Original Article

Nonalcoholic Fatty Liver Disease and Risk of Future Cardiovascular Events Among Type 2 Diabetic Patients

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Nonalcoholic fatty liver disease (NAFLD) is closely correlated to several metabolic syndrome features. We assessed prospectively whether NAFLD predicts future cardiovascular disease (CVD) events among type 2 diabetic individuals, independent of metabolic syndrome features and other classical risk factors. We carried out a prospective nested case-control study in 2,103 type 2 diabetic patients who were free of diagnosed CVD at baseline. During 5 years of follow-up, 248 participants (case subjects) subsequently developed nonfatal coronary heart disease (myocardial infarction and coronary revascularization procedures), ischemic stroke, or cardiovascular death. Using risk-set sampling, 496 patients (control subjects) among those who remained free of diagnosed CVD during follow-up were randomly selected in a 2:1 ratio, matched for age and sex to the case subjects. After adjustment for age, sex, smoking history, diabetes duration, HbA_{1c}, LDL cholesterol, liver enzymes, and use of medications, the presence of NAFLD was significantly associated with an increased CVD risk (odds ratio 1.84, 95% CI 1.4–2.1, $P < 0.001$). Additional adjustment for the metabolic syndrome (as defined by National Cholesterol Education Program Adult Treatment Panel III criteria) appreciably attenuated, but did not abolish, this association (1.53, 1.1–1.7, $P = 0.02$). In conclusion, NAFLD is significantly associated with a moderately increased CVD risk among type 2 diabetic individuals. This relationship is independent of classical risk factors and is only partly explained by occurrence of metabolic syndrome. *Diabetes* 54:3541–3546, 2005

Nonalcoholic fatty liver disease (NAFLD) is currently the most common abnormality observed in hepatology practice. NAFLD is a clinicopathologic syndrome that is closely correlated to visceral obesity, dyslipidemia, insulin resistance, and type 2 diabetes, thus suggesting that NAFLD is another feature of the metabolic syndrome (1–4).

A great deal of evidence suggests that the metabolic syndrome predicts incident cardiovascular disease (CVD) (5–8), so it is possible to hypothesize that NAFLD patients might portend a greater CVD risk and that NAFLD itself might confer a CVD risk above that associated with individual metabolic syndrome risk factors. Recent cross-sectional studies have clearly documented that patients with NAFLD have, other than several features resembling the metabolic syndrome, a markedly greater carotid artery wall thickness than those without NAFLD (9,10). This finding was also validated by the results of a large population-based study (11). However, carotid artery wall thickness is only a marker of early generalized atherosclerosis (12), so currently it is uncertain whether NAFLD is significantly associated with increased risk of future CVD events. Clarification of this aspect may help to explain the underlying mechanisms and may be of clinical importance for undertaking preventive and therapeutic strategies.

We have, therefore, assessed prospectively in a large sample of type 2 diabetic individuals, people in whom the prevalence rates of metabolic syndrome and its associated conditions such as NAFLD are very high (1,2,13), whether NAFLD predicts the risk of future CVD events and whether such an association is independent of traditional risk factors and metabolic syndrome features.

RESEARCH DESIGN AND METHODS

Study subjects were participants in the Valpolicella Heart Diabetes Study, a prospective observational study designed primarily to evaluate associations between type 2 diabetes and incidence of chronic vascular complications. The study initially enrolled all of the outpatients with type 2 diabetes ($n = 2{,}103$, 66.3% of the entire sample of patients who attended our clinic) regularly attending our diabetes clinic in the period between 1 January 2000 and 31 December 2000 who were free of diagnosed CVD (as ascertained by medical history, physical examination, electrocardiograms, and review of hospital records), who were not abusing alcohol, and who did not have other known causes of chronic liver disease (e.g., viral hepatitis, autoimmune hepatitis, use of hepatotoxic medications) as ascertained by medical history and examination, blood testing, and imaging (1). This cohort of patients was substantially comparable for main demographic variables with the entire sample of patients who regularly attended our clinic. All participants were periodically seen (every 4–6 months) for routine medical examinations of their glycemic control and chronic diabetes complications.

