Nonalcoholic Fatty Liver Disease and Vascular Function
Cross-Sectional Analysis in the Framingham Heart Study


**Objective**—Patients with nonalcoholic fatty liver disease (NAFLD) have an increased risk of cardiovascular disease; however, it is not known whether NAFLD contributes to cardiovascular disease independent of established risk factors. We examined the association between NAFLD and vascular function.

**Approach and Results**—We conducted a cross-sectional study of 2284 Framingham Heart Study participants without overt cardiovascular disease who had liver fat attenuation measured on computed tomography and who had measurements of vascular function and covariates. We evaluated the association between NAFLD and vascular function using multivariable partial correlations adjusting for age, sex, cohort, smoking, diabetes mellitus, hyperlipidemia, hypertension, body mass index, and visceral adipose tissue. The prevalence of NAFLD in our sample (mean age, 52±12 years; 51.4% women) was 15.3%. In age-, sex-, and cohort-adjusted analyses, greater liver fat was modestly associated with lower flow-mediated dilation ($r=-0.05; P=0.02$), lower peripheral arterial tonometry ratio ($r=-0.20; P<0.0001$), higher carotid-femoral pulse wave velocity ($r=0.13; P<0.0001$), and higher mean arterial pressure ($r=0.11; P<0.0001$). In multivariable-adjusted models, NAFLD remained associated with higher mean arterial pressure ($r=0.06; P=0.005$) and lower peripheral arterial tonometry ratio ($r=-0.12; P<0.0001$). The association between NAFLD and peripheral arterial tonometry ratio persisted after further adjustment for body mass index and visceral adipose tissue.

**Conclusions**—For multiple measures of vascular function, the relationship with NAFLD appeared largely determined by shared cardiometabolic risk factors. The persistent relationship with reduced peripheral arterial tonometry response beyond established risk factors suggests that NAFLD may contribute to microvascular dysfunction. ([Arterioscler Thromb Vasc Biol. 2015;35:1284-1291. DOI: 10.1161/ATVBAHA.114.305200.]

**Key Words:** multidetector computed tomography obesity risk factors vascular endothelium

Nonalcoholic fatty liver disease (NAFLD) has become the most common chronic liver condition in the United States with a general population prevalence of 20% to 40%. NAFLD is associated with insulin resistance, obesity, type 2 diabetes mellitus, and dyslipidemia. In addition to the risk for advanced liver disease from nonalcoholic steatohepatitis, NAFLD confers an increased risk of cardiovascular disease (CVD). It is not fully established whether NAFLD increases CVD-related morbidity or mortality independent of known cardiovascular risk factors. The mechanisms by which NAFLD may lead to increased CVD are not known. NAFLD may lead to increased CVD by modifying traditional risk factors, such as dyslipidemia and insulin resistance. A leading hypothesis is that hepatic steatosis may lead to the production of proinflammatory cytokines, which accelerate atherosclerosis and lead to progressive endothelial dysfunction. Indeed, NAFLD is associated with an increased C-reactive protein (CRP), a marker of systemic inflammation. Vascular endothelial dysfunction occurs early in the atherosclerosis process. Noninvasive measures
of endothelial function and arterial stiffness, such as brachial artery flow-mediated dilation (FMD), fingertip peripheral arterial tonometry (PAT), and arterial tonometry, are associated with cardiovascular risk factors and with incident CVD. There have been several studies on the association of NAFLD and various markers of vascular structure and function. These studies have been limited by small sample sizes, lack of adequate control for known cardiovascular risk factors, limited evaluation of vascular function, and use of clinical samples.

Thus, the goal of our study was to determine the association between NAFLD, as defined by decreased liver attenuation on multidetector computed tomography (CT), and vascular function, as measured by brachial artery FMD, PAT, and arterial tonometry in a large, unsel ected community-based cohort without apparent CVD. In addition, we explored whether associations remained after adjusting for known cardiovascular risk factors, body mass index (BMI), and visceral adipose tissue.

Materials and Methods

Materials and Methods are available in the online-only Data Supplement.

Results

Study Sample Characteristics

Participant characteristics and mean values for vascular function measures for those with and without NAFLD are summarized in Table 1. Overall, 15.3% of the sample had a liver phantom ratio (LPR) of ≤0.33, consistent with NAFLD.

