmol/L) underwent a standard 2-hour 75 g OGTT (oral glucose tolerance test). Venous blood samples were

collected before and at 15, 30, 45, 60, 90, and 120 minutes after oral glucose load intake. We estimated different aspects of β cell function, i.e. C-peptidogenic index t0-30, C-peptide AUC/ glucose AUC (CPAUC/GAUC), β cell glucose sensitivity, β cell potentiation factor, and β cell rate sensitivity, using formula-based methods and mathematical modeling, as previously described (Den Biggelaar L et al, 2017 and Den Biggelaar L et al, 2020). C-peptidogenic index t0-30, which reflects early phase insulin secretion, was calculated as the ratio of the difference in C-peptide level at 30 minutes post glucose load intake versus baseline (i.e. fasting level; ΔCP30) divided by the difference in glucose level at 30 minutes post glucose load intake versus baseline (i.e. ΔG30). (Den Biggelaar L et al, 2017 and Philips DI et al,1994). CPAUC/GAUC is ratio of the area under the curve (AUC) of C-peptide divided by the AUC of glucose and was calculated to estimate the overall insulin secretion during the OGTT (Den Biggelaar L et al, 2017). B cell glucose sensitivity, which represents the dependence of insulin secretion on absolute glucose concentration at any time point during the OGTT, was calculated as the regression coefficient of the slope of the glucose–insulin secretion dose–response function (Mari A et al, 2002 and Den Biggelaar L et al, 2020). The dose-response function is assumed to be modulated by a time-varying potentiation factor which accounts for effects of sustained hyperglycemia and incretins. This β cell potentiation factor, which represents the relative potentiation of the insulin secretion response to glucose, was calculated as the ratio of the β cell potentiation factor at the end of the 2-h OGTT relative to the β cell potentiation factor at the start of the OGTT (β cell potentiation factor is set as a positive function of time and averages 1 during the OGTT). B cell rate sensitivity, a marker of early phase insulin release, which represents the dynamic dependence of insulin secretion on the rate of change in glucose concentration, was calculated from the glucose and C-peptide data using a previously developed model (Mari A et al, 2002 and Den Biggelaar L et al, 2020).

4.2 THE VERONA NEWLY DIAGNOSED TYPE 2 DIABETES STUDY (VNDS)

Study population and design

The Verona Newly Diagnosed Type 2 Diabetes Study (VNDS) is a study of patients with newly diagnosed type 2 diabetes (Bonetti S et al, 2011 and Bonora E et al 2020). The study has been registered as a clinical trial (ClinicalTrials.gov identifier NCT01526720). As of January 1, 2002, all patients with T2DM referred

to the Diabetes Clinic embedded into the Division of Endocrinology, Diabetes and Metabolic Diseases of the University and Hospital Trust of Verona and whose disease was diagnosed in the past 6 months were offered to participate in this study. Recruitment was ended on December 31, 2015 and a follow-up was then planned and is ongoing. All participants signed an informed consent form. The clinical evidence on which the diagnosis of T2DM had been made was reviewed at the recruitment and the diagnosis was confirmed according to standard criteria. The large majority of patients were drug-naïve (~95%) or, if already treated with antidiabetic drugs (~5%), underwent a treatment washout of at least 1 week before metabolic tests were performed. Exclusion criteria were age >75 years, non-Italian ancestry, current insulin treatment, presence of anti-glutamic acid decarboxylase antibodies and history of malignancies or any condition severely impairing liver and/or kidney function.

Each subject gave informed written consent before participating in the research, which was approved by the Human Investigation Committee of the Verona City Hospital. Standard clinical phenotypes were measured in all patients.

Autonomic function evaluation

Cardiovascular autonomic function was assessed by a computerized system, as previously described (Zoppini G et al), following the criteria presented by Ewing and Clarke (Ewing DJ and Clarke BF, 1986). Patients should be requested to avoid strenuous physical exercise in the 24 h preceding the cardiovascular tests. We recommended not consuming beverages containing caffeine, as well as not smoking or drinking alcohol, at least 2 h before the tests. The three heart rate tests were performed while the patient was fasting or at least 2 h after a light meal. A personal computer collected, stored, and processed R-R intervals and blood pressure (systolic, diastolic, and mean) and analyzed heart rate and blood pressure variations during lying to standing (LS), deep breathing (DB), and Valsalva maneuver (VM). The diagnostic definition of autonomic neuropathy based on this battery of tests allows an indication of the severity of the neuropathy, showing independent parasympathetic and sympathetic functions and reducing the probability of false positives. In the light of the evidence available, experts have proposed that at least two abnormal heart rate tests (below the 5th percentile) are required for the diagnosis of autonomic neuropathy (confirmed autonomic neuropathy). Only one abnormal test or two borderline tests (between the 5^{th} and 10th percentiles) identifies the condition of "early" autonomic neuropathy. The severe form of autonomic neuropathy is diagnosed

when confirmed autonomic neuropathy is associated with orthostatic hypotension (Spallone V et al, 2011).

Laboratory testing

Venous blood was drawn in the morning after an overnight fast in all patients. Plasma glucose and serum creatinine and lipids were assayed by standard laboratory procedures. Hypercholesterolemia was arbitrarily defined when statins were used and/or low-density lipoprotein (LDL) cholesterol was above the current recommended target of <70 mg/dL (<1.8 mmol/L). Hemoglobin A1c (HbA1c) was measured with a high performance liquid chromatography method, standardized according to IFCC. In case of discrepancy between the three tests (fasting plasma glucose, 2-hour plasma glucose, HbA1c), the one documenting diabetes (value above the diagnostic cut-off) was used for diagnosis according to standard criteria (IDF, 8th edn 2017). Glomerular filtration rate (GFR) was estimated from the four- variable Modification of Diet in Renal Disease study equation (Levey AS et al, 1999). Chronic kidney disease (CKD) was established when estimated glomerular filtration rate was <60 mL/min/1.73 m2. Urinary albumin excretion rate was measured from a 24-hour urine sample by an immunonephelometric method on at least two occasions. Microalbuminuria and macroalbuminuria were defined as urinary excretion of 30–300 and >300 mg/day, respectively.

Metabolic studies

Metabolic tests were performed on two separate days in random order.

On 1 day, a frequently sampled, prolonged (240 or 300 min) OGTT (75 g) was carried out and beta-cell function was reconstructed by mathematical modelling, as previously described (Bonadonna et al, 2010). By this method, beta-cell function is described by two parameters of beta cell glucose sensitivity:

1. Derivative (or dynamic) control (DC): the response of the beta cell to the rate of increase of plasma glucose

2. Proportional (or static) control (PC): the response of the beta cell to glucose concentration per se, herein presented as the stimulus–response curve relating insulin secretion rate (ISR, pmoles per min) to glucose concentration (mmol/l).

On a separate day, a euglycaemic insulin clamp was performed to assess insulin sensitivity. The amount of glucose metabolized during the last 60 min of the clamp [M-value, reference insulin sensitivity; units: μmol/min/m2 body surface area (BSA)] was computed with standard formulas (Bonetti S et al, 2011).

4.3 STATISTICAL ANALYSIS

All analyses were performed using the software package SPSS Statistics version 23.0 for Windows (SPSS, IBM Corp., Armonk, NY, USA).

Characteristics of the study population were described as means and standard deviations (SD) for continuous variables or as number and proportions of participants per category for categorical variables (% of study population).

For all analyses, a P-value <0.05 was considered statistically significant.

In the Maastricht Study we used multivariable linear regression analyses to investigate the associations of the overall time domain variable and the overall frequency domain variable (independent variables) entered as z-scores, with the z scores of C-peptidogenic index, overall insulin secretion, β cell glucose sensitivity, β cell potentiation factor (dependent variables).

Logistic regression analysis was performed to assess the association between the overall time domain variable and the overall frequency domain variable with β-cell rate sensitivity. β-Cell rate sensitivity was analyzed in tertiles (of which the highest tertile was considered the reference category), as the distribution of β-cell rate sensitivity was positively skewed and could not be normalized by transformations. Associations are presented as standardized regression coefficients [standardized βs (95% CI)].

Model 1 shows crude results. In model 2, we adjusted for age, sex, educational status (low, medium, high). We chose these variables because they are key demographic confounders. In model 3, we additionally adjusted for Matsuda Index, because of the impact of insulin sensitivity on β cell secretion. In model 4, we additionally adjusted for office systolic blood pressure, use of antihypertensive medication [yes/no], BMI, total cholesterol / HDL cholesterol ratio, lipid-modifying medication [yes/no], smoking status [current, former, never], and alcohol consumption status [none, low, high]). The associations were expressed as standardized regression coefficient (stβ) and corresponding 95% confidence interval (95%CI).

We assessed whether associations differed by sex (i.e. between men and women) or glucose metabolism status (i.e. between individuals with type 2 diabetes, individuals with prediabetes, and individuals with normal glucose metabolism) by including their respective interaction terms in the models.

To assess the robustness of our findings we performed a range of additional analyses. First, to check whether associations of individual HRV measures with

indices of β cell function were not directionally inconsistent, we analyzed the associations of individual measures that were used to compose HRV time and HRV frequency domain. Second, we repeated the analyses with additional adjustment for total energy intake and physical activity. Adjustment for these potential confounders was not included in the main analyses because data were missing for a relatively large number of participants (up to n=311 had missing data on one or more of these variables). Third, we additionally adjusted for kidney function (eGFR) and history of cardiovascular disease. We adjusted for these covariates in a separate model because they may be confounders but may also (in part) be mediators or descendants of the outcome (Soomro QH et al, 2021 & Lahiri MK et al, 2008 & Schisterman EF et al, 2009). Fourth, we replaced waist circumference with BMI; educational status with occupational status or income level; and office systolic BP with office diastolic BP, systolic or diastolic 24-hour ambulatory BP.

In the VNDS the one-way ANOVA and the χ^2 test with Yates correction for continuity were used to analyze the differences among the clinical and biochemical characteristics of participants stratified by status of autonomic neuropathy (absent and early/confirmed). Beta cell secretion indices were skewed and for this reason log transformed.

5. RESULTS

5.1 RESULTS: THE MAASTRICHT STUDY

Selection and characteristics of the study population

Figure 1 shows the study population selection. Participants in whom data on β cell function were missing were excluded first (n=597). Next, individuals with missing data on HRV were excluded (n=712). Last, individuals with missing data on confounders were excluded (n=47).