During an average of 5 years of follow-up (through 31 May 2005), 248 participants (case subjects) subsequently developed nonfatal coronary heart disease (CHD; i.e., myocardial infarction, coronary bypass surgery, or coronary angioplasty), ischemic stroke, or cardiovascular death. Using a risk-set sampling, 496 participants (control subjects) among those who remained free of diagnosed CVD events during follow-up were randomly selected in a 2:1 ratio, matched for age and sex to the case subjects. The response rates for the original cohort and for the NAFLD substudy were 95.6 and 96.9%, respectively, and those who attended the follow-up examinations were similar to those who did not attend in terms of main demographic variables, glycemic control, liver enzymes, and NAFLD status. Written informed consent was obtained from all participants. The study protocol was approved by the local ethics committee.

Cardiovascular outcomes consisted of cardiovascular death and nonfatal

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ALT, alanine aminotransferase; AST, aspartate aminotransferase; ATP III, Adult Treatment Panel III; CHD, coronary heart disease; CVD, cardiovascular disease; GGT, γ-glutamyltransferase; NAFLD, nonalcoholic fatty liver disease.

ischemic stroke or CHD. These events were generally identified by routine clinic visits and periodic electrocardiogram measurements. Nonfatal CHD (as defined above) was confirmed by reviewing medical records of the hospital, including diagnostic symptom patterns and results of laboratory exams and/or electrocardiogram changes (which were interpreted according to Minnesota code). The clinical end points did not include angina pectoris, silent CHD, or possible ischemic electrocardiogram changes alone. Nonfatal ischemic stroke was confirmed if review of medical records showed new-onset neurological symptoms lasting >24 h with diagnostic imaging tests (computed tomography or nuclear magnetic resonance). Physicians who reviewed the records were blind to the risk factor status of participants. Diagnosis of cardiovascular death was confirmed with additional information from hospital records, autopsy reports, and family contact. In no instance was the cause on the death certificate accepted without corroboration.

Laboratory procedures and clinical measurements. Venous blood was drawn in the morning after an overnight fast. Plasma liver function tests and other biochemical blood measurements were determined by standard laboratory procedures. At baseline, most subjects had normal liver enzymes (n = 694, 86.3%); the normal ranges for serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), and γ-glutamyltransferase (GGT) concentrations, in our laboratory, were 10–35 units/l for women and 10–50 units/l for men, respectively. It is known that diabetic patients with NAFLD can have raised or normal aminotransferase levels; aminotransferases fluctuate over time and they may be in the normal range, irrespective of steatosis, when a single measurement is used (1,2). All participants had negative serology for hepatitis B or C. LDL cholesterol was calculated by Friedewald's equation. HbA_{1c} (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%. The urinary albumin excretion rate was measured as the albumin-to-creatinine ratio by an immunonephelometric method (Beckman Analyzer); micro- and macroalbuminuria were defined as an albumin-to-creatinine ratio ≥2.5 and ≥25 mg/mmol.

BMI was calculated by dividing weight in kilograms by height in meters squared. Waist circumference was measured in a standing position at the level of the umbilicus. Blood pressure was assessed with a standard mercury manometer. Information on daily alcohol consumption and other lifestyle characteristics was obtained from all participants by a questionnaire (4). At baseline, most participants were abstainers (n = 558, 75%) or drank minimally (i.e., alcohol consumption <20 g/day; n = 121, 16.3%); only 8.7% (n = 65) of participants drank >50 g/day of alcohol.

Metabolic syndrome was defined according to criteria proposed by the National Cholesterol Education Program Adult Treatment Panel III (ATP III). In accordance with this definition (14), a person with type 2 diabetes was classified as having metabolic syndrome if he/she had at least two of the following risk determinants: *1*) waist circumference >102 cm in men or >88 cm in women, *2*) triglycerides ≥1.70 mmol/l, *3*) HDL <1.0 mmol/l in men and <1.28 mmol/l in women or treatment, and *4*) blood pressure ≥130/85 mmHg or treatment.

