Associations Between Liver Histology and Carotid Intima-Media Thickness in Patients With Nonalcoholic Fatty Liver Disease

To the Editor:

NAFLD is a clinicopathological syndrome that is closely associated with visceral obesity, dyslipidemia, insulin resistance, and type 2 diabetes, thus suggesting that NAFLD is another feature of the metabolic syndrome (MetS). Recent cross-sectional studies have shown that NAFLD is associated with increased carotid intima-media thickness (IMT) as a reliable marker of early atherosclerosis. 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. The aim of this study was to assess whether patients with biopsy-proven NAFLD had greater carotid IMT than control subjects and to evaluate whether there were significant associations between liver histopathology and carotid IMT among NAFLD patients.

Fifty consecutive patients with NAFLD were recruited from clinics. All patients had chronically elevated liver enzymes. The NAFLD diagnosis was based on liver biopsy and exclusion of known etiologic factors of chronic liver disease. Seven men and 4 women had preexisting type 2 diabetes, 8 managed their diabetes with diet alone, and 3 were taking metformin. The control group, recruited from hospital staff member and relatives, consisted of 40 healthy volunteers with normal liver function tests and normal liver ultrasound, who were comparable for age, sex, and body mass index (BMI). The protocol was approved by the local Ethical Committee. Plasma liver function tests and other biochemical blood measurements were determined by standard laboratory procedures. Insulin resistance was estimated by the homeostasis model assessment (HOMA-IR score). The presence of MetS was defined by the Adult Treatment Panel (ATP) III criteria.

Carotid IMT was ultrasonographically measured by a single trained operator, who was blind to clinical features of participants. Carotid IMT was measured at the level of the common carotid artery far wall, as previously reported. Repeated measurements on the same subjects gave coefficients of variation within 8%.

A pathologist blinded to subjects’ details scored liver biopsy specimens using the classification of Brunt et al. Nonalcoholic steatohepatitis (NASH) was defined as the presence of steatosis plus lobular inflammation plus hepatocellular ballooning or steatosis plus any stage of fibrosis. Liver histopathology results were steatosis alone in 7 subjects, NASH with fibrosis stage of 0 in 14 subjects, NASH/fibrosis stage 1 in 16 subjects, NASH/fibrosis stage 2 in 8 subjects, and NASH/fibrosis stage 3 in 5 subjects; none had cirrhosis (a fibrosis stage of 4).

By study design, cases and controls were almost identical in terms of sex (M/F 30/20 versus 26/14), age (mean ± SD; 46 ± 4 versus 46 ± 3 years), and BMI (26.6 ± 1.6 versus 26.2 ± 1.8 kg/m²). NAFLD patients had higher liver enzymes (AST 48 ± 21 versus 22 ± 3 U/L; ALT 102 ± 50 versus 24 ± 4 U/L; P < 0.001), but comparable values of LDL cholesterol concentration (3.20 ± 0.3 versus 3.22 ± 0.3 mmol/L) and smoking status (20% versus 22.5%). As shown in the Table, HOMA-IR score and prevalence of MetS were significantly increased in NAFLD patients. They also had a markedly greater carotid IMT than controls, with no differences between sexes (not shown). Additionally, carotid IMT was significantly different between patients with NASH, patients with simple steatosis, and controls. The marked differences in carotid IMT among the groups were little affected by adjustment for age, sex, HOMA-IR score, and MetS. Results did not change after excluding diabetic