Table from 3 to 8 show general participant characteristics according to tertiles of time and frequency domain HRV. The study population consisted of 2007 individuals with a mean SD age of 59.8 ±8.2 years, of whom 51.8% were men (Table 3). From the first to the third tertile of both time and frequency domain, BMI, waist circumference, HbA1c, total/HDL cholesterol ratio, blood pressure,

and use of glucose lowering, antihypertensive and lipid-modifying medication were lower (table 3 and table 6).

The population with the lowest HRV time and frequency domain, were more often current smokers, more often had a history of cardiovascular disease, and had lower amounts of physical activity, lower estimated glomerular filtration rate, and higher alcohol use (table 3 and table 6).

Furthermore indices of insulin secretion and also Matsuda index improved from the first to the third tertile of both time and frequency domain (table3,5 and table 6,8).

General characteristics of participants included in the study were comparable to those of participants with missing data (Supplemental Table S1).

Time domain HRV and indices of β cell function

As shown in table 9, after adjustment for age, sex and education, and Matsuda index (model 3), greater time domain HRV was significantly associated with greater C-peptidogenic index, greater β cell glucose sensitivity, and greater β cell potentiation factor, but not with overall insulin secretion. Then, further adjustment for cardiovascular risk factors (model 4) did not materially alter these associations, though only the association of HRV with C-peptidogenic remained statistically significant (standardized β [95%CI], for respectively C-peptidogenic index, overall insulin secretion, β cell glucose sensitivity, and β cell potentiation, 0.05 [0.00; 0.09]; 0.04 [-0.00; 0.08]; 0.04 [0.00; 0.08]; and 0.04 [-0.00; 0.08]; Figure 5).

After full adjustment (model 4), time domain HRV was not associated with β cell rate sensitivity (per SD greater time domain HRV, OR [95%CI] for, respectively, low versus high rate sensitivity, or middle versus high rate sensitivity; 0.9 [0.8; 1.0], p=0.688 and 0.9 [0.8;1.0], p= 0.233, respectively) (Table 10).

Frequency domain HRV and indices of β cell function

As shown in table 9, after adjustment for age, sex and education, and Matsuda index (model 3), greater frequency domain HRV was significantly associated with greater C-peptidogenic index, greater β cell glucose sensitivity, and greater β cell potentiation factor, but not with overall insulin secretion. Then, further adjustment for cardiovascular risk factors (model 4) did not materially alter these associations, though only the association of HRV with C-peptidogenic remained

statistically significant (standardized β [95%CI], for respectively C-peptidogenic index, overall insulin secretion, β cell glucose sensitivity, and β cell potentiation, 0.05 [0.00; 0.09]; 0.04 [-0.00; 0.08]; 0.04 [0.00; 0.08] ; and 0.04 [-0.00; 0.08]. Figure 5).

After full adjustment (model 4), frequency domain HRV was not associated with β cell rate sensitivity (per SD greater frequency domain HRV, OR [95%CI] for, respectively, low versus high rate sensitivity, or middle versus high rate sensitivity; 1.0 [0.9; 1.1], p= 0.813 and 0.9 [0.8;1.0], p=0.323, respectively) (table 11).

Interaction analyses

Sex did not modify any of the associations under study and glucose metabolism status did not consistently modify the associations under study. All P-values for interaction are shown in Supplemental Table S2.

Additional analyses

Quantitatively similar results were observed in a range of sensitivity analyses. First, we had numerically similar results when we analyzed the associations of individual measures of HRV with the outcomes under study (Supplemental Table S3). Second, associations did not materially change when we additionally adjusted the associations under study for dietary intake and physical activity; or for kidney function (eGFR) and history of cardiovascular disease (Supplemental Table S4). Fourth, associations did not materially change when we replaced BMI by waist circumference; educational status by occupational status or income level; or office systolic blood pressure by office diastolic blood pressure, systolic or diastolic 24-hour ambulatory blood pressure (Supplemental Table S5).

5.2 RESULTS: THE VERONA NEWLY DIAGNOSED TYPE 2 DIABETES STUDY (VNDS)

The population of VNDS consisted of 844 patients (mean±SD age of 58.2 ±9.8 years of whom 68% were males). To evaluate the associations between cardiautonomic function and β cell secretion we excluded subjects with missing data on cardioautonomic test and parameters of beta cell function. We obtained a sample of 537 patients whose main characteristics are shown in table 12. They had a fairly high mean BMI (30.0 ± 5.1), a good metabolic control (HbA1c: 6.8% ± 1.1) and a low prevalence of micro and macro vascular complications.

Ninety-one subjects (16.9%) showed at least one abnormal test or two borderline tests used to evaluate cardiovascular autonomic function. These subjects were more obese and more frequently smokers (past/current). They showed higher fasting plasma glucose levels and higher albuminuria. They took more frequently antihypertensive drugs and had a higher prevalence of macrovascular complication such as carotid stenosis and coronary heart disease, as reported in table 12. Furthermore they showed a worse first phase of insulin secretion (derivative control) as compared to people with normal cardioautonomic tests. This difference however did not reach statistical significance (p=0.063).

6. DISCUSSION

A better understanding of the pathogenesis of type 2 diabetes is important to develop strategies that aim to prevent and treat this disease. Though type 2 diabetes is predominantly considered as a consequence of a combination of genetic predisposition, improper diet and sedentary lifestyle, emerging evidence also indicates the significance of the autonomic nervous system with sympathetic preponderance in the development and progression of type 2 diabetes and its complications (Wulsin LR et al, 2015 and Alonge KM et al, 2021).

A chronic activation of SNS tone and a withdrawal of PNS one could contribute to the development of metabolic disorders and be important mediators of the associated adverse cardiovascular consequences (Carnagarin R et al, 2018). Exaggerated sympathetic discharge in response to various stress stimuli alters the energy homeostasis and results in adverse metabolic and cardiovascular outcomes (Schlaich M et al, 2015).

Cardiac autonomic function can be noninvasively assessed through different tests. Ewing tests are recommended as the gold standard for clinical testing and are composed of a number of simple bedside tests of short-term RR differences to detect autonomic neuropathy (Ewing DJ and Clarke BF, 1986). Cardiac autonomic function can be measured also through heart rate variability (HRV), which reflects the interaction of the sympathetic and parasympathetic parts of the autonomic nervous system on the sinus node **(**Malik M et al, 1996). HRV can simply be obtained using one lead chest ECG trace from which R to R intervals are measured in milliseconds and plotted in sequence.

Low HRV is a validated index of cardiac autonomic dysfunction and is associated with an increased risk of ventricular arrhythmias and sudden cardiac arrest (Lombardi F et al, 2001).

To gain insight in the mechanisms that underlie the relationships between autonomic dysfunction and development of type 2 diabetes, we explored in the Maastricht study the association between HRV and distinct aspects of β cell function. The associations found between HRV (both the time and the frequency domain) and the parameters of beta cell function went all in the same direction, implying that to a lower HRV corresponds a worse β cell function which is in line with our hypothesis. However only the association with C-peptidogenic index remained significant in the last model. This parameter is a measure of the first phase of insulin secretion and this finding is in line with the known

involvement of the autonomic system in the control of cephalic and first phase of insulin secretion (Teff KL, 2011 and Thorens B, 2014)

Indeed it was demonstrated that the cephalic-phase of insulin release (i.e. insulin secretion in response to feeding cues but before nutrient absorption or increase in blood glucose) is mediated by vagal cholinergic signals which are also stimulated by meal consumption and pharmacological blockade of these signals reduces prandial insulin in humans and animal models (D Alessio DA et al, 2001).

The Maastricht study is population-based cohort and comprise the entire spectrum of glucose metabolism with an oversampling of individuals with type 2 diabetes by design.

We evaluated also the prevalence of signs of cardio autonomic neuropathy in the population of VNDS composed exclusively by subjects with newly diagnosed type 2 diabetes. This drug naïve population is characterized by only moderately high glucose levels removing the significant confounding effect of glucose toxicity. Performing standardized Ewing tests, we found signs of cardioautonomic dysfuntion in nearly 17% of the population. Furthermore, as regards beta cell function, we showed that people with alteration in cardio autonomic tests had a worse derivative control of beta cell function but the difference did not reach statistical significance ($p=0.063$). It must be emphasized that this population did not include subjects with type 2 diabetes for a long-time and that for this reason people analyzed presented a less wide range of insulin secretion and autonomic function compared to the population of the Maastricht Study: this aspect may have attenuated the results in this population. Therefore it will be interesting to follow these patients during the course of the disease and see if this association is maintained and perhaps strengthened.

So far, only few clinical studies investigated the influence of cardio-autonomic function on glicometabolic profile. One large cohort study on more than 3500 subjects without diabetes of the Withehall II study showed that a higher, i.e. less favorable, resting heart rate, which is also controlled by the autonomous nervous system, was associated with increases in both fasting and 2-h insulin levels, during a 5-year follow-up period (Hansen CS et al, 2019). Differently, another study on 450 patients with recently diagnosed type 2 diabetes did not find any association between HRV indices and insulin secretion measured by glucagon-stimulated incremental C-peptide (ΔC-peptide), yielding conflicting results (Ziegler D. et al, 2017).

Taken together our results demonstrate that, a negative impact of autonomic dysfunction on insulin secretion, could add to the other factors already known to influence β cell function such as genetic predisposition, lipotoxicity, glucotoxicity, amyloid formation, defective insulin processing (Bonora E., 2008).

This new knowledge will be important to better understand the pathogenesis of diabetes and develop improved strategies for the treatment of this disease.

Our current findings carefully suggest that prevention of autonomic dysfunction may ameliorate β cell dysfunction. Future clinical trials are warranted to investigate whether early prevention of autonomic dysfunction, such as through lifestyle intervention strategies (loosing weight, increasing physical activity, reducing exposure to harmful factors such as smoking, alcohol and drugs) may reduce β cell dysfunction and, ultimately, prevent and/or slow-down the onset of prediabetes and type 2 diabetes (Fatisson J. et al, 2016).

