Hepatic Steatosis, Obesity, and the Metabolic Syndrome Are Independently and Additively Associated With Increased Systemic Inflammation

Chiadi E. Ndumele, Khurram Nasir, Raquel D. Conceição, Jose A.M. Carvalho, Roger S. Blumenthal, Raul D. Santos

Objective—The goal of this study was to assess the independent and collective associations of hepatic steatosis, obesity, and the metabolic syndrome with elevated high-sensitivity C-reactive protein (hs-CRP) levels.

Methods and Results—We evaluated 2388 individuals without clinical cardiovascular disease between December 2004 and December 2006. Hepatic steatosis was diagnosed by ultrasound, and the metabolic syndrome was defined using National Heart, Lung, and Blood Institute criteria. The cut point of ≥3 mg/L was used to define high hs-CRP. Multivariate logistic regression was used to assess the independent and collective associations of hepatic steatosis, obesity, and the metabolic syndrome with high hs-CRP. Steatosis was detected in 32% of participants, 23% met criteria for metabolic syndrome, and 17% were obese. After multivariate regression, hepatic steatosis (odds ratio [OR] 2.07; 95% CI 1.68 to 2.56), obesity (OR 3.00; 95% CI 2.39 to 3.80), and the metabolic syndrome (2.39; 95% CI 1.88 to 3.04) were all independently associated with high hs-CRP. Combinations of these factors were associated with an additive increase in the odds of high hs-CRP, with individuals with 1, 2, and 3 factors having ORs for high hs-CRP of 1.92 (1.49 to 2.48), 3.38 (2.50 to 4.57), and 4.53 (3.23 to 6.35), respectively.

Conclusion—Hepatic steatosis, obesity, and the metabolic syndrome are independently and additively associated with increased odds of high hs-CRP levels. (Arterioscler Thromb Vasc Biol. 2011;31:1927-1932.)

Key Words: cytokines ■ obesity ■ CRP ■ hepatic steatosis ■ metabolic syndrome

There is growing evidence that hepatic steatosis is associated with increased cardiovascular disease (CVD) risk. Hepatic steatosis has been associated with a greater degree of subclinical atherosclerosis among asymptomatic men.¹ In cross-sectional epidemiological studies, hepatic steatosis has also been associated with an increased prevalence of CVD, independent of traditional risk factors.² Elevations of serum levels of liver enzymes have been shown to independently predict future CVD in prospective epidemiological studies.³ Furthermore, in a case-control study of 2103 patients with type II diabetes mellitus, hepatic steatosis diagnosed by ultrasound was associated with an increased risk of future CVD, despite adjustment for obesity, components of the metabolic syndrome, and other traditional cardiovascular risk factors.⁴ However, the mechanisms underlying the relationship between hepatic steatosis and CVD remain unclear.

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One mechanism that may explain part of the link between hepatic steatosis and CVD is chronic inflammation. Inflammation is central to all stages of atherosclerosis, including fatty streak development, formation of the atherosclerotic plaque, and plaque rupture with associated thrombosis.⁵ Circulating markers of systemic inflammation, measured in the serum, have been shown to predict future CVD in apparently healthy individuals.⁶ Of these, C-reactive protein (CRP) is the most reliable and robust predictor of adverse cardiovascular outcomes.⁷ Elevated levels of CRP, measured in asymptomatic individuals, are strongly and independently predictive of the future development of peripheral artery disease, stroke, and myocardial infarction.⁷-⁹

In patients with nonalcoholic fatty liver disease (NAFLD), the progression from simple steatosis to steatohepatitis and cirrhosis is characterized by cellular injury from oxidative stress and cytokine-driven intrahepatic inflammation.¹⁰ Some studies have suggested that the intrahepatic inflammation associated with NAFLD may also be linked to systemic elevations in inflammatory biomarkers, such as CRP.¹¹-¹³ However, previous studies investigating the relationship between hepatic steatosis and systemic inflammation have been relatively small, have used select patient populations, or have...
used abnormal liver function tests, a much less sensitive marker of steatosis than abdominal imaging.14

Hepatic steatosis is also closely linked with obesity and the metabolic syndrome,15 which are both well established as proinflammatory conditions. It is therefore important to assess the independent relationship between hepatic steatosis and systemic inflammatory markers and to determine the collective impact of combinations of these conditions on systemic inflammation. In this cross-sectional study of a large, community-based cohort of diabetic and nondiabetic men and women, we investigated the relationship between hepatic steatosis, as identified by ultrasound, and systemic levels of CRP, measured with a high-sensitivity assay. We further sought to assess the independent and collective associations of hepatic steatosis, obesity, and the metabolic syndrome with systemic inflammation.

Methods

We evaluated a group of asymptomatic men and women, free of coronary heart disease, who submitted to an obligatory clinical and laboratory health evaluation paid for by their employers from December 2004 to December 2006 at the Preventive Medicine Center of the Albert Einstein Hospital in São Paulo, Brazil. The examination protocol consisted of a clinical consultation, laboratory evaluation, and ultrasonographic abdominal scan. All individuals provided details of their demographics, medical history, quantitative alcohol consumption, smoking status, and medication usage at the time of their clinical consultation. We included all individuals for whom full information was available for all the covariates of interest. We excluded individuals with a known history of liver disease from this analysis, as well as those individuals drinking more than 20 g of alcohol per day.

Information regarding medical history was obtained via questionnaire. Smoking status was defined as current smoker versus current nonsmoker. Diabetes was identified by previous physician diagnosis or by the use of glucose-lowering medication. Hypertension and dyslipidemia were ascertained by a previous history of these conditions or the use of blood pressure–lowering or lipid-lowering medications; those individuals with systolic blood pressure >140 mm Hg or diastolic blood pressure >90 mm Hg at the clinical evaluation were also labeled as having hypertension. During physical examinations, blood pressure was measured with a mercury sphygmomanometer using the method recommended by the American Heart Association.16 Waist circumference was measured at the smallest diameter between the iliac crest and the costal margin using a plastic anthropometric tape held parallel to the floor.

Blood specimens were collected after an overnight fast. Plasma lipid, glucose, and liver transaminase levels (alanine aminotransferase [ALT] and aspartate aminotransferase [AST]) levels were measured by standardized automated laboratory tests using a Vitros platform (Johnson & Johnson Clinical Diagnostics). ALT levels were considered elevated if concentrations were greater than the 90th distribution percentile for the population according to gender. High-sensitivity CRP (hs-CRP) levels were determined by immunonephelometry (Dade-Behring). The previously established cut point of >3 mg/L, a level associated with increased cardiovascular risk in prospective studies, was used to define high hs-CRP levels in our analysis. All tests were performed at the Central Laboratory of the Albert Einstein Hospital.

Hepatic steatosis was diagnosed after at least a 6-hour fast using an ACUSON XP-10 device (Mountain View, CA) and was identified by the presence of an ultrasonographic pattern of a bright liver, with evident contrast between hepatic and renal parenchyma, as has been previously described.17 All hepatic ultrasounds were read by board-certified radiologists. Obesity was defined as a body mass index (BMI) of greater than 30 kg/m². The metabolic syndrome was defined by obesity (≥102 cm [40 inches] for men and ≥88 cm [36 inches] for women), high blood pressure (blood pressure ≥130/85 mm Hg or the use of antihypertensive medications), hyperglycemia (fasting blood glucose ≥100 mg/dL), low high-density lipoprotein cholesterol (HDL-C) (≤40 mg/dL for men and ≤50 mg/dL for women), and hypertriacylglyceridemia (≥150 mg/dL). This study was approved by the local institutional review board, and a waiver for informed consent was obtained.

Baseline characteristics of individuals with and without hepatic steatosis were compared using Wilcoxon’s t test for continuous variables and the Pearson’s χ² test for categorical variables. Because of the skewed distribution of hs-CRP, median values of hs-CRP were used in comparisons of groups of individuals with and without hepatic steatosis, elevated ALT, obesity, and the metabolic syndrome using the nonparametric Kruskal-Wallis test. In multivariate linear regression analyses, we assessed the associations of hepatic steatosis, elevated ALT, obesity, and the metabolic syndrome with continuous levels of natural log–transformed hs-CRP (ln hs-CRP). Multivariate logistic regression was used to evaluate associations of hepatic steatosis, elevated ALT, obesity, and the metabolic syndrome with hs-CRP levels >3 mg/L (high hs-CRP). For all regression analyses, a hierarchical model approach was used, adjusting first for traditional risk factors (age, gender, diabetes mellitus, low-density lipoprotein cholesterol [LDL-C], lipid lowering therapy, smoking, and physical activity) and then simultaneously adjusting for other independent predictors of hs-CRP levels in the multivariate model. Subanalysis testing was performed to estimate the odds of high hs-CRP associated with hepatic steatosis among those with and without other independent predictors of high hs-CRP. To assess the combined effects of hepatic steatosis, obesity, and the metabolic syndrome on systemic inflammation, multivariate logistic regression was used to assess the effect of having any 1, 2, or all 3 of these conditions on the odds of having a high hs-CRP level. All statistical analyses were performed using STATA, version 9.