Correlations Between Liver Attenuation and Vascular Function Measures

In minimally adjusted models, liver attenuation was significantly correlated with all vascular function measures, except for hyperemic mean flow velocity and forward-wave amplitude (Table 2). With more liver fat (lower LPR), the response to ischemia as measured by FMD was lower, which is consistent with conduit vessel dysfunction. More liver fat was also associated with a lower PAT ratio, which is consistent with small vessel dysfunction. For the measures of arterial stiffness, more liver fat was associated with a higher carotid-femoral pulse wave velocity, which is consistent with greater arterial stiffness.

Nonstandard Abbreviations and Acronyms

<table>
<thead>
<tr>
<th>Acronym</th>
<th>Description</th>
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<tr>
<td>BMI</td>
<td>body mass index</td>
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<tr>
<td>CRP</td>
<td>C-reactive protein</td>
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<tr>
<td>CT</td>
<td>computed tomography</td>
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<tr>
<td>CVD</td>
<td>cardiovascular disease</td>
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<td>FMD</td>
<td>flow-mediated dilation</td>
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<td>LFT</td>
<td>liver function test</td>
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<td>LPR</td>
<td>liver phantom ratio</td>
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<td>NAFLD</td>
<td>nonalcoholic fatty liver disease</td>
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<tr>
<td>PAT</td>
<td>peripheral arterial tonometry</td>
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</table>

Multivariable-Adjusted Partial Correlations for NAFLD With Vascular Function Measures

In the multivariable-adjusted model, NAFLD (LPR ≤0.33) was positively associated with mean arterial pressure (r = 0.06; P = 0.005) and inversely associated with the PAT ratio (r = -0.12; P < 0.0001; Table 3). After BMI was added to the multivariable model, the correlation between NAFLD (LPR ≤0.33) and mean arterial pressure was no longer statistically significant (r = 0.03, P = 0.20). NAFLD (LPR ≤0.33) was also positively associated with carotid-femoral pulse wave velocity in the multivariable-adjusted model (r = 0.05; P = 0.03); however, when this model was also adjusted for mean arterial pressure, the correlation was no longer statistically significant (r = 0.02; P = 0.29). The correlation between NAFLD (LPR ≤0.33) and the PAT ratio remained statistically significant after additionally adjusting for BMI (r = -0.09; P = 0.0005) and visceral adipose tissue (r = -0.08; P = 0.004; Table 3). NAFLD (LPR ≤0.33) also was associated with higher baseline brachial artery diameter (multivariable model 1 only), baseline brachial artery mean flow velocity, and baseline peripheral artery pulse amplitude (Table II in the online-only Data Supplement). When we evaluated LPR as a continuous variable, results were largely similar (Table 3). The variance inflation factor was <1.3 for each of these associations, suggesting the lack of severe multicollinearity.

PAT Ratio According to the Presence of NAFLD Overall and Across BMI Categories

The least-square means for the PAT ratio according to the presence of NAFLD overall and by BMI category are shown in Figure. Overall, the PAT ratio was lower among participants with NAFLD compared with those without NAFLD (P < 0.0001). In the overweight BMI category, the PAT ratio in participants with NAFLD was lower compared with those without NAFLD (P = 0.0006), which is consistent with small vessel dysfunction. In the normal weight and obese BMI categories, the findings are in a similar direction; however, not statistically significant (PAT ratio normal weight NAFLD versus non-NAFLD, P = 0.26; PAT ratio obese NAFLD versus non-NAFLD, P = 0.06).

PAT Ratio According to the Presence of NAFLD and CRP Above and Below Median

The least-square means for the PAT ratio according to the presence of NAFLD and CRP above and below the median value are shown in Figure 1 in the online-only Data Supplement. Participants with NAFLD and either CRPmedian or CRP2median had a significantly lower PAT ratio compared with those without NAFLD and CRP < median (P = 0.008 and P = 0.0001, respectively). Among those with NAFLD, there was no difference in the PAT ratio in those with CRP > median versus CRP ≤ median (P = 0.99).

PAT Ratio According to the Presence of NAFLD and Normal or Elevated Liver Function Tests

The least-square means for the PAT ratio according to the presence of NAFLD and elevated liver function tests (LFTs)
are shown in Figure II in the online-only Data Supplement. Participants with NAFLD and either normal or elevated LFTs had a significantly lower PAT ratio compared with those without NAFLD and normal LFTs ($P=0.005$ and $P=0.0003$, respectively). Among those with NAFLD, there was a trend toward a lower PAT ratio among those with elevated LFTs compared with those with normal LFTs; however, this did not meet statistical significance ($P=0.41$).

