Should Nonalcoholic Fatty Liver Disease Be Included in the Definition of Metabolic Syndrome?

A cross-sectional comparison with Adult Treatment Panel III criteria in nonobese nondiabetic subjects

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OBJECTIVE — The ability of the Adult Treatment Panel III (ATP III) criteria of metabolic syndrome to identify insulin-resistant subjects at increased cardiovascular risk is suboptimal, especially in the absence of obesity and diabetes. Nonalcoholic fatty liver disease (NAFLD) is associated with insulin resistance and is emerging as an independent cardiovascular risk factor. We compared the strength of the associations of ATP III criteria and of NAFLD to insulin resistance, oxidative stress, and endothelial dysfunction in nonobese nondiabetic subjects.

RESEARCH DESIGN AND METHODS — Homeostasis model assessment of insulin resistance (HOMA-IR) >2, oxidative stress (nitrotyrosine), soluble adhesion molecules (intracellular adhesion molecule-1, vascular cell adhesion molecule-1, and E-selectin), and circulating adipokines (tumor necrosis factor-α, leptin, adiponectin, and resistin) were cross-sectionally correlated to ATP III criteria and to NAFLD in 197 unselected nonobese nondiabetic subjects.

RESULTS — NAFLD more accurately predicted insulin resistance than ATP III criteria: sensitivity 73 vs. 38% ($P = 0.0001$); positive predictive value: 81 vs. 62% ($P = 0.035$); negative predictive value 87 vs. 74% ($P = 0.012$); positive likelihood ratio 4.39 vs. 1.64 ($P = 0.0001$); and negative likelihood ratio 0.14 vs. 0.35 ($P = 0.0001$). Adding NAFLD to ATP III criteria significantly improved their diagnostic accuracy for insulin resistance. Furthermore, NAFLD independently predicted HOMA-IR, nitrotyrosine, and soluble adhesion molecules on logistic regression analysis; the presence of NAFLD entailed more severe oxidative stress and endothelial dysfunction, independent of adiposity or any feature of the metabolic syndrome in insulin-resistant subjects.

CONCLUSIONS — NAFLD is more tightly associated with insulin resistance and with markers of oxidative stress and endothelial dysfunction than with ATP III criteria in nonobese nondiabetic subjects and may help identify individuals with increased cardiometabolic risk in this population.

Diabetes Care **31**:562–568, 2008

Metabolic syndrome is a cluster of metabolic and cardiovascular risk factors that sharing the hallmark of insulin resistance; its prevalence according to Adult Treatment Panel III (ATP III) criteria is 20% among Western adults (1). Insulin resistance is an independent predictor of cardiovascular disease and type 2 diabetes and should be identified and treated early (2–5).

Nonalcoholic fatty liver disease (NAFLD) is the most common chronic liver disease in Western countries (1). NAFLD predicts incident diabetes independent of classic risk factors and C-reactive protein in large prospective cohort studies and may therefore be an early marker of mechanisms predisposing to future metabolic events (1,6). In a parallel way, NAFLD is emerging as a marker of early atherosclerosis: liver enzymes predicted incident cardiovascular disease independent of traditional risk factors, C-reactive protein and metabolic syndrome, and liver histology correlated with early carotid atherosclerosis in NAFLD, suggesting that the vessels and the liver share common inflammatory mediators (7–9).

Despite the number of studies connecting fatty liver to insulin resistance, it is still unclear whether a diagnosis of NAFLD can help identify apparently healthy individuals with an increased cardiometabolic risk more accurately than current diagnostic criteria. ATP III criteria for metabolic syndrome correlate fairly well with insulin resistance (sensitivity of 46% and specificity of 76% for insulin resistance, respectively) in the general population, and this association is weaker in the absence of obesity and diabetes (10–13).

By directly comparing subjects with NAFLD with insulin-resistant subjects without fatty liver, we tested the hypotheses that *1*) NAFLD might be more tightly associated with insulin resistance than current ATP III criteria in nonobese nondiabetic subjects and *2*) fatty liver per se

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Received for publication 3 August 2007 and accepted in revised form 19 November 2007.