Strengths of our research are firstly the large size of these two populations which enabled us to investigate the associations under study over the entire spectrum of glucose metabolism (i.e. from normal glucose metabolism to prediabetes and type 2 diabetes). Second, the use of state-of-the-art methods to assess all variables included in these studies, and thirdly the extensive phenotyping which enabled us to consider a substantial number of potential confounders and to perform a considerable number of additional analyses, which generally yielded consistent findings. The research also has certain limitations. First, due to the cross-sectional nature of the studies, causal inferences should be made with caution (Rothman KJ and Greenland S, 2008). Mechanistically, hyperglycemia may not only lead to neurodegeneration but the reverse may also be true (Stehower CDA, 2018). Second, we may have underestimated the strength of the associations under study if such associations were similar or stronger in participants that were excluded from the study population (who generally tend to be less healthy). Such range restriction may lead to underestimated associations (Bland JM and Altman DG, 2011). Third, although we took an extensive set of confounders into account, we cannot fully exclude bias due to unmeasured confounding (e.g., environmental factors such as air pollution) (Sharma S et al, 2020). Fourth, we analyzed the data of two different studies that chose different methods for assessing both cardioautonomic function and beta cell secretion, making the results difficult to compare (Tang Z-H, et al, 2014).

Last, we studied Caucasian individuals with access to high-quality diabetes care. Therefore, the generalizability of our results to other populations requires further study.

Concluding remarks

In summary, in the present research we analyzed a possible association between autonomic function and β cell secretion, estimated from OGTT. We found that autonomic dysregulation could contribute to β-cell dysfunction, in particular reducing the first phase of insulin secretion. This mechanism could add to the other factors that lead to the impairment of glucose homeostasis.

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8. FIGURES AND TABLES

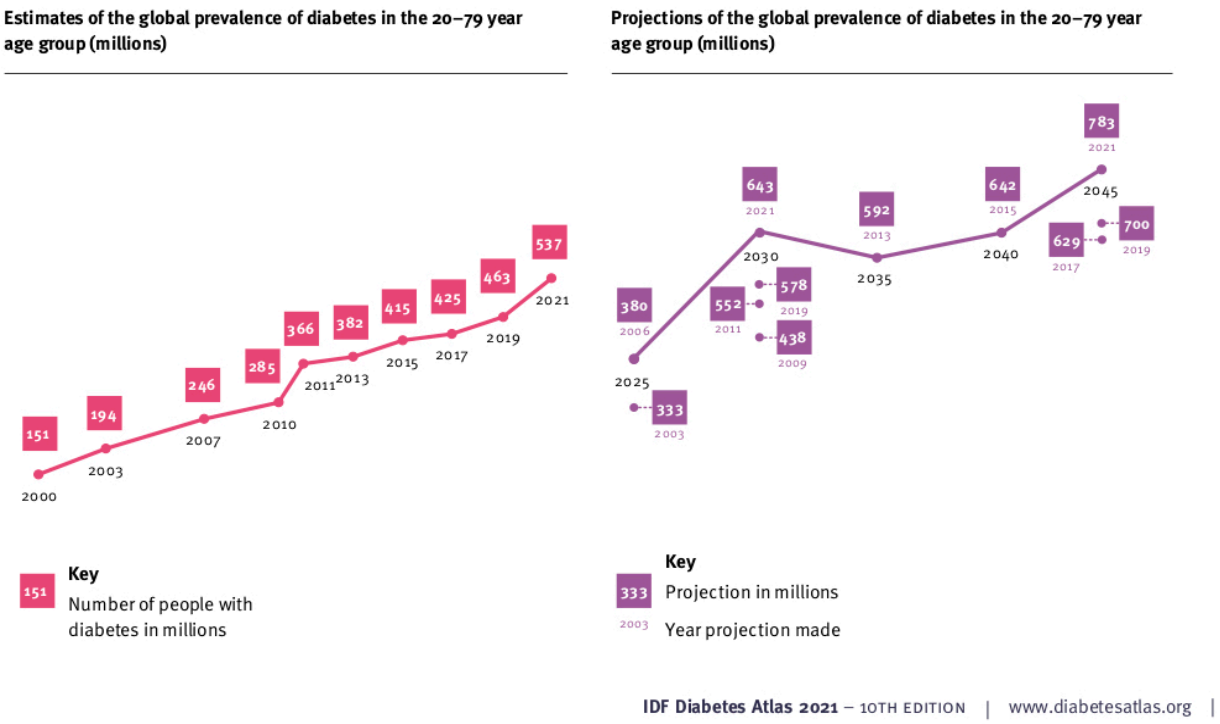

Figure 1. Increasing number of adults with type 2 diabetes during the years (IDF Diabetes Atlas, 10th edn)

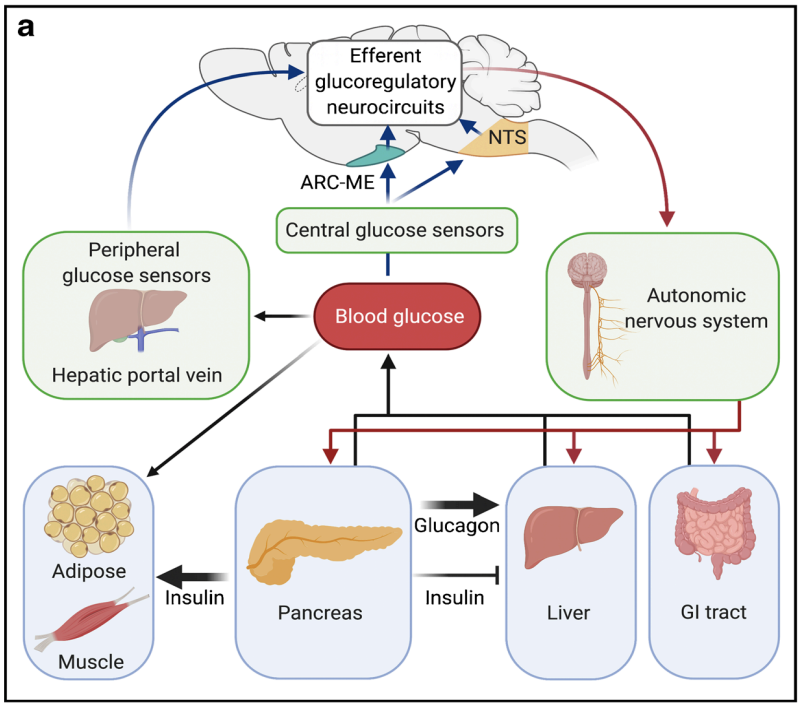

Figure 2. Autonomic Regulation of Glucose Homeostasis (Alonge KM et al, 2021)

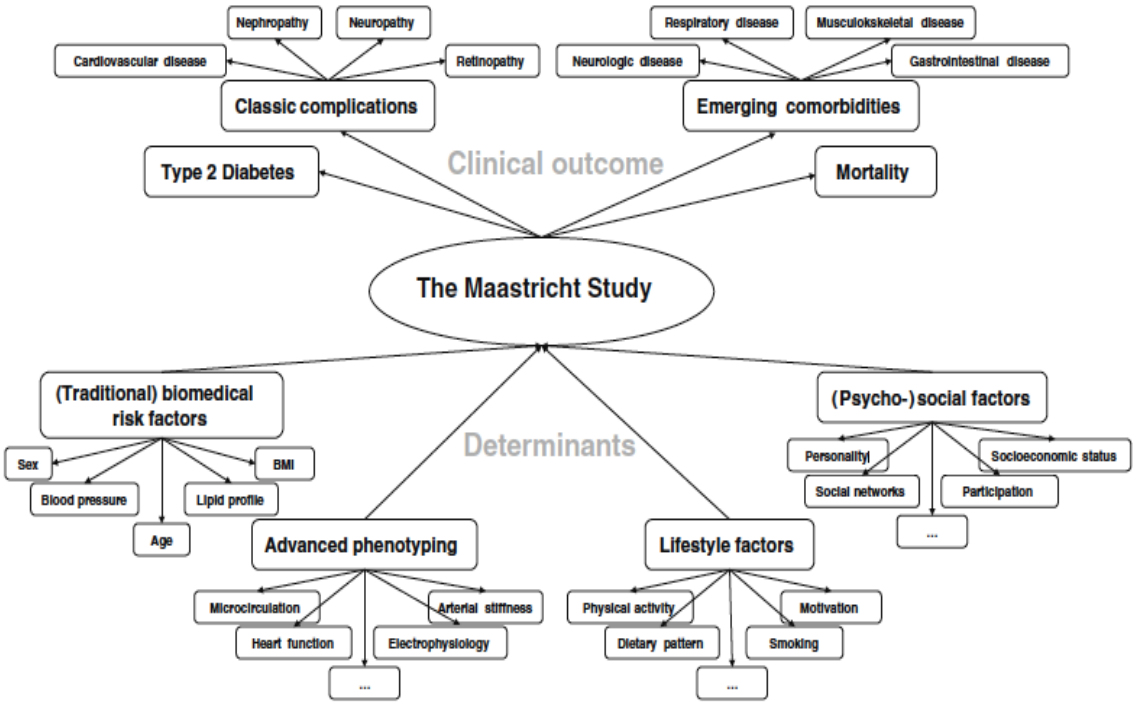

Figure 3. The advanced phenotyping approach of The Maastricht Study (Schram MT et al, 2014).