**Table 1. Participant Characteristics, by the Presence of NAFLD**

<table>
<thead>
<tr>
<th>Clinical Characteristics</th>
<th>NAFLD (LPR≤0.33), n=350</th>
<th>Non-NAFLD (LPR&gt;0.33), n=1934</th>
<th>Total, n=2284</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>54±12</td>
<td>52±12</td>
<td>52±12</td>
</tr>
<tr>
<td>Women, n (%)</td>
<td>157 (44.9)</td>
<td>1017 (52.6)</td>
<td>1174 (51.4)</td>
</tr>
<tr>
<td>Offspring, n (%)</td>
<td>137 (39.1)</td>
<td>675 (34.9)</td>
<td>812 (35.6)</td>
</tr>
<tr>
<td>Current smoking, n (%)</td>
<td>35 (10.0)</td>
<td>208 (10.8)</td>
<td>243 (10.6)</td>
</tr>
<tr>
<td>Heart rate, bpm</td>
<td>64±10</td>
<td>61±10</td>
<td>62±10</td>
</tr>
<tr>
<td>Total/high-density lipoprotein cholesterol</td>
<td>4.46±1.58</td>
<td>3.66±1.19</td>
<td>3.78±1.29</td>
</tr>
<tr>
<td>Triglycerides, mg/dL</td>
<td>170±124</td>
<td>107±62</td>
<td>116±79</td>
</tr>
<tr>
<td>Fasting glucose, mg/dL</td>
<td>108±27</td>
<td>97±18</td>
<td>99±20</td>
</tr>
<tr>
<td>Mean arterial pressure, mm Hg</td>
<td>94±9</td>
<td>90±10</td>
<td>90±10</td>
</tr>
<tr>
<td>Postmenopausal, n (%)</td>
<td>100 (63.7)</td>
<td>516 (50.7)</td>
<td>616 (52.5)</td>
</tr>
<tr>
<td>Hormone replacement therapy, n (%)</td>
<td>14 (8.9)</td>
<td>96 (9.4)</td>
<td>110 (9.4)</td>
</tr>
<tr>
<td>Diabetes mellitus, n (%)</td>
<td>52 (14.9)</td>
<td>71 (3.7)</td>
<td>123 (5.4)</td>
</tr>
<tr>
<td>Hypertension treatment, n (%)</td>
<td>126 (36.0)</td>
<td>355 (18.4)</td>
<td>481 (21.1)</td>
</tr>
<tr>
<td>Lipid-lowering treatment, n (%)</td>
<td>92 (26.3)</td>
<td>336 (17.4)</td>
<td>428 (18.7)</td>
</tr>
<tr>
<td>C-reactive protein, mg/L†</td>
<td>2.57 (1.16–4.76)</td>
<td>0.98 (0.46–2.25)</td>
<td>1.12 (0.50–0.27)</td>
</tr>
<tr>
<td>Alanine aminotransferase, U/L</td>
<td>36.4±27.6</td>
<td>24.5±16.6</td>
<td>26.3±19.2</td>
</tr>
<tr>
<td>Aspartate aminotransferase, U/L</td>
<td>28.6±24.6</td>
<td>23.4±9.6</td>
<td>24.2±13.2</td>
</tr>
<tr>
<td>Adiposity measures</td>
<td></td>
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<tr>
<td>Body mass index, kg/m²</td>
<td>31.0±5.5</td>
<td>26.6±4.7</td>
<td>27.3±5.1</td>
</tr>
<tr>
<td>Waist circumference, cm</td>
<td>106±14</td>
<td>94±13</td>
<td>96±14</td>
</tr>
<tr>
<td>Visceral adipose tissue, cm³</td>
<td>2488±993</td>
<td>1501±876</td>
<td>1652±963</td>
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<tr>
<td>LPR</td>
<td>0.27±0.06</td>
<td>0.38±0.02</td>
<td>0.36±0.05</td>
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<tr>
<td>Vascular measures</td>
<td></td>
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<tr>
<td>Brachial artery measures</td>
<td></td>
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<tr>
<td>Baseline brachial artery diameter, mm</td>
<td>4.47±0.86</td>
<td>4.14±0.83</td>
<td>4.19±0.85</td>
</tr>
<tr>
<td>Flow-mediated dilation, %</td>
<td>4.06±3.65</td>
<td>4.81±3.56</td>
<td>4.69±3.59</td>
</tr>
<tr>
<td>Baseline mean flow velocity, cm/s</td>
<td>9.41±5.03</td>
<td>7.12±4.28</td>
<td>7.49±4.49</td>
</tr>
<tr>
<td>Hyperemic mean flow velocity, cm/s</td>
<td>56±20</td>
<td>59±20</td>
<td>58±20</td>
</tr>
<tr>
<td>Peripheral arterial tonometry measures‡</td>
<td>6.1±0.8</td>
<td>5.6±0.9</td>
<td>5.7±0.9</td>
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<tr>
<td>Peripheral arterial tone ratio, unitless</td>
<td>0.53±0.36</td>
<td>0.75±0.41</td>
<td>0.72±0.41</td>
</tr>
<tr>
<td>Arterial tonometry measures</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Carotid-femoral pulse wave velocity, ms/mm</td>
<td>9.0±2.7</td>
<td>8.1±2.6</td>
<td>8.3±2.6</td>
</tr>
<tr>
<td>1000/carotid-femoral pulse wave velocity, ms/mm</td>
<td>−119±28</td>
<td>−131±29</td>
<td>−129±30</td>
</tr>
<tr>
<td>Forward-wave amplitude, mm Hg</td>
<td>50±14</td>
<td>48±14</td>
<td>48±14</td>
</tr>
<tr>
<td>Augmentation index, %</td>
<td>11.3±12.8</td>
<td>11.6±12.1</td>
<td>11.6±12.2</td>
</tr>
<tr>
<td>Mean arterial pressure, mm Hg</td>
<td>97±10</td>
<td>93±11</td>
<td>93±11</td>
</tr>
</tbody>
</table>