Results

Twenty individuals were excluded from the analysis for missing covariates of interest, 61 individuals for positive hepatitis serologies, and 10 individuals for alcohol use of ≥20 g per day, leaving a study population of 2388 individuals. The characteristics of the study population, stratified by the presence or absence of hepatic steatosis, are displayed in Table 1. In our study population, hepatic steatosis was detected in 32% of study participants. Participants with hepatic steatosis were older (46 versus 43 years, P < 0.0001) and much more likely to be male (94 versus 72%, P < 0.0001) than those without steatosis. Hepatic steatosis was also associated with a worse risk factor profile: individuals with hepatic steatosis had higher systolic blood pressure, LDL-C, triglycerides, fasting glucose, BMI, and waist circumference and lower HDL-C than participants without steatosis. Hepatic steatosis was associated with a higher burden of diabetes (35% versus 12%, P < 0.0001) and hypertension (24 versus 9%, P < 0.0001) and increased use of medications to treat these conditions.

Overall, those with hepatic steatosis were more likely to have the metabolic syndrome (47% versus 11%, P < 0.0001), obesity (38% versus 8%, P < 0.0001), and elevated ALT (19% versus 6%, P < 0.0001) than those without steatosis. The median (interquartile range) of hs-CRP was 2.0 mg/L (1.1 to 3.8 mg/dL) among those with hepatic steatosis compared with 1.2 mg/L (0.6 to 2.5 mg/L) among those without steatosis (P = 0.0001). In a similar fashion, higher hs-CRP levels were
noted among those with high ALT versus normal ALT (1.9 mg/L [1.0 to 3.6 mg/L] versus 1.4 mg/L [0.7 to 2.9 mg/dL], \( P = 0.0002 \)), among those with versus those without the metabolic syndrome (2.4 mg/L [1.2 to 4.2 mg/dL] versus 1.3 mg/L [0.6 to 2.5 mg/L], \( P = 0.0001 \)), and among those with versus those without obesity (2.7 mg/L [1.5 to 4.4 mg/L] versus 1.8 mg/dL [0.9 to 3.1 mg/L], \( P = 0.0001 \)). As Figure 1 demonstrates, participants with steatosis, elevated ALT, the metabolic syndrome, or obesity also had a higher prevalence of high hs-CRP levels (\( \geq 3 \) mg/L) than those without those conditions.

Tables 2 and 3 compare the associations of hepatic steatosis, elevated ALT, the metabolic syndrome, and obesity with hs-CRP, both as a continuous variable (ln hs-CRP) and as a categorical variable (high hs-CRP), in unadjusted and adjusted analysis. Hepatic steatosis, the metabolic syndrome, obesity, and elevated ALT were each associated with higher levels of ln hs-CRP after controlling for traditional cardiovascular risk factors, with obesity demonstrating the strongest association. After additionally adjusting for the other predictors of increased ln hs-CRP (obesity, elevated ALT, and metabolic syndrome components, including abdominal obesity) in our full regression model, the presence of hepatic steatosis was associated with an increase in ln hs-CRP of 0.24 (95% CI 0.14 to 0.33), which corresponds to a 27% higher average hs-CRP level among those with hepatic steatosis. In our full regression model, the metabolic syndrome was also independently associated with an increase in ln hs-CRP of 0.24, and obesity was associated with an increase in ln hs-CRP of 0.42, or 52% higher average hs-CRP levels. Elevated ALT did not demonstrate an independent association with ln hs-CRP (Table 2).

Similarly, after adjustment for traditional cardiovascular risk factors, independent associations with high hs-CRP levels were found for hepatic steatosis (odds ratio [OR] 2.07; 95% CI 1.68 to 2.56), the metabolic syndrome (OR 2.39; 95% CI 1.88 to 3.04), obesity (OR 3.00; 95% CI 2.39 to 3.80), and elevated ALT (OR 1.50; 95% CI 1.12 to 2.00). However, these relationships were attenuated when all of the above predictors of increased hs-CRP were added to our regression model, with significant associations with high hs-CRP re-

### Table 1. Baseline Characteristics of Study Population

<table>
<thead>
<tr>
<th>Variables</th>
<th>Hepatic Steatosis Present (( n = 767 ))</th>
<th>Hepatic Steatosis Absent (( n = 1621 ))</th>
<th>( P )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y (( \pm SD ))</td>
<td>46 (9.4%)</td>
<td>43 (7.2%)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Male</td>
<td>723 (94%)</td>
<td>1172 (72%)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Mean systolic blood pressure, in mm Hg (( \pm SD ))</td>
<td>130 (14)</td>
<td>120 (14)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Subjects with hypertension</td>
<td>184 (24%)</td>
<td>146 (9%)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Mean LDL-C, in mg/dL (( \pm SD ))</td>
<td>129 (35)</td>
<td>121 (34)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Mean HDL-C, in mg/dL (( \pm SD ))</td>
<td>41 (10)</td>
<td>49 (12)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Median triglycerides, in mg/dL (interquartile range)</td>
<td>143 (106 to 196)</td>
<td>96 (73 to 128)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Mean waist circumference, in cm (( \pm SD ))</td>
<td>100 (11)</td>
<td>87 (12)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Mean fasting glucose, in mg/dL (( \pm SD ))</td>
<td>99 (21)</td>
<td>90 (11)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Subjects with diabetes mellitus</td>
<td>267 (35%)</td>
<td>187 (12%)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Subjects with metabolic syndrome</td>
<td>363 (47%)</td>
<td>178 (11%)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Mean BMI, in kg/m(^2) (( \pm SD ))</td>
<td>29 (4)</td>
<td>25 (4)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Subjects with obesity (BMI ( \geq 30 ) kg/m(^2))</td>
<td>288 (38%)</td>
<td>123 (8%)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Median ALT, in U/L (interquartile range)</td>
<td>51 (41 to 47)</td>
<td>40 (33 to 48)</td>
<td>0.0001</td>
</tr>
<tr>
<td>Median ALT/AST ratio (interquartile range)</td>
<td>1.76 (1.53 to 2.04)</td>
<td>1.54 (1.33 to 1.76)</td>
<td>0.0001</td>
</tr>
<tr>
<td>Median hs-CRP, in mg/L (interquartile range)</td>
<td>2.0 (1.1 to 3.8)</td>
<td>1.2 (0.6 to 2.4)</td>
<td>0.0001</td>
</tr>
<tr>
<td>Subjects with high hs-CRP (( \geq 3 ) mg/dL)</td>
<td>271 (35%)</td>
<td>326 (20%)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Subjects using lipid-lowering medications</td>
<td>75 (10%)</td>
<td>120 (7%)</td>
<td>0.048</td>
</tr>
<tr>
<td>Subjects using antihypertensive medications</td>
<td>168 (22%)</td>
<td>130 (8%)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Subjects using hypoglycemic medications</td>
<td>28 (4%)</td>
<td>9 (0.6%)</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

ALT indicates alanine aminotransferase; AST, aspartate aminotransferase.
The interaction of gene expression with traditional cardiovascular risk factors, an independent association persisted between hepatic steatosis and elevated hs-CRP levels. The combined predictors, with 48% of those individuals with hepatic steatosis, the metabolic syndrome, or obesity, whereas 22%, 12%, and 9% had 1, 2, or all 3 of these independent predictors of increased hs-CRP. Among those with none of these independent predictors, elevated CRP was noted in only 17% of study participants. A linear increase in the likelihood of elevated hs-CRP was noted with increasing numbers of the above predictors, with 48% of those individuals with hepatic steatosis, the metabolic syndrome, or obesity, and the metabolic syndrome having high hs-CRP (Figure 2). After taking into account traditional risk factors, compared with those without hepatic steatosis, the metabolic syndrome, or obesity, the likelihood of high hs-CRP increased from an OR of 1.9 with 1 of these conditions to an OR of 4.5 with the presence of all 3 predictors (Table 4).