Hepatic ultrasound scanning was performed in all participants by a trained operator who was blind to all clinical and laboratory characteristics of participants, using an Acuson 128-XP/10 scanner with a 3.5-MHz linear transducer. Hepatic steatosis was diagnosed by characteristic echo patterns, according to conventional criteria (i.e., evidence of diffuse hyperechogenicity of liver relative to kidneys, ultrasound beam attenuation, and poor visualization of intrahepatic structures) (1,2,13). A semiquantitative sonographic evaluation of the degree of steatosis was not available. Repeated measurements (that were performed on a subgroup of 100 patients) on the same subjects gave intra- and interobserver coefficients of variation within 5%. In our previous study, we found a very good agreement between two imaging methods (i.e., ultrasound and computed tomography) in the categorization of subjects with steatosis (9).

Statistical analysis. Data are presented as the means ± SD or frequencies. Skewed variables (serum triglyceride and liver enzyme levels) 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). For prediction of CVD events, 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 multivariate logistic regression analysis was used to assess the independence of the association of NAFLD with incident CVD. In this analysis, CVD was considered as an aggregate end point inclusive of nonfatal ischemic stroke or CHD and cardiovascular death. Separate models were tested, with the individual components of metabolic syndrome simultaneously included as categorical or continuous variables in the same equation or with the metabolic syndrome considered as a single entity. In the fully adjusted logistic regression models, sex, age, smoking status, diabetes duration, A1C, LDL cholesterol, liver enzymes (i.e., GGT, ALT, and AST concentrations or the AST-to-ALT ratio) and use of medications (i.e., hypoglycemic, antihypertensive, lipid-lowering, or antiplatelet drugs) were also included as covariates. In light of the well-known association between alcohol drinking and liver injury (1), we repeated the analyses described above after excluding participants who drank minimally (<20 g/day, n = 121) and who consumed >50 g/day (n = 65). P values <0.05 were considered statistically significant.

RESULTS

During 5 years of follow-up, we documented 248 incident CVD events: 142 were nonfatal CHD (101 myocardial infarction and 41 coronary artery bypass grafting/percutaneous transluminal coronary angioplasty), 29 nonfatal ischemic stroke, and 77 deaths from cardiovascular events.

The clinical and biochemical characteristics of participants are shown in Table 1. Because of the study design, case and control subjects were almost identical in terms of sex and age. The metabolic syndrome and all its individual components were more frequent in case subjects. They also had significantly higher liver enzymes and marginally higher A1C levels. Diabetes duration and treatment, LDL cholesterol, and smoking history did not differ between the groups. Among those treated with oral hypoglycemic agents (~62% of total), about one-third were treated with sulfonylureas and the remaining patients with a combination of sulfonylureas and metformin, with no significant differences between case and control subjects; none of them were taking thiazolidinediones. The proportion using antihypertensive drugs was higher among case subjects (~72 vs. ~58%), whereas the proportion using lipid-lowering or antiplatelet drugs was essentially similar in both groups (~35 and ~50%, respectively). Notably, as shown in Table 1, the proportion of patients with NAFLD was significantly higher in those who subsequently developed CVD events than in those who did not, without differences between sexes (not shown).

As expected, the prevalence of NAFLD was as high as 75% in our population, and when participants were grouped according to NAFLD, those with NAFLD were older ($P < 0.001$), had higher liver enzymes ($P < 0.001$), and tended to have a longer duration of diabetes ($P = 0.064$). The metabolic syndrome and all its individual components were more frequent ($P < 0.001$) among those with NAFLD. Sex, smoking status, LDL cholesterol, A1C, and diabetes treatment did not differ between the groups (not shown).

As shown in Table 2, in univariate regression analysis, NAFLD, metabolic syndrome, age, sex, smoking history, A1C, and GGT levels (and other liver enzymes) (not shown) were significantly associated with incident CVD events, whereas LDL cholesterol concentration, diabetes duration, and use of medications were not. In multivariate regression analyses, the significant association between NAFLD and incident CVD was little affected by adjustment for age and sex (model 1); we additionally controlled for smoking history, diabetes duration, A1C, LDL cholesterol, GGT levels, and use of medications, but NAFLD remained significantly associated with increased CVD risk (model 2). Additional adjustment for metabolic syndrome appreciably attenuated, but did not abolish, the association (model 3). In the fully adjusted regression model, the presence of metabolic syndrome, older age, and cigarette smoking were also independently associated with increased CVD risk. Almost identical results were obtained in models that also adjusted for the AST-to-ALT ratio and

