Nonalcoholic Fatty Liver Disease Is Independently Associated With an Increased Incidence of Chronic Kidney Disease in Patients With Type 1 Diabetes

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OBJECTIVE

There is no information about the role of nonalcoholic fatty liver disease (NAFLD) in predicting the development of chronic kidney disease (CKD) in type 1 diabetes.

RESEARCH DESIGN AND METHODS

We studied 261 type 1 diabetic adults with preserved kidney function and with no macroalbuminuria at baseline, who were followed for a mean period of 5.2 years for the occurrence of incident CKD (defined as estimated glomerular filtration rate [eGFR] <60 mL/min/1.73 m^2 and/or macroalbuminuria). NAFLD was diagnosed by ultrasonography.

RESULTS

At baseline, patients had a mean eGFR of 92 ± 23 mL/min/1.73 m^2; 234 (89.7%) of them had normoalbuminuria and 27 (10.3%) microalbuminuria. NAFLD was present in 131 (50.2%) patients. During follow-up, 61 subjects developed incident CKD. NAFLD was associated with an increased risk of incident CKD (hazard ratio [HR] 2.85 [95% CI 1.59–5.10]; $P < 0.001$). Adjustments for age, sex, duration of diabetes, hypertension, A1C, and baseline eGFR did not appreciably attenuate this association (adjusted HR 2.03 [1.10–3.77], $P < 0.01$). Results remained unchanged after excluding those who had microalbuminuria at baseline (adjusted HR 1.85 [1.03–3.27]; $P < 0.05$). Addition of NAFLD to traditional risk factors for CKD significantly improved the discriminatory capability of the regression models for predicting CKD (e.g., with NAFLD c statistic 0.79 [95% CI 0.73–0.86] vs. 0.76 [0.71–0.84] without NAFLD, $P = 0.002$).

CONCLUSIONS

This is the first study to demonstrate that NAFLD is strongly associated with an increased incidence of CKD. Measurement of NAFLD improves risk prediction for CKD, independently of traditional cardio-renal risk factors, in patients with type 1 diabetes.

Nonalcoholic fatty liver disease (NAFLD) has reached epidemic proportions worldwide (1). Up to 30% of adults in the U.S. and Europe have NAFLD, and the prevalence of this disease is much higher in people with diabetes (1,2). Indeed, the prevalence of NAFLD on ultrasonography ranges from ~50 to 70% in patients with type 2 diabetes (3–5) and

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~40 to 50% in patients with type 1 diabetes (6,7). Notably, patients with diabetes and NAFLD are also more likely to develop more advanced forms of NAFLD that may result in end-stage liver disease (8). However, accumulating evidence indicates that NAFLD is associated not only with liver-related morbidity and mortality but also with an increased risk of developing cardiovascular disease (CVD) and other serious extrahepatic complications (8–10).

In recent years, the possible link between NAFLD and chronic kidney disease (CKD) has also attracted considerable scientific interest (11). CKD is now recognized as a common condition that markedly increases the risk of end-stage renal disease (ESRD), CVD, and other important comorbidities. The number of patients with ESRD is increasing and represents a major public health problem worldwide (12–14). Because kidney disease often progresses to ESRD with its attendant complications, the identification of precursors and risk factors for CKD is essential, with the belief that interventions might prevent or delay progression to ESRD.

Increasing evidence indicates that NAFLD is strongly associated with an increased risk of CKD in people with and without diabetes (11). Indeed, we have previously shown that NAFLD is associated with an increased prevalence of CKD in patients with both type 1 and type 2 diabetes (15–17), and that NAFLD independently predicts the development of incident CKD in patients with type 2 diabetes (18). However, many of the risk factors for CKD are different in patients with type 1 and type 2 diabetes, and to date, it is uncertain whether NAFLD is an independent risk factor for incident CKD in type 1 diabetes or whether measurement of NAFLD improves risk prediction for CKD, taking account of traditional risk factors for CKD.

Therefore, the aim of the current study was to investigate *1*) whether NAFLD is associated with an increased incidence of CKD and *2*) whether measurement of NAFLD improves risk prediction for CKD, adjusting for traditional risk factors, in type 1 diabetic patients.

RESEARCH DESIGN AND METHODS

Patients

Using a retrospective, longitudinal cohort study design, we have initially identified from our electronic database all Caucasian type 1 diabetic outpatients with preserved kidney function (i.e., estimated glomerular filtration rate [eGFR] $\geq$60 mL/min/1.73 m^2) and with no macroalbuminuria (n = 563), who regularly attended our adult diabetes clinic between 1999 and 2001. Type 1 diabetes was diagnosed by the typical presentation of disease, the absolute dependence on insulin treatment for survival, the presence of undetectable fasting C-peptide concentrations, and the presence of anti–islet cell autoantibodies.

