Relations Between Carotid Artery Wall Thickness and Liver Histology in Subjects With Nonalcoholic Fatty Liver Disease

Giovanni Targher, md[1]
Lorenzo Bertolini, md[1]
Roberto Padovani, md[1]
Stefano Rodella, md[2]
Giacomo Zoppini, md[3]
Luciano Zenari, md[1]
Massimo Cigolini, md[4]
Giancarlo Falezza, md[1]
Guido Arcaro, md[1]

OBJECTIVE — Nonalcoholic fatty liver disease (NAFLD) is closely associated with several metabolic syndrome features. We assessed whether NAFLD is associated with carotid artery intima-media thickness (IMT) as a marker of subclinical atherosclerosis and whether such an association is independent of classical risk factors, insulin resistance, and metabolic syndrome features.

RESEARCH DESIGN AND METHODS — We compared carotid IMT, as assessed by ultrasonography, in 85 consecutive patients with biopsy-proven NAFLD and 160 age-, sex-, and BMI-matched healthy control subjects.

RESULTS — NAFLD patients had a markedly greater carotid IMT (1.14 ± 0.20 vs. 0.82 ± 0.12 mm; $P < 0.001$) than control subjects. The metabolic syndrome (according to Adult Treatment Panel III criteria) and its individual components were more frequent in those with NAFLD ($P < 0.001$). The marked differences in carotid IMT observed between the groups were only slightly weakened after adjustment for age, sex, BMI, smoking history, LDL cholesterol, insulin resistance (by homeostasis model assessment), and metabolic syndrome components. Notably, carotid IMT was strongly associated with degree of hepatic steatosis, necroinflammation, and fibrosis among NAFLD patients ($P < 0.001$ for all). Similarly, by logistic regression analysis, the severity of histological features of NAFLD independently predicted carotid IMT ($P < 0.001$) after adjustment for all potential confounders.

CONCLUSIONS — These results suggest that the severity of liver histopathology among NAFLD patients is strongly associated with early carotid atherosclerosis, independent of classical risk factors, insulin resistance, and the presence of metabolic syndrome.

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Nonalcoholic fatty liver disease (NAFLD), the most common cause of abnormal liver function tests in hepatology practice, is frequently associated with visceral obesity, dyslipidemia, insulin resistance, and type 2 diabetes and may represent another component of the metabolic syndrome (1–3).

Recent cross-sectional studies (4–6) have shown that NAFLD is associated with increased carotid artery intima-media thickness (IMT), a marker of early generalized atherosclerosis (7). However, in these studies the NAFLD diagnosis was exclusively based on ultrasound imaging but was not confirmed by liver biopsy, which is the best diagnostic tool for confirming NAFLD (1–3).

Thus, currently it is uncertain whether there is a significant association between early carotid atherosclerosis and the severity of liver histology among NAFLD patients. Clarification of this aspect may help to explain the underlying mechanisms and may be of clinical importance in planning preventive and therapeutic strategies.

We have, therefore, assessed whether patients with biopsy-proven NAFLD have a greater carotid IMT than control subjects and whether there is a significant association between liver histology and carotid IMT among NAFLD patients.

RESEARCH DESIGN AND METHODS

A total of 85 consecutive outpatients with NAFLD were recruited from clinics, 50 of which have been previously included in a small pilot study (8). All patients had chronically elevated liver enzymes and hepatic steatosis detected by ultrasonography. The NAFLD diagnosis was based on liver biopsy and exclusion of other known etiologic factors of chronic liver disease (alcohol abuse or intake ≥20 g/day, viral hepatitis, autoimmune hepatitis, and use of hepato-toxic drugs). No patients had clinical evidence of advanced liver or renal disease, cardiovascular events, or recent history of acute illness. Eight male subjects and four female subjects had preexisting type 2 diabetes; nine managed their diabetes with diet alone, and three were taking metformin.

The control group, recruited from hospital staff members and relatives, consisted of 160 apparently healthy volunteers with normal liver ultrasonography and normal liver function tests who were matched for age, sex, and BMI.

