Nonalcoholic Fatty Liver Disease Is Associated With Carotid Atherosclerosis
A Case–Control Study

Angel Brea, Daniel Mosquera, Eva Martín, Ana Arizti, José L. Cordero, Emilio Ros

Objective—Nonalcoholic fatty liver disease (NAFLD) frequently coexists with obesity, diabetes, and dyslipidemia. We examined whether NAFLD was associated with atherosclerosis, as measured by ultrasound in the carotid arteries.

Methods and Results—Carotid atherosclerosis and cardiovascular risk factors were assessed in 40 patients with an ultrasound diagnosis of primary NAFLD and 40 matched population controls. The metabolic syndrome and all its individual traits, including elevated C-reactive protein, were significantly (P<0.005) more frequent in NAFLD patients than in control subjects. Patients with NAFLD showed more carotid atherosclerosis than controls, with mean intima-media thickness (IMT) of 0.70±0.20 mm and 0.54±0.13 mm (P<0.0001) and plaque prevalence of 50% and 25% (P=0.021), respectively. By multivariate analysis, older age (odds ratio [OR], 2.5 per 10 years; 95% CI, 1.4 to 4.4; P=0.002), the presence of NAFLD (OR, 8.4; 95% CI, 2.49 to 29.4; P=0.001), and elevated serum ferritin (OR, 3.1; 95% CI, 1.2 to 7.9; P=0.016) were independent predictors of an increased IMT.

Conclusions—Patients with NAFLD show a cluster of risk factors of the metabolic syndrome and advanced carotid atherosclerosis. NAFLD appears to be a feature of the metabolic syndrome, and its detection on abdominal ultrasound should alert to the existence of an increased cardiovascular risk. (Arterioscler Thromb Vasc Biol. 2005;25:1045-1050.)

Key words: atherosclerosis ■ metabolic syndrome ■ nonalcoholic fatty liver ■ inflammation ■ cardiovascular risk factors ■ carotid ultrasound.

Nonalcoholic fatty liver disease (NAFLD) is a highly prevalent condition characterized by fatty infiltration of liver cells resembling that of alcohol-induced liver injury but occurring in patients who do not abuse alcohol.1–3 The spectrum of NAFLD ranges from fatty liver alone to steatohepatitis, which is histologically similar to alcoholic hepatitis and may progress to end-stage liver disease, a reason why this entity, long considered an incidental finding, has received increasing attention.4 NAFLD is strongly associated with obesity, type 2 diabetes, and dyslipidemia, and most patients have evidence of central adiposity and are insulin resistant.1–5 Thus, NAFLD shares many features of the metabolic syndrome (MetS), a highly atherogenic condition,6 and its presence could signify a substantial cardiovascular risk above and beyond that conferred by individual risk factors.

The potential cardiovascular risk associated with NAFLD has not been particularly investigated despite the evidence that mortality rates from coronary heart disease (CHD) equaled those attributable to cirrhosis in a large cohort of patients with biopsy-proven NAFLD followed for up to 18 years.7 In a case–control study, we investigated the association of NAFLD with carotid intima-media thickness (IMT) and plaque as surrogate measures of increased cardiovascular risk.8

Methods

Subjects
Between November 2002 and March 2003, we screened all subjects referred for diagnostic abdominal ultrasound to the Radiology Service of Hospital San Millán-San Pedro, Logroño, for fatty liver. A “bright liver” (abnormally intense, high-level echoes arising from the hepatic parenchyma, with an amplitude similar to that of echoes arising from the diaphragm) in the absence of chronic liver disease or cancer was detected in 93 subjects, who were recruited into a protocol approved by the institutional review board. Sixty-six subjects accepted participation and gave signed informed consent. Participants were first given a complete clinical history, during which alcohol consumption was assessed as part of the interview, including the World Health Organization (WHO) Alcohol Use Disorders Identification Test6 and medication use. This was followed...
by blood sampling for liver function tests, including markers for viral, autoimmune, and metabolic liver diseases. Excluded were subjects abusing alcohol or having daily alcohol consumption $>20$ g (n = 13), seropositive for hepatitis B or C viruses (n = 12), or with serum transferrin saturation $>45\%$ (n = 1), thus leaving 40 subjects with “primary” NAFLD. Sex- and age-matched control subjects, randomly chosen from local National Health Service card holders, were contacted by telephone and offered an abdominal ultrasound and participation in the study provided that they did not abuse alcohol and that the liver was ultrasonographically normal. The radiologist was blinded to the identity and clinical and biochemical findings of the subjects. To find 40 controls, 61 persons were called (16 refused participation and 5 were excluded because a fatty liver was detected by ultrasound examination).