We subsequently excluded from analysis *1*) patients for whom a liver ultrasound examination was not available (n = 204), *2*) those with a documented history of cancer, cirrhosis, myocardial infarction, angina, and coronary revascularization procedures (n = 11), and *3*) those with secondary causes of chronic liver disease, such as excessive alcohol consumption (i.e., >30 g/day for men and >20 g/day for women, respectively), viral hepatitis, and drug-induced liver disease (n = 87).

Overall, 261 type 1 diabetic outpatients were included in the final analysis and were tested for the development of incident CKD during the follow-up period (through 31 May 2013). No significant differences were found in main demographic and laboratory data, including eGFR, between patients who did and did not have a liver ultrasound examination (data not shown).

All participants were periodically seen (every 3–6 months) for routine medical examinations of glycemic control and chronic complications of diabetes. No participants were lost to follow-up.

The local ethics committee approved the study protocol. The informed consent requirement for this study was exempted by the ethics committee because researchers only accessed retrospectively a de-identified database for analysis purposes.

Clinical and Laboratory Data

BMI was calculated by dividing weight in kilograms by height in meters squared. Waist circumference was measured at the level of the umbilicus. Blood pressure was measured in duplicate with a standard mercury manometer. Information on smoking, alcohol consumption, and use of medications was obtained from all participants by a validated questionnaire (15,16). In particular, alcohol consumption was assessed on the basis of the self-reported number of drinks consumed per day. The following amounts of alcoholic beverages were considered one drink: 330 mL beer (containing ~5% alcohol), 150 mL wine (containing ~12% alcohol), and 40 mL strong alcohol (containing ~50% alcohol). By study design, most patients included in this analysis were abstainers (n = 208; 79.7%) or drank only minimally (n = 53; 23 women drank <20 g and 30 men drank <30 g of alcohol per day, respectively).

Venous blood was drawn in the morning after an overnight fast. Serum liver enzymes, lipids, creatinine (measured using a Jaffé rate-blanked and compensated assay), and other biochemical blood measurements were determined by standard laboratory procedure (DAX 96; Bayer Diagnostics, Milan, Italy). Normal ranges for serum aminotransferase levels in our laboratory were 10–40 units/L for both men and women. Normal ranges for serum γ-glutamyltransferase (GGT) levels were 5–55 units/L for women and 5–75 units/L for men, respectively. LDL cholesterol was calculated by the Friedewald equation. A1C was measured by a high-performance liquid chromatography analyzer (HA-8140; Menarini Diagnostics, Florence, Italy); the upper limit of normal for our laboratory was 5.6%.

At baseline, eGFR was estimated in all participants from the four-variable Modification of Diet in Renal Disease (MDRD) study equation (19) and from the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation (20). Urinary albumin excretion was also measured from an early morning urine sample as the albumin-to-creatinine ratio (ACR) by an immunonephelometric method; microalbuminuria and macroalbuminuria were defined as ACR >2.5 and >30 mg/mmol for men and ACR >3.5 and >30 mg/mmol for women, respectively (21).

For this study, the development of incident CKD was defined as occurrence of eGFR <60 mL/min/1.73 m^2 and/or macroalbuminuria (21). Both of these outcome measures were confirmed in all participants in a least two consecutive occasions (within 3–6 months after the first examination).

Since waist circumference was available only in a few participants (n = 102), metabolic syndrome was diagnosed by a modified Adult Treatment Panel III definition that used BMI instead of waist circumference. In accordance with this definition (22), a person with type 1 diabetes was classified as having the metabolic syndrome if he/she had at least two of the following components: *1*) BMI $>$28.5 kg/m^2 in men or $>$26.5 kg/m^2 in women; *2*) triglycerides $\geq$1.7 mmol/L; *3*) HDL cholesterol $<$1.0 mmol/L in men and $<$1.29 mmol/L in women, or lipid-lowering treatment; and *4*) blood pressure $\geq$130/85 mmHg or antihypertensive treatment.

At baseline, hepatic ultrasonography was performed (within ~1 month after that blood measurements were taken) at our institution by two experienced radiologists who were blinded to the patient characteristics. Hepatic steatosis was diagnosed on the basis of characteristic ultrasonographic features, i.e., evidence of diffuse hyperechogenicity of the liver relative to the kidneys, ultrasound beam attenuation, and poor visualization of intrahepatic vessel borders and diaphragm (23). It is known that ultrasonography has good sensitivity and specificity for detecting moderate and severe hepatic steatosis (~90–95%), but its sensitivity is reduced when the hepatic fat infiltration upon liver biopsy is $<$30% (23). The intra- and interobserver variabilities for the ultrasound diagnosis of hepatic steatosis were within 5%.