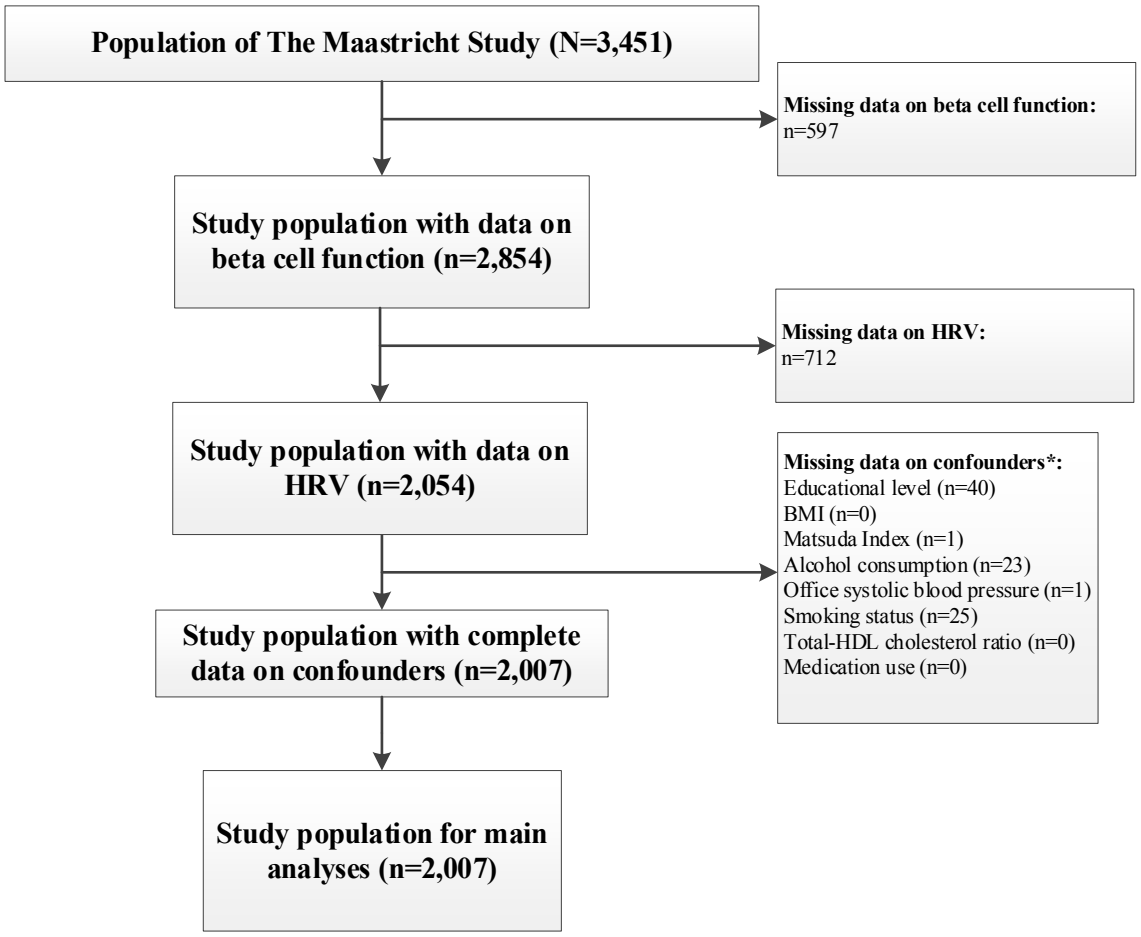

Figure 4. Study population selection

* Not mutually exclusive

Abbreviations: HRV, heart rate variability; HDL, high density lipoprotein; BMI, body-mass index.

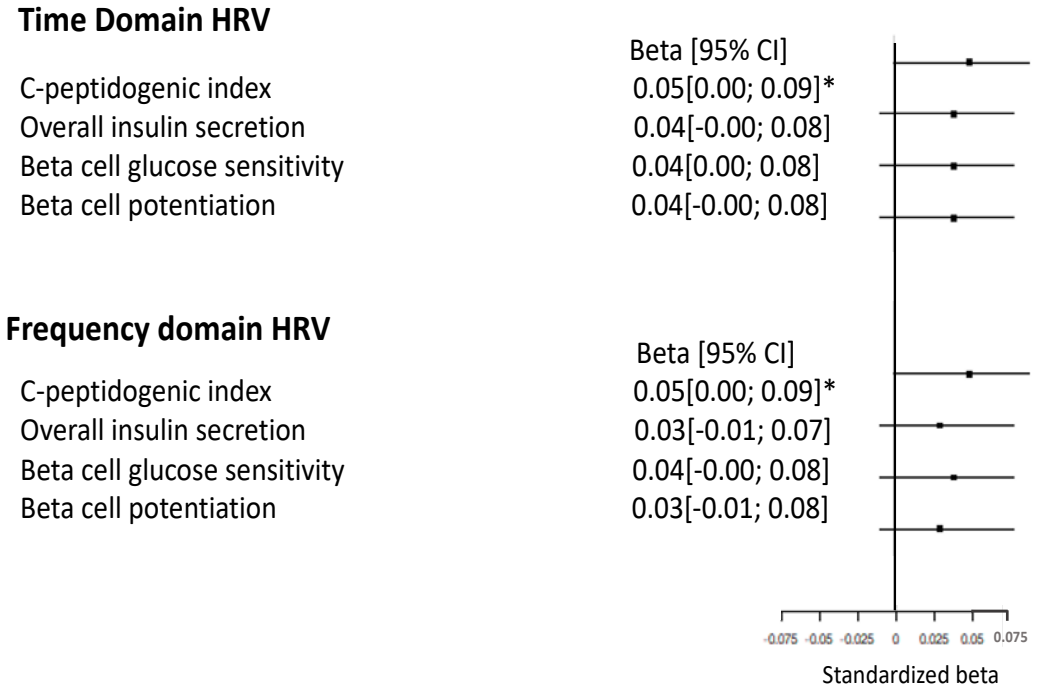

Figure 5. Regression coefficients represent the difference in beta cell index in SD per SD greater HRV.

Time domain HRV was estimated from SDNN, SDANN, RMSSD, SDNN index and pNN50; and frequency domain was estimated from TP, ULF, VLF, LF, and HF.

Variables entered in the models in addition to HRV: age, sex, educational status (low, medium, high), Matsuda Index, office systolic blood pressure, total cholesterol/HDL cholesterol ratio, use of antihypertensive or lipid-modifying medication (yes/no), BMI, smoking status (current, ever, never), and alcohol consumption status (none, low, high). Abbreviations: SD, standard deviation; CI, confidence interval; HRV, heart rate variability, HDL, high density lipid; BMI, body-mass index. All abbreviations for indices of HRV are presented in the Methods section.

Table 1 HRV, Time domain measures

Parameter	Unit	Description
SDNN (ms)	ms	Standard deviation of NN intervals
SDANN (ms)	ms	Standard deviation of the average NN intervals for each 5 min segment of a 24 h HRV recording
RMSSD (ms)	ms	Root mean square of successive RR interval differences
SDNN index (ms)	ms	Mean of the standard deviations of all the NN intervals for each 5 min segment of a 24 h HRV recording
pNN50 (%)	**%**	Percentage of successive RR intervals that differ by more than 50 ms

Table 2 HRV, Frequency domain measures

Parameter	Unit	Description
TP	ms^2	Variance of all NN intervals ≤0.4 Hz
ULF	ms^2	Power of the ultra-low-frequency band (≤0.003 Hz)
VLF	ms^2	Power of the very-low-frequency band (0.0033–0.04 Hz)
LF	ms^2	Power of the low-frequency band (0.04–0.15 Hz)
HF	ms^2	Power of the high-frequency band (0.15–0.4Hz)

Table 3 General study population characteristics of the Maastricht Study according to tertiles of the time domain HRV z-score

		HRV time domain composite score			
Characteristic	Number of participants	Overall, N = 2,007	Tertile 1, N=669	Tertile 2, N=669	Tertile 3, N=669
Age (years)	N=2,007	59.88± 8.25	61.67± 7.47	60.11± 8.01	57.87± 8.76
Sex	N=2,007				
Men		1,044 (52%)	326 (49%)	331 (49%)	387 (58%)
Women		963 (48%)	343 (51%)	338 (51%)	282 (42%)
Educational status	N=2,007				
Low		637 (32%)	236 (35%)	230 (34%)	171 (26%)
Middle		551 (27%)	164 (25%)	178 (27%)	209 (31%)
High		819 (41%)	269 (40%)	261 (39%)	289 (43%)
Occupational status	N=2,007				
Low		637 (32%)	236 (35%)	230 (34%)	171 (26%)
Middle		551 (27%)	164 (25%)	178 (27%)	209 (31%)
High		819 (41%)	269 (40%)	261 (39%)	289 (43%)

Income level (euros)	N=1,554	2,066.08 ± 836.97	2,093.17 ± 881.22	2,083.99 ± 838.65	2,022.57 ± 790.26
Glucose metabolism status	N=2,007				
Normal glucose metabolism		1,208 (60%)	330 (49%)	416 (62%)	462 (69%)
Prediabetes		323 (16%)	113 (17%)	119 (18%)	91 (14%)
Type 2 diabetes		476 (24%)	226 (34%)	134 (20%)	116 (17%)
Other types of diabetes		0 (0%)	0 (0%)	0 (0%)	0 (0%)
Glucose-lowering medication	N=2,007	342 (17%)	172 (26%)	95 (14%)	75 (11%)
Matsuda Index (no unit)	N=2,007	3.46 (2.04, 5.19)	2.92 (1.76, 4.51)	3.62 (2.14, 5.43)	3.93 (2.40, 5.85)
Office systolic blood pressure (mmHg)	N=2,007	134.59 ± 17.94	136.39 ± 17.71	134.64 ± 18.05	132.75 ± 17.91
Office diastolic blood pressure (mmHg)	N=2,007	76.61 ± 9.80	77.31 ± 9.88	76.88 ± 10.02	75.63 ± 9.43
24-hour ambulatory systolic blood pressure (mmHg)	N=1,835	118.68 ± 11.35	119.54 ± 11.75	118.51 ± 10.99	117.99 ± 11.26
24-hour ambulatory diastolic blood pressure (mmHg)	N=1,835	73.61 ± 7.01	74.00 ± 7.23	73.46 ± 6.87	73.36 ± 6.92
Use of antihypertensive medication	N=2,007	744 (37%)	303 (45%)	224 (33%)	217 (32%)
Body-mass index (kg/m^2)	N=2,007	26.72 ± 4.23	27.42 ± 4.70	26.52 ± 3.98	26.23 ± 3.87
waist circumference (cm)	N=2,005	95.05 ± 12.81	97.10 ± 13.52	94.44 ± 12.36	93.61 ± 12.28
Alcohol consumption	N=2,007				
None		335 (17%)	93 (14%)	114 (17%)	128 (19%)

Moderate		1,121 (56%)	350 (52%)	367 (55%)	404 (60%)
High		551 (27%)	226 (34%)	188 (28%)	137 (20%)
Total/HDL cholesterol ratio	N=2,007	3.53 (2.87, 4.36)	3.56 (2.91, 4.40)	3.53 (2.87, 4.38)	3.47 (2.85, 4.29)
Use of lipid-modifying medication (yes/no)	N=2,007	656 (33%)	271 (41%)	206 (31%)	179 (27%)
Smoking status	N=2,007				
Never		687 (34%)	194 (29%)	238 (36%)	255 (38%)
Former		1,063 (53%)	383 (57%)	345 (52%)	335 (50%)
Current		257 (13%)	92 (14%)	86 (13%)	79 (12%)
Physical activity (hours/day)	N=1,788	14.31 ± 8.08	13.83 ± 8.07	14.68 ± 8.38	14.42 ± 7.78
Total caloric intake (KJ/day)	N=1,890	9,215.13 ± 2,553.35	9,095.53 ± 2,565.01	9,093.76 ± 2,408.70	9,456.67 ± 2,665.65
History of cardiovascular disease	N=1,991	316 (16%)	126 (19%)	90 (14%)	100 (15%)
Estimated Glomerular Filtration Rate	N=2,003	88.32 ± 14.31	87.37 ± 14.64	88.12 ± 14.24	89.47 ± 13.99

Abbreviations: HbA1c, hemoglobin A1c; HDL, high-density lipoprotein; NGM, normal glucose metabolism; eGFR, estimated glomerular filtration rate. Data are presented as mean ± standard deviation, median (interquartile range) or number (%).