### Table 2. Comparison of Hepatic Steatosis, High ALT, Metabolic Syndrome, and Obesity With Continuous In hs-CRP in Multivariate Linear Regression Analyses

<table>
<thead>
<tr>
<th></th>
<th>High ALT B Coefficients (95% CI)</th>
<th>Hepatic Steatosis B Coefficients (95% CI)</th>
<th>Metabolic Syndrome B Coefficients (95% CI)</th>
<th>Obesity (BMI $\geq$30 kg/m$^2$) B Coefficients (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Model 1*</td>
<td>0.27 (0.13–0.41)</td>
<td>0.47 (0.37–0.56)</td>
<td>0.57 (0.47–0.67)</td>
<td>0.67 (0.56–0.78)</td>
</tr>
<tr>
<td>Model 2†</td>
<td>0.23 (0.10–0.37)</td>
<td>0.41 (0.32–0.50)</td>
<td>0.50 (0.39–0.61)</td>
<td>0.62 (0.51–0.73)</td>
</tr>
<tr>
<td>Model 3‡</td>
<td>0.03 (–0.10–0.16)</td>
<td>0.24 (0.14–0.33)</td>
<td>0.24 (0.12–0.36)</td>
<td>0.42 (0.31–0.55)</td>
</tr>
</tbody>
</table>

*Model 1: unadjusted.
†Model 2 variables: age, gender, presence of diabetes mellitus, LDL-C, smoking status (current smoker or nonsmoker), use of lipid-lowering medication, and physical activity (assessed by the International Physical Activity Questionnaire as low, moderate, or high physical activity).
‡Model 3 variables: model 2 variables + hepatic steatosis, high ALT, metabolic syndrome components (abdominal obesity, fasting hyperglycemia, low HDL, hypertriglyceridemia, and hypertension/anti-hypertensive medication use), and obesity.

**Discussion**

In this study of 2388 diabetic and nondiabetic men and women without known coronary heart disease, we found a significant association between hepatic steatosis identified by ultrasound and elevated hs-CRP levels. Hepatic steatosis was associated with higher hs-CRP levels among obese and nonobese individuals and among those with and without the metabolic syndrome. As expected, obesity and the metabolic syndrome were also independently associated with increased hs-CRP levels; after adjusting for these and other traditional risk factors, an independent association persisted between hepatic steatosis and elevated hs-CRP levels. The combined

### Table 3. Associations of High ALT, Hepatic Steatosis, Metabolic Syndrome, and Obesity With High CRP ($\geq$3 mg/L) in Multivariate Logistic Regression Analyses

<table>
<thead>
<tr>
<th></th>
<th>High ALT OR (95% CI)</th>
<th>Hepatic Steatosis OR (95% CI)</th>
<th>Metabolic Syndrome OR (95% CI)</th>
<th>Obesity (BMI $\geq$30 kg/m$^2$) OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Model 1*</td>
<td>1.56 (1.17 to 2.07)</td>
<td>2.17 (1.79 to 2.63)</td>
<td>2.64 (2.15 to 3.25)</td>
<td>3.23 (2.58 to 4.04)</td>
</tr>
<tr>
<td>Model 2†</td>
<td>1.50 (1.12 to 2.00)</td>
<td>2.07 (1.68 to 2.56)</td>
<td>2.39 (1.88 to 3.04)</td>
<td>3.00 (2.39 to 3.80)</td>
</tr>
<tr>
<td>Model 3‡</td>
<td>1.07 (0.84 to 1.34)</td>
<td>1.49 (1.18 to 1.88)</td>
<td>1.48 (1.12 to 1.94)</td>
<td>2.21 (1.70 to 2.89)</td>
</tr>
</tbody>
</table>

*Model 1: unadjusted.
†Model 2 variables: age, gender, presence of diabetes-mellitus, LDL-C, smoking status (current smoker or nonsmoker), use of lipid-lowering medication, and physical activity (assessed by the International Physical Activity Questionnaire as low, moderate, or high physical activity).
‡Model 3 variables: model 2 variables + hepatic steatosis, high ALT, metabolic syndrome components (abdominal obesity, fasting hyperglycemia, low HDL, hypertriglyceridemia, and hypertension/anti-hypertensive medication use), and obesity.
presence of hepatic steatosis, obesity, and the metabolic syndrome was associated with an additive increase in the likelihood of high hs-CRP levels, with individuals with all 3 conditions having 4.5 times higher odds of hs-CRP ≥3 mg/dL than those without any of them.

Our findings support the concept of an independent association between hepatic steatosis and systemic inflammation, beyond what is explained by the presence of obesity and the metabolic syndrome. This elevation in hs-CRP among patients with hepatic steatosis may serve as a marker of long-term cardiovascular risk and may explain some of the previously observed associations between hepatic steatosis and CVD. Some small studies have found a relationship between NAFLD and increased levels of inflammatory biomarkers. In 1 study of 77 patients, those with biopsy-proven fatty liver disease had higher serum levels of the inflammatory cytokines CC-chemokine ligand (CCL)2/monocyte chemotactic protein (MCP)-1 and CCL19 than healthy controls, after adjusting for age, sex, and BMI. Similarly, in another study of 135 middle-aged men, those with hepatic steatosis and steatohepatitis had higher levels of hs-CRP and other inflammatory biomarkers than age- and obesity-matched controls after multivariate regression analysis.

Table 4. Associations of Combined Presence of Hepatic Steatosis, Metabolic Syndrome, and Obesity With High hs-CRP (≥3 mg/L) in Multivariate Logistic Regression Analyses

<table>
<thead>
<tr>
<th>No. of Conditions Present</th>
<th>Model 1* OR (95% CI)</th>
<th>Model 2† OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>Reference group‡</td>
<td>Reference group‡</td>
</tr>
<tr>
<td>1</td>
<td>1.80 (1.42 to 2.29)</td>
<td>1.92 (1.49 to 2.48)</td>
</tr>
<tr>
<td>2</td>
<td>3.29 (2.50 to 4.34)</td>
<td>3.38 (2.50 to 4.57)</td>
</tr>
<tr>
<td>3</td>
<td>4.44 (3.27 to 6.01)</td>
<td>4.53 (3.23 to 6.35)</td>
</tr>
</tbody>
</table>

*Model 1: unadjusted.  †Model 2: adjusted for age, gender, diabetes mellitus, LDL-C, smoking status (current smoker or nonsmoker), use of lipid-lowering medication, and physical activity (assessed by the International Physical Activity Questionnaire as low, moderate, or high physical activity). ‡Reference group: No hepatic steatosis, metabolic syndrome, or obesity.

Figure 2. Prevalence of high hs-CRP (≥3 mg/dL) associated with combinations of hepatic steatosis, metabolic syndrome, and obesity. The presence of a greater number of factors was associated with a higher prevalence of elevated hs-CRP levels.

Table 4. Associations of Combined Presence of Hepatic Steatosis, Metabolic Syndrome, and Obesity With High hs-CRP (≥3 mg/L) in Multivariate Logistic Regression Analyses

This study has some limitations. Although ultrasound is a very useful noninvasive tool for identifying hepatic steatosis, its sensitivity for detecting fatty changes within the liver is impaired fatty acid oxidation, increased oxidative stress, and local inflammation that can fuel a transition from simple steatosis to steatohepatitis. It is also noteworthy that the liver is the primary source of CRP production, and recent studies indicate that the degree of hepatic steatosis and inflammation by histology correlates with systemic levels of inflammatory biomarkers. In 1 study of 85 patients, increasing grades of hepatic steatosis, necroinflammation, and fibrosis on biopsy samples were each associated with sequentially increasing hs-CRP levels, well into the high risk range. Other studies have found a direct association between NAFLD severity and hepatocyte expression of inflammatory mediators.

Abdominal obesity and the metabolic syndrome predispose to hepatic steatosis, both via increased delivery of free fatty acids to the liver and through increases in hepatic lipogenesis associated with hyperinsulinemia. In turn, the worsening insulin resistance associated with hepatic steatosis may also exacerbate the metabolic syndrome. The close associations among hepatic steatosis, obesity, and cardiometabolic risk factors have led to the suggestion that hepatic steatosis may be a novel component of the metabolic syndrome. However, even among patients with obesity and those with the metabolic syndrome as currently defined, the presence of hepatic steatosis in this study was associated with higher levels of hs-CRP. This study suggests that in these already high-risk populations, the finding of hepatic steatosis could be a marker for an even greater degree of systemic inflammation. Furthermore, combinations of hepatic steatosis, obesity, and the metabolic syndrome were associated with an increasing likelihood of elevated hs-CRP in our analysis. Given their physiological interrelatedness, it is certainly conceivable that these conditions could be reinforcing each other in an inflammatory cascade that predisposes to increased cardiovascular risk.

