Prevalence of Nonalcoholic Fatty Liver Disease and Its Association With Cardiovascular Disease Among Type 2 Diabetic Patients

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OBJECTIVE — To determine the prevalence of nonalcoholic fatty liver disease (NAFLD) in type 2 diabetic population and to compare the prevalence of cardiovascular disease (CVD) and its risk factors between people with and without NAFLD.

RESEARCH DESIGN AND METHODS — The entire sample of type 2 diabetic outpatients ($n = 2,839$) who regularly attended our clinic was screened. Main outcome measures were NAFLD (by patient history and liver ultrasound) and manifest CVD (by patient history, review of patient records, electrocardiogram, and echo-Doppler scanning of carotid and lower limb arteries).

RESULTS — The unadjusted prevalence of NAFLD was 69.5% among participants, and NAFLD was the most common cause (81.5%) of hepatic steatosis on ultrasound examination. The prevalence of NAFLD increased with age (65.4% among participants aged 40–59 years and 74.6% among those aged ≥60 years; $P < 0.001$) and the age-adjusted prevalence of NAFLD was 71.1% in men and 68% in women. NAFLD patients had remarkably ($P < 0.001$) higher age and sex-adjusted prevalences of coronary (26.6 vs. 18.3%), cerebrovascular (20.0 vs. 13.3%), and peripheral (15.4 vs. 10.0%) vascular disease than their counterparts without NAFLD. In logistic regression analysis, NAFLD was associated with prevalent CVD independent of classical risk factors, glycemic control, medications, and metabolic syndrome features.

CONCLUSIONS — NAFLD is extremely common in people with type 2 diabetes and is associated with a higher prevalence of CVD. Follow-up studies are needed to determine whether NAFLD predicts the development and progression of CVD.

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Nonalcoholic fatty liver disease (NAFLD) is the most common cause of abnormal liver function tests among adults in Western countries (1–4). The spectrum of NAFLD ranges from simple steatosis to nonalcoholic steatohepatitis (NASH), which can progress to end-stage liver disease. NAFLD is commonly associated with obesity, type 2 diabetes, dyslipidemia, and insulin resistance, all of which are components of the metabolic syndrome, strongly supporting the notion that NAFLD is the hepatic manifestation of the syndrome (1–4). The prevalence of NAFLD has been reported to be in the 15–30% range in the general population in various countries (5–7) and is almost certainly increasing. Accordingly, a huge number of individuals are at risk of developing advanced liver disease.

Compared with nondiabetic subjects, people with type 2 diabetes appear to have an increased risk of developing NAFLD and certainly have a higher risk of developing fibrosis and cirrhosis (1–4). It has been estimated that ~70–75% of type 2 diabetic patients may have some form of NAFLD (8); however, the "precise" prevalence of NAFLD in type 2 diabetes is unknown. The few available studies have been small and performed in highly selected populations or have estimated only the prevalence of abnormal aminotransferase levels (9–12), which are a poor proxy measure of NAFLD (1–3).

Recent data suggest that the presence of NAFLD in type 2 diabetes may also be linked to increased cardiovascular disease (CVD) risk independently of components of the metabolic syndrome (13,14), although this hypothesis needs verification in larger studies. However, if correct, these data suggest that the identification of NAFLD in type 2 diabetes may help in CVD risk prediction with important management implications. Identifying people with NAFLD would also highlight a subgroup of diabetic patients who should be targeted with more intensive therapy to decrease their risk of future CVD events. The main purpose of this study was to determine the prevalence of NAFLD, as diagnosed by patient history and liver ultrasound, which is the most widely used imaging test for detecting hepatic steatosis, and to establish whether there is an association between NAFLD and CVD in a large cohort of type 2 diabetic adults.

RESEARCH DESIGN AND METHODS

— All of the outpatients ($n = 3,166$) with type 2 diabetes who regularly attended our clinic during the pe-

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Abbreviations: ALT, alanine aminotransferase; ATP III, Adult Treatment Panel III; CVD, cardiovascular disease; NAFLD, nonalcoholic fatty liver disease; NASH, nonalcoholic steatohepatitis.

