Nonalcoholic Fatty Liver Disease Is Independently Associated With an Increased Incidence of Cardiovascular Events in Type 2 Diabetic Patients

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Recent data suggest that the presence of nonalcoholic fatty liver disease (NAFLD) in type 2 diabetes may be linked to increased cardiovascular disease (CVD) independent of components of the metabolic syndrome (1–3), although this hypothesis needs verification in larger studies. We assessed whether NAFLD, as diagnosed by ultrasound, predicts the risk of incident CVD events in a large cohort of type 2 diabetic adults.

RESEARCH DESIGN AND METHODS

— Study subjects were participants in the Valpolicella Heart Diabetes Study (1). Briefly, we enrolled all of the type 2 diabetic outpatients (n = 2,103) who regularly attended our clinic in the period January–December 2000 after excluding those who had manifest CVD and/or secondary causes of chronic liver disease (alcohol abuse, viral infection, or medications). The local ethics committee approved the study. All participants provided written informed consent.

During 6.5 years of follow-up (through December 2006; follow-up range: 5–84 months), 384 participants subsequently developed CVD events (myocardial infarction, ischemic stroke, coronary revascularization, or cardiovascular death), whereas 1,719 patients remained free of diagnosed CVD. These events were ascertained by patient history, chart review, autopsy reports, and family contact (1).

Plasma liver enzymes, A1C, and other biochemical blood measurements were determined by standard procedures. At baseline, most participants (~86%) had normal liver enzymes (reference ranges for aminotransferases were 10–35 and 10–50 units/l for female and male subjects, respectively) and were abstainers (77%) or drank minimally (13%); only 10% of participants drank >20 g/day of alcohol. No participants had seropositivity for viral hepatitis.

Hepatic ultrasonography scanning was performed in all participants by an experienced radiologist who was blind to subjects' details. Hepatic steatosis was diagnosed by characteristic sonographic features (4,5). Metabolic syndrome was diagnosed by a recently modified Adult Treatment Panel III definition (6).

Statistical analyses included unpaired t test, χ^2 test, and multivariate Cox proportional hazards analysis. In this latter analysis, CVD was considered a composite end point inclusive of nonfatal coronary heart disease, ischemic stroke, and cardiovascular death.

RESULTS

— During follow-up, we documented 384 CVD events: 219 cases of nonfatal coronary heart disease (151 myocardial infarction and 68 revascularization procedures), 44 cases of nonfatal ischemic stroke, and 121 cardiovascular deaths.

As shown in Table 1, subjects who developed CVD events during follow-up were older, had higher liver enzymes and A1C, and had greater prevalence of metabolic syndrome than those who did not develop CVD events. Sex, smoking, LDL cholesterol, diabetes duration, and treatment did not differ between the groups. The frequency of NAFLD was markedly higher in those who developed CVD events than in those who did not, without significant sex differences (not shown).

In univariate regression analysis, NAFLD (hazard ratio [HR] 2.01 [95% CI 1.4–2.9]), metabolic syndrome (1.74 [1.3–3]), age (1.11 [1.05–1.2]), male sex (1.52 [1.3–1.8]), smoking (1.48 [1.2–2.2]), A1C (1.44 [1.4–2.9]), LDL cholesterol (1.37 [1.1–1.8]), alanine aminotransferase (1.47 [1.2–1.9]), and other liver enzymes were significantly ($P < 0.01$) associated with incident CVD, whereas diabetes duration and medications were not. In multivariate regression analysis, the significant association between NAFLD and incident CVD was little affected (1.96 [1.4–2.7], $P < 0.001$) by adjustment for sex, age, smoking, diabetes duration, A1C, LDL cholesterol, and medications (hypoglycemic, antihypertensive, lipid-lowering, or antiplatelet drugs); further adjustment for the metabolic syndrome did not appreciably change the association (1.87 [1.2–2.6], $P < 0.001$).

Almost identical results were obtained in models that also adjusted for individual components of the metabolic syndrome and/or liver enzymes. No liver enzymes were independently associated with incident CVD after controlling for the metabolic syndrome and/or ultrasound-diagnosed NAFLD. Exclusion of participants who were light/moderate drinkers did not alter the association be-

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Abbreviations: CVD, cardiovascular disease; NAFLD, nonalcoholic fatty liver disease.