Statistical Analysis

Data are expressed as means ± SD, medians (interquartile ranges), or proportions. Skewed variables (diabetes duration, liver enzymes, triglycerides, eGFR, urinary ACR, and daily insulin dose) were logarithmically transformed to improve normality prior to analysis. The unpaired Student t test and the χ^2 test with Yates correction for continuity (for categorical variables) were used to compare baseline characteristics of patients between those who developed CKD at follow-up and those who did not (Table 1) or between those with and without NAFLD at baseline (Supplementary Table 1).

Cox regression analysis was used to evaluate the independent association of NAFLD with the risk of incident CKD (i.e., defined as occurrence of macroalbuminuria or eGFR $<$60 mL/min/1.73 m^2 as estimated by either the MDRD study equation or the CKD-EPI equation) after adjustment for potential confounders (Table 2 and Supplementary Table 2). For prediction of incident CKD, men and women were combined and first-order interaction terms for sex-by-NAFLD interactions on risk for CKD were examined. Because the interactions were not statistically significant (P = 0.38), a sex-pooled multivariate Cox regression analysis was used. Three forced-entry multivariate Cox regression models were performed. The first regression model was adjusted for age, sex, duration of diabetes, A1C, and hypertension (i.e., blood pressure $\geq$140/90 mmHg or drug treatment) (model 1), the second model was additionally adjusted for baseline eGFR (i.e., a strong risk factor for CKD) (model 2), and, finally, the third model was adjusted for the same covariates as model 2 after excluding those (n = 27) with microalbuminuria at baseline (model 3). Covariates included in the regression models were chosen as potential confounding based on their biological plausibility or statistical association with CKD in univariate analyses. We also performed multivariate Cox regression analyses for each of the components of the renal outcome, i.e., macroalbuminuria and eGFR $<$60 mL/min/1.73 m^2, separately.

Concordance Harrell c index, defined as the proportion of all usable patient pairs in which predictors and outcomes are concordant, was calculated for different Cox regression models. The c index estimates the probability of concordance between predicted and observed CKD-free survival probability. A value of 0.5 indicates no predictive discrimination, and a value of 1.0 indicates a perfect separation of patients with different outcomes. By comparing c statistics with a nonparametric approach, we evaluated the discriminatory capability of the above-mentioned regression models 1 and 2 with the use of NAFLD as compared with the same models without NAFLD (Table 3).

We also evaluated risk reclassification for regression models 1 and 2 with the inclusion and exclusion of NAFLD, according to the method developed by Pencina et al. (24) for determining net reclassification improvement (NRI) (Supplementary Tables 1 and 4).

A Kaplan-Meier analysis of incidence curves for CKD during the follow-up was also undertaken in patients with and without NAFLD at baseline (Fig. 1). Differences between groups were tested by the log-rank test.

All analyses were performed using statistical package SPSS 19 and STATA 10. Statistical significance was assessed at the two-tailed 0.05 threshold.

RESULTS

Table 1 shows the baseline characteristics of the whole cohort of type 1 diabetic adults with preserved kidney function and without macroalbuminuria. At baseline, the mean $eGFR_{MDRD}$ was 92 ± 23 mL/min/1.73 m^2 (median 87.9 [IQR 74–104]), or $eGFR_{EPI}$ was 98.6 ± 19 mL/min/1.73 m^2 (median 99.7 [84–112]). Most patients (n = 234; 89.7%) had normal albuminuria, whereas 27 patients (10.3%) had microalbuminuria. NAFLD was present in 131 patients (50.2%).

Table 1 also shows the baseline characteristics of the cohort stratified by the presence of incident CKD_{MDRD} at follow-up. At baseline, patients who developed CKD at follow-up were older, more likely to be female and obese, and had a longer duration of diabetes than those who did not. These patients also had higher values of systolic blood pressure, A1C, triglycerides, serum GGT, and urinary ACR and lower values of $eGFR_{MDRD}$ and $eGFR_{EPI}$. Moreover, there was a higher percentage of patients with hypertension, metabolic syndrome, microalbuminuria, and some degree of diabetic retinopathy in patients who developed CKD at follow-up compared with those remaining free from CKD. The proportion using antihypertensive drugs (that always included the use of ACE inhibitors or angiotensin receptor blockers) was higher in those who progressed to CKD. Notably, as shown in Table 1, this patient group also had a substantially higher frequency of NAFLD on ultrasonography.