Table 4 Time and frequency measurements of HRV in the population of the Maastricht Study according to tertiles of the time domain HRV z-score

Characteristic	Number of participants	Overall, N = 2,007	Tertile 1, N=669	Tertile 2, N=669	Tertile 3, N=669
HRV, Time domain composite score (SD)	N=2,007	0.00 ± 1.00	-0.93 ± 0.30	-0.15 ± 0.21	1.07 ± 0.89
SDNN (ms)		135.54 ± 37.55	100.38 ± 17.52	134.89 ± 17.50	171.36 ± 33.14
SDANN (ms)		122.09 ± 36.13	90.63 ± 17.96	122.66 ± 19.76	152.97 ± 35.50
RMSSD (ms)		29.88 ± 17.98	18.99 ± 5.12	26.18 ± 6.79	44.48 ± 23.50
SDNN index (ms)		54.30 ± 18.31	39.32 ± 7.40	51.51 ± 7.60	72.07 ± 18.59
SDSD (ms)		29.88 ± 17.98	18.99 ± 5.12	26.18 ± 6.79	44.48 ± 23.50
pNN50 (%)		6.16 (2.66, 12.24)	2.19 (1.09, 3.91)	6.01 (3.85, 9.04)	15.14 (10.02, 24.16)
HRV, Frequency domain composite score (SD)	N=2,007	0.00 ± 1.00	-0.54 ± 0.28	-0.13 ± 0.28	0.67 ± 0.70
TP (ms^2)		11,589.40 (7,873.16, 16,499.85)	6,814.82 (5,047.99, 8,417.24)	12,083.60 (9,973.33, 14,188.90)	18,732.40 (14,987.20, 23,342.00)
ULF (ms^2)		9,840.92 (6,481.91, 13,973.95)	5,862.03 (4,170.16, 7,395.05)	10,429.70 (8,374.72, 12,625.10)	16,042.00 (11,901.40, 20,353.70)
VLF (ms^2)		1,075.88 (736.36, 1,556.57)	644.88 (489.58, 839.21)	1,073.34 (882.15, 1,312.46)	1,859.36 (1,410.70, 2,473.42)
LF (ms^2)		347.03 (207.59, 591.74)	192.20 (133.26, 274.39)	343.04 (244.30, 479.55)	691.66 (494.01, 966.11)
HF (ms^2)		84.26 (47.72, 147.19)	42.84 (29.88, 64.23)	83.69 (57.15, 121.17)	173.71 (109.62, 280.54)

Abbreviation: HRV, heart rate variability. Data are presented as mean ± standard deviation, median (interquartile range)

Table 5 β cell function parameters in the population of the Maastricht Study according to tertiles of the time domain HRV z-score

			HRV time domain composite score		
Characteristic	Number of participants	Overall, N = 2,007	Tertile 1, N=669	Tertile 2, N=669	Tertile 3, N=669
C-peptidogenic index (no unit)	N=2,007	471.29 ± 1,003.20	413.50 ± 589.52	456.00 ± 1,204.82	544.37 ± 1,101.91
Overall Insulin secretion (no unit)	N=2,007	193.60 ± 79.46	189.74 ± 83.38	196.95 ± 79.61	194.11 ± 75.13
B cell glucose sensitivity (pmol/m^2/mM)	N=2,007	27.39 ± 18.43	26.09 ± 19.09	27.99 ± 18.07	28.10 ± 18.07
B cell potentiation factor (no unit)	N=2,007	1.63 ± 0.69	1.53 ± 0.67	1.68 ± 0.73	1.68 ± 0.67
B cell rate sensitivity (pmol/m^2/mM)	N=2,007	239.72 ± 312.06	216.94 ± 247.33	239.76 ± 285.85	262.45 ± 385.39

Data are presented as mean ± standard deviation

Table 6 General population characteristics of the Maastricht Study according to tertiles of the frequency domain HRV z-score

		HRV frequency domain composite score			
Characteristic	Number of participants	Overall, N = 2,007	Tertile 1, N=669	Tertile 2, N=669	Tertile 3, N=669
Age (years)	N=2,007	59.88± 8.25	62.11± 7.29	60.16± 8.05	57.38± 8.65
Sex	N=2,007				
Men		1,044 (52%)	323 (48%)	332 (50%)	389 (58%)
Women		963 (48%)	346 (52%)	337 (50%)	280 (42%)
Educational status	N=2,007				
Low		637 (32%)	242 (36%)	234 (35%)	161 (24%)
Middle		551 (27%)	162 (24%)	168 (25%)	221 (33%)
High		819 (41%)	265 (40%)	267 (40%)	287 (43%)
Occupational level	N=2,007				
Low		637 (32%)	242 (36%)	234 (35%)	161 (24%)
Middle		551 (27%)	162 (24%)	168 (25%)	221 (33%)
High		819 (41%)	265 (40%)	267 (40%)	287 (43%)
Income level (euros)	N=1,554	2,066.08 ± 836.97	2,085.92 ± 883.16	2,082.45 ± 837.32	2,031.61 ± 790.60

Glucose metabolism status	N=2,007				
Normal glucose metabolism		1,208 (60%)	321 (48%)	425 (62%)	462 (69%)
Prediabetes		323 (16%)	124 (19%)	111 (15%)	102 (15%)
Type 2 diabetes		476 (24%)	224 (34%)	142 (22%)	105 (16%)
Other types of diabetes		0 (0%)	0 (0%)	0 (0%)	0 (0%)
Glucose-lowering medication	N=2,007	342 (17%)	169 (25%)	102 (15%)	71 (11%)
Matsuda Index	N=2,007	3.46 (2.04, 5.19)	2.88 (1.74, 4.46)	3.59 (2.18, 5.45)	3.98 (2.42, 5.82)
Office systolic blood pressure (mmHg)	N=2,007	134.59 ± 17.94	136.91 ± 17.75	134.40 ± 18.42	132.48 ± 17.39
Office diastolic blood pressure (mmHg)	N=2,007	76.61 ± 9.80	77.38 ± 9.77	76.66 ± 10.09	75.79 ± 9.48
24-hour ambulatory systolic blood pressure (mmHg)	N=1,835	118.68 ± 11.35	119.76 ± 11.82	118.24 ± 11.05	118.04 ± 11.10
24-hour ambulatory diastolic blood pressure (mmHg)	N=1,835	73.61 ± 7.01	73.92 ± 7.31	73.33 ± 6.81	73.57 ± 6.90
Use of antihypertensive medication (yes/no)	N=2,007	744 (37%)	319 (48%)	229 (34%)	196 (29%)
Body-mass index (kg/m2)	N=2,007	26.72 ± 4.23	27.52 ± 4.68	26.54 ± 4.10	26.11 ± 3.73
waist circumference (cm)	N=2,005	95.05 ± 12.81	97.44 ± 13.62	94.31 ± 12.34	93.40 ± 12.09
Alcohol consumption	N=2,007				
None		335 (17%)	103 (15%)	105 (16%)	127 (19%)
Moderate		1,121 (56%)	342 (51%)	372 (56%)	407 (61%)

High		551 (27%)	224 (33%)	192 (29%)	135 (20%)
Total/HDL cholesterol ratio	N=2,007	3.53 (2.87, 4.36)	3.56 (2.89, 4.40)	3.50 (2.85, 4.38)	3.50 (2.89, 4.29)
Use of lipid-modifying medication (yes/no)	N=2,007	656 (33%)	279 (42%)	202 (30%)	175 (26%)
Smoking status	N=2,007				
Never		687 (34%)	191 (29%)	236 (35%)	260 (39%)
Former		1,063 (53%)	379 (57%)	346 (52%)	338 (51%)
Current		257 (13%)	99 (15%)	87 (13%)	71 (11%)
Physical activity (hours/day)	N=1,788	14.31 ± 8.08	13.74 ± 7.90	14.91 ± 8.40	14.29 ± 7.91
Total caloric intake (KJ/day)	N=1,890	9,215.13 ± 2,553.35	9,052.41 ± 2,564.97	9,115.10 ± 2,401.69	9,480.60 ± 2,670.02
History of cardiovascular disease	N=1,991	316 (16%)	128 (19%)	100 (15%)	88 (13%)
Estimated Glomerular Filtration Rate	N=2,003	88.32 ± 14.31	86.64 ± 14.84	88.34 ± 13.89	89.98 ± 14.00

Abbreviations: HbA1c, hemoglobin A1c; HDL, high-density lipoprotein; NGM, normal glucose metabolism; eGFR, estimated glomerular filtration rate; HRV, heart rate variability.