The protocol was approved by the local ethics committee. All participants gave written informed consent.

Clinical measurements and laboratory procedures

BMI was calculated by dividing weight in kilograms by the square of height in meters. Waist circumference was mea-

From the [1]Division of Internal Medicine, "Sacro Cuore" Hospital, Negrar, Italy; the [2]Department of Radiology, "Sacro Cuore" Hospital, Negrar, Italy; the [3]Division of Endocrinology, University of Verona, Verona, Italy; and the [4]Observatory of Clinical Epidemiology "sen. Giacometti," Arzignano, Italy.

Address correspondence and reprint requests to Giovanni Targher, MD, Division of Internal Medicine and Diabetes Unit, Ospedale "Sacro Cuore – don Calabria," Via Sempreboni, 5, 37024 Negrar (VR), Italy. E-mail: targher@sacrocuore.it.

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Abbreviations: ATP, Adult Treatment Panel; CVD, cardiovascular disease; HOMA-IR, homeostasis model assessment for insulin resistance; IMT, intima-media thickness; 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.

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sured in a standing position at the level of the umbilicus. Blood pressure was measured with a standard mercury manometer. Information on daily alcohol consumption and other lifestyle characteristics was obtained from all participants by questionnaire (4).

Venous blood was drawn in the morning after an overnight fast. Plasma liver function tests, insulin, and other biochemical blood measurements were determined by standard laboratory procedures. Normal ranges for aspartate aminotransferase, alanine aminotransferase, and γ-glutamyltransferase, in our laboratory, were 10–35 units/l for female subjects and 10–50 units/l for male subjects, respectively. All participants had negative serology for viral hepatitis B and C. LDL cholesterol was calculated by the Friedewald's equation. HbA_{1c} (A1C) was measured by a high-performance liquid chromatography analyzer (HA-8140; Arkray-Menarini, Florence, Italy) in NAFLD patients with diabetes ($n = 12$); mean ± SD A1C levels were 6.3 ± 0.2%. Insulin resistance was calculated by homeostasis model assessment for insulin resistance (HOMA-IR) score (9). An oral glucose tolerance test was performed in all participants, except for those with preexisting diabetes; only two patients with NAFLD had impaired glucose tolerance (i.e., 2-h glucose >7.8 and <11.1 mmol/l), whereas the remaining participants had normal glucose tolerance. Metabolic syndrome was diagnosed by a modified Adult Treatment Panel (ATP) III definition that was recently proposed by the American Heart Association and the National Heart, Lung, and Blood Institute (10). In accordance with this definition, a person was classified as having metabolic syndrome if he/she had at least three of the following risk determinants: *1*) waist circumference >102 cm in men or >88 cm in women, *2*) fasting glucose ≥5.6 mmol/l or drug treatment, *3*) triglycerides ≥1.70 mmol/l or drug treatment, *4*) HDL cholesterol <1.0 mmol/l in men and <1.29 mmol/l in women or drug treatment, and *5*) blood pressure ≥130/85 mmHg or drug treatment.

Carotid IMT was measured with ultrasonography by a single trained operator who was blind to clinical characteristics of participants. Carotid IMT measurements were made bilaterally at the level of the common carotid artery far wall and always in stenotic-free segments, as previously reported (4,11). For each subject, three measurements on both sides were performed, i.e., the anterior, lateral, and posterior projection of the near and far wall. All readings were then averaged. Repeated measurements on the same subjects (that were done in a subgroup of 100 subjects) gave CVs <9%. A carotid plaque was defined as a focal thickening ≥1.2 mm at the level of carotid artery (5–7); none of the study participants had clinically relevant carotid stenosis (i.e., ≥60%).