Continuous variables expressed as mean±SD and categorical variables as n (%) unless noted. LPR indicates liver phantom ratio; and NAFLD, nonalcoholic fatty liver disease.

*Clinical characteristics were assessed for the offspring participants at examination 7. Adiposity measures were assessed for the offspring participants between examinations 7 and 8. Peripheral arterial tonometry and arterial tonometry variables were assessed for the offspring participants at examination 8. Third-generation participants had clinical characteristics and all vascular function measurements assessed at examination 1.

†Values represent median (interquartile range).

‡Peripheral artery tone measures are natural logarithm transformed.
both are markers of vasodilator function, brachial and digital microvascular dysfunction. Consistent with a potential association between NAFLD and CVD risk factors and adiposity measures. These findings are peripheral artery pulse amplitude even in models adjusted for ratio, baseline brachial artery mean flow velocity, and baseline arterial tonometry measures. The associations with measures of microvascular dysfunction, including PAT with low digital vascular function being associated with metabolic risk factors, including BMI, cholesterol, and the presence of diabetes mellitus. Relevant to NAFLD, one previous selected study of patients with obstructive sleep apnea identified an association between hepatic steatosis and lower PAT. The present investigation demonstrates, in a large community-based sample, an association between PAT ratio and NAFLD after adjusting for cardiovascular and metabolic risk factors. Furthermore, across categories of obesity, the presence of NAFLD was associated with lower PAT hyperemic response. High baseline brachial flow is also gaining appreciation as a metabolic disease–associated vascular alteration. In this study, we observed higher resting flow velocity in participants with NAFLD that may contribute to microvascular damage. These results emphasize the association of NAFLD with abnormalities in the microcirculation.

Previous smaller studies have evaluated the relationship of NAFLD with several individual measures of vascular function. In small, clinically selected samples, patients with biopsy-proven NAFLD had conduit artery dysfunction, as measured by brachial artery FMD, compared with age- and sex-matched controls. The association of clinical NAFLD with reduced FMD persisted when accounting for a limited set of metabolic risk factors. NAFLD determined by ultrasound also has been associated with lower endothelium-dependent dilation in clinical samples. Similarly, one study showed higher aortic stiffness in patients with biopsy-proven NAFLD. In community-based cohorts, an association was observed between sonographically defined NAFLD and higher arterial stiffness measured using global stiffness measures, brachial-ankle pulse wave velocity, and carotid-femoral pulse wave velocity. However, in a study of adolescents with ultrasound-defined NAFLD, the association between NAFLD and higher arterial stiffness was limited to participants with high-risk metabolic features, including greater waist circumference, triglycerides, insulin, systolic blood pressure, and lower high-density lipoprotein. Thus, whether NAFLD is associated with abnormal vascular function beyond the associated cardiometabolic risk factors remained unclear.