<p>| Values of Carotid Artery Wall Thickness, HOMA-Estimated Insulin Resistance, and Presence of Metabolic Syndrome in Participants Grouped According to Their Status of NAFLD and Severity of Hepatic Fibrosis |</p>
<table>
<thead>
<tr>
<th>Carotid IMT (mm)</th>
<th>HOMA-IR Score</th>
<th>MetS Prevalence (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control subjects (n=40)</td>
<td>0.84±0.13</td>
<td>1.70±1.0</td>
</tr>
<tr>
<td>NAFLD patients (n=50)</td>
<td>1.10±0.20</td>
<td>4.14±2.0</td>
</tr>
<tr>
<td>P values*</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>P values†</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>P values‡</td>
<td>=0.005</td>
<td>Not determinable</td>
</tr>
<tr>
<td>Control subjects (n=40)</td>
<td>0.84±0.13</td>
<td>1.70±1.0</td>
</tr>
<tr>
<td>Simple steatosis (n=7)</td>
<td>0.96±0.15</td>
<td>2.99±1.4</td>
</tr>
<tr>
<td>Steato-hepatitis (NASH) (n=43)</td>
<td>1.26±0.24</td>
<td>5.19±2.5</td>
</tr>
<tr>
<td>P values*</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>P values†</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>P values‡</td>
<td>=0.001</td>
<td>Not determinable</td>
</tr>
<tr>
<td>NASH/fibrosis stage</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 (n=14)</td>
<td>1.09±0.2</td>
<td>3.74±1.2</td>
</tr>
<tr>
<td>1 (n=16)</td>
<td>1.21±0.2</td>
<td>4.61±1.6</td>
</tr>
<tr>
<td>2 (n=8)</td>
<td>1.32±0.3</td>
<td>5.73±2.4</td>
</tr>
<tr>
<td>3 (n=5)</td>
<td>1.46±0.3</td>
<td>6.81±3.3</td>
</tr>
<tr>
<td>P values*</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>P values†</td>
<td>=0.001</td>
<td>Not determinable</td>
</tr>
</tbody>
</table>

Data are means ± SD or proportions.

*P values by analysis of variance for unadjusted differences or trends.
†P values by analysis of covariance for differences or trends adjusted for age and sex.
‡P values by analysis of covariance for differences or trends adjusted for age, sex, HOMA-IR score, and presence of ATP-III defined MetS.
patients from analysis, or when the MetS was replaced by its individual components in multivariate analysis.

Interestingly, the severity of hepatic fibrosis was strongly associated with increased carotid IMT and HOMA-IR score and higher prevalence of MetS. These associations were only modestly attenuated by adjustment for likely confounders (Table). By logistic regression analysis, hepatic fibrosis stage independently predicted carotid IMT ($P<0.001$) after adjustment for MetS and other confounders.

This study has shown, for the first time, that (1) NAFLD patients have, other than several features resembling the MetS, a marked increase in carotid IMT compared with matched controls, (2) carotid IMT is significantly higher for individuals with NASH than for those with simple steatosis, and (3) liver histopathology (hepatic fibrosis stage) independently predicts carotid IMT after adjustment for MetS and other potential confounders.

These results confirm and extend the observations of some recent studies documenting that patients with NAFLD, as assessed by ultrasonography, had a greater carotid IMT and increased prevalence of carotid plaques compared with controls.2–4 Overall, therefore, the evidence from this and other studies3 supports the possibility that NAFLD is atherogenic beyond its close relationship to the MetS phenotype, possibly through increased oxidative stress, subclinical inflammation, postprandial lipemia, and decreased adiponectin concentrations.9–12

It is known that the ATP III definition for identifying the MetS includes waist circumference among its diagnostic criteria. However, it is also known that waist circumference provides only an indirect and crude estimation of visceral fat,13 so we cannot be certain that these results completely exclude an effect of visceral fat. This could be done by controlling for a more accurate measure of visceral fat obtained by computed tomography or MRI.

Our findings might have important clinical implications. Because NAFLD patients are at increased cardiovascular risk, the casual detection of NAFLD on an ultrasound examination should alert to the possible coexistence of multiple underlying cardiovascular risk factors warranting evaluation and treatment as much as the risk for advancing liver disease.

In conclusion, these results suggest that the severity of histopathologic features in NAFLD is closely associated with early carotid atherosclerosis, independent of classical risk factors, insulin resistance, and MetS. These findings will need clearly to be replicated in future studies using larger cohorts of patients.

Giovanni Targher
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Letter to the Editor: Associations Between Liver Histology and Carotid Intima-Media
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