TABLE 1
Baseline characteristics of the study participants (n = 744)

Variables	Control subjects	Case subjects	P
n	496	248	—
Sex (% men)	62	62	NS*
Age (years)	65 ± 3	66 ± 4	NS*
BMI (kg/m^2)	26 ± 3	29 ± 4	<0.001
Waist circumference (cm)	93 ± 13	101 ± 14	<0.001
Duration of diabetes (years)	13 ± 3	14 ± 3	NS
Oral hypoglycemic agents (%)	61.9	63.3	NS
Insulin treatment only (%)	12	14	NS
Current smokers (%)	20	22	NS
Systolic blood pressure (mmHg)	124 ± 13	131 ± 15	<0.001
Diastolic blood pressure (mmHg)	79 ± 12	83 ± 10	<0.001
A1C (%)	6.9 ± 0.8	7.2 ± 0.9	0.059
Triglycerides (mmol/l)	1.24 ± 0.6	1.62 ± 0.9	<0.001
HDL cholesterol (mmol/l)	1.39 ± 0.3	1.25 ± 0.4	<0.001
LDL cholesterol (mmol/l)	3.29 ± 0.4	3.27 ± 0.5	NS
AST (units/l)	20 ± 10	26 ± 12	<0.01
ALT (units/l)	23 ± 12	33 ± 14	<0.001
GGT (units/l)	27 ± 14	38 ± 16	<0.001
Microalbuminuria (%)	20	23	NS
ATP III–defined metabolic syndrome (%)	52	73	<0.001
NAFLD (%)	56	94	<0.001

Data are the means ± SD, unless otherwise indicated. Differences were assessed by the unpaired t test (for normally distributed variables) and by the χ^2 test (for categorical variables). *Matched variables.

in models in which serum AST, ALT, and GGT concentrations were simultaneously included (not shown); none of individual liver enzymes was independently associated with incident CVD after controlling for NAFLD and other potential confounders. In separate models in which the individual components of metabolic syndrome were entered as categorical or continuous variables (instead of the metabolic syndrome as a single entity), only hypertension and dyslipidemia (P = 0.001 for both), along with age, smoking history, and presence of NAFLD, showed independent associations with CVD. Exclusion of participants who were light or moderate/heavy drinkers did not alter the observed associations between NAFLD and CVD risk (multiple-adjusted odds ratio 1.62, 95% CI 1.2–1.9, P = 0.01).

DISCUSSION

In this prospective nested case-control study, we have shown, for the first time, that NAFLD is associated with an increased risk of future CVD events among type 2 diabetic individuals. Importantly, this association is independent of classical risk factors, liver enzymes, and the metabolic syndrome, a highly atherogenic condition that is strongly correlated to NAFLD.

These results are supported by previous prospective studies reporting strong associations of elevated liver enzymes (particularly serum GGT levels), as surrogate markers of NAFLD (1,2,13), with the occurrence of CVD events in both nondiabetic subjects and type 2 diabetic patients. In a study of 14,874 middle-aged Finnish men and women, mildly elevated GGT levels were independently associated with an increased risk of ischemic stroke in both sexes (15). Among 7,613 middle-aged British men followed for 11.5 years, elevated GGT levels were independently associated with a significant increase in mortality from all causes and from CHD (16). Our results are also supported by a prospective study of 132 patients with biopsy-proven NAFLD followed for 18 years, demonstrating that CVD deaths were the second most common cause of death in NAFLD patients, with rates equaling those of liver-related deaths and trailing only cancer-related deaths (17). Finally, our results extend recent cross-sectional observations documenting a marked increase in carotid artery wall thickness and elevated prevalence of atherosclerotic carotid plaques among diabetic and nondiabetic individuals with NAFLD (9–11).