In this study, we had the opportunity to evaluate multiple measures reflecting distinct aspects of vascular health in a community-based sample with a comprehensive assessment of cardiometabolic risk factors. In contrast to previous work, we observed that the NAFLD was no longer associated with conduit artery function or aortic stiffness after adjusting for concurrent risk factors. Thus, our findings suggest that the risk factors that cluster with NAFLD account for the observed vascular dysfunction, particularly in the conduit and large arteries. There are several possible explanations for these apparently discrepant results. First of all, several of the previous studies used hospital-based patient samples, which may have a higher prevalence of comorbid conditions that contribute to conduit artery dysfunction and arterial stiffness compared with our study in a community-based sample. It is also

Sex Interactions

In multivariable models of the main outcome measures, there was no evidence of effect modification by sex (P value range, 0.16–0.90).

Analysis Limited to the Third-Generation Cohort

In a secondary analysis limited to participants in the Third-Generation Cohort who had all vascular function measurements and the multidetector CT scans at the same study visit, the results were not substantively changed (Table III in the online-only Data Supplement).

Analysis Limited to the Participants With All 3 Vascular Measures

In a secondary analysis limited to participants who had all 3 vascular measures (n=1005), the results were not substantively changed (Table IV in the online-only Data Supplement).

Discussion

In this large (n=2284) community-based cohort of participants without apparent CVD, we observed modest associations between lower liver attenuation (more liver fat) and FMD, a measure of conduit artery vasodilator function; PAT ratio, a measure of microvascular function; mean arterial pressure, a measure of systemic perfusion; and carotid-femoral pulse wave velocity, a measure of arterial stiffness. The associations of NAFLD with conduit artery function and arterial stiffness were attenuated and no longer significant after adjusting for CVD risk factors. However, NAFLD remained correlated with measures of microvascular dysfunction, including PAT ratio, baseline brachial artery mean flow velocity, and baseline peripheral artery pulse amplitude even in models adjusted for CVD risk factors and adiposity measures. These findings are consistent with a potential association between NAFLD and microvascular dysfunction.

We advance the previous literature by evaluating microvascular dysfunction in NAFLD as measured by PAT. Although both are markers of vasodilator function, brachial and digital measures of vasodilation evaluate distinct vascular beds and previous work suggests they reflect distinct aspects of vascular function.
possible that large artery dysfunction and arterial stiffness occur later in the pathogenesis of NAFLD. In addition, the large sample size and detailed assessment of cardiovascular risk factors, including adiposity measures, allowed for a more complete adjustment for potential confounding in our study. Thus, NAFLD may be associated with vascular dysfunction through processes that also drive the occurrence of traditional risk factors, including dyslipidemia, hyperglycemia, insulin resistance, and blood pressure.

Several potential factors may underlie the association of NAFLD with vascular dysfunction. Liver fat accumulation occurs in a complex of metabolic disturbances, including abdominal obesity and dyslipidemia. Thus, isolating the association of fatty liver from the coexistent metabolic disruptions is not straightforward. Endothelial dysfunction may also contribute to the development of fatty liver. It has been proposed that the liver is both exposed to an abnormal metabolic environment and a source of substances that promote vascular damage. Fatty liver has been associated with increased proinflammatory cytokine production and heightened oxidative stress. Thus, reduced vascular function associated with NAFLD may reflect systemic inflammation. Both conduit and small vessel FMD depend on endothelial production of nitric oxide that may be reduced in the setting of inflammation. However, it may be that small vessel vasodilator responses have greater susceptibility to metabolic insults, including NAFLD, before the development of atherosclerotic disease. Further longitudinal studies are needed to define the precise mechanisms linking NAFLD to microvascular damage.

The major strengths of our investigation include our use of a large community-based sample that has not been selected for NAFLD and the detailed assessment of multiple measures of vascular function. In this well-characterized sample with a thorough assessment of covariates using standardized measurements, we are able to add to the present literature by adjusting for several important confounders in exploring the association with endothelial dysfunction and NAFLD in multivariable models.