A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.

riod between 1 January 2005 and 1 January 2006 were screened. Excluding those for whom a liver ultrasound examination was not available (n = 327, 10.3%), 2,839 type 2 diabetic outpatients were studied; 800 of them were previously included in a study (14). The local ethics committee approved the study. All participants provided written informed consent.

BMI was calculated by dividing weight in kilograms by the square of height in meters. Waist circumference was measured in a standing position at the level of the umbilicus. Blood pressure was measured with a standard mercury manometer. Information on daily alcohol consumption, smoking status, and use of medications (including also hepatotoxic drugs such as glucocorticoids, amiodarone, methotrexate, or antineoplastic drugs) was obtained from all participants by a questionnaire (13). Most participants were abstainers (n = 2,169, 76.4%) or drank minimally (alcohol consumption <20 g/day; n = 312, 11%); only 12.6% of participants drank >20 g/day of alcohol.

Venous blood was drawn in the morning after an overnight fast. Plasma liver tests and other biochemical blood measurements were determined by standard laboratory procedures. Normal ranges for serum aminotransferase levels, in our laboratory, were 10–35 units/l for women and 10–50 units/l for men, respectively. In this study, a single measurement of liver enzymes that was obtained within ~1 month of liver ultrasonography was used in statistical analyses. However, as all participants attended our clinic regularly, repeated aminotransferase measurements were available for each participant (>2 per year); none of those without NAFLD had raised serum aminotransferase levels at any time. Serology for viral hepatitis B and C was assessed in all participants. 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 the laboratory was 5.9%.

Metabolic syndrome was diagnosed by the Adult Treatment Panel III (ATP III) definition. In accordance with this definition (15), a person with type 2 diabetes was classified as having the syndrome if he or she had at least two of the following four components: *1*) waist circumference >102 cm in men or >88 cm in women, *2*) triglycerides ≥1.7 mmol/l, *3*) HDL <1.0 mmol/l in men and <1.29 mmol/l in women or receiving treatment, and *4*) blood pressure ≥130/85 mmHg or receiving treatment.

Hepatic ultrasonography scanning was performed in all participants by a single experienced radiologist, who was blinded to subjects' details. The diagnosis of hepatic steatosis was made on the basis of characteristic sonographic features (1–3). It is known that ultrasonography has a sensitivity of ~90% and a specificity of ~95% in detection of moderate and severe hepatic steatosis (1,2), although ultrasonography is not totally sensitive, particularly when hepatic fat infiltration on liver biopsy is <30% (16). Semiquantitative sonographic scoring for the degree of hepatic steatosis was not available. Repeated measurements that were done on a random subgroup of 150 of the same subjects gave coefficients of variation within 3% (13,14).

The presence of CVD was assessed as follows. A detailed medical history was collected by a questionnaire (14) administered by a trained physician to record previous or current coronary (myocardial infarction, angina, or revascularization procedures), cerebrovascular (ischemic stroke, recurrent transient ischemic attacks, carotid endarterectomy, or carotid stenosis ≥70% as diagnosed by echo-Doppler scanning), and peripheral (intermittent claudication, rest pain, as confirmed by echo-Doppler scanning, lower extremity amputation, or revascularization procedures) vascular disease. The presence of CVD was confirmed by reviewing hospital medical records of all patients and by a thorough physical examination by one of the investigators that also included vascular laboratory studies (electrocardiogram and echo-Doppler scanning of carotid and lower limb arteries, which were performed for all participants). Data on CVD were collected only for those with and without NAFLD but not for those with other causes of chronic liver disease.