This study has some limitations. Although ultrasound is a very useful noninvasive tool for identifying hepatic steatosis, its sensitivity for detecting fatty changes within the liver is reduced when the steatosis is less than moderate in severity. Therefore, it is likely that some cases of hepatic steatosis, particularly of mild severity, were not detected in this cohort. In addition, more direct measures of visceral adiposity, such as computed tomography, may be even more accurate than anthropometric measurements in assessing and controlling for the impact of abdominal obesity on inflammatory biomarkers. Because this is an observational study, there is larger study of 1740 individuals, abnormal liver function tests were associated with elevated hs-CRP independent of cardiometabolic risk factors. Additionally, in a study of 832 Chilean subjects, increased hs-CRP was 1 of the variables independently associated with ultrasound-diagnosed hepatic steatosis. This study extends these findings in a large, community-based cohort of middle-aged asymptomatic men and women, among whom hepatic steatosis was identified via abdominal imaging.
always the possibility of residual bias. Finally, as this is a
cohort of Brazilian men and women, the extent to which these
findings can be generalized to other populations is unclear.

Strengths of the present study include a large number of
participants of both genders, each of whom had undergone
extensive cardiovascular risk factor assessment. This allowed
for adjustment for potentially confounding risk factors in
assessing the associations of hepatic steatosis, obesity, and
the metabolic syndrome with high hs-CRP levels. The broadly
representative cohort also allowed for evaluation of the
relationship between hepatic steatosis and hs-CRP within
demographic and clinical subgroups. The use of abdominal
imaging to identify steatosis is also a strength of this study, as
abnormal liver function tests—which have been used in
previous analyses—are known to have poor sensitivity for the
detection of NAFLD.14 Finally, our use of individuals without
known CVD may help make this data most relevant, as
hs-CRP measurement is most commonly performed as part of a
primary prevention strategy.

In summary, this study demonstrated an association be-
 tween hepatic steatosis and elevated hs-CRP levels among
asymptomatic men and women, independent of obesity, the
metabolic syndrome, and other cardiovascular risk factors.
The combined presence of hepatic steatosis, obesity, and
the metabolic syndrome was associated with an additive increase
in the odds of high hs-CRP. Additional research is needed to
further elucidate the mechanisms underlying the interrela-
tionships between hepatic steatosis, obesity, the metabolic
syndrome, and systemic inflammation and to determine the
impact of these associations on cardiovascular risk.

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Disclosures
None.

References
1. Santos RD, Nasir K, Conceicao RD, Sarwar A, Carvalho JA, Blumenthal
RS. Hepatic steatosis is associated with a greater prevalence of coronary
artery calcification in asymptomatic men. Atherosclerosis. 2007;194:
517–519.
C, Arcaro G. Prevalence of nonalcoholic fatty liver disease and its
association with cardiovascular disease among type 2 diabetic patients.
Benjamin EJ, D’Agostino RB, Vasan RS. γ-Glutamyl transferase and
metabolic syndrome, cardiovascular disease, and mortality risk: the Fra-
Falezza G. Nonalcoholic fatty liver disease and risk of future cardiovas-
cular events among type 2 diabetic patients. Diabetes. 2005;54:
3541–3546.
340:115–126.
6. Blake GJ, Ridker PM. Novel clinical markers of vascular wall inflam-
7. Ridker PM, Hennekens CH, Buring JE, Rifai N. C-reactive protein and
other markers of inflammation in the prediction of cardiovascular disease
8. Ridker PM, Cushman M, Stampfer MJ, Tracy RP, Hennekens CH.
Plasma concentration of C-reactive protein and risk of developing pe-
9. Sakkinen P, Abbott RD, Curb JD, Rodriguez BL, Yano K, Tracy RP.
C-reactive protein and myocardial infarction. J Clin Epidemiol. 2002;55:
149–151.
10. Browning JD, Horton JD. Molecular mediators of hepatic steatosis and
I, Torjesen PA, Birkeland K, Bjoro K, Aukrust P. Systemic inflammation
in nonalcoholic fatty liver disease is characterized by elevated levels of
Frook GJ, Aromson Y. The evaluation of hepatic steatosis in patients with
elevated liver enzymes and C-reactive protein: possible hepatic contribu-
13. Targher G. Relationship between high-sensitivity C-reactive protein
levels and liver histology in subjects with non-alcoholic fatty liver
function tests in a population of Japanese male bank employees. J Clin
Epidemiol. 2001;54:945–951.
1221–1231.
16. Perloff D, Grim C, Flack J, Frohlich ED, Hill M, McDonald M, Mor-
genstern BZ. Human blood pressure determination by sphygmoman-
Cristinni G, Tiberielli C. Prevalence of and risk factors for hepatic
18. Grundy SM, Cleeman JI, Daniels SR, Donato KA, Eckel RH, Franklin
BA, Gordon DJ, Krauss RM, Savage PJ, Smith SC Jr, Spertus JA, Costa
F. Diagnosis and management of the metabolic syndrome: an American
Heart Association/National Heart, Lung, and Blood Institute Scientific
Maggeo M, Day CP. NASH predicts plasma inflammatory biomarkers
independently of visceral fat in men. Obesity (Silver Spring). 2008;16:
1394–1399.
RM, Gonzalez R, Alvarez M, Hernandez V, Garcia-Zattera MJ, Otarola
fatty liver disease and its association with obesity, insulin resistance and
erased serum levels of C-reactive protein in Hispanics. Liver Int.
Takahashi H, Kirikoshi H, Inamori M, Nozaki Y, Abe Y, Kubota K, Saito
S, Iwasaki T, Terauchi Y, Takahashi H, Kirikoshi H, Inamori M, Nozaki Y,
Abe Y, Kubota K, Saito S, Iwasaki T, Terauchi Y, Togo S, Maeyama S,
Nakajima A. High sensitivity C-reactive protein is an independent clinical feature of non-
alcoholic steatohepatitis (NASH) and also of the severity of fibrosis in
22. Kotronin A, Yki-Jarvinen H. Fatty liver: a novel component of the
23. Saadeh S, Younossi ZM, Remer EM, Gramlich T, Ong JP, Hurley M,
Mullen KD, Cooper JN, Sheridan MJ. The utility of radiological imaging
in nonalcoholic fatty liver disease. Gastroenterology. 2002;123:745–750.
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지방간, 비만, 대사증후군은 독립적으로 그리고 부가적으로 전신 염증 반응과 연관이 있다.

임수 교수
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Summary

여러 연구에서 비만, 대사증후군, 당뇨병 환자에서 hs-CRP (high-sensitivity C-reactive protein)과 같은 염증인자가 증가한다는 사실이 보고되고 있다. 특히 CRP는 IL-6, TNF-α와 같은 사이토카인의 자극을 받아 간에서 만들어지는 제2형 당뇨병에서 흔히 볼 수 있는 고혈당지반혈증, 고밀도지단백 콜레스테롤 높도의 감소와 같은 지질 이상도 급성기 반응의 한 특징이라고 할 수 있다. 또한 최근에는 비만, 지방간, 대사증후군 등이 심혈관질환과 밀접한 관련을 있는 기전의 하나로 IL-6 및 TNF-α와 같은 proinflammatory cytokine이 중요한 역할을 할 것으로 여겨지며, 이중에서도 대표적으로 CRP가 인슐린 저항성과 관련이 있다는 많은 연구 결과가 있다. 본 연구는 같은 연구 결과가 있다. 본 연구는 지방간, 지방, 대사증후군과 염증 지표인 hs-CRP의 관계를 조사하였다. 심혈관질환에 없는 총 2,388명을 환자를 대상으로 비만, 지방간, 대사증후군을 평가하였으며, hs-CRP는 3mg/L 이상을 의미 있는 것으로 정의하였다. 다중 회귀 분석을 통하여, 지방간, 비만, 대사증후군의 높은 hs-CRP와의 관계를 독립적인, 그리고 집합적인 방법으로 분석하였다. 대상자의 32%에서 지방간이 있었고, 23%가 대사증후군에 해당되었고, 17%가 비만에 해당하였다. 지방간(odds ratio[OR] 2.07; 95% CI 1.68 to 2.56), 비만(OR 3.00; 95% CI 2.39 to 3.80), 대사증후군(OR 2.39; 95% CI 1.88 to 3.04)이 각각 독립적으로 높은 hs-CRP와 관련이 있었다. 지방간, 비만, 대사증후군의 누적효과를 조사한 결과, 하나를 가진 경우, hs-CRP(3ml/L 이상)에 대한 각각의 OR은 1.92(1.49 to 2.48), 3.38(2.50 to 4.57), 4.53(3.23 to 6.35)로 의미 있게 증가하였다. 결론적으로 지방간, 비만, 대사증후군은 독립적으로, 그리고 같이 존재하는 경우는 더 강하게 높은 hs-CRP와 연관이 있었다.
Commentary

최근 들어 지방간(hepatic steatosis)이 심혈관질환과 연관이 있다는 보고가 늘고 있다. 지방간은 증상이 없는 동맥경화(subclinical atherosclerosis)와 관련이 있다고 보고된 바 있으며, 많은 역학연구에서는 지방간이 다른 위험인자와는 독립적으로 심혈관질환의 유병률과 연관이 있다고 제시하고 있다. 이외에도 전향적인 연구를 통하여 지방간이 심혈관질환의 발생을 예측할 수 있다는 보고가 있으며, 2,103명의 제2형 당뇨병 환자를 대상으로 한 연구에서는 초음파로 평가된 지방간이 비만, 대사증후군 등의 전통적인 위험인자를 보정한 다음에도 심혈관질환의 발생과 연관이 있다는 보고가 있었다. 하지만 이들의 연관성에 대한 기전에 대해서는 정확히 알려진 바 없다.