There are several important limitations to our investigation that warrant mention. First, the cross-sectional design of this observational study precludes any inferences on causality or...
temporality. The Framingham Heart Study is largely white; so, results may not be generalizable to individuals of non-European ancestry. In addition, we defined NAFLD based on CT imaging, which likely under-represents the burden of NAFLD in the population, which may have led to nondifferential misclassification biasing our results to the null. Also, CT imaging cannot accurately detect steatohepatitis; so, we are unable to determine the association of vascular function and NAFLD disease severity. We also lack information about viral hepatitis status and other chronic liver conditions, which can cause the appearance of liver fat on CT scan. However, these findings would likely lead to misclassification and would bias our findings toward the null. Thus, they are unlikely to account for our positive results. For the Offspring Cohort participants, there were temporal differences between the vascular measures and CT scans. However, in a sensitivity analysis limited to the Third-Generation Cohort participants who had vascular measures and CT scans at the same study visit, our results were similar. In addition, those participants with available PAT measurements were older and had more cardiometabolic risk factors compared with those with missing data. This may have led to a selection bias away from the null. However, when the analysis was repeated in participants who had all vascular measures, the results were largely unchanged. Overall, although statistically significant, the magnitude of the association between NAFLD and the PAT ratio is modest. Future longitudinal studies are necessary to explore the clinical significance of our findings.

We observed an association between NAFLD and markers of endothelial dysfunction and arterial stiffness. Future longitudinal studies are required to further explore the association between NAFLD and microvascular dysfunction and how this relates to cardiovascular risk.

**Acknowledgments**


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Disclosures
Dr Mitchell is the owner of Cardiovascular Engineering, Inc. a company that designs and manufactures devices that measure vascular stiffness. The company uses these devices in clinical trials that evaluate the effects of diseases and interventions on vascular stiffness. The other authors report no conflicts.

References


Significance

Patients with nonalcoholic fatty liver disease (NAFLD) are at risk of cardiovascular disease. We evaluated the association between NAFLD and multiple measures of vascular function in a large (n=2284) community-based cohort of participants without apparent cardiovascular disease. Overall NAFLD is associated with multiple aspects of vascular function; however, this association is largely attributed to coexisting cardiometabolic risk factors. However, NAFLD remained correlated with measures of microvascular dysfunction in adjusted models. Future longitudinal studies should further explore the association between NAFLD and microvascular dysfunction and how this relates to cardiovascular risk.
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Materials and Methods

Study sample

The Framingham Heart Study (FHS), is a large, multi-generational, community cohort established in 1948 to prospectively identify risk factors for CVD. The design of the FHS Offspring and Third Generation Cohorts has been previously described. This study sample was drawn from participants in the Offspring Cohort and Third Generation Cohort who participated in the multi-detector CT sub-study to evaluate adipose tissue deposits between 2002 and 2005. For the Offspring Cohort participants, brachial artery ultrasound studies were performed at the seventh examination (1998-2001) and PAT and arterial tonometry measurements were performed at the eighth examination (2005-2008). For the Offspring Cohort, the brachial artery measures were obtained on average 4 years before the CT sub-study and the PAT and arterial tonometry measurements were obtained on average 3 years after the CT sub-study. For the Third Generation Cohort participants, all vascular function measurements were performed during the first examination (2002-2005) contemporaneously with the CT sub-study. PAT testing started part way through the examination cycle; thus, PAT studies were not performed in participants who were examined during the first 14 months of the cycle.

For the brachial artery analysis, our study sample was derived from a total of 3,170 Offspring and Third Generation Cohort participants who had measurement of liver attenuation on multi-detector CT scan. Individuals were excluded from this analysis if the CT scan was not interpretable for liver attenuation (1 participant) or VAT (98 participants), they had significant alcohol use defined as > 7 drinks per week for women and > 14 drinks per week for men (440 participants), prevalent clinical CVD (angina, coronary insufficiency, myocardial infarction, stroke, transient ischemic attack, intermittent claudication, heart failure) as determined by a panel of three investigators using previously published criteria (138 participants), missing
covariate measurements (24 participants) or incomplete brachial artery measurements (408 participants), yielding a total sample of 2,061 (603 Offspring, 1458 Third Generation) for the FMD analysis. For the peripheral artery and arterial tonometry analysis, out of the 3,124 participants who underwent CT scans, 99 participants were excluded for missing adiposity measures, 392 had significant alcohol use, 201 had prevalent CVD, 17 had missing covariates, 1,022 did not have peripheral artery tonometry performed and 131 did not have arterial tonometry performed, resulting in a sample size of 1,393 (757 Offspring, 636 Third Generation) for the PAT analysis and 2,284 (812 Offspring, 1472 Third Generation) for the arterial tonometry analysis. Participants who had available PAT data were older, with a higher fasting glucose, higher mean blood pressure, higher BMI, higher waist circumference, higher VAT and were more likely to be on hypertensive medications, on lipid lowering medications, have diabetes and be postmenopausal compared to those participants without PAT data (Supplementary Table I).