Statistical analysis

Data are means ± SD or proportions. Skewed variables (triglycerides and liver enzymes) were logarithmically transformed to improve normality before analysis. Statistical analyses included the unpaired t test and the χ^2 test with Yates' correction for continuity (for categorical variables). The independence of the associations of variables with prevalent CVD, included as the dependent variable, was assessed by multivariable logistic regression analysis and expressed as odds ratios (ORs). In this analysis, men and women were combined and first-order interaction terms for sex-by-NAFLD interactions on risk for CVD were examined. Because the interactions were not statistically significant, sex-pooled multivariable logistic regression analysis was used to assess the independence of the association of NAFLD with CVD. In that analysis, CVD was considered as the composite end point inclusive of those patients with coronary, cerebrovascular, or peripheral vascular disease (as defined above). In the fully adjusted regression models, NAFLD, sex, age, BMI, smoking status, diabetes duration, A1C, LDL cholesterol, use of medications (hypoglycemic, antihypertensive, lipid-lowering, or antiplatelet drugs), and presence of ATP III–defined metabolic syndrome were also included as covariates. Separate models were also tested with the four individual components of the metabolic syndrome included as categorical or continuous measures. $P < 0.05$ was considered statistically significant.

RESULTS — As shown in Fig. 1, 3,166 type 2 diabetic outpatients, who regularly attended our clinic, were initially screened. Excluding those (n = 327) who did not have a liver ultrasound examination left 2,839 participants, aged 40–86 years, who were included in analyses. There were no significant differences in main study variables between those who did and those who did not have a liver ultrasound examination. Of the 2,839 participants, 2,421 had hepatic steatosis on ultrasound, whereas 418 had negative liver ultrasound tests as well as normal liver tests and the absence of viral hepatitis or excessive alcohol consumption. Among those with hepatic steatosis, 358 participants admitted alcohol abuse or drank >20 g/day and 89 had other causes of chronic liver disease (viral hepatitis or medications), whereas the remaining 1,974 participants met the criteria for diagnosis of NAFLD, i.e., hepatic steatosis among individuals without excessive alcohol consumption or other causes of chronic liver disease. Thus, the unadjusted prevalence of NAFLD was 69.5% (95% CI 68.5–70.5), and NAFLD represented the most common cause (81.5%) of hepatic steatosis on ultrasound. The prevalence of NAFLD increased with increasing age (65.4% among participants aged 40–59 years and 74.6% among

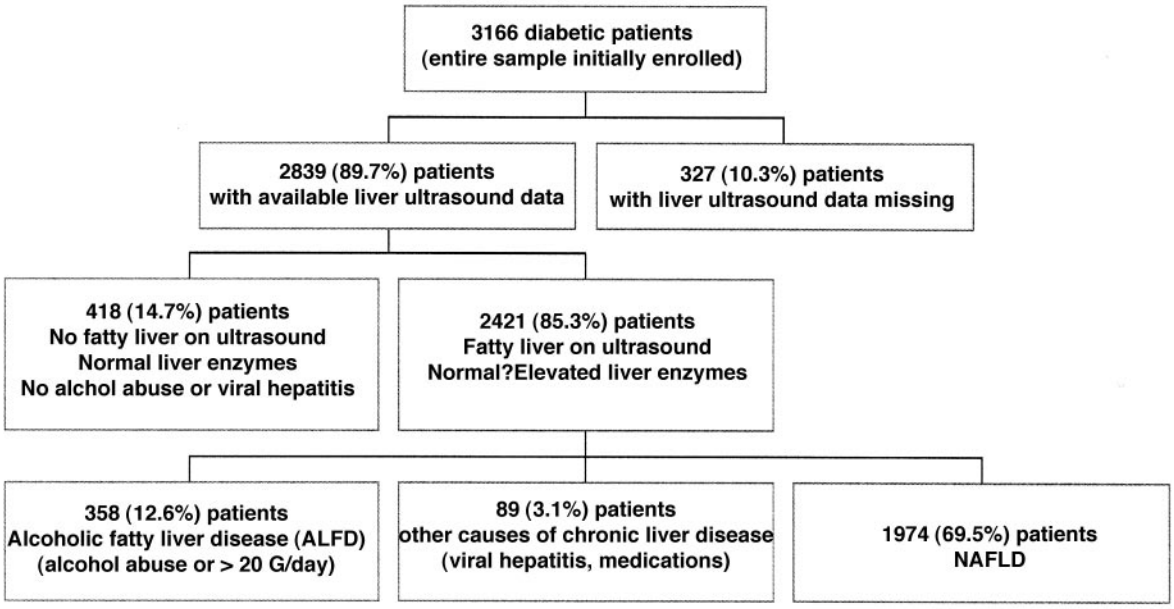

Figure 1—*Details of the study design.*

those aged ≥60 years; $P < 0.001$), and the age-adjusted prevalence of NAFLD was 71.1% in men and 68.0% in women ($P = 0.20$).