많은 연구자들이 지방간과 심혈관질환을 연계짓는 연결고리의 하나로 만성 염증(chronic inflammation)을 들고 있다. 염증 반응은 특히 fatty streak development, 동맥경화반의 형성, 혈전을 동반한 플라크(plaque)의 파열 등 모든 동맥경화에 있어서 핵심적이다. 혈액에서 측정할 수 있는 염증 반응인자의 대표는 hs-CRP이다. 특히 증상이 없는 hs-CRP의 상승은 말초혈관질환, 뇌졸중, 심근 경색과 연관이 있다. 비알코올성 지방간(nonalcoholic fatty liver disease) 환자의 경과 중 단순 지방 침착에서 지방간염 내지 간경화로의 진행은 산화 스트레스와 간내 염증 반응에 기인한 다. 몇몇 연구에서 비알코올성 지방간과 연관된 간내 염증 반응이 혈액 내 염증 마커인 hs-CRP 상승과 연관이 있다고 보고하였다.

지방간은 또한 비만 및 대사증후군과 연관이 있다. 이 둘 역시 염증 반응과 연관이 있다. 이러한 사실들을 바탕으로 볼 때, 지방간, 비만, 대사증후군의 연관은 미치는 독립적인 영향과 이에 더하여 이들이 공존하였을 때 어떠한 부가적인 영향이 있을지를 보는 것은 의미가 있다고 하겠다. 참고적으로 hs-CRP 연구 논문을 볼 때, 측정 방법과 높고 낮음의 기준을 어떻게 정하였는지를 주의 깊게 보아야 한다. 본 논문에서 hs-CRP 농도는 immunonephelometry(Dade-Behring) 방법으로 측정되었고, 이전 여러 연구 결과를 바탕으로 3mg/L를 기준으로 이상과 이하로 나누어 분석하였다.

전체적으로 지방간에 해당되는 사람이 대사증후군(47% vs 11%, P < 0.01) 및 비만(38% vs 8%, P < 0.01)에 훨씬 많이 해당되었다. 지방간이 있는 사람의 평균 hs-CRP 농도는 2.0mg/L이었고, 지방간이 없는 사람의 평균 hs-CRP는 1.2mg/L이었다. 다중 회귀 분석에서 지방간(OR 2.07), 비만(3.00), 대사증후군(2.39)이 각각 독립적으로 높은 hs-CRP와 관련이 있었다(Figure 1). 지방간, 비만, 대사증후군의 누적효과를 조사한 결과 각각은 두 개, 세 개를 가진 경우, 높은 hs-CRP에 대한 각각의 OR은 1.92, 3.38, 4.53로 의미 있게 증가하였다. 

Figure 1. hsCRP 농도 3 mg/dL 이상에서의 ALT 상승, 지방간, 대사증후군, 비만의 유병률.

<table>
<thead>
<tr>
<th>Percentage of hs-CRP &gt; 3 mg/L</th>
<th>Absent</th>
<th>Present</th>
</tr>
</thead>
<tbody>
<tr>
<td>High ALT</td>
<td>24</td>
<td>33</td>
</tr>
<tr>
<td>Hepac Steatosis</td>
<td>20</td>
<td>35</td>
</tr>
<tr>
<td>Metabolic Syndrome</td>
<td>20</td>
<td>40</td>
</tr>
<tr>
<td>Obesity</td>
<td>21</td>
<td>46</td>
</tr>
</tbody>
</table>
가하였다. 결론적으로 지방간, 비만, 대사증후군은 독립적으로, 그리고 누적효과를 가지며 높은 hs-CRP와 연관이 있었다(Figure 2).

본 연구 결과는 대사증후군 내지 비만의 영향을 배제하고 지방간과 연중 반응과의 관련성을 입증한 것에 의미가 있다. 지방간 환자에서 hs-CRP의 상승은 잠재적으로 심혈관질환의 위험인자로 작용할 수 있으며, 이전의 여러 연구 결과를 뒷받침한다고 볼 수 있다. 본 연구를 포함하여 지방간과 hs-CRP의 상승과의 연관성이 인과관계를 입증하지는 않는다는 점을 염두에 두어야 한다. 그러나 간내 침착되는 중성지방이 지방산 산화의 장애를 초래하고, 산화 스트레스를 증가시키며, 이는 단순 지방간(simple steatosis)을 지방간염(steatohepatitis)으로 진행시키는 역할을 한다. 또한 간이 hs-CRP의 생산 장소이며, 이전의 연구 결과들에서 지방간의 정도가 전반적인 연중상태와 관련이 있다는 것을 입증하였다.

복부 비만과 대사 증후군은 지방간을 선형하며, 특히 지방산을 간으로 이동시켜, 간내 지방합성 및 고인슐린혈증을 유발한다. 지방간과 함께 인슐린 저항성이 심해지면 대사증후군을 악화시킬 수 있다. 이러한 상호관계를 통해 지방간, 비만, 대사 증후군이 독립적으로뿐만 아니라, 협력하여 hs-CRP의 상승을 초래하며, 나아가 심혈관질환의 발생과 연관이 있을 수 있다. 특히 이러한 관계가 중대성이 없는 사람들에서 입증되었다는 점을 고려할 때, hs-CRP 측정이 심혈관질환의 일차 예방목적으로 측정될 수 있을 것이다.

요약하면 증상이 없는 상대적으로 건강한 대규모 사람을 대상으로 지방간, 비만, 대사증후군이 독립적이면서도, 서로 연계하여 대표적인 연중 수치인 hs-CRP와 연관이 있음을 알 수 있다. 이들 관계에 대해 먼저 대사증후군과 연중 반응과의 관련성을 살펴 보면, 특히 hs-CRP 농도는 대사증후군 인자들 중에서도 허리 둘레와 가장 밀접한 관련성이 있는 것으로 보고되고 있으며, 이는 체내 연중 반응 정도가 복부 비만과 관련성이 있음을 시사한다고 할 수 있다. 대사증후군 구성요소가 증가할수록 hs-CRP 값도 증가하는 것으로 알려져 있어 대사증후군의 정도가 심할수록 hs-CRP가 상승하는 것으로 인지된다. 인슐린 저항성이 심하지 않은 한국인에서도 hs-CRP 농도와 대사증후군의 요소들 사이에 밀접한 연관성이 있음을 확인할 수 있었는데, 만성 연중 반응이 대사증후군의 중요한 요인이 될 수 있을음을 뒷받침해주는 것이라 하겠다.

이러한 만성 연중 반응과 대사증후군 또는 인슐린 저항성 사이의 연관성에 대해 다양한 기전이 존재한다. 만성 연중이 대사증후군 및 제2형 당뇨 발생의 초기 유발인자이며 특히 연중이 증가함에 따라 또는 고지방 고훈련 식습관에 따라 연중 유발성 사이토카인인 IL-1, IL-6, TNF-α의 분비가 증
가되며, 이는 결국 인슐린 저항성이 당뇨병이 발생
한다. 결론적으로 지방간, 비만, 대사증후군이 독
립적이면서도 서로 협력하여 hs-CRP 농도의 상승
에 기여하고, 이는 인슐린 저항성과 밀접한 관련
이 있음을 알 수 있으며, 나아가 심혈관질환의 발
생으로 이어질 수 있다.