Table 7 Time and frequency measurements of HRV in the population of the Maastricht Study according to tertiles of the frequency HRV z-score

HRV, Frequency domain composite score (SD)					
Characteristic	Number of participants	Overall, N = 2,007	Tertile 1, N=669	Tertile 2, N=669	Tertile 3, N=669
HRV, Time domain composite score (SD)	N=2,007	0.00 ± 1.00	-0.69 ± 0.33	-0.11 ± 0.35	0.80 ± 0.82
SDNN (ms)		135.54 ± 37.55	100.24 ± 17.41	134.10 ± 17.07	172.29 ± 32.21
SDANN (ms)		122.09 ± 36.13	90.55 ± 17.90	121.48 ± 19.09	154.23 ± 34.69
RMSSD (ms)		29.88 ± 17.98	19.51 ± 6.01	27.10 ± 8.78	43.04 ± 23.85
SDNN index (ms)		54.30 ± 18.31	39.07 ± 7.16	51.64 ± 7.23	72.18 ± 18.54
SDSD (ms)		29.88 ± 17.98	19.51 ± 6.01	27.10 ± 8.78	43.04 ± 23.85
pNN50 (%)		6.16 (2.66, 12.24)	2.26 (1.11, 4.03)	6.16 (3.81, 9.73)	13.97 (9.04, 22.85)
HRV, Frequency domain composite score (SD)	N=2,007	0.00 ± 1.00	-0.61 ± 0.17	-0.13 ± 0.14	0.73 ± 0.64
TP (ms2)		11,589.40 (7,873.16, 16,499.85)	6,792.09 (5,018.46, 8,410.98)	11,958.30 (9,837.80, 13,882.00)	18,924.20 (15,496.70, 23,474.60)
ULF (ms2)		9,840.92 (6,481.91, 13,973.95)	5,850.61 (4,165.43, 7,360.11)	10,304.80 (8,061.78, 12,509.10)	16,284.00 (12,436.30, 20,458.90)
VLF (ms2)		1,075.88 (736.36, 1,556.57)	633.38 (485.93, 810.70)	1,071.67 (884.47, 1,283.38)	1,864.39 (1,428.75, 2,473.42)
LF (ms2)		347.03 (207.59, 591.74)	189.50 (132.12, 257.22)	344.69 (262.36, 471.07)	712.31 (513.60, 975.19)

HF (ms2)	84.26 (47.72, 147.19)	43.44 (30.16, 65.20)	86.59 (57.68, 127.73)	162.02 (102.58, 275.97)

Abbreviation: HRV, heart rate variability.
Data are presented as mean ± standard deviation, median (interquartile range)

Table 8 B cell function parameters in the population of the Maastricht Study according to tertiles of the frequency domain HRV z-score

		HRV frequency domain composite score			
Characteristic	Number of participants	Overall, N = 2,007	Tertile 1, N=669	Tertile 2, N=669	Tertile 3, N=669
C-peptidogenic index (no unit)	N=2,007	471.29 ± 1,003.20	412.39 ± 613.12	444.55 ± 1,171.54	556.92 ± 1,123.52
Overall Insulin secretion (no unit)	N=2,007	193.60 ± 79.46	190.45 ± 82.62	195.56 ± 80.55	194.78 ± 75.05
B cell glucose sensitivity (pmol/m^2/mM)	N=2,007	27.39 ± 18.43	25.94 ± 18.78	27.72 ± 18.08	28.52 ± 18.36
B cell potentiation factor (no unit)	N=2,007	1.63 ± 0.69	1.55 ± 0.67	1.64 ± 0.72	1.70 ± 0.68
B cell rate sensitivity (pmol/m^2/mM)	N=2,007	239.72 ± 312.06	216.89 ± 251.43	236.92 ± 271.61	265.33 ± 392.77

Data are presented as mean ± standard deviation

Table 9 Associations of heart rate variability (HRV) with C-peptidogenic index, overall insulin secretion, β cell glucose sensitivity and β cell potentiation factor.

	C-peptidogenic index	*P-value*	Overall insulin secretion	*P-value*	B cell glucose sensitivity	*P-value*	B cell potentiation	*P-value*
HRV time domain, per SD	stβ [95% CI]		stβ [95% CI]		stβ [95% CI]		stβ [95% CI]	
Crude	0.06[0.02; 0.10]	**0.007**	0.01[-0.03; 0.05]	0.664	0.04[-0.00; 0.08]	0.055	0.07[0.03; 0.12]	**0.001**
2	0.06[0.01; 0.10]	**0.008**	0.00[-0.04; 0.05]	0.876	0.04[-0.00; 0.09]	**0.042**	0.07[0.03; 0.12]	**0.001**
3	0.06[0.01; 0.10]	**0.010**	0.03[-0.00; 0.08]	0.091	0.05[0.00; 0.09]	**0.022**	0.04[0.00; 0.09]	**0.033**
4	0.05[0.00; 0.09]	**0.036**	0.04[-0.00; 0.08]	0.102	0.04[0.00; 0.08]	0.077	0.04[-0.00; 0.08]	0.078
HRV frequency domain, per SD	stβ [95% CI]		stβ [95% CI]		stβ [95% CI]		stβ [95% CI]	
Crude	0.06[0.02; 0.10]	**0.006**	0.01[-0.03; 0.05]	0.585	0.04[0.00; 0.09]	**0.042**	0.07[0.03; 0.12]	**0.001**
2	0.06[0.02; 0.10]	**0.009**	0.00[-0.04;0.05]	0.873	0.05[0.00; 0.09]	**0.034**	0.07[0.03; 0.12]	**0.001**
3	0.06[0.01; 0.10]	**0.011**	0.03[-0.01; 0.07]	0.112	0.05[0.00; 0.10]	**0.019**	0.05[0.00; 0.09]	**0.030**
4	0.05[0.00; 0.09]	**0.042**	0.03[-0.01; 0.07]	0.137	0.04[-0.00; 0.08]	0.091	0.03[-0.01; 0.08]	0.107

Standardized regression coefficient (stβ) represents the difference in β cell index (in SD) per 1-SD increment in HRV measure, where time domain HRV was estimated from SDNN, SDANN, RMSSD, SDNN index and pNN50; and frequency domain was estimated from TP, ULF, VLF, LF, and HF.
Bold indicates $p<0.05$.
Variables entered in the models in addition to HRV: model 1: none (crude results); model 2: age, sex, and educational status (low, medium, high); model 3: model 2 + Matsuda Index; model 4: model 3 + office systolic blood pressure, total cholesterol/HDL cholesterol ratio, use of antihypertensive or lipid-modifying medication (yes/no), BMI, smoking status (current, ever, never), and alcohol consumption status (none, low, high).
Abbreviations: SD, standard deviation; CI, confidence interval; HRV, heart rate variability, HDL, high density lipid; BMI, body-mass index. All abbreviations for indices of HRV are presented in the Methods section.

Table 10 Associations of HRV time domain with β-cell rate sensitivity tertiles (odds ratios and 95% confidence intervals)

HRV time domain	RATE SENSITIVITY-Tertiles		
	1	2	3
Crude model			
OR (95%CI)	0.92 (0.83; 1.03)	0.92 (0.83; 1.03)	1 (ref)
P trend	0.152	0.137	-
Model 2			
OR (95%CI)	0.96(0.86; 1.07)	0.94 (0.85; 1.05)	1 (ref)
P trend	0.464	0.319	-
Model 3			
OR (95%CI)	0.96 (0.86; 1.08)	0.93 (0.83; 1.04)	1 (ref)
P trend	0.522	0.202	-
Model 4			
OR (95%CI)	0.97(0.87; 1.09)	0.93(0.83; 1.05)	1 (ref)
P trend	0.688	0.233	-

CI, confidence interval. Third tertile of β-cell rate sensitivity is the reference group. Values <1.00 indicate a better β-cell rate sensitivity, values >1.00 indicate worse β-cell rate sensitivity.

Table 11 Associations of HRV frequency domain with β-cell rate sensitivity tertiles (odds ratios and 95% confidence intervals)

HRV frequency domain	RATE SENSITIVITY-Tertiles		
	1	2	3
Crude Model			
OR (95%CI)	0.94 (0.85; 1.04)	0.93 (0.84; 1.04)	1 (ref)
P trend	0.259	0.192	-
Model 2			
OR (95%CI)	0.99 (0.88; 1.10)	0.96 (0.85; 1.07)	1 (ref)
P trend	0.827	0.455	-
Model 3			
OR (95%CI)	0.99 (0.89; 1.10)	0.94 (0.84; 1.05)	1 (ref)
P trend	0.900	0.305	-
Model 4			
OR (95%CI)	1.01 (0.90; 1.13)	0.94 (0.84; 1.06)	1 (ref)
P trend	0.813	0.323	-

CI, confidence interval. Third tertile of β-cell rate sensitivity is the reference group. Values <1.00 indicate a better β-cell rate sensitivity, values >1.00 indicate lower β-cell rate sensitivity.

Table 12 General characteristics and metabolic phenotype of the population of the VNDS according to absence/presence of signs of cardio autonomic neuropathy

Characteristic	Overall, n=537	Subjects without alteration of cardioautonomic tests, n=446	Subjects with alteration of cardioautonomic tests, n=91	p value
Age (years)	58.3 ± 9.6	58.3 ± 9.7	58.2 ± 9.1	0.907
Males (%)	66.3	66.1	67.0	0.904
BMI (Kg/m^2)	30.0 ± 5.1	29.6 ± 4.9	31.5 ± 5.5	**0.001**
Waist (cm)	101.1 ± 11.9	100.4 ± 11.6	104.5 ± 12.9	**0.003**
HbA1c (%)	6.8 ± 1.1	6.8 ± 1.1	6.9 ± 1.1	0.174
Smokers (past/current) (%)	50.7	48.3	61.8	**0.026**
Fasting plasma glucose (mmol/L)	7.2 ± 1.6	7.1 ± 1.5	7.5 ± 2.1	**0.043**
2-hour OGTT plasma glucose (mmol/L)	13.0 ± 4.1	13.0 ± 4.0	13.4 ± 4.1	0.400
Systolic blood pressure (mmHg)	135.9 ± 16.5	135.6 ± 16.3	137.3 ± 17.7	0.382
Diastolic blood pressure (mmHg)	83.7 ± 8.9	83.5 ± 8.5	84.5 ± 10.7	0.342
Total cholesterol (mmol/l)	4.9 ± 1.0	4.8 ± 1.0	4.8 ± 1.0	0.720
LDL cholesterol (mmol/L)	3.0 ± 0.9	3.0 ± 0.9	3.0 ± 0.9	0.779
HDL cholesterol (mmol/L)	1.1 ± 0.3	1.1 ± 0.3	1.2 ± 0.3	0.125
TGs (mmol/L)	1.6 ± 0.9	1.6 ± 1.0	1.6 ± 0.9	0.695
Basal Cpeptide (nmol/l)	1.4 ± 0.4	1.0 ± 0.4	1.1 ± 0.4	0.283
eGFR MDRD (mL/min/1.73 m2)	85.0 ± 21.4	84.5 ± 21.2	87.5 ± 22.6	0.218
Albuminuria (mcg/min)	6.0 [2-12.8]	5.9 [1.9-11.0]	8.0 [3.7-19.8]	**0.003**
Insulin sensitivity (μmol/min/m^2 BSA)	614.0 [387.7-873.0]	618 [398-873]	554 [320-895]	0.202