The biological mechanisms by which NAFLD could contribute to accelerated atherosclerosis are still poorly known. Our data suggest that in people with type 2 diabetes, the relationship between NAFLD and increased CVD risk most likely reflects the overall atherogenic impact of the metabolic syndrome phenotype, principally hypertension and dyslipidemia, as supported by our multivariate analyses. However, because in this study NAFLD correlated to CVD events, independent of metabolic syndrome and classic risk factors, it is conceivable that other atherogenic mechanisms could be involved. Unfortunately, we did not directly measure insulin resistance in our population. Recent evidence has raised concern that the current ATP III definition of the metabolic syndrome has low sensitivity for identifying insulin resistance in subjects (18). Conversely, several studies have consistently documented that insulin resistance predicts incident CVD events (19,20) and plays a pivotal role in the development of poor clinical outcomes in NAFLD patients (1,2,13). Thus, NAFLD in its more advanced forms might act as a stimulus for further increased whole-body insulin resistance and dyslipidemia, leading to accelerated atherosclerosis. This hypothesis is also partly validated by recent prospective studies demonstrating that raised liver enzymes independently predict the development of type 2 diabetes and other metabolic syndrome features (21–23).

Another possible atherogenic mechanism linking NAFLD and increased CVD risk is represented by in-

TABLE 2
Univariate and multivariate logistic regression analyses of factors associated with incident CVD events among type 2 diabetic patients

Variables	Univariate	Multivariate model 1	Multivariate model 2	Multivariate model 3
NAFLD				
OR	1.91	1.9	1.84	1.53
95% CI	1.4–2.2	1.4–2.2	1.4–2.1	1.1–1.7
P values	0.001	0.001	0.001	0.02
ATP III–defined metabolic syndrome				
OR	1.64	1.64	1.62	1.58
95% CI	1.3–2.5	1.3–2.3	1.3–2.1	1.3–2
P values	0.001	0.001	0.001	0.01
Age				
OR	1.14	1.13	1.13	1.12
95% CI	1.07–1.16	1.07–1.14	1.07–1.14	1.06–1.14
P values	0.001	0.001	0.001	0.001
Sex				
OR	1.50	1.48	1.46	1.46
95% CI	1.2–2	1.2–2	1.2–1.9	1.2–1.9
P values	0.001	0.001	0.001	0.001
Diabetes duration				
OR	1.08	NS	NS	NS
95% CI	0.8–1.3	NS	NS	NS
P values	NS	NS	NS	NS
A1C				
OR	1.51	1.46	NS	NS
95% CI	1.1–4.9	1.02–4.2	NS	NS
P values	0.02	0.05	NS	NS
Smoking status				
OR	1.44	1.42	1.40	1.40
95% CI	1.1–2.0	1.1–2.0	1.1–1.9	1.1–1.9
P values	0.01	0.01	0.01	0.01
LDL cholesterol				
OR	1.18	NS	NS	NS
95% CI	0.9–1.4	NS	NS	NS
P values	NS	NS	NS	NS
GGT				
OR	1.42	1.40	1.27	NS
95% CI	1.1–2.0	1.1–1.9	1.05–1.7	NS
P values	0.001	0.001	0.01	NS
Use of medications				
OR	1.0	NS	NS	NS
95% CI	0.7–1.2	NS	NS	NS
P values	NS	NS	NS	NS

Model 1: adjustment for age and sex; model 2: further adjustment for smoking history, diabetes duration, A1C, LDL cholesterol, GGT levels, and use of medications (i.e., hypoglycemic, antihypertensive, lipid-lowering, or antiplatelet drugs); model 3: additional adjustment for the presence of metabolic syndrome or NAFLD (for the first and second variables in the table) or additional adjustment for the presence of metabolic syndrome and NAFLD (for all other variables in the table).