REFERENCES
Hepatic Steatosis, Obesity, and the Metabolic Syndrome Are Independently and Additively Associated With Increased Systemic Inflammation

Chiadi E. Ndumele, Khurram Nasir, Raquel D. Conceição, Jose A.M. Carvalho, Roger S. Blumenthal, Raul D. Santos

Objective—The goal of this study was to assess the independent and collective associations of hepatic steatosis, obesity, and the metabolic syndrome with elevated high-sensitivity C-reactive protein (hs-CRP) levels.

Methods and Results—We evaluated 2388 individuals without clinical cardiovascular disease between December 2004 and December 2006. Hepatic steatosis was diagnosed by ultrasound, and the metabolic syndrome was defined using National Heart, Lung, and Blood Institute criteria. The cut point of ≥3 mg/L was used to define high hs-CRP. Multivariate logistic regression was used to assess the independent and collective associations of hepatic steatosis, obesity, and the metabolic syndrome with high hs-CRP. Steatosis was detected in 32% of participants, 23% met criteria for metabolic syndrome, and 17% were obese. After multivariate regression, hepatic steatosis (odds ratio [OR] 2.07; 95% CI 1.68 to 2.56), obesity (OR 3.00; 95% CI 2.39 to 3.80), and the metabolic syndrome (2.39; 95% CI 1.88 to 3.04) were all independently associated with high hs-CRP. Combinations of these factors were associated with an additive increase in the odds of high hs-CRP, with individuals with 1, 2, and 3 factors having ORs for high hs-CRP of 1.92 (1.49 to 2.48), 3.38 (2.50 to 4.57), and 4.53 (3.23 to 6.35), respectively.

Conclusion—Hepatic steatosis, obesity, and the metabolic syndrome are independently and additively associated with increased odds of high hs-CRP levels. (Arterioscler Thromb Vasc Biol. 2011;31:1927-1932.)

Key Words: cytokines ■ obesity ■ CRP ■ hepatic steatosis ■ metabolic syndrome

There is growing evidence that hepatic steatosis is associated with increased cardiovascular disease (CVD) risk. Hepatic steatosis has been associated with a greater degree of subclinical atherosclerosis among asymptomatic men.1 In cross-sectional epidemiological studies, hepatic steatosis has also been associated with an increased prevalence of CVD, independent of traditional risk factors.2 Elevations of serum levels of liver enzymes have been shown to independently predict future CVD in prospective epidemiological studies.3 Furthermore, in a case-control study of 2103 patients with type II diabetes mellitus, hepatic steatosis diagnosed by ultrasound was associated with an increased risk of future CVD, despite adjustment for obesity, components of the metabolic syndrome, and other traditional cardiovascular risk factors.4 However, the mechanisms underlying the relationship between hepatic steatosis and CVD remain unclear.

One mechanism that may explain part of the link between hepatic steatosis and CVD is chronic inflammation. Inflammation is central to all stages of atherosclerosis, including fatty streak development, formation of the atherosclerotic plaque, and plaque rupture with associated thrombosis.5 Circulating markers of systemic inflammation, measured in the serum, have been shown to predict future CVD in apparently healthy individuals.6 Of these, C-reactive protein (CRP) is the most reliable and robust predictor of adverse cardiovascular outcomes.7 Elevated levels of CRP, measured in asymptomatic individuals, are strongly and independently predictive of the future development of peripheral artery disease, stroke, and myocardial infarction.7–9

In patients with nonalcoholic fatty liver disease (NAFLD), the progression from simple steatosis to steatohepatitis and cirrhosis is characterized by cellular injury from oxidative stress and cytokine-driven intrahepatic inflammation.10 Some studies have suggested that the intrahepatic inflammation associated with NAFLD may also be linked to systemic elevations in inflammatory biomarkers, such as CRP.11–13 However, previous studies investigating the relationship between hepatic steatosis and systemic inflammation have been relatively small, have used select patient populations, or have
used abnormal liver function tests, a much less sensitive marker of steatosis than abdominal imaging.\textsuperscript{14} Hepatic steatosis is also closely linked with obesity and the metabolic syndrome,\textsuperscript{15} which are both well established as proinflammatory conditions. It is therefore important to assess the independent relationship between hepatic steatosis and systemic inflammatory markers and to determine the collective impact of combinations of these conditions on systemic inflammation. In this cross-sectional study of a large, community-based cohort of diabetic and nondiabetic men and women, we investigated the relationship between hepatic steatosis, as identified by ultrasound, and systemic levels of CRP, measured with a high-sensitivity assay. We further sought to assess the independent and collective associations of hepatic steatosis, obesity, and the metabolic syndrome with systemic inflammation.

**Methods**

We evaluated a group of asymptomatic men and women, free of coronary heart disease, who submitted to an obligatory clinical and laboratory health evaluation paid for by their employers from December 2004 to December 2006 at the Preventive Medicine Center of the Albert Einstein Hospital in São Paulo, Brazil. The examination protocol consisted of a clinical consultation, laboratory evaluation, and ultrasonographic abdominal scan. All individuals provided details of their demographics, medical history, quantitative alcohol consumption, smoking status, and medication usage at the time of their clinical consultation. We included all individuals for whom full information was available for all the covariates of interest. We excluded individuals with a known history of liver disease from this analysis, as well as those individuals drinking more than 20 g of alcohol per day.

Information regarding medical history was obtained via questionnaire. Smoking status was defined as current smoker versus current nonsmoker. Diabetes was identified by previous physician diagnosis or by the use of glucose-lowering medication. Hypertension and dyslipidemia were ascertained by a previous history of these conditions or the use of blood-pressure-lowering or lipid-lowering medications; those individuals with systolic blood pressure >140 mm Hg or diastolic blood pressure >90 mm Hg at the clinical evaluation were also labeled as having hypertension. During physical examinations, blood pressure was measured with a mercury sphygmomanometer using the method recommended by the American Heart Association.\textsuperscript{16} Waist circumference was measured at the smallest diameter between the iliac crest and the costal margin using a plastic anthropometric tape held parallel to the floor.

Blood specimens were collected after an overnight fast. Plasma lipid, glucose, and liver transaminase levels (alanine aminotransferase [ALT] and aspartate aminotransferase [AST]) levels were measured by standardized automated laboratory tests using a Vitros platform (Johnson & Johnson Clinical Diagnostics). ALT levels were considered elevated if concentrations were greater than the 90th distribution percentile for the population according to gender. High-sensitivity CRP (hs-CRP) levels were determined by immunonephelometry (Dade-Behring). The previously established cut point of >3 mg/L, a level associated with increased cardiovascular risk in prospective studies, was used to define high hs-CRP levels in our analysis. All tests were performed at the Central Laboratory of the Albert Einstein Hospital.

Hepatic steatosis was diagnosed after at least a 6-hour fast using an ACUSON XP-10 device (Mountain View, CA) and was identified by the presence of an ultrasonographic pattern of a bright liver, with evident contrast between hepatic and renal parenchyma, as has been previously described.\textsuperscript{17} All hepatic ultrasounds were read by board-certified radiologists. Obesity was defined as a body mass index (BMI) of greater than 30 kg/m\textsuperscript{2}. The metabolic syndrome was defined using criteria from the American Heart Association/National Heart, Lung, and Blood Institute scientific statement on the metabolic syndrome.\textsuperscript{18} Patients with >3 of the following metabolic risk factors were determined to have the metabolic syndrome: truncal obesity (≥102 cm [40 inches] for men and ≥88 cm [36 inches] for women), high blood pressure (blood pressure ≥130/85 mm Hg or the use of antihypertensive medications), hyperglycemia (fasting blood glucose ≥100 mg/dL), low high-density lipoprotein cholesterol (HDL-C) (≤40 mg/dL for men and ≤50 mg/dL for women), and hypertriglyceridemia (≥150 mg/dL). This study was approved by the local institutional review board, and a waiver for informed consent was obtained.