Beta-cell function-derivative control LOG (pmol/m^2 BSA/(mmol/L/min)	4.8 ± 2.9	4.9 ± 2.8	4.3 ± 3.1	0.063
Beta-cell function-proportional control LOG (pmol/m^2 BSA)/(mmol/L/min)	3.8 ± 0.8	3.8 ± 0.8	3.7 ± 0.9	0.108
l-s	1.18 ± 0.15	1.20 ± 0.15	1.12 ± 0.12	**<0.001**
DB	19.4 ± 8.8	20.9 ± 8.5	12.3 ± 7.0	**<0.001**
VM	1.50 ± 0.47	1.55 ± 0.50	1.27 ± 0.19	**<0.001**
Statins (%)	19.7	19.0	23.1	0.386
Antihypertensive drugs (%)	53.3	50.2	68.1	**0.002**
Antiplatelet drugs (%)	14.4	13.0	20.9	0.070
Retinopathy (any) (%)	4.9	4.4	7.7	0.247
Carotid stenosis >40% (%)				
No stenosis	31,4	34.6	15.9	
Intimal thickening	43,5	42.2	50.0	**0.007**
Stenosis <40%	20.9	19.2	29.3	
Stenosis >40%	4.2	4.0	4.9	
ECG (%)				
No alteration	75.7	85.6	62.7	
CHD certain	3.6	3.3	4.8	**0.005**
CHD probable	2.5	2.8	1.2	
Other alterations	18.2	15.4	31.3	
Lower limb stenosis (any) (%)				
No stenosis	47.6	47.6	47.6	
Intimal thickening	28.7	28.9	28.0	0.818
Atherogenic plaques	18.4	18.7	17.1	
Stenosis	5.2	4.8	7.3	

Abbreviation: BMI, Body Mass Index; HbA1c, hemoglobin A1c; LDL, low-density lipoprotein ; HDL, high-density lipoprotein; TGs, triglycerides; eGFR, estimated glomerular filtration rate (MDRD: Modification of Diet in Renal Disease); BSA, Body Surface Area; l-s, lying to standing; DB, deep breathing; VM, Valsalva maneuver; CHD: Coronary Heart Disease.

Data are presented as mean ± standard deviation, median (interquartile range)

9. SUPPLEMENTAL MATERIAL

Supplemental Table S1 General study population characteristics of the included and excluded participants

Characteristic	Included, N=2,007	Excluded, N=1,444
Age (years)	59.88± 8.25	59.60± 8.30
Sex		
Men	1,044 (52%)	731 (51%)
Women	963 (48%)	713 (49%)
Educational status		
Low	637 (32%)	498 (36%)
Middle	551 (27%)	403 (29%)
high	819 (41%)	469 (34%)
Occupational status		
Low	637 (32%)	498 (36%)
Middle	551 (27%)	403 (29%)
high	819 (41%)	469 (34%)
Income level (euros)	2,066.08 ± 836.97	1,934.71 ± 786.12
Glucose metabolism status		
Normal glucose metabolism status	1,208 (60%)	716 (50%)

Characteristic	Included, N=2,007	Excluded, N=1,444
Prediabetes	323 (16%)	188 (13%)
Type 2 diabetes	476 (24%)	499 (35%)
Other types of diabetes	0 (0%)	41 (2%)
Glucose-lowering medication	342 (17%)	465 (32%)
Matsuda Index (no unit)	3.46 (2.04, 5.19)	3.49 (2.01, 5.39)
Office systolic blood pressure (mmHg)	134.59 ± 17.94	135.75 ± 18.57
Office diastolic blood pressure (mmHg)	76.61 ± 9.80	75.55 ± 9.90
24-hour ambulatory systolic blood pressure (mmHg)	118.68 ± 11.35	119.38 ± 12.18
24-hour ambulatory diastolic blood pressure (mmHg)	73.61 ± 7.01	72.91 ± 7.08
Use of antihypertensive medication (yes/no)	744 (37%)	648 (45%)
Body-mass index (kg/m^2)	26.72 ± 4.23	27.60 ± 4.94
waist circumference (cm)	95.05 ± 12.81	97.21 ± 14.99
Alcohol consumption		
None	335 (17%)	301 (22%)
Moderate	1,121 (56%)	767 (55%)
High	551 (27%)	328 (23%)
Total/HDL cholesterol ratio	3.53 (2.87, 4.36)	3.38 (2.75, 4.20)
Use of lipid-modifying medication (yes/no)	656 (33%)	603 (42%)

Characteristic	Included, N=2,007	Excluded, N=1,444
Smoking status		
Never	687 (34%)	485 (35%)
Former	1,063 (53%)	696 (50%)
Current	257 (13%)	213 (15%)
Physical activity (hours/day)	14.31 ± 8.08	13.67 ± 8.17
Total caloric intake (KJ/day)	9,215.13 ± 2,553.35	9,053.90 ± 2,516.81
History of cardiovascular disease	316 (16%)	252 (18%)
Estimated Glomerular Filtration Rate	88.32 ± 14.31	87.84 ± 15.80
SDNN (ms)	135.54 ± 37.55	124.04 ± 37.99
SDANN (ms)	122.09 ± 36.13	111.83 ± 36.62
RMSSD (ms)	29.88 ± 17.98	29.01 ± 17.58
SDNN index (ms)	54.30 ± 18.31	49.78 ± 17.87
pNN50 (%)	6.16 (2.66, 12.24)	5.66 (2.14, 12.53)
TP (ms^2)	11,589.40 (7,873.16, 16,499.85)	9,570.26 (6,238.30, 14,006.20)
ULF (ms^2)	9,840.92 (6,481.91, 13,973.95)	8,154.15 (5,049.28, 12,047.40)
VLF (ms^2)	1,075.88 (736.36, 1,556.57)	896.47 (580.99, 1,380.89)
LF (ms^2)	347.03 (207.59, 591.74)	290.82 (162.51, 499.96)
HF (ms^2)	84.26 (47.72, 147.19)	73.61 (38.95, 139.65)

Characteristic	Included, N=2,007	Excluded, N=1,444
C-peptidogenic index	471.29 ± 1,003.20	441.40 ± 1,268.47
Overall Insulin secretion	193.60 ± 79.46	197.94 ± 81.10
B cell glucose sensitivity	27.39 ± 18.43	27.11 ± 17.97
B cell potentiation factor	1.63 ± 0.69	1.63 ± 0.66
B cell rate sensitivity	239.72 ± 312.06	264.29 ± 341.54

Data are presented as mean ± standard deviation, median (interquartile range) or number (%).

Abbreviations: BMI, body-mass index; HbA1c, hemoglobin A1c; HDL, high-density lipoprotein; NGM, normal glucose metabolism; eGFR, estimated glomerular filtration rate. All abbreviations for indices of HRV are presented in the Methods section.

Supplemental Table S2 P-values of interaction terms with glucose metabolism status and sex in the associations of HRV with indices of β cell function

	N	Prediabetes P-value for interaction	Type 2 diabetes P-value for interaction	Sex P-value for interaction
Main independent: HRV time domain				
Dependent:				
C-peptidogenic index	2,007	0.20	0.40	0.10
Overall insulin secretion	2,007	0.30	0.40	0.40
B cell glucose sensitivity	2,007	0.20	0.40	0.60
B cell potentiation	2,007	**0.01**	0.93	0.70
B cell rate sensitivity	2,007	0.30	0.70	0.80
HRV frequency domain				
C-peptidogenic index	2,007	0.20	0.40	0.08
Overall insulin secretion	2,007	0.30	0.70	0.30
B cell glucose sensitivity	2,007	0.20	0.50	0.60
B cell potentiation	2,007	**0.01**	0.92	0.50
Rate sensitivity	2,007	0.30	0.80	0.90

Interaction terms were calculated for glucose metabolism status (i.e., prediabetes versus normal glucose metabolism status and type 2 diabetes versus normal glucose metabolism status) or sex with the main determinants (i.e. time or frequency domain HRV score) and included in the respective model 4s of table 2 (main results). P value for interaction were in the associations of time or frequency domain HRV with indices of β cell function. Time domain HRV was estimated from SDNN, SDANN, RMSSD, SDNN index and pNN50; and frequency domain was estimated from TP, ULF, VLF, LF, and HF.

Variables in the model in addition to determinants and interaction term(s) with sex, glucose metabolism status and are: age, sex, educational status, Matsuda Index, office systolic blood pressure, total cholesterol/HDL cholesterol ratio, use of antihypertensive or lipid-modifying medication, BMI, smoking status, and alcohol consumption status.

P value < 0.05 denotes statistically significant interaction.

Abbreviations: HRV, heart rate variability, HDL, high density lipid; BMI, body-mass index. All abbreviations for indices of HRV are presented in the Methods section.

Supplemental Table S3 Associations of individual HRV indices with C-peptidogenic index, overall insulin secretion, β cell glucose sensitivity, β cell potentiation, β cell rate sensitivity (model 3)

	C-peptidogenic index		Overall insulin secretion		B cell glucose sensitivity		B cell potentiation factor		B cell rate sensitivity	
HRV time domain, per SD	stβ [95% CI]	*P-value*	stβ [95% CI]	*P-value*	stβ [95% CI]	*P-value*	stβ [95% CI]	*P-value*	stβ [95% CI]	*P-value*
SDNN, per SD lower	-0.06 (-0.12 to -0.01)	**0.025**	-0.05 (-0.10 to 0.00)	0.054	-0.05 (-0.09 to 0.00)	**0.047**	-0.02 (-0.07 to 0.04)	0.53	-0.01 (-0.06 to 0.05)	0.77
RMSSD, per SD lower	-0.03 (-0.07 to 0.02)	0.21	-0.02 (-0.06 to 0.02)	0.33	-0.02 (-0.06 to 0.02)	0.33	-0.01 (-0.05 to 0.03)	0.60	0.00 (-0.04 to 0.05)	0.90
SDANN, per SD lower	-0.05 (-0.09 to 0.00)	**0.044**	-0.04 (-0.08 to 0.01)	0.10	-0.03 (-0.08 to 0.01)	0.13	-0.04 (-0.09 to 0.00)	0.060	-0.02 (-0.07 to 0.02)	0.36
SDNN index, per SD lower	-0.05 (-0.09 to 0.00)	**0.044**	-0.04 (-0.08 to 0.01)	0.10	-0.03 (-0.08 to 0.01)	0.13	-0.04 (-0.09 to 0.00)	0.060	-0.02 (-0.07 to 0.02)	0.36
pNN50, per SD lower	-0.04 (-0.08 to 0.01)	0.11	-0.03 (-0.07 to 0.02)	0.24	-0.03 (-0.07 to 0.01)	0.19	-0.02 (-0.06 to 0.03)	0.47	0.00 (-0.04 to 0.04)	0.98
HRV frequency domain, per SD	stβ [95% CI]		stβ [95% CI]		stβ [95% CI]		stβ [95% CI]		stβ [95% CI]	
TP, per SD lower	-0.07 (-0.12 to -0.01)	**0.015**	-0.05 (-0.10 to 0.01)	0.084	-0.04 (-0.09 to 0.00)	0.063	-0.02 (-0.07 to 0.04)	0.55	0.00 (-0.06 to 0.05)	0.86
ULF, per SD lower	-0.05 (-0.09 to 0.00)	**0.040**	-0.03 (-0.08 to 0.01)	0.13	-0.04 (-0.08 to 0.01)	0.11	-0.04 (-0.09 to 0.00)	**0.043**	-0.01 (-0.06 to 0.03)	0.54
VLF, per SD lower	-0.04 (-0.09 to 0.00)	0.069	-0.02 (-0.07 to 0.02)	0.30	-0.03 (-0.07 to 0.02)	0.27	-0.02 (-0.07 to 0.02)	0.32	0.00 (-0.05 to 0.04)	0.90
LF, per SD lower	-0.02 (-0.07 to 0.02)	0.36	-0.02 (-0.06 to 0.03)	0.41	-0.03 (-0.07 to 0.02)	0.22	-0.01 (-0.05 to 0.04)	0.78	0.01 (-0.03 to 0.06)	0.62
HF, per SD lower	-0.03 (-0.08 to 0.01)	0.16	-0.03 (-0.07 to 0.01)	0.14	-0.02 (-0.06 to 0.02)	0.34	-0.02 (-0.06 to 0.02)	0.32	-0.01 (-0.06 to 0.03)	0.57

