Nonalcoholic Fatty Liver Disease Is Associated With Carotid Atherosclerosis

A Case–Control Study

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

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

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

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

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

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

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

Methods

Subjects

Between November 2002 and March 2003, we screened all subjects referred for diagnostic abdominal ultrasound to the Radiology Service of Hospital San Millán-San Pedro, Logroño, for fatty liver. A "bright liver" (abnormally intense, high-level echoes arising from the hepatic parenchyma, with an amplitude similar to that of echoes arising from the diaphragm) in the absence of chronic liver disease or cancer was detected in 93 subjects, who were recruited into a protocol approved by the institutional review board. Sixty-six subjects accepted participation and gave signed informed consent. Participants were first given a complete clinical history, during which alcohol consumption was assessed as part of the interview, including the World Health Organization (WHO) Alcohol Use Disorders Identification Test[9] and medication use. This was followed

Original received October 25, 2004; final version accepted February 14, 2005.

From the Lipid Clinic, Internal Medicine Service (A.B., D.M., E.M., A.A.) and Radiology Service (J.L.C.), Hospital San Millán-San Pedro, Logroño, Spain; and Lipid Clinic, Endocrinology and Nutrition Service, Institut d'Investigacions Biomèdiques August Pi Sunyer, Hospital Clínico, Barcelona (E.R.), Spain.

Since submission of this manuscript, the carotid ultrasound findings of a case–control study in men with and without NAFLD have been published[40] and show a higher IMT in the former. The high frequency of MetS components in NAFLD was confirmed. The results support the atherogenic potential of NAFLD observed in our study.

Correspondence to Angel Brea, MD, Lipid Clinic, Internal Medicine Service, Hospital San Millán-San Pedro, Autonomía de la Rioja 4, 26004 Logroño, Spain. E-mail a.brea@arrakis.es

Arterioscler Thromb Vasc Biol. **is available at http://www.atvbaha.org** **DOI: 10.1161/01.ATV.0000160613.57985.18**

by blood sampling for liver function tests, including markers for viral, autoimmune, and metabolic liver diseases. Excluded were subjects abusing alcohol or having daily alcohol consumption >20 g (n=13), seropositive for hepatitis B or C viruses (n=12), or with serum transferrin saturation >45% (n=1), thus leaving 40 subjects with "primary" NAFLD. Sex- and age-matched control subjects, randomly chosen from local National Health Service card holders, were contacted by telephone and offered an abdominal ultrasound and participation in the study provided that they did not abuse alcohol and that the liver was ultrasonographically normal. The radiologist was blinded to the identity and clinical and biochemical findings of the subjects. To find 40 controls, 61 persons were called (16 refused participation and 5 were excluded because a fatty liver was detected by ultrasound examination).

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

Clinical and Laboratory Measurements

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

Subjects fasted overnight before phlebotomy. Serum glucose, both fasting and 120 minutes after an oral glucose challenge (75 g in 200 mL water), was measured using a glucose dehydrogenase method. Serum insulin was determined by standard radioimmunoassay. Cholesterol and triglycerides were measured using enzymatic procedures. High-density lipoprotein (HDL) cholesterol was quantified after precipitation with manganese chloride. Low-density lipoprotein (LDL) cholesterol was calculated with the Friedewald equation. Apolipoprotein B (apoB) was determined by the use of turbidimetry. The index of insulin resistance was calculated using the fasting values of serum glucose and insulin according to the homeostasis model assessment (HOMA) method.[10] The top quartile of the control sample (>2.649) was used to define insulin resistance. Serum ferritin and transferrin were determined by nephelometric methods, and serum iron was measured with a centrifugal analyzer with ferrozine as chromogen. AAT and CRP were determined by immunonephelometry.

Pertinent data on adiposity, blood pressure, glycemic control, and blood lipids were used to classify subjects as having MetS by National Cholesterol Education Program (NCEP) Adult Treatment Panel III (ATP-III)[11] and WHO[12] criteria.

Carotid Ultrasound

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

Statistical Analyses

Comparisons of patients and control subjects were made with unpaired *t* tests or the Mann–Whitney *U* test, when appropriate, for continuous variables and by χ^2 analyses for categorical variables. Values with a skewed distribution were transformed to their natural logarithm (ln) for analyses. Pearson's correlation coefficients were constructed to test the relationship between continuous variables. The independence of the association of variables with the presence of NAFLD or atherosclerosis (abnormal IMT, defined as the top quartile of control values, or presence of plaque) was assessed by multivariate logistic regression and expressed as odds ratios (ORs). An ANOVA statistic was used to compare sex- and age-adjusted IMT values between different groups of NAFLD and MetS. Two-sided *P* values <0.05 were considered. Analyses were performed with SPSS 10.0 software.