Baseline characteristics of individuals with and without hepatic steatosis were compared using Wilcoxon's t test for continuous variables and the Pearson's \(\chi^2\) test for categorical variables. Because of the skewed distribution of hs-CRP, median values of hs-CRP were used in comparisons of groups of individuals with and without hepatic steatosis, elevated ALT, obesity, and the metabolic syndrome using the nonparametric Kruskal-Wallis test. In multivariate linear regression analyses, we assessed the associations of hepatic steatosis, elevated ALT, obesity, and the metabolic syndrome with continuous levels of natural log-transformed hs-CRP (ln hs-CRP). Multivariate logistic regression was used to evaluate associations of hepatic steatosis, elevated ALT, obesity, and the metabolic syndrome with ln hs-CRP levels >3 mg/L (high hs-CRP). For all regression analyses, a hierarchical model approach was used, adjusting first for traditional risk factors (age, gender, diabetes mellitus, low-density lipoprotein cholesterol [LDL-C], lipid lowering therapy, smoking, and physical activity) and then simultaneously adjusting for other independent predictors of hs-CRP levels in the multivariate model. Subanalysis testing was performed to estimate the odds of high hs-CRP associated with hepatic steatosis among those with and without other independent predictors of high hs-CRP. To assess the combined effects of hepatic steatosis, obesity, and the metabolic syndrome on systemic inflammation, multivariate logistic regression was used to assess the effect of having any 1, 2, or all 3 of these conditions on the odds of having a high hs-CRP level. All statistical analyses were performed using STATA, version 9.

**Results**

Twenty individuals were excluded from the analysis for missing covariates of interest. 61 individuals for positive hepatitis serologies, and 10 individuals for alcohol use of ≥20 g per day, leaving a study population of 2388 individuals. The characteristics of the study population, stratified by the presence or absence of hepatic steatosis, are displayed in Table 1. In our study population, hepatic steatosis was detected in 32% of study participants. Participants with hepatic steatosis were older (46 versus 43 years, \(P<0.0001\)) and much more likely to be male (94 versus 72%, \(P<0.0001\)) than those without steatosis. Hepatic steatosis was also associated with a worse risk factor profile: individuals with hepatic steatosis had higher systolic blood pressure, LDL-C, triglycerides, fasting glucose, BMI, and waist circumference and lower HDL-C than participants without steatosis. Hepatic steatosis was associated with a higher burden of diabetes (35% versus 12%, \(P<0.0001\)) and hypertension (24 versus 9%, \(P<0.0001\)) and increased use of medications to treat these conditions.

Overall, those with hepatic steatosis were more likely to have the metabolic syndrome (47% versus 11%, \(P<0.0001\)), obesity (38% versus 8%, \(P<0.0001\)), and elevated ALT (19% versus 6%, \(P<0.0001\)) than those without steatosis. The median (interquartile range) of hs-CRP was 2.0 mg/L (1.1 to 3.8 mg/dL) among those with hepatic steatosis compared with 1.2 mg/L (0.6 to 2.5 mg/L) among those without steatosis (\(P=0.0001\)). In a similar fashion, higher hs-CRP levels were
noted among those with high ALT versus normal ALT (1.9 mg/L [1.0 to 3.6 mg/L] versus 1.4 mg/L [0.7 to 2.9 mg/dL], P = 0.0002), among those with versus those without the metabolic syndrome (2.4 mg/L [1.2 to 4.2 mg/dL] versus 1.3 mg/L [0.6 to 2.5 mg/L], P = 0.0001), and among those with versus those without obesity (2.7 mg/L [1.5 to 4.4 mg/L] versus 1.8 mg/dL [0.9 to 3.1 mg/L], P = 0.0001). As Figure 1 demonstrates, participants with steatosis, elevated ALT, the metabolic syndrome, or obesity also had a higher prevalence of high hs-CRP levels (≥3 mg/dL) than those without those conditions.

Tables 2 and 3 compare the associations of hepatic steatosis, elevated ALT, the metabolic syndrome, and obesity with hs-CRP, both as a continuous variable (ln hs-CRP) and as a categorical variable (high hs-CRP), in unadjusted and adjusted analysis. Hepatic steatosis, the metabolic syndrome, obesity, and elevated ALT were each associated with higher levels of ln hs-CRP after controlling for traditional cardiovascular risk factors, with obesity demonstrating the strongest association. After additionally adjusting for the other predictors of increased ln hs-CRP (obesity, elevated ALT, and metabolic syndrome components, including abdominal obesity) in our full regression model, the presence of hepatic steatosis was associated with an increase in ln hs-CRP of 0.24 (95% CI 0.14 to 0.33), which corresponds to a 27% higher average hs-CRP level among those with hepatic steatosis. In our full regression model, the metabolic syndrome was also independently associated with an increase in ln hs-CRP of 0.24, and obesity was associated with an increase in ln hs-CRP of 0.42, or 52% higher average hs-CRP levels. Elevated ALT did not demonstrate an independent association with ln hs-CRP (Table 2).

Similarly, after adjustment for traditional cardiovascular risk factors, independent associations with high hs-CRP levels were found for hepatic steatosis (odds ratio [OR] 2.07; 95% CI 1.68 to 2.56), the metabolic syndrome (OR 2.39; 95% CI 1.88 to 3.04), obesity (OR 3.00; 95% CI 2.39 to 3.80), and elevated ALT (OR 1.50; 95% CI 1.12 to 2.00). However, these relationships were attenuated when all of the above predictors of increased hs-CRP were added to our regression model, with significant associations with high hs-CRP re-
and women. In multivariate analyses, the association of icant (der and hepatic steatosis for high hs-CRP was not signif-
nonobese individuals, respectively. The interaction of gen-
(1.11 to 2.32) and 1.79 (1.21 to 2.65) among obese and
regression model.
found similar associations with ln hs-CRP 0.45 (0.34 to 0.57)
and increased hs-CRP levels found in this study does not
prove a causal relationship. However, excess triglyceride
and women, among whom hepatic steatosis was identified via
mainiing only for hepatic steatosis (OR 1.49; 95% CI 1.18 to
1.88), the metabolic syndrome (OR 1.48; 95% CI 1.12 to
1.94), and obesity (OR 2.21; 95% CI 1.70 to 2.89) (Table 3).
When using the metabolic syndrome criteria for abdominal
obesity rather than the BMI-based definition of obesity, we
found similar associations with ln hs-CRP 0.45 (0.34 to 0.57)
and high hs-CRP (OR 2.36; 95% CI 1.81 to 3.09) in our full
regression model.
In subanalyses, we demonstrated that the presence of
steatosis was associated with high hs-CRP levels among
individuals with and without the metabolic syndrome, as well
as among those with and without obesity, even after control-
ning for traditional cardiovascular risk factors. Among partic-
ients with the metabolic syndrome, the OR for high hs-CRP
associated with hepatic steatosis was 1.83 (1.40 to 2.40),
wheras the respective OR was 1.67 (1.13 to 2.46) in the
absence of the metabolic syndrome. In a similar fashion, the
ORs for high hs-CRP associated with steatosis were 1.61
(1.11 to 2.32) and 1.79 (1.21 to 2.65) among obese and
nonobese individuals, respectively. The interaction of gen-
eral and hepatic steatosis for high hs-CRP was not signif-
ificant (P = 0.80), indicating similar associations among men
and women. In multivariate analyses, the association of
hepatic steatosis with high hs-CRP was similar among
individuals with high ALT (OR 1.53; 95% CI 1.20 to 1.92)
and those without elevated liver enzymes (OR 1.52; 95%
CI 1.20 to 1.93).
Because hepatic steatosis, the metabolic syndrome, and
obesity were independent predictors of elevated hs-CRP, we
also assessed whether a combination of these factors was
associated with a higher burden of inflammation. In our
study, 58% of participants were unaffected by hepatic steato-
sis, the metabolic syndrome, or obesity, whereas 22%, 12%,
and 9% had 1, 2, or all 3 of these independent predictors of
increased hs-CRP. Among those with none of these indepen-
dent predictors, elevated CRP was noted in only 17% of study
participants. A linear increase in the likelihood of elevated
hs-CRP was noted with increasing numbers of the above
predictors, with 48% of those individuals with hepatic steato-
sis, obesity, and the metabolic syndrome having high hs-CRP
(Figure 2). After taking into account traditional risk factors,
compared with those without hepatic steatosis, the metabolic
syndrome, or obesity, the likelihood of high hs-CRP in-
creased from an OR of 1.9 with 1 of these conditions to an
OR of 4.5 with the presence of all 3 predictors (Table 4).