Baseline characteristics of the cohort stratified by NAFLD status at baseline are listed in Supplementary Table 1. As expected, NAFLD patients were older and more likely to be men compared with their counterparts without NAFLD. This group also had a higher prevalence of the metabolic syndrome and its individual components, a longer duration of

Table 1—Baseline clinical and biochemical characteristics of the cohort stratified by presence of incident CKD at follow-up

Variables	Whole cohort (*N* = 261)	No incident CKD (*n* = 200)	Incident CKD (*n* = 61)	*P* value*
Sex (male/female)	116/145	96/104	20/41	<0.05
Age (years)	41 ± 12	39 ± 12	48 ± 13	<0.001
Diabetes duration (years)	18 (10–29)	16 (10–24)	28 (18–36)	<0.001
BMI (kg/m^2)	24.5 ± 4.6	24.2 ± 4.3	25.9 ± 5.4	<0.05
Waist circumference (cm)§	90.2 ± 18	89.3 ± 19	92.8 ± 18	0.43
Current smokers (%)	24	26	18	0.18
Systolic blood pressure (mmHg)	128 ± 17	126 ± 16	136 ± 20	<0.001
Diastolic blood pressure (mmHg)	78 ± 9	78 ± 8	79 ± 10	0.14
Fasting glucose (mmol/L)	10.6 ± 4.2	10.6 ± 3.9	10.4 ± 4.7	0.71
A1C (%)	8.0 ± 1.1	7.9 ± 1.0	8.4 ± 1.3	<0.005
A1C (mmol/mol)	63.9 ± 7	62.8 ± 7	68.3 ± 8	<0.005
Triglycerides (mmol/L)	1.03 (0.73–1.43)	1.01 (0.73–1.37)	1.23 (0.84–1.97)	<0.01
HDL cholesterol (mmol/L)	1.41 ± 0.4	1.38 ± 0.3	1.46 ± 0.5	0.20
LDL cholesterol (mmol/L)	2.64 ± 0.8	2.61 ± 0.7	2.76 ± 0.9	0.19
$eGFR_{MDRD}$ (mL/min/1.73 m^2)	87.9 (74–104)	92.3 (84–110)	67.1 (62–83)	<0.001
$eGFR_{EPI}$ (mL/min/1.73 m^2)	99.2 (84–112)	105.2 (94–116)	76.1 (69–90)	<0.001
Urinary ACR (mg/mmol)	1.0 (0.3–2.9)	1.2 (0.3–2.0)	3.1 (0.8–10.0)	<0.001
Microalbuminuria (%)	10	4	31	<0.001
Diabetic retinopathy, any degree (%)	53	49	66	<0.001
Metabolic syndrome (%)	35	31	48	<0.01
Hypertension (%)	44	36	70	<0.001
Antihypertensive drug users (%)	40	25	61	<0.001
Insulin dose (units/day)	40 (28–55)	39 (27–54)	43 (30–62)	0.08
AST (units/L)	17 (13–24)	17 (12–23)	19 (13–25)	0.20
ALT (units/L)	19 (14–26)	19 (14–26)	20 (14–26)	0.66
GGT (units/L)	16 (11–29)	16 (11–25)	20 (12–39)	<0.05
NAFLD (%)	50	42	75	<0.001

Cohort size: *N* = 261. Data are expressed as means ± SD, medians (IQR), or proportions. Incident CKD was defined as occurrence of macroalbuminuria and/or $eGFR_{MDRD}$ <60 mL/min/1.73 m^2 as estimated by the MDRD study equation. The metabolic syndrome was defined by a modified Adult Treatment Panel III definition. Hypertension was defined as blood pressure ≥140/90 mmHg or antihypertensive treatment. ALT, alanine aminotransferase; AST, aspartate aminotransferase. **P* values for differences between those with and those without incident CKD_{MDRD} at follow-up that were assessed by the unpaired Student *t* test (for continuous variables) and by the χ^2 test (for categorical measures). §Measurement of waist circumference was available in 102 patients only. All other parameters were available in all patients.

diabetes, and higher serum liver enzymes (although the majority of NAFLD patients had serum liver enzymes within the normal range). Additionally, NAFLD patients also had a higher frequency of microalbuminuria and lower values of $eGFR_{MDRD}$ and $eGFR_{EPI}$ at baseline.

During follow-up (mean duration 5.2 ± 1.7 years, range 2–10), 61 patients developed CKD using the MDRD study equation to estimate eGFR (i.e., ~4.5% of participants progressed every year to eGFR <60 mL/min/1.73 m^2 or macroalbuminuria). Of these, 28 developed an $eGFR_{MDRD}$ <60 mL/min/1.73 m^2 with abnormal albuminuria (micro- or macroalbuminuria), 21 developed a reduced $eGFR_{MDRD}$ with normal albuminuria (but 9 of them had some degree of diabetic retinopathy at baseline), and 12 developed macroalbuminuria alone. None of them developed kidney failure requiring chronic dialysis.