Standardized regression coefficient (stβ) represents the difference in β cell index (in SD) per SD lower SDNN, SDANN, RMSSD, SDNN index and pNN50, TP, ULF, VLF, LF, and HF. Values per SD are reported in the legend of Table 2.
Bold indicates P<0.05.
Variables entered in the models in addition to HRV: age, sex, and educational status (low, medium, high), Matsuda Index, office systolic blood pressure, total cholesterol/HDL cholesterol ratio, use of antihypertensive or lipid-modifying medication (yes/no), BMI, smoking status (current, ever, never), and alcohol consumption status (none, low, high).
Abbreviations: SD, standard deviation; CI, confidence interval; HRV, heart rate variability, HDL, high density lipid; BMI, body-mass index. All abbreviations for indices of HRV are presented in the Methods section.

Supplemental Table S4 Associations of HRV with C-peptidogenic index, overall insulin secretion, β cell glucose sensitivity, β cell potentiation, and β cell rate sensitivity after additional adjustment for total caloric intake and physical activity (model 4), kidney function (eGFR; model 5), or history of CVD (model 6)

	C-peptidogenic index	*P-value*	Overall insulin secretion	*P-value*	B cell glucose sensitivity	*P-value*	B cell potentiation factor	*P-value*	B cell rate sensitivity	*P-value*
HRV time domain, per SD	stβ [95% CI]		stβ [95% CI]		stβ [95% CI]		stβ [95% CI]		stβ [95% CI]	
4, n=1,699	-0.06 (-0.11 to -0.01)	**0.026**	-0.04 (-0.08 to 0.01)	0.13	-0.04 (-0.09 to 0.01)	0.089	-0.03 (-0.08 to 0.02)	0.24	-0.01 (-0.06 to 0.04)	0.71
5, n=2,003	-0.05 (-0.09 to 0.00)	**0.047**	-0.03 (-0.07 to 0.02)	0.21	-0.03 (-0.08 to 0.01)	0.15	-0.03 (-0.08 to 0.01)	0.13	-0.01 (-0.06 to 0.03)	0.55
6, n=1,991	-0.05 (-0.09 to 0.00)	**0.039**	-0.03 (-0.08 to 0.01)	0.13	-0.04 (-0.08 to 0.01)	0.11	-0.04 (-0.08 to 0.01)	0.10	-0.01 (-0.06 to 0.03)	0.63
HRV frequency domain, per SD	stβ [95% CI]		stβ [95% CI]		stβ [95% CI]		stβ [95% CI]		stβ [95% CI]	
4, n=1,699	-0.05 (-0.10 to 0.00)	**0.032**	-0.03 (-0.08 to 0.02)	0.19	-0.04 (-0.09 to 0.01)	0.11	-0.03 (-0.08 to 0.02)	0.26	0.00 (-0.05 to 0.05)	0.92
5, n=2,003	-0.05 (-0.09 to 0.00)	0.054	-0.03 (-0.07 to 0.02)	0.25	-0.03 (-0.08 to 0.01)	0.16	-0.03 (-0.07 to 0.01)	0.17	-0.01 (-0.05 to 0.04)	0.69
6 n=1,991	-0.05 (-0.09 to 0.00)	**0.045**	-0.03 (-0.08 to 0.01)	0.14	-0.04 (-0.08 to 0.01)	0.11	-0.03 (-0.08 to 0.01)	0.13	-0.01 (-0.05 to 0.04)	0.77

Standardized regression coefficient (stβ) represents the difference in β cell index in SD per SD greater individual HRV index. Values per SD are numerically similar to the numbers reported in the legend of Table 2.
Bold indicates P<0.05.
Variables entered in the models in addition to HRV: age, sex, and educational status (low, medium, high), Matsuda Index, office systolic blood pressure, total cholesterol/HDL cholesterol ratio, use of antihypertensive or lipid-modifying medication (yes/no), BMI, smoking status (current, ever, never), and alcohol consumption status (none, low, high).
Abbreviations: OR, odds ratio; SD, standard deviation; CI, confidence interval; HRV, heart rate variability, HDL, high density lipid; BMI, body-mass index. All abbreviations for indices of HRV are presented in the Methods section.

Supplemental Table S5 Associations of HRV with C-peptidogenic index, overall insulin secretion, β cell glucose sensitivity, β cell potentiation factor and β cell rate sensitivity, where BMI was replaced with waist circumference (model 3A); educational level was replaced with occupational status (model 3B) or income level (model 3C); and office systolic blood pressure was replaced with office diastolic blood pressure (model 3D), 24-hour ambulatory systolic blood pressure (model 3E), or 24-hour ambulatory diastolic blood pressure (model 3F)

	C-peptidogenic index	*P-value*	Overall insulin secretion	*P-value*	B cell glucose sensitivity	*P-value*	B cell potentiation factor	*P-value*	B cell rate sensitivity	*P-value*
HRV time domain, per SD	stβ [95% CI]		stβ [95% CI]		stβ [95% CI]		stβ [95% CI]		stβ [95% CI]	
3A, n=2,005	-0.05 (-0.09 to 0.00)	**0.035**	-0.04 (-0.08 to 0.01)	0.088	-0.04 (-0.08 to 0.01)	0.10	-0.04 (-0.08 to 0.01)	0.10	-0.01 (-0.06 to 0.03)	0.59
3B, n=										
3C, n=1,440	-0.06 (-0.12 to -0.01)	**0.025**	-0.05 (-0.10 to 0.00)	0.054	-0.05 (-0.09 to 0.00)	**0.047**	-0.02 (-0.07 to 0.04)	0.53	-0.01 (-0.06 to 0.05)	0.77
3D, n=2,007	-0.05 (-0.10 to 0.00)	**0.034**	-0.04 (-0.08 to 0.00)	0.078	-0.04 (-0.08 to 0.00)	0.081	-0.04 (-0.08 to 0.01)	0.10	-0.01 (-0.06 to 0.03)	0.60
3E. n=1,836	-0.05 (-0.10 to 0.00)	**0.050**	-0.04 (-0.09 to 0.00)	0.064	-0.04 (-0.09 to 0.00)	0.059	-0.04 (-0.09 to 0.01)	0.085	-0.01 (-0.06 to 0.03)	0.55
3F, n=1,836	-0.05 (-0.10 to 0.00)	**0.045**	-0.05 (-0.09 to 0.00)	**0.043**	-0.05 (-0.09 to 0.00)	**0.047**	-0.04 (-0.09 to 0.00)	0.080	-0.02 (-0.06 to 0.03)	0.46
HRV frequency domain, per SD	stβ [95% CI]		stβ [95% CI]		stβ [95% CI]		stβ [95% CI]		stβ [95% CI]	
3A, n=2,005	-0.05 (-0.09 to 0.00)	**0.041**	-0.04 (-0.08 to 0.01)	0.11	-0.04 (-0.08 to 0.01)	0.11	-0.03 (-0.08 to 0.01)	0.13	-0.01 (-0.05 to 0.04)	0.74
3B, n=										
3C, n=1,440	-0.07 (-0.12 to -0.01)	**0.015**	-0.05 (-0.10 to 0.01)	0.084	-0.04 (-0.09 to 0.00)	0.063	-0.02 (-0.07 to 0.04)	0.55	0.00 (-0.06 to 0.05)	0.86
3D, n=2,007	-0.05 (-0.09 to 0.00)	**0.039**	-0.04 (-0.08 to 0.01)	0.10	-0.04 (-0.08 to 0.01)	0.093	-0.03 (-0.08 to 0.01)	0.14	-0.01 (-0.05 to 0.04)	0.74
3E. n=1,836	-0.05 (-0.10 to 0.00)	**0.037**	-0.04 (-0.08 to 0.01)	0.12	-0.04 (-0.09 to 0.01)	0.081	-0.04 (-0.08 to 0.01)	0.10	-0.01 (-0.06 to 0.04)	0.68
3F, n=1,836	-0.05 (-0.10 to 0.00)	**0.033**	-0.04 (-0.09 to 0.00)	0.079	-0.04 (-0.09 to 0.00)	0.079	-0.04 (-0.09 to 0.00)	0.063	-0.04 (-0.09 to 0.01)	0.093

Standardized regression coefficient (stβ) represents the difference in β cell index in SD per SD greater individual HRV index. Values per SD are numerically similar to the numbers reported in the legend of Table 2.
Bold indicates P<0.05.
Variables entered in the models in addition to HRV: age, sex, and educational status (low, medium, high), Matsuda Index, office systolic blood pressure, total cholesterol/HDL cholesterol ratio, use of antihypertensive or lipid-modifying medication (yes/no), BMI, smoking status (current, ever, never), and alcohol consumption status (none, low, high).
Abbreviations: OR, odds ratio; SD, standard deviation; CI, confidence interval; HRV, heart rate variability, HDL, high density lipid; BMI, body-mass index. All abbreviations for indices of HRV are presented in the Methods section.