creased oxidative stress and subclinical inflammation, which are thought to be causal factors in the progression from simple steatosis to more advanced forms of NAFLD (1,2,24). Reactive oxygen species derived from steatosis-stimulated fatty acid oxidation, attendant hepatocyte injury and cytokine release, and the ensuing proinflammatory milieu are likely to perpetuate the liver damage of NAFLD and add further atherogenic stimuli to the already high oxidative/proinflammatory status that is closely related to the metabolic syndrome (19,20). We have recently shown significant associations of NAFLD with impaired fibrinolytic activity and elevated plasma C-reactive protein and fibrinogen concentrations in nondiabetic individuals; these associations were independent of age, BMI, blood pressure, lipids, and insulin resistance (25). Decreased plasma levels of adiponectin, which is an adipose-secreted cytokine with antiatherogenic properties (26), may represent another possible mechanism linking NAFLD and CVD risk. We have recently reported that hypoadiponectinemia closely correlated to NAFLD in obese individuals, independent of insulin resistance and other metabolic syndrome variables (27). This was also validated by a recent study by Hui et al. (28). Finally, accumulating evidence also exists that NAFLD could be linked to accelerated atherogenesis through the presence of abnormal lipoprotein metabolism. In NAFLD, hepatic apolipoprotein B-100 synthesis, a rate-determining step in hepatic VLDL formation and in hepatocyte lipid export, is markedly reduced, and postprandial apolipoprotein B-100 responses are flat and strikingly dissociated from the concomitant increases of postprandial triglycerides (29,30). Disturbances of VLDL assembly are an important factor in the natural history of NAFLD and can also result in increased levels of atherogenic triglyceride- and cholesterol-rich remnant particles (1,2, 19). Small dense LDL particles, which are thought to be more atherogenic (19,20), could also be increased in

NAFLD patients. However, detailed compositional lipoprotein studies should be performed in patients with NAFLD to prove this contention.

A limitation of this study is that the diagnosis of NAFLD was based on ultrasonography and exclusion of known etiologic factors of chronic liver disease, but it was not confirmed by liver biopsy. It is known that none of the radiological features can distinguish between steatohepatitis and other types of NAFLD and that only liver biopsy can assess the severity of damage and the prognosis (1,2). However, liver biopsy is not easily applied in large epidemiological studies. Conversely, ultrasonography is by far the most common method of diagnosing NAFLD in clinical practice and has a very good sensitivity and specificity in detecting moderate and severe steatosis in patients with biopsy-proven disease (1,2,13,31). Indeed, it has been reported that the presence of >33% fat on liver biopsy is optimal for ultrasound detection of steatosis, although ultrasonography is not completely sensitive, particularly when hepatic fat infiltration is <33% (32).

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 a negative ultrasound), 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 increased CVD risk.

Because liver biopsies were not available in this study, we cannot obviously exclude the possibility of a differential relationship between the broad spectrum of NAFLDs and CVD risk. Recent studies have partially substantiated this showing that the severity of liver histology in NAFLD patients is strongly correlated to the increasing number of metabolic syndrome features (33), greater carotid artery wall thickness (34), and lower endothelial flow-mediated vasodilation (35).

These findings might have important clinical and public health implications. Our results support the implication that type 2 diabetic individuals with NAFLD should be considered at high risk for CVD. Thus, the casual detection of NAFLD on an ultrasound examination in these patients should alert to the coexistence of multiple underlying CVD risk factors warranting evaluation and treatment as much as the risk for advancing liver disease. It is worth emphasizing that our findings are likely to be limited to people with type 2 diabetes and NAFLD and that we cannot be certain that identical results could be obtained in nondiabetic individuals, i.e., a people who have much lower rates of CVD and lower risk of having more advanced NAFLD and more disease progression as compared with patients with type 2 diabetes (1,2,36). Moreover, we recognize that this cohort does not represent a random sample of all diabetic patients, but it is a representative sample of type 2 diabetic patients who are usually seen in clinics; they frequently have a positive liver ultrasound, despite normal or mildly elevated liver enzymes. 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 among type 2 diabetic patients, including weight reduction and treatment with insulin-sensitizing oral antidiabetic agents, also improve NAFLD (1,2,13).

Because we studied a large representative sample of type 2 diabetic individuals with low reported alcohol intake and with no exposure to hepatotoxic chemical or viral agents, and because a liver ultrasound in diagnosing NAFLD was performed in all participants, we believe that the validity of our findings is enhanced.

In conclusion, our findings support the hypothesis that NAFLD is associated with a moderately increased risk for future CVD events among type 2 diabetic individuals, independent of classical risk factors, liver enzymes, and the presence of metabolic syndrome. Future follow-up studies using larger cohorts of patients are necessary to validate these results and to extend these findings among NAFLD patients without type 2 diabetes.

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