**Discussion**

In this study of 2388 diabetic and nondiabetic men and
women without known coronary heart disease, we found a
significant association between hepatic steatosis identified by
ultrasound and elevated hs-CRP levels. Hepatic steatosis was
associated with higher hs-CRP levels among obese and
nonobese individuals and among those with and without the
metabolic syndrome. As expected, obesity and the metabolic
syndrome were also independently associated with increased
hs-CRP levels; after adjusting for these and other traditional
risk factors, an independent association persisted between
hepatic steatosis and elevated hs-CRP levels. The combined

<table>
<thead>
<tr>
<th>Table 2. Comparison of Hepatic Steatosis, High ALT, Metabolic Syndrome, and Obesity With Continuous In hs-CRP in Multivariate Linear Regression Analyses</th>
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<tbody>
<tr>
<td><strong>High ALT B</strong> Coefficients (95% CI)</td>
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<tr>
<td>-----------------------------------------</td>
</tr>
<tr>
<td>Model 1*</td>
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<tr>
<td>Model 2†</td>
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<tr>
<td>Model 3‡</td>
</tr>
</tbody>
</table>

*Model 1: unadjusted.
†Model 2 variables: age, gender, presence of diabetes-mellitus, LDL-C, smoking status (current smoker or nonsmoker), use of lipid-lowering medication, and physical activity (assessed by the International Physical Activity Questionnaire as low, moderate, or high physical activity).
‡Model 3 variables: model 2 variables + hepatic steatosis, high ALT, metabolic syndrome components (abdominal obesity, fasting hyperglycemia, low HDL, hyperglyceridemia, and hypertension/anti-hypertensive medication use), and obesity.


<table>
<thead>
<tr>
<th>Table 3. Associations of High ALT, Hepatic Steatosis, Metabolic Syndrome, and Obesity With High CRP (≥3 mg/L) in Multivariate Logistic Regression Analyses</th>
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<tbody>
<tr>
<td>**High ALT OR (95% CI)</td>
</tr>
<tr>
<td>-----------------------------------------</td>
</tr>
<tr>
<td>Model 1*</td>
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<tr>
<td>Model 2†</td>
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*Model 1: unadjusted.
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‡Model 3 variables: model 2 variables + hepatic steatosis, high ALT, metabolic syndrome components (abdominal obesity, fasting hyperglycemia, low HDL, hyperglyceridemia, and hypertension/anti-hypertensive medication use), and obesity.
presence of hepatic steatosis, obesity, and the metabolic syndrome was associated with an additive increase in the likelihood of high hs-CRP levels, with individuals with all 3 conditions having 4.5 times higher odds of hs-CRP ≥3 mg/dl than those without any of them.

Our findings support the concept of an independent association between hepatic steatosis and systemic inflammation, beyond what is explained by the presence of obesity and the metabolic syndrome. This elevation in hs-CRP among patients with hepatic steatosis may serve as a marker of long-term cardiovascular risk and may explain some of the previously observed associations between hepatic steatosis and CVD. Some small studies have found a relationship between NAFLD and increased levels of inflammatory biomarkers. In 1 study of 77 patients, those with biopsy-proven fatty liver disease had higher serum levels of the inflammatory cytokines CC-chemokine ligand (CCL)2/monocyte chemoattractant protein (MCP)-1 and CCL19 than healthy controls, after adjusting for age, sex, and BMI.11 Similarly, in another study of 135 middle-aged men, those with hepatic steatosis and steatohepatitis had higher levels of hs-CRP and other inflammatory biomarkers than age- and obesity-matched controls after multivariate regression analysis.19 In a larger study of 1740 individuals, abnormal liver function tests were associated with elevated hs-CRP independent of cardiovascular risk factors.12 Additionally, in a study of 832 Chilean subjects, increased hs-CRP was 1 of the variables independently associated with ultrasound-diagnosed hepatic steatosis.20 This study extends these findings in a large, community-based cohort of middle-aged asymptomatic men and women, among whom hepatic steatosis was identified via abdominal imaging.

The epidemiological association between hepatic steatosis and increased hs-CRP levels found in this study does not prove a causal relationship. However, excess triglyceride accumulation in hepatocytes is known to be associated with impaired fatty acid oxidation, increased oxidative stress, and local inflammation that can fuel a transition from simple steatosis to steatohepatitis.10 It is also noteworthy that the liver is the primary source of CRP production, and previous studies indicate that the degree of hepatic steatosis and inflammation by histology correlates with systemic levels of inflammatory biomarkers. In 1 study of 85 patients, increasing grades of hepatic steatosis, necroinflammation, and fibrosis on biopsy samples were each associated with sequentially increasing hs-CRP levels, well into the high risk range.13 Other studies have found a direct association between NAFLD severity and hepatocyte expression of inflammatory mediators.21

Abdominal obesity and the metabolic syndrome predispose to hepatic steatosis, both via increased delivery of free fatty acids to the liver and through increases in hepatic lipogenesis associated with hyperinsulinemia.10 In turn, the worsening insulin resistance associated with hepatic steatosis may also exacerbate the metabolic syndrome. The close associations among hepatic steatosis, obesity, and cardiometabolic risk factors have led to the suggestion that hepatic steatosis may be a novel component of the metabolic syndrome.22 However, even among patients with obesity and those with the metabolic syndrome as currently defined, the presence of hepatic steatosis in this study was associated with higher levels of hs-CRP. This suggests that in these already high-risk populations, the finding of hepatic steatosis could be a marker for an even greater degree of systemic inflammation. Furthermore, combinations of hepatic steatosis, obesity, and the metabolic syndrome were associated with an increasing likelihood of elevated hs-CRP in our analysis. Given their physiological interrelatedness, it is certainly conceivable that these conditions could be reinforcing each other in an inflammatory cascade that predisposes to increased cardiovascular risk.

This study has some limitations. Although ultrasound is a very useful noninvasive tool for identifying hepatic steatosis, its sensitivity for detecting fatty changes within the liver is reduced when the steatosis is less than moderate in severity.23 Therefore, it is likely that some cases of hepatic steatosis, particularly of mild severity, were not detected in this cohort. In addition, more direct measures of visceral adiposity, such as computed tomography, may be even more accurate than anthropometric measurements in assessing and controlling for the impact of abdominal obesity on inflammatory biomarkers. Because this is an observational study, there is

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**Figure 2.** Prevalence of high hs-CRP (≥3 mg/dL) associated with combinations of hepatic steatosis, metabolic syndrome, and obesity. The presence of a greater number of factors was associated with a higher prevalence of elevated hs-CRP levels. *Factors: hepatic steatosis, metabolic syndrome, and obesity.*

**Table 4. Associations of Combined Presence of Hepatic Steatosis, Metabolic Syndrome, and Obesity With High hs-CRP (≥3 mg/L) in Multivariate Logistic Regression Analyses**

<table>
<thead>
<tr>
<th>No. of Conditions Present</th>
<th>Model 1 * OR (95% CI)</th>
<th>Model 2 † OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>Reference group‡‡</td>
<td>Reference group‡‡</td>
</tr>
<tr>
<td>1</td>
<td>1.80 (1.42 to 2.29)</td>
<td>1.92 (1.49 to 2.48)</td>
</tr>
<tr>
<td>2</td>
<td>3.29 (2.50 to 4.34)</td>
<td>3.38 (2.50 to 4.57)</td>
</tr>
<tr>
<td>3</td>
<td>4.44 (3.27 to 6.01)</td>
<td>4.53 (3.23 to 6.35)</td>
</tr>
</tbody>
</table>

*Model 1: unadjusted.
†Model 2: adjusted for age, gender, diabetes mellitus, LDL-C, smoking status (current smoker or nonsmoker), use of lipid-lowering medication, and physical activity (assessed by the International Physical Activity Questionnaire as low, moderate, or high physical activity).
‡Reference group: No hepatic steatosis, metabolic syndrome, or obesity.
always the possibility of residual bias. Finally, as this is a cohort of Brazilian men and women, the extent to which these findings can be generalized to other populations is unclear.

Strengths of the present study include a large number of participants of both genders, each of whom had undergone extensive cardiovascular risk factor assessment. This allowed for adjustment for potentially confounding risk factors in assessing the associations of hepatic steatosis, obesity, and the metabolic syndrome with high hs-CRP levels. The broadly representative cohort also allowed for evaluation of the relationship between hepatic steatosis and hs-CRP within demographic and clinical subgroups. The use of abdominal imaging to identify steatosis is also a strength of this study, as abnormal liver function tests—which have been used in previous analyses—are known to have poor sensitivity for the detection of NAFLD. Finally, our use of individuals without known CVD may help make this data most relevant, as hs-CRP measurement is most commonly performed as part of a primary prevention strategy.

In summary, this study demonstrated an association between hepatic steatosis and elevated hs-CRP levels among asymptomatic men and women, independent of obesity, the metabolic syndrome, and other cardiovascular risk factors. The combined presence of hepatic steatosis, obesity, and the metabolic syndrome was associated with an additive increase in the odds of high hs-CRP. Additional research is needed to further elucidate the mechanisms underlying the interrelationships among hepatic steatosis, obesity, the metabolic syndrome, and systemic inflammation and to determine the impact of these associations on cardiovascular risk.

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Disclosures
None.

References