At follow-up, patients who developed CKD had a mean $eGFR_{MDRD}$ of 59 ± 13 mL/min/1.73 m^2, whereas those who did not develop CKD had a mean $eGFR_{MDRD}$ of 85 ± 15 mL/min/1.73 m^2. The annual $eGFR_{MDRD}$ decline for the whole cohort was 2.68 ± 3.5 mL/min/1.73 m^2 per year. Interestingly, NAFLD patients had a greater annual decline in $eGFR_{MDRD}$ than those without NAFLD at baseline (3.28 ± 3.8 vs. 2.10 ± 3.0 mL/min/1.73 m^2 per year, $P < 0.005$). Similarly, the frequency of a renal functional decline (arbitrarily defined as ≥25% loss of baseline $eGFR_{MDRD}$) was greater among those with NAFLD than among those without the disease (26 vs. 11%, $P = 0.005$).

Figure 1 shows a Kaplan-Meier analysis of incidence curves for CKD_{MDRD} during the follow-up in patients with and without NAFLD at baseline. The difference between the two groups was statistically significant ($P < 0.001$ by the log-rank test). Almost identical results were found when we used the CKD-EPI formula for estimating eGFR (not shown).

Table 2 shows the effect of the adjustment for known renal risk factors on the relationship between NAFLD and incident CKD. In univariate regression analysis, NAFLD was significantly associated with an increased risk of incident CKD (hazard ratio [HR] 2.85 [95% CI 1.59–5.10], $P < 0.001$). Higher age, longer diabetes duration, higher A1C, higher serum triglycerides, higher serum GGT level, higher urinary ACR, hypertension, and lower baseline eGFR were also significantly associated with incident CKD. Interestingly, BMI was not significantly associated with CKD.

In multivariate regression analyses (Table 2, models 1 and 2), NAFLD maintained a significant association with the risk of incident CKD after adjusting for age, sex, diabetes duration,

Table 2—Univariate and multivariate Cox regression analyses showing associations of NAFLD and other factors with risk of incident CKD among type 1 diabetic adults

	Univariate analysis	*P* value	Multivariate model 1	*P* value	Multivariate model 2	*P* value	Multivariate model 3	*P* value
NAFLD (yes vs. no)	2.85 (1.59–5.10)	<0.001	2.26 (1.22–4.20)	<0.01	2.03 (1.10–3.77)	<0.01	1.85 (1.03–3.27)	<0.05
Sex (female vs. male)	1.43 (0.84–2.43)	0.19	1.54 (0.90–2.68)	0.14	0.75 (0.40–1.41)	0.37	0.93 (0.42–2.18)	0.86
Age (years)	1.03 (1.01–1.04)	<0.001	1.01 (0.98–1.04)	0.37	1.01 (0.97–1.03)	0.96	1.01 (0.97–1.05)	0.65
Diabetes duration (years)	1.02 (1.01–1.04)	<0.001	1.01 (0.98–1.03)	0.74	1.01 (0.97–1.02)	0.71	1.0 (0.97–1.03)	0.94
Hypertension (yes vs. no)	2.32 (1.40–4.38)	<0.001	1.96 (1.06–3.61)	<0.05	1.90 (1.02–3.39)	<0.05	1.75 (0.99–2.97)	0.06
A1C (%)	1.17 (1.02–1.37)	<0.05	1.11 (0.93–1.34)	0.26	1.09 (0.92–1.31)	0.32	1.15 (0.92–1.41)	0.29
$eGFR_{MDRD}$ (mL/min/1.73 m^2)	0.94 (0.91–0.96)	<0.001			0.95 (0.92–0.97)	<0.001	0.94 (0.91–0.97)	<0.001
Urinary ACR (mg/mmol)	1.08 (1.05–1.10)	<0.001						
BMI (kg/m^2)	1.03 (0.99–1.08)	0.09						
Smokers (yes vs. no)	1.22 (0.63–2.38)	0.56						
Triglycerides (mmol/L)	1.01 (1.01–1.03)	<0.01						
HDL cholesterol (mmol/L)	1.0 (0.98–1.01)	0.76						
LDL cholesterol (mmol/L)	1.0 (0.99–1.01)	0.32						

Cohort size: *N* = 261. Data are presented as HRs (±95% CIs) by Cox regression analysis. Incident CKD was defined as occurrence of macroalbuminuria and/or $eGFR_{MDRD}$ <60 mL/min/1.73 m^2 as estimated by the MDRD study equation. CKD_{MDRD} identified 61 subjects who developed CKD during follow-up. Multivariable regression model 1, adjustment for age, sex, duration of diabetes, A1C, and hypertension (blood pressure ≥140/90 mmHg or drug treatment); multivariable regression model 2, adjustment for age, sex, duration of diabetes, A1C, hypertension, and baseline $eGFR_{MDRD}$; multivariable regression model 3, adjustment for the same covariates as model 2 after excluding those (*n* = 27) with microalbuminuria at baseline.