An experienced hepatopathologist blinded to subjects' details scored liver biopsy specimens using the semiquantitative classification of Brunt et al. (12). Briefly, the severity of steatosis was graded on the basis of the extent of involved parenchyma: grade 1, <33% of hepatocytes affected; grade 2, 33–66% of hepatocytes affected; and grade 3, >66% of hepatocytes affected. Nonalcoholic steatohepatitis (NASH) was defined as the presence of steatosis plus lobular inflammation plus hepatocellular ballooning or steatosis plus any stage of fibrosis. The stages of fibrosis were graded as follows: stage 1, zona three perivenular, perisinusoidal, or pericellular fibrosis; stage 2, as above with focal or extensive periportal fibrosis; stage 3, bridging fibrosis, focal or extensive; and stage 4, cirrhosis.

Statistical analysis

Data are presented as means ± SD or percentages. Skewed variables (plasma triglycerides and HOMA-IR score) were logarithmically transformed to improve normality before analysis. Statistical analyses included unpaired *t* test, one-way ANOVA, χ^2 test with Yates' correction for continuity (for categorical variables), univariate linear correlations, and ANCOVA. The independence of the associations of variables with carotid IMT, considered as the dependent variable, was also assessed by multiple logistic regression analyses and expressed as odds ratio (OR). For this purpose, carotid IMT was modeled as a categorical variable and subjects stratified into those belonging to the top quartile versus the other three quartiles. In the fully adjusted multivariate logistic regression models, along with the histological features of NAFLD (i.e., steatosis grade, necroinflammatory grade, or fibrosis stage included in these models separately), sex, age, BMI, smoking history, LDL cholesterol, HOMA-IR score, and metabolic syndrome (considered as a single clinical entity) were included as covariates. Separate regression models were also tested with the individual components of metabolic syndrome that were simultaneously included as either continuous or categorical variables in the same equation. *P* values <0.05 were considered statistically significant.

RESULTS — Liver histopathology results were steatosis alone in 16 subjects, steatohepatitis (NASH) with fibrosis score 0 in 23 subjects, NASH/fibrosis score 1 in 25 subjects, NASH/fibrosis score 2 in 13 subjects, and NASH/fibrosis score 3 in 8 subjects. None had cirrhosis (a fibrosis score of 4).

The baseline characteristics of participants are shown in Table 1. Because of the study design, case and control subjects were almost identical in terms of age, sex, and BMI. Metabolic syndrome and its individual components occurred more frequently in NAFLD patients, with no differences between sexes (not shown). These patients also had significantly higher liver enzymes and HOMA-IR score. Smoking history, plasma LDL cholesterol, creatinine, and 2-h glucose concentrations did not differ between the groups.

As shown in Table 2, NAFLD patients had a markedly greater carotid IMT than control subjects, with no differences between men and women (not shown). Accordingly, compared with control subjects, those with NAFLD also had a greater prevalence of carotid plaques (69.1 vs. 34.2%, $P < 0.001$), defined as a focal thickening ≥1.2 mm at the level of common carotid artery. As also shown in Table 2, carotid IMT was significantly different between patients with NASH, patients with simple steatosis, and control subjects; the lowest levels were in control subjects, intermediate in patients with steatosis, and highest in those with NASH. Importantly, the severity of histological features of NAFLD (steatosis, necroinflammation, and fibrosis) strongly correlated with increased carotid IMT as well as with increased HOMA-IR score and higher prevalence of metabolic syndrome. The marked differences in carotid IMT observed among the groups were little affected by adjustment for age, sex, and BMI; additional adjustment for smoking history, LDL cholesterol concentration, HOMA-IR score, and the presence of metabolic syndrome did not substantially modify these results. Similar results were found after excluding type 2 diabetic patients.