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

Table 1—*Baseline characteristics of the diabetic cohort* (n = 2,103) *by CVD status*

Variables	Control subjects	Case subjects	*P*
n	1,719	384	
Sex (% men)	62%	63%	0.80
Age (years)	59 ± 3	61 ± 4	0.001
BMI (kg/m^2)	26 ± 3	28 ± 4	0.001
Waist circumference (cm)	93 ± 11	99 ± 13	0.001
Duration of diabetes (years)	14 ± 3	16 ± 3	0.60
Diabetes treatment			
Diet only	21	15	0.20
Oral hypoglycemic drugs	62	65	0.30
Insulin only	17	20	0.20
Antihypertensive users	60	73	0.001
Aspirin users	49	48	0.80
Lipid-lowering users	34	36	0.60
Current smokers	22	23	0.70
Systolic blood pressure (mmHg)	127 ± 12	131 ± 16	0.001
Diastolic blood pressure (mmHg)	80 ± 12	83 ± 14	0.001
A1C (%)	6.9 ± 0.8	7.3 ± 1.0	0.001
Triglycerides (mmol/l)	1.32 ± 0.6	1.62 ± 1.0	0.001
HDL cholesterol (mmol/l)	1.40 ± 0.3	1.32 ± 0.4	0.001
LDL cholesterol (mmol/l)	3.35 ± 0.4	3.32 ± 0.5	0.80
AST (units/l)	20 ± 6	26 ± 12	0.001
ALT (units/l)	24 ± 6	32 ± 13	0.001
GGT (units/l)	23 ± 10	34 ± 14	0.001
Metabolic syndrome	59	75	0.001
NAFLD	61	96	0.001

Data are means ± SD or percentages unless otherwise indicated. Differences are assessed by the unpaired *t* test (for normally distributed variables) and by the χ^2 test (for categorical variables). ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT, γ-glutamyl transferase.

tween NAFLD and CVD risk (HR 1.80 [95% CI 1.2–2.7], $P < 0.005$).

CONCLUSIONS — The major finding of this study is that NAFLD, as diagnosed by ultrasound, is associated with an increased incidence of CVD in a large cohort of type 2 diabetic adults. Notably, this association appears to be independent of a broad spectrum of risk factors, thus suggesting that NAFLD might confer an excess of CVD risk over and above what would be expected because of increased prevalence of the underlying metabolic risk factors. Moreover, this study provides further evidence that normal liver enzymes provide little diagnostic or prognostic value when assessing patients for NAFLD (3–5,7) because more than four-fifths (~86%) of our NAFLD patients had liver enzymes within the normal range.

Our findings complement recent observations that the severity of NAFLD histology is associated with greater carotid intima-media thickness (8) and lower endothelial flow-mediated vasodilation (9), independent of underlying metabolic abnormalities, and that NAFLD is associated with higher all-cause death (10,11) and higher prevalence (2,3,12–15) and incidence (1,11) of CVD in nondiabetic and type 2 diabetic individuals. Others have shown that individuals with slightly elevated liver enzymes, as surrogate markers of NAFLD, have an increased CVD risk (16–19).

There is therefore now growing evidence suggesting that NAFLD is not merely a marker of CVD but may also be involved in its pathogenesis. The possible molecular mediators linking NAFLD and CVD have been extensively reviewed elsewhere (20) but include the release of proatherogenic mediators from the liver, including C-reactive protein, fibrinogen, and plasminogen activator inhibitor-1.

This study has some limitations. First, we did not directly measure abdominal visceral fat (by computed tomography) or insulin resistance (by euglycemic clamp), so we cannot be certain that these data completely exclude an independent contribution of insulin resistance and visceral fat to accelerated CVD in NAFLD. Second, NAFLD diagnosis was based on ultrasonography but was not confirmed by biopsy. It is known that ultrasonography has a good sensitivity/specificity in detecting moderate and severe liver steatosis, but its sensitivity is reduced when hepatic fat infiltration on biopsy is <33% (5). Thus, although some nondifferential misclassification of NAFLD on the basis of ultrasonography is likely (some of the diabetic control subjects could have underlying NAFLD despite normal liver enzymes and negative ultrasonography), this limitation would serve to attenuate the magnitude of our effect measures toward the null; thus, our results can probably be considered conservative estimates of the relationship between NAFLD and CVD risk.

In conclusion, our findings suggest that NAFLD is associated with an increased incidence of CVD in type 2 diabetic patients, independent of traditional CVD risk factors and metabolic syndrome components. These findings support the hypothesis that the identification of NAFLD in type 2 diabetes may help in CVD risk prediction, with important management implications. Further studies are needed to extend these findings to NAFLD patients without type 2 diabetes.

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