hypertension, A1C, and baseline eGFR (the latter being the strongest known risk factor for CKD). In model 1, hypertension and lower eGFR at baseline were also independently associated with incident CKD. As also shown in Table 2, the significant association between NAFLD and incident CKD was only slightly weakened when we excluded those (*n* = 27) with microalbuminuria at baseline (model 3) or when we further adjusted the results of model 2 for BMI and serum triglycerides (adjusted HR 2.02 [95% CI 1.08–3.83], $P < 0.01$).

In light of the well-known association between alcohol consumption and liver injury, we repeated all analyses described above after excluding participants who drank minimally (*n* = 53). Notably, the main results remained unchanged (not shown).

Similar results were found for each of the components of the renal outcome, i.e., macroalbuminuria and eGFR <60 mL/min/1.73 m^2, separately. The presence of NAFLD significantly predicted subsequent development of either $eGFR_{MDRD}$ <60 mL/min/1.73 m^2 (adjusted HR 1.92 [95% CI 1.1–6.7], $P < 0.01$) or macroalbuminuria (adjusted HR 5.41 [2.3–12.1], $P < 0.001$) after adjusting for age, sex, diabetes duration, A1C, and hypertension. However, given the relatively small number of events, the results of these latter multivariable regression models should be interpreted with some caution.

We undertook a sensitivity analysis, by repeating the regression models shown in Table 2, having estimated eGFR according to the CKD-EPI equation (that identified a lower number of incident CKD cases, *n* = 38). The results of this supplementary analysis were very similar to that obtained with eGFR calculated from MDRD study equation, and the presence of NAFLD was again independently associated with an increased risk of CKD (Supplementary Table 2).

Table 3 shows the discriminatory capability of the regression models with NAFLD as compared with the models without NAFLD for predicting the risk of incident CKD. Notably, all regression models that included NAFLD resulted in a better risk prediction for CKD (i.e., a significantly better c statistic) than the models that did not include NAFLD.

We also undertook net reclassification of risk based on regression model 1 and model 2, according to the inclusion or exclusion of NAFLD, for subjects in whom CKD_{MDRD} developed and for those in whom CKD_{MDRD} did not develop (Supplementary Table 3*A* and *B*, models 1 and 2, respectively). We repeated these analyses with CKD_{EPI} as the outcome (Supplementary Table 4*A* and *B*). In the regression model 1 that adjusted for age, sex, diabetes duration, A1C, and hypertension, the inclusion of NAFLD reclassified, respectively, 10.5% of participants when eGFR was estimated according to the MDRD equation and 10.2% of participants when eGFR was estimated according to the CKD-EPI equation ($P < 0.001$ for both), primarily by reclassifying lower-risk people into higher-risk categories. In the regression model 2 that additionally adjusted the results for baseline eGFR, the inclusion of NAFLD appropriately reclassified smaller proportions of participants (NRI = 4.6% and $P = 0.073$ for regression models with CKD_{MDRD} as outcome; NRI = 2.5% and $P = 0.01$ for regression models with CKD_{EPI} as outcome).

CONCLUSIONS

Our novel findings indicate that NAFLD is strongly associated with an increased incidence of CKD during a mean follow-up

Table 3—Discriminatory capability and NRI* of the regression models with NAFLD as compared with the models without NAFLD for predicting the risk of developing incident CKD in adult patients with type 1 diabetes

	Prediction model	
	Without NAFLD	With NAFLD
Regression models with CKD_{MDRD} as outcome		
Regression model 1		
Discriminatory capability		
c statistic	0.66	0.70
95% CI	0.58–0.74	0.62–0.78
P value for difference		<0.001
NRI (%)		10.5
P value		<0.001
Regression model 2		
Discriminatory capability		
c statistic	0.76	0.79
95% CI	0.71–0.84	0.73–0.86
P value for difference		0.002
NRI (%)		4.6
P value		0.073
Regression models with CKD_{EPI} as outcome		
Regression model 1		
Discriminatory capability		
c statistic	0.73	0.81
95% CI	0.65–0.81	0.75–0.87
P value for difference		<0.001
NRI (%)		10.2
P value		<0.005
Regression model 2		
Discriminatory capability		
c statistic	0.81	0.87
95% CI	0.75–0.88	0.82–0.92
P value for difference		0.002
NRI (%)		2.5
P value		0.01