Concordantly, in the fully adjusted multiple logistic regression models, age

Table 1—*Clinical and biochemical characteristics of NAFLD patients and control subjects*

	NAFLD patients	Control subjects	*P*
Sex (male/female)	50/35	95/65	NS*
Age (years)	45 ± 2	45 ± 2	NS*
Weight (kg)	75 ± 3	75 ± 2	NS*
BMI (kg/m^2)	26.1 ± 2	26.0 ± 2	NS*
Waist circumference (cm)	94 ± 6	90 ± 3	<0.01
Systolic blood pressure (mmHg)	127 ± 6	120 ± 3	<0.01
Diastolic blood pressure (mmHg)	83 ± 4	79 ± 2	<0.01
Current smokers (%)	18.8 (*n* = 16)	20.0 (*n* = 32)	NS
Fasting glucose (mmol/l)†	6.6 ± 0.5	5.2 ± 0.3	<0.05
HOMA insulin resistance score†	4.05 ± 2.1	1.83 ± 1.0	<0.001
2-h glucose (mmol/l)‡	6.5 ± 0.6	6.1 ± 0.3	NS
Triglycerides (mmol/l)	1.46 ± 0.7	0.97 ± 0.5	<0.001
HDL cholesterol (mmol/l)	1.28 ± 0.3	1.43 ± 0.2	<0.01
LDL cholesterol (mmol/l)	3.20 ± 0.3	3.18 ± 0.3	NS
Creatinine (μmol/l)	89 ± 6	85 ± 5	NS
Aspartate aminotransferase (units/l)	46 ± 22	19 ± 3	<0.001
Alanine aminotransferase (units/l)	100 ± 40	20 ± 3	<0.001
γ-Glutamyl transferase (units/l)	57 ± 21	21 ± 2	<0.001

Data are means ± SD unless otherwise indicated. Differences were assessed by the unpaired *t* test (for normally distributed variables) and the χ^2 test (for categorical variables). *Matched variables; †including type 2 diabetic patients (*n* = 12); ‡excluding type 2 diabetic patients.

(OR 1.13 [95% CI 1.05–1.15]; $P < 0.001$), HOMA-IR score (1.61 [1.2–2.4]; $P < 0.001$), presence of ATP III–defined metabolic syndrome (2.01 [1.5–2.9]; $P < 0.001$), and the severity of hepatic fibrosis (1.71 [1.4–2.2]; $P < 0.001$), either steatosis or necroinflammation ($P < 0.001$ for both), independently predicted carotid IMT (quartile 4 vs. 1–3) after adjustment for sex, BMI, smoking history, and LDL cholesterol concentrations. Almost identical results were obtained in models that also adjusted for the individual components of metabolic syndrome or in models in which diabetic patients were removed from analysis (not shown).

As expected, carotid IMT was significantly correlated with age, male sex, waist circumference, blood pressure, HOMA-IR score, plasma insulin, and triglyceride and LDL cholesterol concentrations, whereas it was only marginally correlated with BMI and smoking history both among NAFLD patients ($r = 0.26$–0.48, $P < 0.01$, or less) and control subjects ($r = 0.23$–0.36). However, after pooling subjects, the correlations of carotid IMT to cigarette smoking and BMI also reached statistical significance ($r = 0.17$ and $r = 0.14$, respectively, $P < 0.01$). Carotid IMT was positively correlated with A1C levels ($r = 0.39$, $P < 0.001$) and was significantly higher in NAFLD patients with type 2 diabetes ($n = 12$) than in their nondiabetic counterparts ($n = 73$) (1.20 ± 0.3 vs. 1.11 ± 0.2 mm; $P < 0.05$).

CONCLUSIONS — This study has shown for the first time that *1)* patients with biopsy-proven NAFLD have a marked increase in carotid IMT in comparison with age-, sex-, and BMI-matched control subjects; *2)* carotid IMT is higher for individuals with NASH than for those with simple steatosis; and *3)* the histological severity of NAFLD independently predicts carotid IMT after adjustment for a broad spectrum of potential confounders, including the metabolic syndrome, a highly atherogenic condition that is strongly correlated to NAFLD.

These results are supported by previous prospective studies reporting strong associations between elevated serum liver enzymes as surrogate markers of NAFLD (1–3) and the incidence of cardiovascular disease (CVD) in both nondiabetic and diabetic individuals (13,14). Our results are also supported by a recent prospective study of 2,103 type 2 diabetic patients followed for ~5 years, demonstrating that NAFLD, as diagnosed by ultrasound, was associated with a moderately increased risk for future CVD events, independent of classical risk factors, liver enzymes, and the presence of metabolic syndrome (15). Moreover, these results extend recent cross-sectional observations documenting a significant increase in carotid IMT among patients with ultrasonographically diagnosed NAFLD (4–6). Finally, our results are in line with those of a recent small study showing that NAFLD patients had a significant decrease in endothelium-dependent vasodilation compared with control subjects and that this decrease was significantly associated with the histological severity of NAFLD (16).

The biological mechanisms by which NAFLD could contribute to accelerated atherosclerosis are still poorly understood. Our data suggest the possibility that the putative elevated CVD risk associated with NAFLD most likely reflects the overall atherogenic impact of the metabolic syndrome phenotype as also partly supported by our multivariate analyses. Several studies have consistently documented that insulin resistance predicts incident CVD (17) and plays a pivotal role in the development of poor clinical outcomes in NAFLD patients (1–3). 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 the metabolic syndrome (18,19). However, since in this study NAFLD was associated with increased carotid IMT independent of classical risk factors, insulin resistance, and the metabolic syndrome, it is conceivable that other atherogenic mechanisms could be involved. A possible atherogenic mechanism linking NAFLD and carotid IMT could be represented by increased oxidative stress and subclinical inflammation, which are thought to be causal factors in the progression from simple steatosis to more advanced forms of NAFLD (1,3,20). 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 closely related to the metabolic syndrome (17). We have recently reported significant associations of NAFLD with impaired fibrinolytic activity and increased plasma C-reactive protein and fibrinogen concentrations in nondiabetic individuals; these associations were independent of age, BMI, blood pressure, lipids, and in-

Table 2—*Values of carotid IMT, HOMA-estimated insulin resistance, and presence of metabolic syndromen (MetS) in participants grouped according to NAFLD and the severity of liver histology*

	n	Carotid IMT (mm)	HOMA-IR score	Metabolic syndrome prevalence (%)
Control subjects	160	0.82 ± 0.1	1.83 ± 1.0	1.3 (*n* = 2)
NAFLD patients	85	1.14 ± 0.2	4.05 ± 2.2	48.2 (*n* = 41)
P values*		0.001	0.001	0.001
P values†		0.001	0.001	0.001
P values‡		0.001	NA	NA
Simple steatosis	16	0.99 ± 0.2	3.12 ± 1.7	25.0 (*n* = 4)
Steatohepatitis (NASH)	69	1.30 ± 0.2	4.95 ± 2.5	53.6 (*n* = 37)
P values*		0.001	0.001	0.001
P values†		0.001	0.001	0.001
P values‡		0.001	NA	NA
After excluding type 2 diabetics	12			
Control subjects	160	0.82 ± 0.1	1.82 ± 1.0	1.3 (*n* = 2)
Simple steatosis	14	0.98 ± 0.1	2.70 ± 1.3	14.3 (*n* = 2)
Steatohepatitis (NASH)	59	1.28 ± 0.2	4.01 ± 2.0	45.8 (*n* = 27)
P values*		0.001	0.001	0.001
P values†		0.001	0.001	0.001
P values‡		0.001	NA	NA
Grading for steatosis§	85			
Grade 1	22	0.99 ± 0.1	3.67 ± 1.6	27.3 (*n* = 6)
Grade 2	35	1.20 ± 0.2	4.55 ± 2.1	45.7 (*n* = 16)
Grade 3	28	1.36 ± 0.3	5.85 ± 3.6	67.9 (*n* = 19)
P values*		0.001	0.001	0.001
P values†		0.001	0.001	0.001
P values‡		0.001	NA	NA
Grading for necroinflammation	69			
Grade 1	18	1.03 ± 0.2	3.77 ± 1.6	27.8 (*n* = 5)
Grade 2	23	1.26 ± 0.2	4.88 ± 2.3	47.8 (*n* = 11)
Grade 3	28	1.39 ± 0.3	6.01 ± 3.5	75.0 (*n* = 21)
P values*		0.001	0.001	0.001
P values†		0.001	0.001	0.001
P values‡		0.001	NA	NA
Staging for fibrosis	69			
Stage 0	23	1.08 ± 0.2	3.82 ± 1.7	30.5 (*n* = 7)
Stage 1	25	1.23 ± 0.2	4.85 ± 1.8	52.0 (*n* = 13)
Stage 2	13	1.34 ± 0.3	5.58 ± 2.5	69.2 (*n* = 9)
Stage 3	8	1.46 ± 0.3	6.67 ± 3.6	100 (*n* = 8)
P values*		0.001	0.001	0.001
P values†		0.001	0.001	0.001
P values‡		0.001	NA	NA

Data are means ± SD unless otherwise indicated. **P* values by ANOVA or χ^2 test for unadjusted differences or trends; †*P* values by ANCOVA for differences or trends adjusted for age, sex, and BMI; ‡*P* values by ANCOVA for differences or trends additionally adjusted for smoking history, LDL cholesterol, HOMA-IR score, and presence of ATP III–defined metabolic syndrome; §including patients with simple steatosis and those with NASH.

sulin resistance (21). Decreased plasma levels of adiponectin, an adipose-secreted cytokine with anti-atherogenic properties (22), may represent another possible mechanism linking NAFLD and carotid IMT. We have recently shown that hypoadiponectinemia closely correlates to NAFLD in obese individuals, independent of insulin resistance and other metabolic syndrome components (23). This was also validated by the recent study of Hui et al. (24). 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 (25,26). Disturbances of VLDL assembly are an important factor in the natural history of NAFLD (1–3) and can also result in increased levels of atherogenic triglyceride- and cholesterol-rich remnant particles. Small dense LDL particles, which are thought to be more atherogenic (17), could also be increased in NAFLD patients. However, detailed lipoprotein compositional studies should be performed in patients with NAFLD to prove this contention.

This study has some limitations that should be kept in mind. Because our study was cross-sectional, the causative nature of the associations cannot be estab-

lished. Prospective studies will be required to sort out the time sequence of events. However, it is important to emphasize that the evidence from this and our recent prospective study demonstrating an independent association between NAFLD and incident CVD in NAFLD patients with type 2 diabetes (15) strongly supports the possibility that NAFLD could also be atherogenic among NAFLD patients without diabetes. Another possible limitation of this study is that among the control subjects, the exclusion of NAFLD was based on medical history, blood testing, and ultrasound imaging but was not confirmed by liver biopsy. However, although some nondifferential misclassification of NAFLD on the basis of ultrasound is likely (i.e., some of the 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 carotid IMT.

Interestingly, O'Leary et al. (27) have previously reported that a carotid IMT value ≤0.86 mm carries a low risk of developing CVD, whereas an IMT value ≥1.10 carries a high risk of developing CVD. It is worth emphasizing that the mean carotid IMT values we found among control subjects and NAFLD patients were of 0.82 and 1.14 mm, respectively. Thus, our findings might have important clinical and public health implications. Our data further emphasize the importance of evaluating the CVD risk in patients diagnosed with NAFLD. Patients with NAFLD having increased carotid IMT could be candidates not only for aggressive treatment of the liver disease, but also for cholesterol lowering and aggressive treatment of underlying CVD risk factors; this would help to modify and potentially decrease the global CVD risk of these patients.

Currently, it is not known whether improving NAFLD will ultimately prevent the development of CVD. However, it is notable that interventions known to be effective in preventing CVD, including weight reduction and treatment with insulin-sensitizing oral agents (thiazolidinediones or metformin), also improve NAFLD (1–3).

In conclusion, our findings support the hypothesis that the severity of histopathological features in NAFLD is strongly associated with early carotid atherosclerosis, independent of classical risk factors, insulin resistance, and the presence of metabolic syndrome. Future follow-up studies are necessary to validate these findings and better estimate the risk of incident CVD among patients with biopsy-proven NAFLD.

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