The baseline characteristics of the study participants, grouped according to NAFLD status, after exclusion of those with other known causes of chronic liver disease ($n = 447$), are presented in Table 1. Individuals with NAFLD were older, more likely to be male, and had longer diabetes duration than those without NAFLD. They also had higher values of A1C and liver enzymes, although the vast majority of patients with NAFLD (86%) had normal serum alanine aminotransferase (ALT) levels. Metabolic syndrome and its individual components occurred more frequently among patients with NAFLD. Smoking history, plasma LDL cholesterol, and creatinine concentrations were not significantly different between the groups. The proportion using insulin or antihypertensive or antiplatelet drugs was higher among patients with NAFLD, whereas the proportion using lipid-lowering drugs was similar in both groups.

Overall, 1,074 (44.4% [95% CI 43.4–45.4]) of 2,421 participants with and without NAFLD were coded positive for CVD (as composite end point). Of these, 546 patients (22.6% [21.6–23.6]) had coronary heart disease (398 with myocardial infarction and 148 subjects with angina or revascularization procedures), 398 patients (16.4% [15.4–17.4]) had cerebrovascular disease (298 with ischemic stroke, recurrent transient ischemic attack, or carotid endarterectomy and 100 with carotid stenosis ≥70% as ascertained by echo-Doppler scanning), and 307 patients (12.7% [11.7–13.7]) had peripheral vascular disease (182 of them with rest pain or claudication as confirmed by echo-Doppler scanning and 125 subjects with prior lower extremity

Table 1—*Baseline characteristics of the study participants, grouped according to NAFLD status*

Variables	Without fatty liver	With NAFLD	*P* value
n	418	1,974	
Sex (% men)	54	57	<0.001
Age (years)	60 ± 4	65 ± 6	<0.001
BMI (kg/m^2)	26.5 ± 3	28.3 ± 4	<0.001
Diabetes duration (years)	7 ± 2	12 ± 3	<0.001
Oral hypoglycemic users (%)	67	66	0.80
Insulin users (%)	17	25	<0.001
Antihypertensive drug users (%)	60	73	<0.001
Aspirin users (%)	48	57	<0.001
Lipid-lowering drug users (%)	41	43	0.60
Current smokers (%)	25	27	0.60
Systolic blood pressure (mmHg)	135 ± 10	139 ± 12	<0.001
Diastolic blood pressure (mmHg)	83 ± 7	85 ± 10	<0.001
A1C (%)	6.7 ± 0.6	7.3 ± 1.1	<0.001
Triglycerides (mmol/l)	1.40 ± 0.6	1.68 ± 1.0	<0.001
HDL cholesterol (mmol/l)	1.41 ± 0.3	1.34 ± 0.4	<0.001
LDL cholesterol (mmol/l)	3.40 ± 0.4	3.37 ± 0.4	0.40
Creatinine (μmol/l)	90 ± 12	92 ± 14	0.40
Aspartate aminotransferase (units/l)	23 ± 3	28 ± 10	<0.001
ALT (units/l)	25 ± 3	33 ± 12	<0.001
Elevated ALT (men >50 units/l; women >35 units/l) (%)	0	14	<0.001
ATP III–defined metabolic syndrome (%)	70	86	<0.001

Data are means ± SD or proportions. $n = 2,392$.