Published ahead of print at http://care.diabetesjournals.org on 4 December 2007. DOI: 10.2337/dc07-1526.

Additional information for this article can be found in an online appendix at http://dx.doi.org/10.2337/dc07-1526.

Abbreviations: ALT, alanine aminotransferase; ATP III, Adult Treatment Panel III; HOMA-IR, homeostasis model assessment of insulin resistance; ICAM-1, intercellular adhesion molecule-1; NAFLD, nonalcoholic fatty liver disease; OGTT, oral glucose tolerance test; TNF-α, tumor necrosis factor-α; VCAM-1, vascular cell adhesion molecule-1.

conveys a higher cardiovascular risk in otherwise healthy insulin-resistant subjects, independent of insulin resistance, metabolic syndrome, and circulating adipokines.

RESEARCH DESIGN AND METHODS

— A total of 197 Caucasians (87 women, age range 24–63 years, BMI range 19.9–29.9 kg/m^2) were selected from a population-based cohort participating in previous institutional studies over the past 5 years (14). All were nondiabetic and nonobese, in good general health, and with normal findings on medical history, physical examination, blood count, and chemical screening battery. Insulin resistance was defined by a homeostasis model assessment of insulin resistance (HOMA-IR) index >2. The HOMA-IR index closely correlated with clamp measures in nondiabetic Northern Italian subjects (15) and with oral glucose tolerance test (OGTT)-derived indexes of insulin sensitivity in our subjects with NAFLD (G.M., unpublished data); furthermore, a cutoff value >2 predicted insulin resistance and increased cardiovascular risk in the general population independent of traditional risk factors (2–4,14,15).

NAFLD was diagnosed by persistently (>6 months) elevated aminotransferases (defined by alanine aminotransferase [ALT] ≥30 units/l in men and ≥20 units/l in women, based on recently proposed cutoff values, which increase the sensitivity for detection of NAFLD) (16,17) and ultrasonographic bright liver with no other liver disease; histological confirmation was available for 66% of the subjects. Exclusion criteria were a history of alcohol consumption >70 g/week (assessed by a detailed inquiry of patients and relatives and a validated questionnaire filled in daily for 1 week by the patients); diabetes (fasting plasma glucose ≥126 mg/dl or ≥200 mg/dl at +2 h with a standard oral glucose load on an OGTT); obesity (BMI ≥30 kg/m^2); positive serum markers of viral disease; and exposure to occupational hepatotoxins or to drugs known to be steatogenic or to affect glucose metabolism.

Modified ATP III criteria for the diagnosis of metabolic syndrome were the following: hypertension (systolic/diastolic blood pressure ≥130/85 mmHg or receiving antihypertensive therapy); hypertriglyceridemia (fasting plasma triglycerides ≥150 mg/dl [1.7 mmol/l] or receiving lipid-lowering therapy; low plasma HDL cholesterol (<40 mg/dl [1.03 mmol/l] in men and <50 mg/dl [1.29 mmol/l]) in women]); impaired glucose regulation (impaired fasting glycemia, i.e., fasting plasma glucose ≥100 but <126 mg/dl [5.6–7.0 mmol/l] or impaired glucose tolerance, i.e., plasma glucose ≥140 mg/dl [7.8 mmol/l] at +2 h on an OGTT]; or abdominal obesity, modified according to ethnic-specific cutoff values to increase sensitivity for "metabolically obese" lean subjects (for Europeans, cutoff ranges were a waist circumference >94 cm [37 inches] in men and >80 cm [31 inches] in women) (12). A diagnosis of metabolic syndrome required fulfillment of at least three criteria.