Incident CKD was defined as occurrence of macroalbuminuria and/or eGFR <60 mL/min/1.73 m^2 (using either the MDRD study equation or the CKD-EPI equation). Multivariable regression model 1, adjustment for age, sex, diabetes duration, A1C, and hypertension; multivariable regression model 2, adjustment for age, sex, diabetes duration, A1C, hypertension, and baseline eGFR. *Complete data on net reclassification of risk according to the inclusion or exclusion of NAFLD for subjects in whom CKD developed and for those in whom CKD did not develop are reported in Supplementary Table 3 (regression models with CKD_{MDRD} as outcome) and Supplementary Table 4 (regression models with CKD_{EPI} as outcome).

of 5 years and that measurement of NAFLD improves risk prediction for CKD, independently of traditional risk factors (age, sex, diabetes duration, A1C, hypertension, baseline eGFR, and microalbuminuria [i.e., the last two factors being the strongest known risk factors for CKD]), in type 1 diabetic adults. Additionally, although NAFLD was strongly associated with obesity, obesity (or increased BMI) did not explain the association between NAFLD and CKD.

Since it remains uncertain which of the two eGFR equations is superior in patients with type 1 diabetes, we used both the MDRD and CKD-EPI equations to estimate eGFR. As shown in Table 3, it is worth noting that the two eGFR equations identified a different number of incident CKD cases (n = 61 using the MDRD equation and n = 38 using the CKD-EPI equation). However, with both the MDRD and CKD-EPI equations, the discriminatory capability (c statistic) and the NRI of the regression models that included NAFLD were substantially comparable (for predicting incident CKD). Since there were relatively few incident CKD events in our cohort, we did not undertake formal statistical analyses to compare the NRI data obtained with both eGFR equations. We suggest that further follow-up studies on larger cohorts of patients with type 1 diabetes are now needed to improve understanding of this issue.

The annual cumulative incidence rate of CKD in our cohort of patients (i.e., ~4.5% per year) was essentially comparable to that previously described in other European populations with type 1 diabetes and similar baseline characteristics (~2.5–9% of patients who progressed every year to CKD) (25,26). In line with previously published information (25–28), we also found that hypertension, microalbuminuria, and lower eGFR at baseline were strong predictors of incident CKD in type 1 diabetic patients.

Another interesting finding of our study that corroborates our previously published observations (15,16) is that the frequency of NAFLD on ultrasonography was high in type 1 diabetic adults (i.e., ~50% of our patients had hepatic steatosis), and that the majority of patients with NAFLD had serum liver enzymes within the "normal" reference ranges. This further suggests that serum liver enzyme levels are insensitive markers for the detection of NAFLD, and that the "normal" reference values for serum liver enzymes currently used to exclude NAFLD need to be revised (1,2,8).

There is a pressing and unmet need to determine whether NAFLD is associated with a higher risk of CKD in people with type 1 diabetes. It has only recently been recognized that NAFLD represents an important burden of disease for type 2 diabetic patients (11,17,18), but the magnitude of the problem of NAFLD and its association with risk of CKD in type 1 diabetes is presently poorly recognized. Although there is clear evidence that NAFLD is closely associated with a higher prevalence of CKD both in those without diabetes (11) and in those with type 1 and type 2 diabetes (15–17), only four prospective studies have examined the association between NAFLD and risk of incident CKD (18,29–31), and only one of these studies was published in patients with type 2 diabetes (18). Indeed, in this prospective study, involving 1,760 type 2 diabetic individuals with preserved kidney function and not macroalbuminuria at baseline, we found that ultrasound-diagnosed NAFLD was associated with an increased incidence of CKD, independently of several established cardio-renal risk factors (18). Similarly, Chang et al. (29), following an occupational cohort of 8,329 nondiabetic men with normal kidney function and no proteinuria at baseline for a mean period of ~3.5 years, showed