Results

Clinical Features and Laboratory Data

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

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

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

Criteria for MetS

Whether assessed by ATP III or WHO criteria, all the risk factors related to MetS (visceral adiposity, hypertension,

TABLE 1. Clinical and Laboratory Data of Patients With NAFLD and Control Subjects

Variables	Patients (n=40)	Controls (n=40)	*P* Value*
Clinical			
Men/women	20/20	20/20†	
Age, years	53.2±12.6	51.6±10.9†	
History of high blood pressure	20 (50)	6 (15)	0.001
History of diabetes	7 (17.5)	0‡	0.012
History of CHD	4 (10)	0‡	0.12
Current smoker	11 (27.5)	10 (25)	0.80
BMI, kg/m^2	31.8±5.1	26.3±3.6	<0.001
Waist circumference, cm	104±13	91±13	<0.001
Waist/hip ratio	0.93±0.07	0.89±0.07	0.015
Systolic blood pressure, mm Hg	141±16	124±16	<0.001
Diastolic blood pressure, mm Hg	87±9	77±10	<0.001
Laboratory			
Fasting glucose, mmol/L	6.4±1.3	5.5±0.7	<0.001
120-minute glucose, mmol/L	8.1±3.2	5.7±1.9	0.001
Fasting insulin, μU/mL	17.4±9.6	8.7±4.2	<0.001
Insulin resistance (HOMA) index	4.12 (2.80–5.78)	1.63 (1.42–2.65) §	<0.001
Insulin resistance	28 (70)	10 (25)	<0.001
Total cholesterol, mmol/L	5.9±1.1	5.8±1.0	0.65
LDL cholesterol, mmol/L	3.8±0.9	3.9±0.9	0.55
HDL cholesterol, mmol/L	1.1±0.3	1.3±0.3	0.013
Triglycerides, mmol/L	1.6 (1.0–2.4)	0.9 (0.7–1.2) §	0.005
ApoB, g/L	1.29±0.32	1.20±0.27	0.18
ALT, mU/mL	43±23	22±10	<0.001
GGT, mU/mL	47±45	20±10	<0.001
AAT, g/L	1.71±0.28	1.62±0.31	0.24
CRP, mg/L	1.85 (1.33–6.28)	1.10 (0.60–1.61)	<0.001
Serum iron, ng/mL	83±36	78±29	0.53
Total transferrin, ng/mL	263±32	239±40	0.004
Transferrin saturation, percent	25.4±10.8	25.8±10.8	0.89
Ferritin, ng/mL	106 (68–163)	76 (39–121)	0.035

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

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

abnormal glucose metabolism, insulin resistance, hypertriglyceridemia, and low HDL cholesterol) were significantly ($P<0.005$) more prevalent in NAFLD patients than in control subjects, resulting in a 4-fold higher frequency of WHO-MetS (80% versus 20%, respectively) and a nearly 5-fold prevalence of ATP-MetS (72.5% versus 15%, respectively) in NAFLD. The prevalence of ATP-MetS and WHO-MetS in NAFLD was higher in women than in men (80% versus 70% and 85% versus 55%, respectively), whereas depending on the definition, control men had a 3- to 5-fold excess of MetS compared with control women (25% versus 5% by ATP criteria and 30% versus 10% by WHO criteria, respectively).

Findings of Carotid Ultrasound Studies

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

TABLE 2. Carotid IMT and Plaque in Patients With NAFLD and Control Subjects

Variables	Patients (n=40)	Controls (n=40)	*P* Value*
Mean IMT, mm	0.70±0.20	0.54±0.13	<0.0001
Maximum IMT, mm	0.75±0.22	0.58±0.13	<0.001
Mean IMT above top quartile	27 (67.5)	10 (25)	<0.001
Carotid plaque	20 (50)	10 (25)	0.021

Data as mean±SD or No. (percentage).

*Group comparisons by unpaired *t* test or chi-square test.