The Institutional Review Boards of the Boston University Medical Center and Massachusetts General Hospital approved the study protocol. All subjects provided written informed consent.

Multi-detector CT scan protocol and measurement of VAT and liver attenuation

The multi-detector CT scan cohort and protocol are described in detail elsewhere. In brief, while in the supine position, a total of 25 contiguous 5-mm-thick slices (120 kVp, 400mA, gantry rotation time 500 ms, and table feed 3:1) covering 125 mm above S1 were obtained using an 8-slice multi-detector abdominal CT scanner (LightSpeed Ultra, General Electric, Milwaukee, WI). A calibration phantom (Image Analysis, Lexington, KY) with a water equivalent compound (CT-Water, Light Speed Ultra; General Electric, Milwaukee, WI) and calcium hydroxyapatite at 0, 75, and 150 mg/cm³ was placed under each participant. The phantom was visualized on each image obtained. An image display window of -195 to -45 Hounsfield Units (HU) and a window center of -120 HU was used to identify pixels containing fat. The VAT compartment was separated from the subcutaneous adipose tissue (SAT) compartment by a single reader who
manually traced the muscular abdominal wall separating these depots. VAT and SAT volumes were subsequently quantified using a semiautomatic segmentation technique at a dedicated offline workstation (Aquarius 3D Workstation; TeraRecon, San Mateo, CA) as described. The correlation coefficients between 2 independent readers on a subset of 100 randomly selected participants were 0.992 and 0.997 for VAT and SAT, respectively.

The measurement of liver attenuation on the multi-detector CT scans of the abdomen has been previously described. Briefly, the liver attenuation in HU was measured in three areas from the liver, two areas from the spleen and one area from the external phantom and averaged to create liver spleen ratios and liver phantom ratios (LPR). The liver phantom ratio was chosen as the indexed standard since the spleen was not visualized on all scans. A liver spleen ratio of 1.1 corresponds to 30% hepatic steatosis based on studies in liver donors. We defined NAFLD as a liver phantom ratio of ≤ 0.33 which was shown in our prior work to have a sensitivity of 70% and specificity of 98% for determining significant steatosis using a liver spleen ratio < 1.1 as the gold standard cut-off.

Measurement of brachial artery flow-mediated dilation and reactive hyperemia

The protocol for measuring FMD and reactive hyperemia at the brachial artery has been described in detail elsewhere. In brief, images of the brachial artery were obtained using a high resolution ultrasound system. Arterial flow is interrupted for 5 minutes by the inflation of a cuff (Hokanson AG101) placed on the proximal forearm. Baseline brachial artery diameter and brachial artery diameter 1 minute after cuff deflation were measured by blinded sonographers. Resting and hyperemic flow velocities were measured as previously described. FMD induced by reactive hyperemia is expressed as the relative change from baseline (%).
Measurement of peripheral artery tonometry

The protocol for measuring peripheral artery tonometry has been previously described. Briefly, digital pulse amplitude was measured using a PAT device placed on the tip of each index finger (Endo-PAT2000, Itamar-Medical, Caesarea, Israel). Pulse amplitude in the finger before and after induction of hyperemia by 5 minute forearm cuff inflation was measured. The PAT ratio was calculated as the index of the hyperemic to control index finger in the 90-120 seconds following cuff deflation.

Measurement of arterial tonometry

The protocol for measuring arterial tonometry has been previously described. In brief, supine brachial systolic and diastolic blood pressures were obtained after a 5 minute rest period. Using a commercially available tonometer (SPT-301, Millar Instruments, Houston, TX), arterial tonometry was obtained from each participant’s right brachial, radial, femoral and carotid arteries with simultaneous electrocardiograms. To measure the pulse wave velocity, the transit distances from the suprasternal notch to each pulse recording site were assessed by body surface measurements. All data was digitized and transferred to the core laboratory (Cardiovascular Engineering, Inc, Norwood, MA) for analyses that were performed blinded to clinical data.