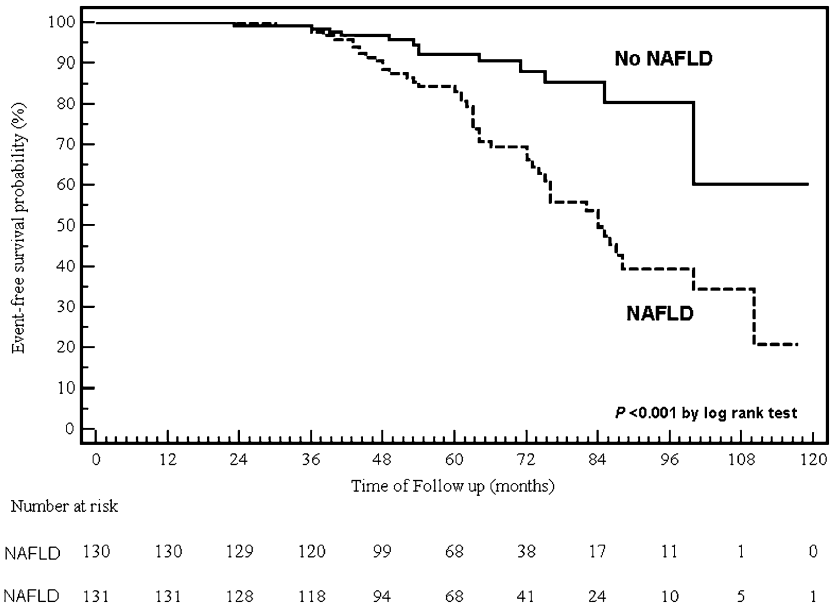

Figure 1—Incidence curves for CKD_{MDRD} during the follow-up in type 1 diabetic patients with (dotted line) and without (solid line) NAFLD on ultrasonography at baseline.

that ultrasound-diagnosed NAFLD was associated with an increased incidence of CKD, even after adjusting for several potential confounders, including insulin resistance and C-reactive protein. Two other large community-based cohort studies, using elevated serum GGT levels as proxy markers for NAFLD, have also shown that NAFLD was independently associated with an increased incidence of kidney disease (30,31).

The underlying mechanisms responsible for the observed association between NAFLD and CKD are not well understood. Speculatively, the most plausible explanation for our findings is that the association between NAFLD and risk of incident CKD is simply a consequence of shared cardio-renal risk factors and comorbidities. However, since the association between NAFLD and CKD was independent of shared risk factors, and inclusion of NAFLD improved the discriminatory capability of regression models for predicting CKD, our findings suggest that NAFLD is not only a marker of CKD but may also be partly involved in its pathogenesis. Experimental evidence suggests that NAFLD itself releases a variety of proinflammatory, procoagulant, prooxidant, and profibrogenic mediators that may play important roles in the development and progression of CKD (11,32–35).

The possible clinical implication for these findings is that type 1 diabetic patients with NAFLD may benefit from more intensive surveillance or early treatment interventions to decrease the risk for CKD. Currently, there is no approved treatment for NAFLD. However, NAFLD and CKD share numerous cardiometabolic risk factors, and treatment strategies for NAFLD and CKD should be similar and aimed primarily at modifying the associated cardiometabolic risk factors.

The major limitations of this study are its retrospective, longitudinal design (which does not allow us to draw any firm conclusion about causality) and a possible selection bias of excluding the patients who had missing liver ultrasonographic data at baseline. In addition, we used an eGFR instead of a directly measured GFR to define kidney function. However, current GFR estimates facilitate the evaluation and management of CKD, and many organizations recommend the use of prediction equations for the evaluation of kidney function in clinical practice (14,21,36). Another possible limitation of our study was that the diagnosis of NAFLD was based on ultrasonography (that is relatively insensitive to the presence of smaller amounts of hepatic steatosis, i.e., $<$30% liver fat infiltration) and the exclusion of other secondary causes of chronic liver disease, but was not confirmed by liver biopsy, which would be unethical to perform in our patients who had normal or only slightly elevated serum liver enzyme levels. Thus, although some nondifferential misclassification of NAFLD on the basis of ultrasonography is likely (some of the diabetic control patients could have underlying NAFLD despite normal serum liver enzymes and negative ultrasonography examination), this limitation would serve to attenuate the magnitude of our effect measures toward null; thus, our results can probably be considered to be conservative estimates of the relationship between NAFLD and risk of CKD. It is also important to underline that self-reporting of alcohol consumption may be unreliable and often underestimates the true risk. However, the main results of our study remained unchanged when participants who were light-to-moderate drinkers were excluded from analysis. Finally, whether these observations can also be extended to non-Caucasian ethnic groups remains to be determined.

Notwithstanding these limitations, our study has several strengths, such as the relatively large sample size, the long duration of follow-up, the completeness of the dataset, and the ability to adjust for multiple established cardio-renal risk factors and potential confounders. In addition, our patients were free from diagnosed CVD and cirrhosis; the evaluation

of patients with such complications would almost certainly have confounded interpretation of the data.

In conclusion, this is the first study to demonstrate that NAFLD is strongly associated with an increased incidence of CKD, and that measurement of NAFLD improves the risk prediction of CKD, independently of traditional cardio-renal risk factors, in type 1 diabetic adults. Further large, long-term prospective studies are needed to confirm our results (before suggesting a routine liver ultrasound examination in all patients with type 1 diabetes to better predict the future development of CKD) and to determine whether improvement in NAFLD (or future treatments for NAFLD) will ultimately delay or prevent the development and progression of CKD in patients with type 1 diabetes.

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