After signing informed consent, participants entered a study protocol consisting of clinical evaluation for cardiovascular risk factors: sampling of fasting blood for measurement of glucose, insulin, lipids, liver function tests, $\alpha$-1 antitrypsin (AAT), and high-sensitivity C-reactive protein (CRP); an oral glucose tolerance test; and carotid ultrasound for determination of IMT and presence of plaque.

**Clinical and Laboratory Measurements**

Body mass index (BMI) was calculated as weight in kilograms divided by the square of height in meters. Obesity was defined as a BMI $\geq 30$ kg/m$^2$. Waist circumference was measured after expiration at the midpoint between the lowest rib and the iliac crest. Hip circumference was obtained at the widest point between hip and buttock. Blood pressure was measured with a random-zero mercury sphygmomanometer. We used the mean of 2 measurements of systolic and diastolic blood pressure taken while subjects were sitting after a 5-minute rest.

Subjects fasted overnight before phlebotomy. Serum glucose, both fasting and 120 minutes after an oral glucose challenge (75 g in 200 mL water), was measured using a glucose dehydrogenase method. Serum insulin was determined by standard radioimmunoassay. Cholesterol and triglycerides were measured using enzymatic procedures. High-density lipoprotein (HDL) cholesterol was quantified after precipitation with manganese chloride. Low-density lipoprotein (LDL) cholesterol was calculated with the Friedewald equation. Apolipoprotein B (apoB) was determined by the use of turbidimetry. Apolipoprotein A1 (apoA1) was determined by enzymatic methods with a chromogen. AAT and CRP were determined by immunonephelometry. Pertinent data on adiposity, blood pressure, glycemic control, and blood lipids were used to classify subjects as having MetS by National Cholesterol Education Program (NCEP) Adult Treatment Panel III (ATP-III)$^{11}$ and WHO$^{12}$ criteria.

**Carotid Ultrasound**

A General Electric Logic 500 Pro apparatus equipped with a 9-MHz multifrequency transducer was used for B-mode carotid ultrasound. An experienced sonographer (J.L.C.) who was unaware of the individuals’ disease status scanned the right and left carotid arteries and recorded images on videotape for off-line assessment. The present analysis used the average of 10 electronic caliper IMT measurements from the far wall of the distal 10 mm of left and right common carotid arteries at a site free from any discrete plaque. A plaque was defined as a focal thickening of $\pm 1.2$ mm in any of 12 carotid segments (near and far walls of right and left common carotid artery, bifurcation, and internal carotid artery).

**Statistical Analyses**

Comparisons of patients and control subjects were made with unpaired $t$ tests or the Mann–Whitney U test, when appropriate, for continuous variables and by $\chi^2$ analyses for categorical variables.

Values with a skewed distribution were transformed to their natural logarithm (ln) for analyses. Pearson’s correlation coefficients were constructed to test the relationship between continuous variables. The independence of the association of variables with the presence of NAFLD or atherosclerosis (abnormal IMT, defined as the top quartile of control values, or presence of plaque) was assessed by multivariate logistic regression and expressed as odds ratios (ORs). An ANOVA statistic was used to compare sex- and age-adjusted IMT values between different groups of NAFLD and MetS. Two-sided $P$ values $<0.05$ were considered. Analyses were performed with SPSS 10.0 software.

**Results**

**Clinical Features and Laboratory Data**

Compared with control subjects, patients with NAFLD had a similar prevalence of smoking, but they had a higher frequency of high blood pressure and history of diabetes (Table 1). Four patients with NAFLD had a history of CHD. BMI, central adiposity measures, and systolic and diastolic blood pressure were higher in NAFLD than in control subjects. Obesity was present in 24 (60%) patients with NAFLD and in 7 (17.5%) control subjects ($P<0.001$).

Table 1 shows that patients with NAFLD had higher fasting and 120-minute glucose and were more insulin resistant than control subjects. Metabolic testing uncovered 6 additional cases of diabetes among NAFLD patients. Two new cases of diabetes were also detected in the control group. Subjects with known and newly discovered diabetes had fair glycemic control, as judged by HbA1c levels (mean 7.4%; range 6.4% to 9.1%). Total cholesterol, LDL cholesterol, and apoB levels were similar, whereas HDL cholesterol was lower and triglycerides were higher in NAFLD than in controls. The levels of serum alanine aminotransferase (ALT) and $\gamma$-glutamyl transpeptidase (GGT) were nearly double in NAFLD. The AAT level was similar in the 2 groups, whereas CRP was higher in the NAFLD group than in control subjects. Regardless iron status, serum levels of total transferrin and ferritin were also higher in NAFLD than in controls.

Taking the upper quartiles of control values as normality limits, elevated serum CRP was present in 24 (60%) NAFLD patients and 10 (25%) control subjects ($P=0.003$), whereas a CRP $>3.0$ mg/L, the level above which cardiovascular risk is substantially increased,$^{13}$ was detected in 17 (42.5%) and 2 (5%), respectively ($P<0.001$). In CRP level was strongly ($P<0.001$) correlated with BMI ($r=0.604$), waist circumference ($r=0.431$), ln HOMA ($r=0.604$), and the serum ALT level ($r=0.384$). Other correlations of ln CRP were AAT ($r=0.337$; $P=0.003$) and systolic blood pressure ($r=0.258$; $P=0.021$). A serum ferritin above the upper quartile of control values was present in 16 (40%) NAFLD patients and 10 (25%) control subjects ($P=0.23$). In ferritin correlated strongly ($P<0.001$) with fasting glucose ($r=0.358$) and ALT ($r=0.389$); weaker but significant ($P<0.05$) correlations of ln ferritin were the serum GGT level ($r=0.332$), ln HOMA ($r=0.280$), waist circumference ($r=0.221$), and carotid IMT ($r=0.254$).

**Criteria for MetS**

Whether assessed by ATP III or WHO criteria, all the risk factors related to MetS (visceral adiposity, hypertension,
abnormal glucose metabolism, insulin resistance, hypertriglyceridemia, and low HDL cholesterol) were significantly more prevalent in NAFLD patients than in control subjects, resulting in a 4-fold higher frequency of WHO-MetS (80% versus 20%, respectively) and a nearly 5-fold prevalence of ATP-MetS (72.5% versus 15%, respectively) in NAFLD. The prevalence of ATP-MetS and WHO-MetS in NAFLD was higher in women than in men (80% versus 70% and 85% versus 55%, respectively), whereas depending on the definition, control men had a 3- to 5-fold excess of MetS compared with control women (25% versus 5% by ATP criteria and 30% versus 10% by WHO criteria, respectively).

Findings of Carotid Ultrasound Studies

Compared with control subjects, patients with NAFLD also showed increased mean and maximum IMT and a 2-fold higher frequency of plaque (Table 2). The mean differences (95% CI) between NAFLD and controls were 0.16 mm (0.08 to 0.23 mm) for mean IMT and 0.17 mm (0.09 to 0.25 mm) for maximum IMT (P<0.0001 for both). Figure 1 shows that case–control differences in IMT and plaque frequency were more marked in women than in men. When subdividing the study population into

<table>
<thead>
<tr>
<th>TABLE 1. Clinical and Laboratory Data of Patients With NAFLD and Control Subjects</th>
</tr>
</thead>
<tbody>
<tr>
<td>Variables</td>
</tr>
<tr>
<td>Clinical</td>
</tr>
<tr>
<td>Men/women                                    20/20</td>
</tr>
<tr>
<td>Age, years                                   53.2±12.6</td>
</tr>
<tr>
<td>History of high blood pressure               20 (50)</td>
</tr>
<tr>
<td>History of diabetes                          7 (17.5)</td>
</tr>
<tr>
<td>History of CHD                               4 (10)</td>
</tr>
<tr>
<td>Current smoker                              11 (27.5)</td>
</tr>
<tr>
<td>BMI, kg/m²                                   31.8±5.1</td>
</tr>
<tr>
<td>Waist circumference, cm                      104±13</td>
</tr>
<tr>
<td>Waist/hip ratio                              0.93±0.07</td>
</tr>
<tr>
<td>Systolic blood pressure, mm Hg               141±16</td>
</tr>
<tr>
<td>Diastolic blood pressure, mm Hg              87±9</td>
</tr>
<tr>
<td>Laboratory</td>
</tr>
<tr>
<td>Fasting glucose, mmol/L                      6.4±1.3</td>
</tr>
<tr>
<td>120-minute glucose, mmol/L                   8.1±3.2</td>
</tr>
<tr>
<td>Fasting insulin, μU/mL                       17.4±9.6</td>
</tr>
<tr>
<td>Insulin resistance (HOMA) index              4.12 (2.80–5.78)</td>
</tr>
<tr>
<td>Insulin resistance                           28 (70)</td>
</tr>
<tr>
<td>Total cholesterol, mmol/L                    5.9±1.1</td>
</tr>
<tr>
<td>LDL cholesterol, mmol/L                      3.8±0.9</td>
</tr>
<tr>
<td>HDL cholesterol, mmol/L                      1.1±0.3</td>
</tr>
<tr>
<td>Triglycerides, mmol/L                        1.6 (1.0–2.4)</td>
</tr>
<tr>
<td>ApoB, g/L                                    1.29±0.32</td>
</tr>
<tr>
<td>ALT, µU/mL                                   43±23</td>
</tr>
<tr>
<td>GGT, µU/mL                                   47±45</td>
</tr>
<tr>
<td>AAT, g/L                                     1.71±0.28</td>
</tr>
<tr>
<td>CRP, mg/L                                    1.85 (1.33–6.28)</td>
</tr>
<tr>
<td>Serum iron, ng/mL                            83±36</td>
</tr>
<tr>
<td>Total transferrin, ng/mL                     263±32</td>
</tr>
<tr>
<td>Transferrin saturation, percent              25.4±10.8</td>
</tr>
<tr>
<td>Ferritin, ng/mL                              106 (68–163)</td>
</tr>
</tbody>
</table>

Data are mean±SD or No. (percent) or median (interquartile range) for variables with skewed distribution.

*Group comparisons by unpaired t test or chi-square test, with Fisher’s exact test (‡), and Whitney U test (§), when appropriate; †selection criteria not tested for statistical significance.

<table>
<thead>
<tr>
<th>TABLE 2. Carotid IMT and Plaque in Patients With NAFLD and Control Subjects</th>
</tr>
</thead>
<tbody>
<tr>
<td>Variables</td>
</tr>
<tr>
<td>Mean IMT, mm                                  0.70±0.20</td>
</tr>
<tr>
<td>Maximum IMT, mm                               0.75±0.22</td>
</tr>
<tr>
<td>Mean IMT above top quartile                   27 (67.5)</td>
</tr>
<tr>
<td>Carotid plaque                               20 (50)</td>
</tr>
</tbody>
</table>

Data as mean±SD or No. (percentage).

*Group comparisons by unpaired t test or chi-square test.
NAFLD and Carotid Atherosclerosis Predictors by Multivariate Analyses

After adjustment for sex, age, the risk factors listed in Table 1 showing a significant bivariate relationship, the serum level of CRP and AAT categorized as abnormal when above the respective top quartiles, and the frequency of ATP-MetS or WHO-MetS components, independent associations of NAFLD by multivariate logistic regression were visceral obesity (OR, 4.65; 95% CI, 1.43 to 14.54; \( P = 0.010 \)) and MetS (OR, 8.67; 95% CI, 2.65 to 28.33; \( P = 0.0001 \)) when considering ATP-III criteria, and hyperlipidemia (OR, 4.50; 95% CI, 0.96 to 21.05; \( P = 0.056 \)) and MetS (OR, 5.91; 95% CI, 1.74 to 20.09; \( P = 0.004 \)) when using the WHO definition.

Logistic regression with similar adjustments, including the presence of NAFLD, and with abnormal IMT as dependent variable showed independent associations with older age (OR, 2.49 per 10 years; 95% CI, 1.41 to 4.39; \( P = 0.002 \)), the presence of NAFLD (OR, 8.38; 95% CI, 2.39 to 29.43; \( P = 0.001 \)), and serum ferritin above the top quartile of control values (OR, 3.14; 95% CI, 1.24 to 7.94; \( P = 0.016 \)). When considering plaque occurrence as the dependent variable, similar associations with age (\( P = 0.001 \)) and serum ferritin (\( P = 0.012 \)) were observed, but NAFLD was excluded from the equation and replaced by MetS, whether defined by ATP-III or WHO criteria (\( P = 0.001 \)). Exclusion of the 4 cases with previous CHD or the 7 patients with known diabetes did not appreciably change either the predictive variables or the ORs for abnormal IMT or plaque (data not shown).

Discussion

This case–control study assessed the frequency and magnitude of cardiovascular risk factors and measured carotid atherosclerosis in unselected patients with an ultrasound diagnosis of primary NAFLD. Confirming previous evidences,1–5 patients with NAFLD showed a cluster of abnormalities related to MetS. A novel finding of this study is that patients with NAFLD had advanced carotid atherosclerosis. NAFLD was an independent predictor of an increased IMT, whereas MetS, present in 72.5% (ATP-III) or 80% (WHO) of NAFLD patients, was independently associated with carotid plaque. The findings support the view of NAFLD as a hepatic manifestation of MetS.5,14,15 They also suggest that hepatic fat accumulation is atherogenic beyond its association with insulin resistance.

The diagnosis of NAFLD was based on the exclusion of known etiologic factors of liver disease and on ultrasound examination but was not confirmed by liver biopsy for ethical reasons. However, ultrasound examination is by far the commonest way of diagnosing NAFLD in clinical practice16 and is very sensitive in the detection of significant hepatic steatosis in patients with biopsy-proven disease.17 Indeed, Saadeh et al17 reported that the presence of \( >33\% \) fat on liver biopsy was optimal for radiological detection of steatosis; that is, moderate to severe fatty infiltration has to be present for the liver ultrasound pattern to become altered and suggest the diagnosis of NAFLD. Insulin resistance was not measured by the euglycemic clamp technique but by the simpler HOMA method. However, HOMA has been reported as a very reliable technique to assess insulin sensitivity.10,18
Likewise, an increased incidence and progression of carotid atherosclerosis, was independently associated with MetS in our study. Increased risk for cardiovascular and all-cause mortality. Cardiovascular mortality observed in NAFLD.\textsuperscript{7} The probable existence of multiple underlying cardiovascular risk factors warranting evaluation and treatment as much as the risk for advancing liver disease.

In summary, NAFLD is a strong risk factor for carotid atherosclerosis beyond its association with MetS. As illustrated by the frequency of previous CHD and uncovered diabetes in unselected patients with NAFLD, the clinical corollary to our findings is that the casual detection of a fatty liver on abdominal ultrasound examination should alert to the probable existence of multiple underlying cardiovascular risk factors warranting evaluation and treatment as much as the risk for advancing liver disease.

**Acknowledgments**

Financial support was provided by grants from Instituto de Estudios Riojanos (1928/2003) and Spanish Ministry of Health (ISCIII G03/181). The authors sincerely thank the patients participating in the study; Nuria Aristimuno, RN, for careful anthropometric measurements and extraction of blood samples; and Enrique Ramalle, Consejería de Salud de La Rioja, for expert advice.
References

Nonalcoholic Fatty Liver Disease Is Associated With Carotid Atherosclerosis: A Case–Control Study
Angel Brea, Daniel Mosquera, Eva Martín, Ana Arizti, José L. Cordero and Emilio Ros

Arterioscler Thromb Vasc Biol. 2005;25:1045-1050; originally published online February 24, 2005;
doi: 10.1161/01.ATV.0000160613.57985.18
Arteriosclerosis, Thrombosis, and Vascular Biology is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2005 American Heart Association, Inc. All rights reserved.
Print ISSN: 1079-5642. Online ISSN: 1524-4636

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://atvb.ahajournals.org/content/25/5/1045

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Arteriosclerosis, Thrombosis, and Vascular Biology can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Arteriosclerosis, Thrombosis, and Vascular Biology is online at:
http://atvb.ahajournals.org//subscriptions/