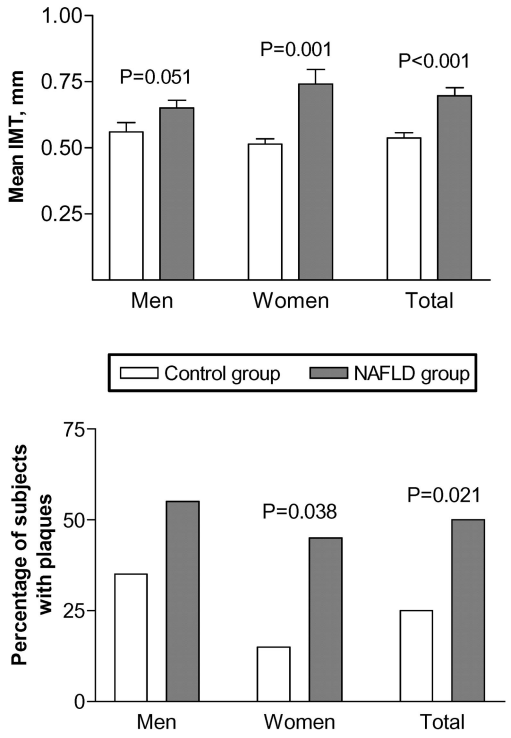

Figure 1. Comparisons of mean carotid IMT (top) and plaque frequency (bottom) in patients with NAFLD and control subjects. Error bars represent SEM.

subjects with and without MetS, by any definition, and with and without NAFLD, IMT progressed in the order: control without MetS<control with MetS<NAFLD without MetS<NAFLD with MetS (Figure 2).

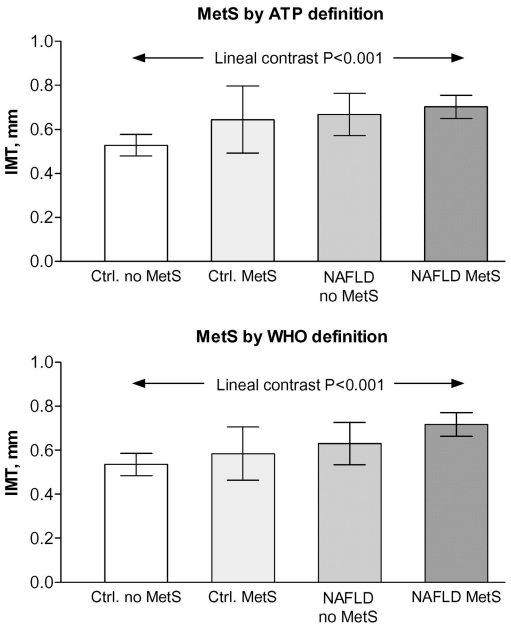

Figure 2. Sex- and age-adjusted mean carotid IMT in control (Ctrl) subjects and NAFLD patients with and without MetS by 2 definitions. Error bars represent 95% CIs.

NAFLD and Carotid Atherosclerosis Predictors by Multivariate Analyses

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

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

Discussion

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

The diagnosis of NAFLD was based on the exclusion of known etiologic factors of liver disease and on ultrasound examination but was not confirmed by liver biopsy for ethical reasons. However, ultrasound examination is by far the commonest way of diagnosing NAFLD in clinical practice[16] and is very sensitive in the detection of significant hepatic steatosis in patients with biopsy-proven disease.[17] Indeed, Saadeh et al[17] reported that the presence of >33% fat on liver biopsy was optimal for radiological detection of steatosis; that is, moderate to severe fatty infiltration has to be present for the liver ultrasound pattern to become altered and suggest the diagnosis of NAFLD. Insulin resistance was not measured by the euglycemic clamp technique but by the simpler HOMA method. However, HOMA has been reported as a very reliable technique to assess insulin sensitivity.[10,18]

MetS, whether defined by ATP-III[11] or WHO criteria,[12] was the strongest determinant of NAFLD in a multivariate model with adjustment for various confounders. This finding agrees with previous evidences of a strong association of NAFLD with individual features of MetS, such as obesity, type 2 diabetes, and dyslipidemia, or with the complete syndrome.[1–5,14,15] As shown in recent reports from different populations,[19–21] adults with MetS are at consistently increased risk for cardiovascular and all-cause mortality. Carotid plaque incidence, a measure of advanced atherosclerosis, was independently associated with MetS in our study. Likewise, an increased incidence and progression of carotid plaque in subjects with MetS has been reported recently from the prospective Bruneck Study.[22] Thus, the close association of NAFLD with MetS might explain the high cardiovascular mortality observed in NAFLD.[7]

In our study, carotid IMT was noticeably higher in NAFLD patients than in sex- and age-matched control subjects. Furthermore, by logistic regression with adjustment for various confounders, the presence of NAFLD was associated with an abnormal IMT independently of MetS and all its traits, regardless of definition. Moreover, sex- and age-adjusted IMT increased in the sequence: control without MetS<control with MetS<NAFLD without MetS<NAFLD with MetS (Figure 2). These findings suggest that NAFLD is atherogenic beyond its association with MetS. However, to prove this contention, larger numbers of subjects with and without NAFLD and with and without MetS need to be studied for carotid atherosclerosis or other cardiovascular risk markers.

As opposed to control subjects, women with NAFLD had carotid atherosclerosis to a similar or even higher extent than men with NAFLD (Figure 1). These observations agree with studies showing that several MetS traits[23,24] or MetS by itself[21] have a stronger effect on CHD risk among women than men.

A probable mechanistic explanation for the marked proatherogenic effect of NAFLD is the enhanced oxidative stress characteristic of this condition, which is believed to play a role in the progression from hepatic steatosis to steatohepatitis, fibrosis, and cirrhosis.[1–4,25] Reactive oxygen species derived from steatosis-stimulated fatty acid oxidation, attendant hepatocyte injury and cytokine release, and the ensuing inflammatory milieu are likely to perpetuate the liver disease of NAFLD and add additional atherogenic stimuli to the already high oxidative/inflammatory status associated with MetS and epitomized by an elevated CRP serum level.[26–28] CRP was higher in NAFLD patients than in control subjects in our study. As expected, the CRP level was strongly associated with adiposity measures and insulin resistance. However, CRP was also associated with ALT, the best serum marker of hepatic inflammation, and with AAT, a serine proteinase inhibitor and acute-phase reactant predominantly synthesized in the liver.[29] Together, these findings suggest that hepatic injury contributed to the inflammatory status in NAFLD.

Another potential mechanism by which NAFLD may increase cardiovascular risk beyond that imposed by MetS is abnormal lipoprotein metabolism. In NAFLD, hepatic apoB synthesis, a limiting step in very LDL (VLDL) formation, is reduced[30] and postprandial apoB responses are flat and strikingly dissociated from triglyceride increases.[31] Disturbances of VLDL assembly in NAFLD could be causal to the development of hepatic steatosis. Importantly, impaired VLDL secretion also results in a lower number of circulating particles that are large, triglyceride-rich, and highly atherogenic.[32,33] Other conditions characterized by hepatic steatosis or impaired liver function, such as preeclampsia[34] and fatty liver of pregnancy,[35] also feature an accumulation of triglyceride-rich VLDL and remnants in the circulation. Our patients with NAFLD had elevated triglycerides, but the total serum apoB level was similar to control values (Table 1), suggesting the presence of triglyceride-rich lipoproteins. However, detailed lipoprotein compositional studies should be performed in NAFLD to prove this contention.

Ferritin serum levels were moderately increased in NAFLD patients compared with control subjects. The main function of ferritin is the storage and delivery of iron for cellular use, and the serum ferritin concentration reflects the level of total body iron stores. However, ferritin is also an acute-phase reactant that may increase in response to infection, inflammation, and other stimuli.[36] Subjects with iron overload suggestive of hereditary hemochromatosis were excluded by study design. There are no evidences for a role of excess iron in the pathogenesis of NAFLD[3,5,15]; presumably, a higher ferritin level in these patients could be linked to the existence of an inflammatory milieu associated with liver cell steatosis and necrosis. The finding in our study that the ferritin level was strongly correlated with markers of liver cell injury supports this theory.

Elevated serum ferritin was strongly and independently associated with an abnormal IMT and carotid plaque in our study. After much research and debate, the results to date do not support the theory that iron status is related to CHD.[37,38] Although the observed association between ferritin and carotid atherosclerosis might add to the controversy, an alternate explanation for this finding involves the inflammatory state intimately linked to atherosclerosis: because ferritin genes are upregulated by inflammatory cytokines and are susceptible to induction in the course of plaque formation,[39] the elevated serum ferritin level might just be reactive to the atherogenic process.

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

Acknowledgments

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

References

1. Angulo P. Nonalcoholic fatty liver disease. *N Engl J Med*. 2002;346: 1221–1231.
2. McCullough AJ. Update on nonalcoholic fatty liver disease. *J Clin Gastroenterol*. 2002;34:255–262.
3. Brunt EM. Nonalcoholic steatohepatitis. *Semin Liver Dis*. 2004;24:3–20.
4. Younossi Z, Diehl AM, Ong JP. Nonalcoholic fatty liver disease: an agenda for clinical research. *Hepatology*. 2002;35:746–752.
5. Marchesini G, Brizi M, Bianchi G, Tomassetti S, Bugianesi E, Lenzi M, McCullough AJ, Natale S, Forlani G, Melchionda **N.** Nonalcoholic fatty liver disease. A feature of the metabolic syndrome. *Diabetes*. 2001;50: 1844–1850.
6. Grundy SM, Brewer HB, Cleeman JI, Smith SC, Lenfant C, for the Conference participants. Definition of metabolic syndrome. Report of the National Heart, Lung, and Blood Institute/American Heart Association Conference on Scientific Issues Related to Definition. *Circulation*. 2004; 109:433–438.
7. Matteoni CA, Younossi ZM, Gramlich T, Boparai N, Liu YC, McCullough AJ. Nonalcoholic fatty liver disease: a spectrum of clinical and pathological severity. *Gastroenterology*. 1999;116:1413–1419.
8. O'Leary DH, Polak JF. Intima-media thickness: a tool for atherosclerosis imaging and event prediction. *Am J Cardiol*. 2002;90(suppl 10C): 18L–21L.
9. World Health Organization. *AUDIT. The Alcohol Use Disorders Identification Test: Guidelines for use in Primary Health Care*. Geneva, Switzerland: WHO; 1992.
10. Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. Homeostasis model assessment: insulin resistance and beta-cell function from plasma fasting glucose and insulin concentrations in man. *Diabetologia*. 1985;28:412–419.
11. Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults. Executive Summary of the Third Report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III). *J Am Med Assoc*. 2001;285: 2486–2497.
12. World Health Organization. *Definition, Diagnosis, and Classification of Diabetes Mellitus and Its Complications. Part 1: Diagnosis and Classification of Diabetes Mellitus.* Geneva, Switzerland: WHO; 1999.
13. Ridker PM. Clinical application of C-reactive protein for cardiovascular disease detection and prevention. *Circulation*. 2003;107:363–369.
14. Pagano G, Pacini G, Musso G, Gambino R, Mecca F, Depetris N Cassader M, David E, Cavallo-Perin P, Rizzetto M. Nonalcoholic steatohepatitis, insulin resistance and metabolic syndrome: further evidence for an etiologic association. *Hepatology*. 2002;35:367–372.
15. Marchesini G, Bugianesi E, Forlani G, Cerrelli F, Lenzi M, Manini R, Natale S, Vanni E, Villanova N, Melchionda N, Rizzetto M. Nonalcoholic fatty liver, steatohepatitis, and the metabolic syndrome. *Hepatology*. 2003;37:917–923.
16. Caturelli E, Squillante MM, Andriulli A, Cedrone A, Cellerino C, Pompili M, Manoja ER, Rapaccini GL. Hypoechoic lesions in the "bright liver": a reliable indicator of fatty change. A prospective study. *J Gastroenterol Hepatol*. 1992;7:469–472.
17. 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.
18. Bonora E, Targher G, Alberiche M, Bonadonna RC, Saggiani F, Zenere MB, Monauni T, Muggeo M. Homeostasis model assessment closely mirrors the glucose clamp technique in the assessment of insulin sensitivity: studies in subjects with various degrees of glucose tolerance and insulin sensitivity. *Diabetes Care*. 2000;23:57–63.
19. Lakka HM, Laaksonen DE, Lakka TA, Niskanen LK, Kumpusalo E, Tuomilehto J, Salonen JT. The metabolic syndrome and total and cardiovascular disease mortality in middle-aged men. *J Am Med Assoc*. 2002; 288:2709–2716.
20. Malik S, Wong ND, Franklin SS, Kamath TV, L'Italien GJ, Pio JR, Williams GR. Impact of the metabolic syndrome on mortality from coronary heart disease, cardiovascular disease, and all causes in United States adults. *Circulation*. 2004;110:1245–1250.
21. Hunt KJ, Resendez RG, Williams K, Haffner SM, Stern MP. National Cholesterol Education Program versus World Health Organization metabolic syndrome in relation to all-cause and cardiovascular mortality in the San Antonio Heart Study. *Circulation*. 2004;110:1251–1257.
22. Bonora E, Kiechl S, Willeit J, Oberhollenzer F, Egger G, Bonadonna RC, Muggeo M. Carotid atherosclerosis and coronary heart disease in the metabolic syndrome. *Diabetes Care*. 2003;26:1251–1257.
23. Kannel WB, McGee DL. Diabetes and glucose intolerance as risk factors for cardiovascular disease: the Framingham Study. *Diabetes Care*. 1979; 2:120–126.
24. Austin MA, Hokanson JE, Edwards KL. Hypertriglyceridemia as a cardiovascular risk factor. *Am J Cardiol*. 1998;81:7B–12B.
25. Sanyal AJ, Campbell-Sargent C, Mirshahi F, Rizzo WB, Contos MJ, Sterling RK, Luketic VA, Shiffman ML, Clore JN. Nonalcoholic steatohepatitis: association of insulin resistance and mitochondrial abnormalities. *Gastroenterology*. 2001;120:1183–1192.
26. Yudkin JS, Stehouwer CD, Emeis JJ, Coppack SW. C-reactive protein in healthy subjects: associations with obesity, insulin resistance, and endothelial dysfunction: a potential role for cytokines originating from adipose tissue? *Arterioscler Thromb Vasc Biol*. 1999;19:972–978.
27. Festa A, D'Agostino, Howard G, Mykkanen L, Tracy RP, Haffner SM. Chronic subclinical inflammation as part of the insulin resistance syndrome: the Insulin Resistance Atherosclerosis Study (IRAS). *Circulation*. 2000;102:42–47.
28. Ridker PM, Buring JE, Cook NR, Rifai N. C-reactive protein, the metabolic syndrome, and risk of incident cardiovascular events. An 8-year follow-up of 14 719 initially healthy American women. *Circulation*. 2003;107:391–397.
29. Janciauskiene S. Conformational properties of serine proteinase inhibitors (serpins) confer multiple pathophysiological roles. *Biochim Biophys Acta*. 2001;1535:221–235.
30. Charlton M, Sreekumar R, Rasmussen D, Lindor K, Nair KS. Apolipoprotein synthesis in non-alcoholic steatohepatitis. *Hepatology*. 2002; 35:898–904.
31. Musso G, Gambino R, de Michieli F, Cassader M, Rizetto M, Durazzo M, Fagà E, Silli B, Pagano G. Dietary habits and their relations to insulin resistance and postpradial lipemia in non-alcoholic steatohepatitis. *Hepatology*. 2003;37:909–916.
32. Gianturco SH, Bradley WA. Lipoprotein-mediated cellular mechanisms for atherogenesis in hypertriglyceridemia. *Semin Thromb Hemostasis*. 1988;14:165–169.
33. Packard CJ, Shepherd J. Lipoprotein heterogeneity and apolipoprotein B metabolism. *Arterioscler Thromb Vasc Biol*. 1997;17:3542–3556.
34. Winkler K, Wetzka B, Hoffmann MM, Friedrich I, Kinner M, Baumstark MW, Zahradnik HP, Wieland H, März W. Triglyceride-rich lipoproteins are associated with hypertension in preeclampsia. *J Clin Endocrinol Metab*. 2003;88:1162–1166.
35. Wetzka B, Hoffmann MM, Friedrich I, Baumstark MW, Zahradnik HP, März W, Winkler K. Transient remnant removal disease in acute fatty liver of pregnancy. *Hypertens Pregnancy*. 2004;23:143–153.
36. Worwood M. Laboratory determination of iron status. In: Brock JH, Halliday JW, Pippard MJ, Powell LW, eds. *Iron Metabolism in Health and Disease*. London, UK: WB Saunders; 1994:449–476.
37. Danesh J, Appleby P. Coronary heart disease and iron status: meta-analyses of prospective studies. *Circulation*. 1999;99:852–854.
38. Ma J, Stampfer MJ. Body iron stores and coronary heart disease. *Clin Chem*. 2002;48:601–603.
39. Pang JHS, Jiang MJ, Chen YL, Wang FW, Wang DL, Chu SH, Chau LY. Increased ferritin gene expression in atherosclerotic lesions. *J Clin Invest*. 1996;97:2204–2212.
40. Targher G, Bertolini L, Padovani R, Zenari L, Zoppini G, Falezza G. Relation of nonalcoholic hepatic steatosis to early carotid atherosclerosis in healthy men. *Diabetes Care*. 2004;27:2498–2500.