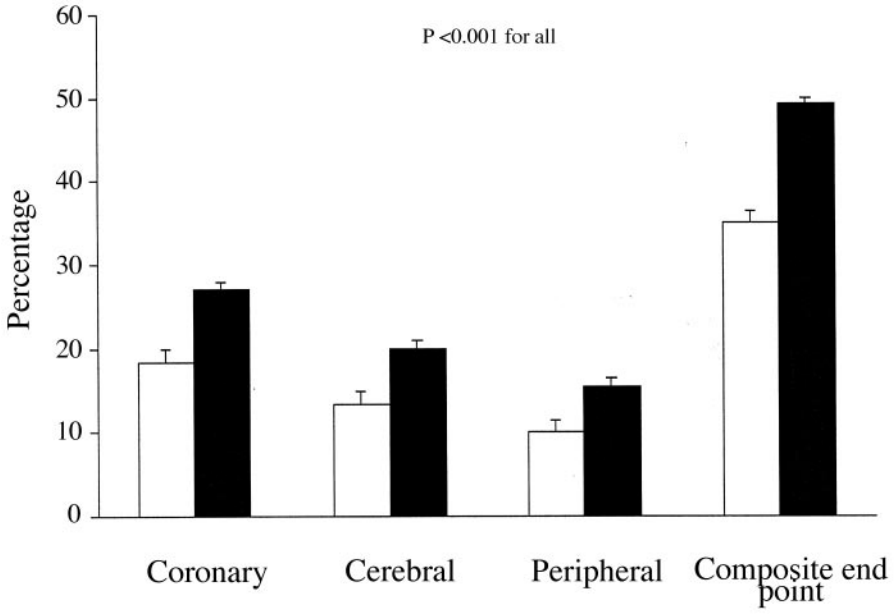

Figure 2—*Age- and sex-adjusted prevalence of CVD in type 2 diabetic adults with (■) and without (□) NAFLD. Data are expressed as percentages ± SE. P < 0.001 for differences between the groups.*

amputation or revascularization procedures); many subjects had CVD in multiple sites. Interestingly, compared with previous reports, in which comparable diagnostic noninvasive measures were used, the prevalence of CVD in this study was similar to that described in other populations with comparable age, diabetes duration, glycemic control, and smoking status (17–20). As shown in Fig. 2, the age- and sex-adjusted prevalences of coronary, cerebrovascular, and peripheral vascular disease were remarkably higher in patients with NAFLD than in those without NAFLD.

In univariate logistic regression analysis, together with NAFLD (Fig. 3, *top bar*), age, male sex, BMI, smoking, diabetes duration, A1C, LDL cholesterol, presence of ATP III–defined metabolic syndrome, and use of medications (particularly the aspirin use) were significantly associated with CVD (not shown). The relationship between NAFLD and CVD was little affected by adjustment for age, sex, BMI, smoking, diabetes duration, A1C, LDL cholesterol, and medications (Fig. 3, *second* and *third bars*); additional adjustment for the metabolic syndrome did not appreciably change this relationship (Fig. 3, *bottom bar*). In this fully adjusted regression model, together with NAFLD, male sex (OR 1.5 [95% CI 1.2–1.9]), older age (1.08 [1.06–1.1]), smoking (1.4 [1.1–1.8]), and the metabolic syndrome (1.6 [1.3–2.6]) were also independently ($P < 0.001$) associated with CVD.

Almost identical results were obtained in models that also adjusted for the individual components of the metabolic syndrome (not shown). Exclusion of participants who have asymptomatic CVD (carotid stenosis ≥70%) or who were light-to-moderate drinkers (alcohol consumption <20 g/day) did not alter the observed associations between NAFLD and CVD (OR 1.49 [95% CI 1.1–2.0], $P = 0.032$).

CONCLUSIONS — There is a pressing unmet need to determine the prevalence of NAFLD in the type 2 diabetic population and to evaluate its association with CVD. It has only recently been recognized that NAFLD represents an important burden of disease for patients with type 2 diabetes (1–4,21), but the magnitude of the problem of NAFLD in patients with type 2 diabetes is currently unknown. It is also becoming evident that NAFLD is related to CVD in people with type 2 diabetes (13,14), but further research in this area is required to ascertain whether NAFLD is a (independent) CVD risk factor. Indeed, the impact of NAFLD on CVD risk deserves particular attention in view of the implications for screening/surveillance strategies in the growing number of patients with NAFLD.