Covariates and baseline measurements

Routine medical history, physical examination and laboratory evaluations are performed at each FHS examination. For each analysis, the covariates were used from the same examination cycle at which the vascular function tests were measured. Self-reported data on smoking status and menopausal status were assessed on the basis of physician-administrated questionnaires. Participants were considered current smokers if they had smoked at least one cigarette per day.
in the year preceding the FHS examination. Women were considered post-menopausal if menstrual cycle had stopped for >1 year. Trained technicians used standard protocols for measuring heart rate, blood pressure, height, weight, and waist circumference. BMI was defined as weight (kg)/height\(^2\) (m\(^2\)). Serum total and high density lipoprotein cholesterol, triglycerides, glucose, C-reactive protein (CRP), alanine aminotransferase (ALT), and aspartate aminotransferase (AST) were measured on fasting morning blood samples.\(^{15}\) We defined elevated CRP at greater than the median value. We defined elevated liver function tests (LFT) as presence of elevated ALT (defined as > 31 U/L for women and > 40 U/L for men) or elevated AST (defined as > 31 U/L for women and >37 U/L for men) using standard laboratory definitions for the upper limits of normal as has been done in prior studies.\(^{16}\) Diabetes was defined as a fasting plasma glucose ≥126 mg/dL or treatment with a hypoglycemic agent or insulin. Hypertension was defined as systolic blood pressure ≥140 mm Hg, diastolic blood pressure ≥90 mm Hg, or on treatment with an antihypertensive agent.

**Statistical analysis**

The primary exposure of interest was NAFLD defined by a LPR ≤ 0.33. The primary dependent variables included FMD, hyperemic mean flow velocity, PAT ratio, carotid-femoral pulse wave velocity (CFPWV), forward wave amplitude and mean arterial pressure. Due to a heteroskedastic error structure and skewed distribution respectively, the PAT ratio was natural logarithmically transformed and the CFPWV was transformed to -1,000/CFPWV. Secondary dependent variables included baseline brachial artery diameter, baseline flow velocity, baseline mean pulse amplitude and the augmentation index.

Partial Pearson correlations, adjusted for age, sex and cohort, were examined to assess the cross-sectional relations between vascular function measures and LPR. We examined multivariable-adjusted partial correlations for vascular function measures and LPR as a
continuous variable or as a dichotomous variable (LPR ≤ 0.33 consistent with NAFLD) for the vascular function measures that were significant in the age-, sex- and cohort-adjusted analysis. The first multivariable model included age, sex, cohort, smoking status, mean arterial pressure (except in models with mean arterial pressure as the dependent variable), heart rate, 6-minute walk test before vascular test (yes/no), total/high density lipoprotein cholesterol, triglycerides, fasting glucose level, diabetes, menopausal status, use of hormone replacement therapy, hypertension treatment and lipid lowering treatment. Model 2 included the covariates in model 1 and additionally adjusted for BMI. Model 3 included model 2 and additionally adjusted for VAT.

In secondary analyses, we tested for effect modification of the association between the liver measures and vascular function by sex in the base model.

Since the PAT ratio has been previously shown to be associated with obesity, we further characterized the association between the PAT ratio, NAFLD and BMI, by stratifying participants by BMI category (normal BMI (BMI < 25 kg/m²), overweight (25 kg/m² ≤ BMI < 30 kg/m²) and obese (BMI ≥ 30 kg/m²)). Using multivariable regression, we calculated the least-square means with standard errors for the PAT ratio among those with NAFLD (LPR ≤ 0.33) and those without NAFLD (LPR > 0.33) overall and across the BMI categories adjusting for the same covariates in model 1 of the correlation analysis above.

To further explore the hypothesis that systemic inflammation may contribute to endothelial dysfunction, we conducted post-hoc analyses on the PAT ratio among those with and without NAFLD and CRP > median value or elevated LFTs. Using multivariable regression, we calculated the least-square means with standard errors for the PAT ratio among participants in each of the following categories: No NAFLD and CRP < median (reference), No NAFLD and CRP ≥ median, NAFLD and CRP < median, and NAFLD and CRP ≥ median. We adjusted for
the same covariates in model 1 of the correlation analysis above and used the Tukey-Kramer adjustment for multiple comparisons. A similar approach was done for the PAT ratio for participants with and without NAFLD and with and without elevated LFTs.

Because of the time separation between the measurement of liver attenuation and vascular measures for the Offspring Cohort participants, we performed a sensitivity analysis limited to the Third-Generation Cohort participants in whom multi-detector CT and vascular measures were performed at the same study visit. Additionally, since a large minority of participants did not have PAT measurements, we performed a sensitivity analysis limited to those participants with data from all three vascular function measures available.

All analyses were performed with SAS version 9.3 for Windows (SAS Institute, Cary, NC). Two-tailed p-values of < 0.05 were considered statistically significant for the main analysis.
References:


