

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

GIOVANNI TARGHER, MD^{1,2}
LORENZO BERTOLINI, MD¹
STEFANO RODELLA, MD³
ROBERTO TESSARI, MD¹

LUCIANO ZENARI, MD¹
GIUSEPPE LIPPI, MD⁴
GUIDO ARCARO, MD¹

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 re-

mained 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-

From the ¹Unit of Internal Medicine and Diabetes, "Sacro Cuore" Hospital of Negrar, Negrar, Italy; the ²Section of Endocrinology, Biomedical and Surgical Sciences, University Hospital of Verona, Verona, Italy; the ³Department of Radiology, "Sacro Cuore" Hospital of Negrar, Negrar, Italy; and the ⁴Section of Clinical Chemistry, Biomedical and Morphological Sciences, University Hospital of Verona, Verona, Italy.

Address correspondence and reprint requests to Dr. Giovanni Targher, Endocrinology and Metabolic Diseases, Ospedale Civile Magglore, Piazzale Stefani, 1, 37126 Verona, Italy. E-mail: giovanni.targher@univr.it.

Received for publication 21 February 2007 and accepted in revised form 13 May 2007.

Published ahead of print at <http://care.diabetesjournals.org> on 22 May 2007. DOI: 10.2337/dc07-0349.

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.

© 2007 by the American Diabetes Association.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

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.

References

1. Targher G, Bertolini L, Poli F, Rodella S, Scala L, Tessari R, Zenari L, Falezza G: Nonalcoholic fatty liver disease and risk of future cardiovascular events among type 2 diabetic patients. *Diabetes* 54:3541–3546, 2005
2. Targher G, Bertolini L, Padovani R, Poli F, Scala L, Tessari R, Zenari L, Falezza G: Increased prevalence of cardiovascular disease among type 2 diabetic patients with non-alcoholic fatty liver disease. *Diabet Med* 23:403–409, 2006
3. Targher G, Bertolini L, Padovani R, Rodella S, Tessari R, Zenari L, Day C, Arcaro G: Prevalence of nonalcoholic fatty liver disease and its association with cardiovascular disease among type 2 diabetic patients. *Diabetes Care* 30:1212–1218, 2007
4. Angulo P: Nonalcoholic fatty liver disease. *N Engl J Med* 346:1221–1231, 2002
5. Saadeh S, Younossi ZM, Remer EM, Gramlich T, Ong JP, Hurley M, Mullen KD, Cooper JN, Sheridan MJ: The utility of radiological imaging in nonalcoholic fatty liver disease. *Gastroenterology* 123:745–750, 2002
6. Grundy SM, Cleeman JI, Daniels SR, Donato KA, Eckel RH, Franklin BA, Gordon DJ, Krauss RM, Savage PJ, Smith SC Jr, Spertus JA, Costa F; American Heart Association; National Heart, Lung, and Blood Institute: Diagnosis and management of the metabolic syndrome: an

- American Heart Association/National Heart, Lung, and Blood Institute scientific statement. *Circulation* 112:2735–2752, 2005
7. Day CP: Nonalcoholic fatty liver disease: current concepts and management strategies. *Clin Med* 6:19–25, 2006
 8. Targher G, Bertolini L, Padovani R, Rodella S, Zoppini G, Zenari L, Cigolini M, Falezza G, Arcaro G: Relations between carotid artery wall thickness and liver histology in subjects with nonalcoholic fatty liver disease. *Diabetes Care* 29: 1325–1330, 2006
 9. Villanova N, Moscatiello S, Ramilli S, Bugianesi E, Magalotti D, Vanni E, Zoli M, Marchesini G: Endothelial dysfunction and cardiovascular risk profile in nonalcoholic fatty liver disease. *Hepatology* 42: 473–478, 2005
 10. Adams LA, Lymp JF, St Sauver J, Sander-son SO, Lindor KD, Feldstein A, Angulo P: The natural history of nonalcoholic fatty liver disease: a population-based cohort study. *Gastroenterology* 129:113–121, 2005
 11. Ekstedt M, Franzen LE, Mathiesen UL, Thorelius L, Holmqvist M, Bodemar G, Kechagias S: Long-term follow-up of patients with NAFLD and elevated liver enzymes. *Hepatology* 44:865–873, 2006
 12. Lin YC, Lo HM, Chen JD: Sonographic fatty liver, overweight and ischaemic heart disease. *World J Gastroenterol* 11: 4838–4842, 2005
 13. Kessler A, Levy Y, Roth A, Zelber-Sagi S, Leshno M, Blendis L, Halpern Z, Oren R: Increased prevalence of NAFLD in patients with acute myocardial infarction independent of BMI (Abstract). *Hepatology* 42:623A, 2005
 14. Volzke H, Robinson DM, Kleine V, Deutscher R, Hoffmann W, Ludemann J, Schminke U, Kessler C, John U: Hepatic steatosis is associated with an increased risk of carotid atherosclerosis. *World J Gastroenterol* 11:1848–1853, 2005
 15. Targher G, Bertolini L, Padovani R, Zenari L, Zoppini G, Falezza G: Relation of non-alcoholic hepatic steatosis to early carotid atherosclerosis in healthy men: role of visceral fat accumulation. *Diabetes Care* 27: 1498–1500, 2004
 16. Wannamethee G, Ebrahim S, Shaper AG: Gamma-glutamyltransferase: determinants and association with mortality from ischaemic heart disease and all causes. *Am J Epidemiol* 142:699–708, 1995
 17. Ruttman E, Brant LJ, Concin H, Diem G, Rapp K, Ulmer H: Gamma-glutamyltransferase as a risk factor for cardiovascular disease mortality: an epidemiological investigation in a cohort of 163,944 Austrian adults. *Circulation* 112:2130–2137, 2005
 18. Schindhelm RK, Dekker JM, Nijpels G, Bouter LM, Stehouwer CD, Heine RJ, Diamant M: Alanine aminotransferase predicts coronary heart disease events: a 10-year follow-up of the Hoorn Study. *Atherosclerosis* 191:391–396, 2007
 19. Ioannou GN, Weiss NS, Boyko EJ, Mozafarian D, Lee SP: Elevated serum alanine aminotransferase activity and calculated risk of coronary heart disease in the United States. *Hepatology* 43:1145–1151, 2006
 20. Targher G, Arcaro G: Non-alcoholic fatty liver disease and increased risk of cardiovascular disease. *Atherosclerosis* 191:235–240, 2007