To our knowledge, this is the largest cross-sectional study with the specific aims of establishing the prevalence of NAFLD in a large cohort of type 2 diabetic patients and assessing the association of NAFLD with CVD. A major finding of this

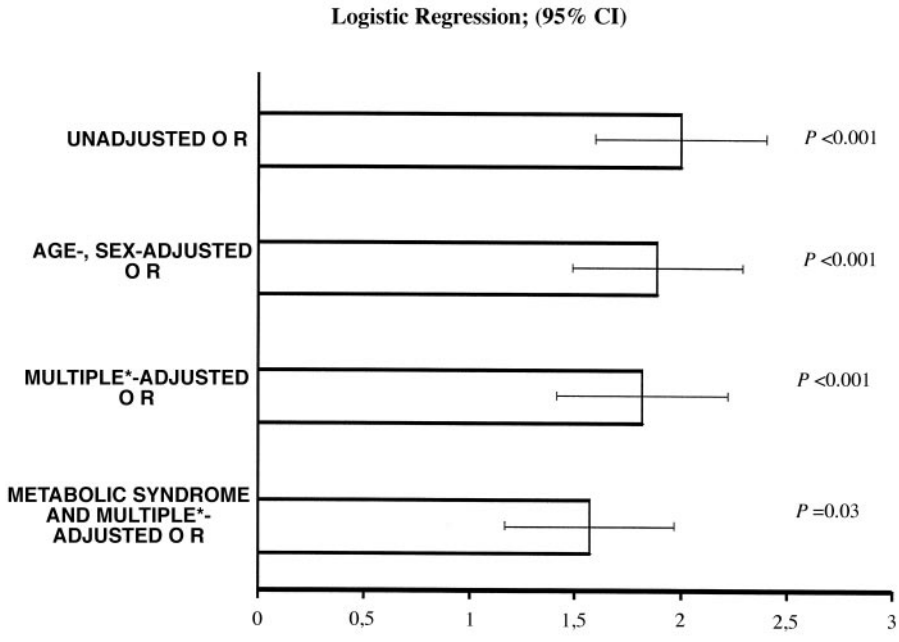

Figure 3—*Association between NAFLD and prevalent CVD in type 2 diabetic adults with and without NAFLD* (n = *2,392). Data are expressed as ORs ± 95% CI. *The multiple adjustment reported in the* third *and* fourth bars *was as follows: age, sex, BMI, smoking status, diabetes duration, A1C, LDL cholesterol, and current use of medications (hypoglycemic, antihypertensive, lipid-lowering, or antiplatelet drugs).*

study is that the prevalence of NAFLD, as diagnosed by patient history and characteristic sonographic features, in the type 2 diabetic population is very high. Indeed, NAFLD is present in ~70% of our population and represents the most common explanation (81.5%) for any form of hepatic steatosis on ultrasound examination.

Interestingly, this study provides further evidence that a normal serum ALT level provides little diagnostic or prognostic value when assessing patients for NAFLD, because more than four-fifths (86%) of our patients with NAFLD had normal ALT levels. Even when more stringent criteria were used (i.e., ALT >30 units/l in men and >19 units/l in women) (22), most patients with NAFLD (63%) had normal ALT levels. Therefore, serum ALT levels appear to be insensitive markers for NAFLD. Indeed, it is known that the full histological spectrum of NAFLD may be present among patients with normal liver enzymes (23), which therefore cannot be reliably used to exclude the presence of more advanced stages of NAFLD (1–4).

Another major finding of this study is that NAFLD is associated with a higher prevalence of CVD in multiple sites (coronary, cerebrovascular, and peripheral vascular disease). Importantly, this association remains statistically significant after adjustment for a broad spectrum of prognostic factors, including the metabolic syndrome, a chronic inflammatory cardiovascular condition that is closely associated with NAFLD (as also confirmed by our results).

Given our study design, we are unable to draw conclusions about causality in the relation between NAFLD and CVD and to determine whether the higher CVD prevalence among patients with NAFLD affects long-term mortality. The most likely explanation for our findings could be that the relationship between NAFLD and CVD mainly reflects the overall, adverse, impact of the metabolic syndrome phenotype, principally insulin resistance. Although our results have been adjusted for the metabolic syndrome, a condition that is closely associated with insulin resistance, we did not directly measure insulin resistance (by glucose clamp) in our diabetic population, so we cannot be certain that identical results could be obtained after additional adjustment for this CVD risk factor. However, the glucose clamp would be impossible to perform routinely in a large epidemiological study. On the other hand, the homeostasis model assessment score is not a reliable method for determining insulin resistance in diabetic patients treated with antidiabetes agents, particularly in those receiving insulin treatment (24).

A significant association between NAFLD and increased CVD prevalence in type 2 diabetic individuals has been previously shown in our smaller study (14). In that study, however, the association between NAFLD and CVD was no longer apparent after adjustment for the metabolic syndrome. These apparently discrepant results can be principally explained by the larger sample size of this study, which provided a greater statistical power and permitted more complete adjustment for potential confounders. Moreover, our findings are validated by a follow-up study demonstrating that NAFLD is associated with increased CVD incidence in type 2 diabetic patients independent of traditional risk factors and the presence of the metabolic syndrome (13). Others have cross-sectionally shown that individuals with modestly elevated serum ALT levels, as surrogate measures of NAFLD, have an increased CVD risk (as calculated by the Framingham risk score) (25). Currently, it is not known whether improving NAFLD will ultimately prevent the development of CVD. However, it is notable that interventions that are known to be effective in preventing CVD in type 2 diabetic people, including weight reduction and treatment with insulin-sensitizing antidiabetes agents (26–32), may possibly improve NAFLD.

Overall, these findings might have possible clinical and public health implications. Our results indicate that the majority of patients with type 2 diabetes have NAFLD, and previous studies showing that type 2 diabetes is an independent predictor of advanced liver disease in NAFLD suggest that consideration should be given to referring patients to a hepatologist for further evaluation. This will be particularly important once an effective treatment for NASH has been established, and better noninvasive methods for assessing disease severity are validated. Additionally, our findings complement recent observations that the severity of NAFLD histology is associated with greater carotid intima-media thickness and plaques (33) and lower endothelial flow-mediated vasodilation (34) independent of underlying metabolic abnormalities and that NAFLD is associated with higher all-cause death (35–37) and predicts the risk of future CVD events (13,37). There is therefore now growing evidence that NAFLD is not simply a marker of CVD but may also be, directly or indirectly, involved in its pathogenesis. The possible molecular mediators linking NAFLD and CVD have been extensively reviewed elsewhere but include the release of proatherogenic mediators from the liver including C-reactive protein, interleukin-6, and plasminogen activator inhibitor-1 (38).

The present study has some limitations that should be noted. The cross-sectional design of our study precludes the establishment of causal or temporal relations among NAFLD, metabolic syndrome, and CVD. Another limitation of this study is that the diagnosis of NAFLD was based on ultrasound imaging and exclusion of other causes of chronic liver disease but was not confirmed by liver biopsy. It is known that none of the radiological features can distinguish between NASH and other forms of NAFLD and that only liver biopsy can assess the severity of damage and the prognosis (1–3). However, liver biopsy would be impossible to perform routinely in a large epidemiological study. Conversely, ultrasonography is by far the most common way of diagnosing NAFLD in clinical practice and has good sensitivity and specificity in detecting moderate and severe steatosis (1,2). Indeed, it has been reported that the presence of >30% fat on liver biopsy is optimal for ultrasound detection of steatosis, whereas ultrasonography is not totally sensitive, particularly when hepatic fat infiltration is <30% (16). Thus, although some nondifferential misclassification of NAFLD on the basis of ultrasonography is likely (i.e., some of the diabetic control subjects could have underlying NAFLD despite normal liver enzymes and negative ultrasonography examination), this limitation would serve to attenuate the magnitude of our effect measures toward the null; thus, our results can probably be considered as conservative estimates of the relationship between NAFLD and CVD.

In conclusion, our results suggest that NAFLD is extremely common in people with type 2 diabetes and is associated with a higher prevalence of CVD. The association between NAFLD and CVD appears to be independent of classical risk factors, glycemic control, medications, and presence of the metabolic syndrome. Thus, these results further confirm the hypothesis that the identification of NAFLD in

type 2 diabetes may help in CVD risk prediction. Future experimental and follow-up studies are needed to elucidate the possible molecular mechanisms linking NAFLD and CVD and to determine whether NAFLD predicts the development and progression of CVD.

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