Table 1—*Main characteristics of study subjects according to HOMA-IR index*

	Insulin sensitive: HOMA-IR ≤2	Insulin resistant: HOMA-IR >2	*P* value
n	129	68	
Age (years)	47 ± 12	44 ± 10	0.083
Sex (male/female)	72/57	38/30	0.928
BMI (kg/m^2)	25.0 ± 2.0	25.8 ± 2.2	0.030
Overweight (% subjects)	39	71	0.000
% Smokers	40	38	0.931
Waist (cm)	88 ± 8	92 ± 8	0.005
Systolic BP (mmHg)	130 ± 14	131 ± 16	0.700
Diastolic BP (mmHg)	81 ± 8	87 ± 9	0.009
Triglycerides (mmol/l)	1.32 ± 0.59	1.47 ± 0.75	0.198
Total cholesterol (mmol/l)	5.53 ± 0.96	5.69 ± 1.06	0.126
HDL cholesterol (mmol/l)	1.53 ± 0.28	1.29 ± 0.28	0.0002
Triglyceride-to-HDL ratio	2.2 ± 1.2	2.7 ± 1.6	0.037
LDL cholesterol (mmol/l)	3.39 ± 0.85	3.52 ± 0.93	0.406
Glucose (mmol/l)	4.83 ± 0.68	5.08 ± 0.62	0.032
Insulin (pmol/l)	32.2 ± 12.0	116.7 ± 55.2	0.0002
HOMA-IR	0.90 ± 0.67	3.74 ± 2.01	0.0001
AST (units/l)	17 ± 9	29 ± 16	0.0001
ALT (units/l)	24 ± 11	61 ± 16	0.0001
GGT (units/l)	27 ± 31	76 ± 63	0.0001
Resistin (ng/ml)	3.35 ± 0.22	3.62 ± 0.24	0.729
Adiponectin (ng/ml)	9851 ± 639	5401 ± 349	0.0002
Leptin (pg/ml)	7988 ± 1289	8247 ± 1502	0.863
TNF-α (pg/ml)	1.35 ± 0.18	1.49 ± 0.24	0.546
C-reactive protein (mg/l)	1.11 ± 1.01	2.96 ± 1.86	0.002
Nitrotyrosine(mmol/ml)	5.1 ± 6.0	19.9 ± 17.6	0.00001
E-selectin (mg/ml)	22.7 ± 11.5	36.1 ± 10.9	0.0001
ICAM-1 (mg/ml)	203.6 ± 46.4	241.8 ± 28.9	0.0002
VCAM-1 (mg/ml)	448.6 ± 155.4	486.7 ± 138.6	0.043
Metabolic syndrome (ATP III) (% subjects)	11	38	0.0003
NAFLD (% subjects)	8	73	0.0001

Data are means ± SD unless indicated otherwise. AST, aspartate aminotransferase; BP, blood pressure; GGT, γ-glutamyl transferase.

Oxidative stress

Nitrosative stress is believed to be involved in diabetic endothelial dysfunction and cardiovascular complications (1) and in liver oxidative injury in NAFLD (18,19). Fasting plasma nitrotyrosine, as a marker of nitrosative stress, was determined by a commercial ELISA kit product by HyCult Biotechnology (Pantec, Turin, Italy).

Endothelial dysfunction

Soluble adhesion molecules E-selectin, vascular cell adhesion molecule-1 (VCAM-1), and intercellular adhesion molecule 1 (ICAM-1) have been associated with endothelial dysfunction and early cardiovascular disease (20,21). Serum E-selectin, VCAM-1, and ICAM-1 levels were measured by a solid-phase ELISA (R&D Systems, Minneapolis, MN). Minimal detectable doses and intra- and interassay coefficients of variation (CVs) were, respectively, <0.1 ng/ml, 4.7–5.0%, and 7.4–8.8%, 0.17–1.26 pg/ml,

Table 2—*Comparison of the ability of the individual components of the metabolic syndrome, of metabolic syndrome overall, and of NAFLD to identify insulin-resistant subjects*

	No. of case subjects								
	Total	IR	IS	Sensitivity × IR	Specificity × IR	PPV × IR	NPV × IR	LR+ × IR	LR− × IR
Obesity	77	35	42	0.54 (0.40–0.68)	0.68 (0.59–0.77)	0.46 (0.33–0.59)	0.75 (0.66–0.84)	0.84 (0.71–0.97)	0.33 (0.29–0.49)
IGR	76	35	41	0.54 (0.40–0.68)	0.69 (0.59–0.78)	0.46 (0.33–0.59)	0.75 (0.66–0.84)	0.87 (0.66–0.84)	0.33 (0.26–0.40)
Triglycerides	46	22	24	0.33 (0.20–0.47)	0.82 (0.75–0.90)	0.48 (0.31–0.66)	0.71 (0.63–0.80)	0.94 (0.73–1.15)	0.40 (0.32–0.48)
HDL	16	13	3	0.21 (0.09–0.32)	0.98 (0.96–1.00)	0.83 (0.67–0.99)	0.71 (0.64–0.79)	5.00 (4.00–6.00)	0.40 (0.33–0.47)
Hypertension	103	47	57	0.73 (0.60–0.85)	0.57 (0.47–0.67)	0.45 (0.34–0.57)	0.81 (0.72–0.90)	0.83 (0.70–0.95)	0.23 (0.19–0.27)
Overall cases of MS:									
ATP III	40	26	14	0.38 (0.24–0.51)	0.89 (0.82–0.95)	0.62 (0.44–0.80)	0.74 (0.66–0.82)	1.64 (1.16–2.12)	0.35 (0.30–0.40)
NAFLD	59	49	10	0.73 (0.60–0.85)*	0.92 (0.86–0.97)	0.81 (0.70–0.93)†	0.87 (0.81–0.94)†	4.90 (4.01–5.79)*	0.14 (0.12–0.16)*
Overall cases of MS: ATP III + NAFLD	64	47	17	0.69 (0.58–0.84)*	0.87 (0.80–0.93)	0.72 (0.59–0.85)	0.85 (0.78–0.92)‡	2.53 (2.07–2.99)†	0.18 (0.16–0.20)*

95% CIs are reported in parentheses. *$P < 0.001$ vs. ATP III criteria. †$P < 0.01$ vs. ATP III criteria. ‡$P < 0.05$ vs. ATP III criteria. IGR, impaired glucose regulation; IR, insulin resistance; IS, insulin sensitive; LR+, positive likelihood ratio; LR−, negative likelihood ratio; MS, metabolic syndrome; NPV, negative predictive value; PPV, positive predictive value.

2.3–3.6%, and 5.5–7.8%, and <0.1 ng/ml, 4.7–5.0%, and 7.4–8.8%.

Adipokines

Serum tumor necrosis factor-α (TNF-α), leptin, and adiponectin were measured by a sandwich ELISA (R&D Systems Europe, Abingdon, UK). For TNF-α the kit has a sensitivity of 0.12 pg/ml in a 200-μl sample size and a range of 0.5 to 32 pg/ml. Intra- and interassay CVs were 5.9 and 12.6%, respectively. For adiponectin, the kit has a sensitivity of 0.25 pg/ml in a 50-μl sample size and a range of 3.9 to 250 ng/ml. The intra- and interassay CVs were 3.4 and 5.8%, respectively. Resistin was measured by a biotin-labeled antibody-based sandwich enzyme immunoassay (BioVendor Laboratory Medicine, Brno, Czech Republic).

Statistics

Data are expressed as means ± SD. Differences between groups were analyzed by ANOVA when variables were normally distributed; otherwise (for triglycerides, insulin, HOMA-IR, adipokines, nitrotyrosine, and adhesion molecules), the Mann-Whitney test was used. A χ^2 test was used to compare categorical variables. Normality was evaluated by the Shapiro-Wilk test. For multiple comparisons, ANOVA and the Kruskal-Wallis test, followed by the Bonferroni correction or a Dunn test, were used, as appropriate. Spearman correlation coefficients were used to estimate the relationship between variables. Logistic regression analysis was applied when multiple associations were detected on univariate analysis. Differences were considered statistically significant if $P < 0.05$.

Sensitivity, specificity, positive and negative predictive value, and likelihood ratios of different criteria for the presence of insulin resistance were calculated. The positive likelihood ratio is the true-positive rate divided by the false-positive rate; the negative likelihood ratio is the inverse of the true-negative rate divided by the false-negative rate. For each parameter 95% CIs were provided.

RESULTS — Of the subjects, 50% were overweight (39% of insulin-sensitive and 71% of insulin-resistant subjects) (Table 1). The prevalence of NAFLD was 30% (8% in insulin-sensitive and 73% in insulin-resistant subjects). Insulin-resistant subjects also had higher circulating markers of oxidative stress and

Table 3—*Main characteristics of insulin-resistant subjects grouped according to the presence of NAFLD*

	IR, NAFLD	IR, no NAFLD	*P* value
n			
Age (years)	45 ± 10	48 ± 7	0.313
Sex (male/female)	29/5	10/6	0.277
BMI (kg/m^2)	25.6 ± 2.1	26.3 ± 2.5	0.350
Overweight (% subjects)	61	74	0.786
Smokers (%)	35	42	0.921
Waist (cm)	92 ± 7	94 ± 8	0.112
Systolic BP (mmHg)	130 ± 14	128 ± 14	0.468
Diastolic BP (mmHg)	87 ± 8	87 ± 12	0.920
Triglycerides (mmol/l)	1.34 ± 0.69	1.78 ± 0.83	0.091
Total cholesterol (mmol/l)	6.39 ± 1.24	5.64 ± 0.98	0.282
HDL cholesterol (mmol/l)	1.27 ± 0.21	1.42 ± 0.39	0.173
Triglyceride-to-HDL ratio	2.5 ± 1.4	3.2 ± 2.3	0.252
LDL cholesterol (mmol/l)	3.69 ± 0.72	3.36 ± 0.98	0.195
Glucose (mmol/l)	5.14 ± 0.61	4.89 ± 0.83	0.189
Insulin (pmol/l)	125.6 ± 68.9	105.7 ± 59.1	0.279
HOMA-IR	3.78 ± 2.27	2.88 ± 2.06	0.178
AST (units/l)	35 ± 12	15 ± 5	0.0001
ALT (units/l)	78 ± 21	17 ± 9	0.0001
GGT (units/l)	97 ± 89	21 ± 12	0.0003
C-reactive protein (mg/l)	2.91 ± 1.25	2.02 ± 1.12	0.391
Nitrotyrosine(mmol/ml)	27.1 ± 18.9	9.9 ± 7.6	0.002
E-selectin (mg/ml)	51.3 ± 17.1	32.7 ± 16.6	0.004
ICAM-1(mg/ml	261.2 ± 38.5	225.6 ± 24.0	0.002
VCAM-1(mg/ml)	512.5 ± 141.5	474.2 ± 113.9	0.191
Abdominal obesity (% subjects)	50	64	0.559
Impaired glucose regulation (% subjects)	78	44	0.115
Hypertension (% subjects)	74	71	0.835
Hypertriglyceridemia (% subjects)	29	43	0.217
Low HDL cholesterol (% subjects)	24	14	0.745
Metabolic syndrome (ATP III) (% subjects)	32	50	0.412

Data are means ± SD unless indicated otherwise. AST, aspartate aminotransferase; GGT, γ-glutamyl transferase; IR, insulin resistance.

endothelial dysfunction and lower adiponectin levels.

The prevalence of different features of the metabolic syndrome and of NAFLD were higher in insulin-resistant subjects. Seventy-three percent of insulin-resistant versus 43% of insulin-sensitive subjects were hypertensive ($P = 0.001$), 54% of insulin-resistant versus 32% of insulin-sensitive subjects had abdominal obesity ($P = 0.017$), 33% of insulin-resistant versus 18% of insulin-sensitive subjects were hypertriglyceridemic ($P = 0.054$), 21% of insulin-resistant versus 2% of insulin-sensitive subjects had low HDL cholesterol, and 54% of insulin-resistant versus 31% of insulin-sensitive control subjects had impaired glucose regulation ($P = 0.012$). The prevalence of metabolic syndrome was 20% according to ATP III criteria (12).

Relationship of ATP III criteria and NAFLD to insulin resistance

Table 2 shows the relationship of different ATP III criteria and of NAFLD to insulin resistance. Hypertension and NAFLD were the most sensitive (73% for both) and had the highest negative predictive value (81 and 87%, respectively) for insulin resistance. The negative likelihood ratio (i.e., the extent to which the odds of insulin resistance decrease if the test result is negative) was greatest with hypertension (0.23) and NAFLD (0.14). HDL cholesterol and NAFLD were most specific for insulin resistance (98 and 92%, respectively) and had the highest positive predictive value (83 and 81%, respectively) and positive likelihood ratio (i.e., the odds of insulin resistance if the test result is positive: 5.00 and 4.39, respectively).

ATP III criteria had a positive predictive value of 62% for the presence of insulin resistance; the addition of NAFLD to ATP criteria yielded a prevalence of metabolic syndrome of 32%, with a positive predictive value for insulin resistance of 72%. The sensitivity of ATP III criteria for insulin resistance was 38%, but rose to 69% with the inclusion of NAFLD as a criterion ($P = 0.002$). The specificity of ATP III criteria for insulin resistance was 89% without NAFLD and 87% with NAFLD ($P = 0.002$). The positive likelihood ratio for insulin resistance increased from 1.64 to 2.53 when NAFLD was included ($P = 0.010$), whereas the negative likelihood ratio, i.e., the odds of insulin resistance in the absence of a certain condition, decreased from 35 to 18% with the inclusion of NAFLD in the diagnostic criteria of metabolic syndrome ($P = 0.0002$).

Insulin resistance with NAFLD versus insulin resistance without fatty liver

With subgrouping of insulin-resistant subjects according to the presence of NAFLD, patients with NAFLD had higher levels of nitrotyrosine and of soluble adhesion molecules than subjects without fatty liver (Table 3).

Correlative analysis

The main Spearman correlation coefficients are presented in Table 1 of the online appendix (available at http://dx.doi.org/10.2337/dc07-1526). HOMA-IR correlated with age, ALT (Fig. 1 of the online appendix), HDL cholesterol, triglyceride-to-HDL cholesterol ratio, C-reactive protein, adiponectin, nitrotyrosine, waist, adhesion molecules, and the number of ATP criteria.

On logistic regression analysis, HOMA-IR >2 was independently predicted by NAFLD, adiponectin, and nitrotyrosine (Table 4). Nitrotyrosine was independently predicted by NAFLD and C-reactive protein, NAFLD ($\beta = 0.36$; $P = 0.008$) and HOMA-IR independently predicted E-selectin, ICAM-1 was independently predicted by ALT and adiponectin, and adiponectin predicted VCAM-1 (Table 4).

CONCLUSIONS — By directly comparing NAFLD with ATP III criteria for metabolic syndrome in nonobese nondiabetic subjects, we found that *1)* fatty liver, as diagnosed by ALT levels and ul-

Table 4—*Logistic regression analysis for factors associated with insulin resistance (HOMA-IR >2), plasma nitrotyrosine, and soluble adhesion molecule levels in study subjects*

Factor	Odds ratio	95% CI	*P* value
Insulin resistance (HOMA-IR >2)			
Age (quartiles)	1.4	0.5–2.5	0.791
NAFLD (present vs. absent)	2.8	1.9–4.3	0.007
HDL cholesterol (quartiles)	1.7	0.6–6.2	0.100
Triglyceride-to-HDL cholesterol ratio (quartiles)	1.5	0.6–3.9	0.231
C-reactive protein (quartiles)	1.6	0.7–2.8	0.072
Adiponectin (quartiles)	1.8	1.0–3.4	0.045
Nitrotyrosine (quartiles)	2.1	1.6–3.1	0.021
Waist (quartiles)	1.1	0.5–2.2	0.692
No. ATP III criteria	1.5	0.4–2.9	0.571
Nitrotyrosine (upper quartile)			
Age (quartiles)	1.2	0.8–3.1	0.680
NAFLD (present vs. absent)	3.2	2.0–4.8	0.002
HOMA-IR (quartiles)	1.5	1.2–2.8	0.112
No. ATP III criteria	1.7	1.0–2.9	0.213
HDL cholesterol (quartiles)	1.4	0.6–3.2	0.427
C-reactive protein (quartiles)	2.0	1.5–3.9	0.038
E-selectin (upper quartile)			
NAFLD (present vs. absent)	1.9	1.5–2.6	0.009
BMI (quartiles)	1.3	0.6–2.3	0.238
TNF-α (quartiles)	1.1	0.4–2.1	0.85
Triglyceride-to-HDL cholesterol ratio (quartiles)	1.0	0.6–1.5	0.871
No. ATP III criteria	1.3	0.8–2.1	0.276
HOMA-IR (quartiles)	1.7	1.4–2.3	0.01
C-reactive protein (quartiles)	1.4	0.9–1.9	0.457
ICAM-1 (upper quartile)			
NAFLD (present vs. absent)	1.8	1.4–2.5	0.010
BMI (quartiles)	0.9	0.4–1.8	0.348
Adiponectin (quartiles)	1.7	1.3–2.1	0.015
HOMA-IR (quartiles)	1.5	1.2–2.4	0.079
No. ATP III metabolic syndrome criteria	1.2	0.9–2.9	0.575
VCAM-1 (upper quartile)			
NAFLD(present vs. absent)	1.7	1.3–2.9	0.069
Adiponectin (quartiles)	1.9	1.5–2.7	0.008
HOMA-IR (quartiles)	1.5	0.9–3.2	0.134
C-reactive protein (quartiles)	1.6	1.2–3.5	0.397

$n = 197$.

trasonography, is more closely associated to insulin resistance than to ATP III criteria and 2) the presence of NAFLD in insulin-resistant subjects implies more severe systemic oxidative stress and endothelial dysfunction, independently of metabolic syndrome, adiposity, and adipokines.

The aim of the ATP III criteria is to identify individuals at increased risk for cardiovascular disease and diabetes to allow early treatment. Current ATP III criteria were selected because they tend to cluster together, share insulin resistance as their common denominator, and have individually been associated with an increased cardiovascular risk. However, they correlate weakly with the presence of insulin resistance, having a sensitivity and positive predictive value of 46 and 76%, respectively, in the general population (10). Because of their low sensitivity, many cases of insulin resistance remain undiagnosed, particularly in nonobese nondiabetic subjects, in whom the diagnosis of metabolic syndrome is less assisted by obesity and glucose criteria (10–13).

ATP III criteria consistently identified only 39% of insulin-resistant subjects in our population, with a positive predictive value of 62% and a positive likelihood ratio of 1.64. The presence of NAFLD alone doubled the sensitivity and significantly elevated positive predictive value and positive likelihood ratio, keeping the same specificity for insulin resistance (92% vs. 89%). The addition of NAFLD to the ATP III criteria improved their sensitivity by 72%, from 39 to 69%, with only a slight decrease in specificity, from 89 to 87%. Pathogenetically, these findings suggest that in nonobese nondiabetic subjects hepatic fat accumulation is more tightly related to insulin resistance than visceral adiposity, as estimated by waist circumference or any other feature of the metabolic syndrome, as defined by ATP III criteria.

The mechanism(s) underlying the association between NAFLD and insulin resistance are under investigation, but impaired hepatic lipid and lipoprotein handling and increased oxidative stress may enhance liver fat accumulation and lead to insulin resistance by nuclear factor-κB pathway activation (22–26). Increased nitrosative stress, in particular, seems to be operating in NAFLD even in the absence of insulin resistance (27).

The second intriguing finding relates to the additive information that NAFLD carries in the setting of insulin resistance. Patients with NAFLD displayed more severe oxidative stress and endothelial dysfunction than nonsteatotic insulin-resistant subjects, despite similar HOMA-IR and adiposity, and NAFLD independently entailed more severe oxidative stress and endothelial dysfunction.

Nitrotyrosine is an index of peroxynitrite formation, which is believed to play a key role in diabetic endothelial dysfunction and in liver oxidative injury in NAFLD (18,19). Mechanisms underlying increased oxidative stress in NAFLD cannot be elucidated in our study, but impaired mitochondrial β-oxidation, dietary saturated fat excess, and reduced antioxidant intake have been proposed (22,23,26). Increased oxidative stress can induce not only steatosis but also necroinflammation and fibrosis in NAFLD: high oxidative stress experimentally impaired VLDL secretion, leading to hepatocyte triglyceride accumulation (24,28); oxidation end products trigger the inflammatory cascade and extracellular matrix deposition, paralleling the severity of liver fibrosis in NAFLD (23).

Soluble adhesion molecule levels were higher in NAFLD than in insulin resistance without fatty liver, and fatty liver predicted increased E-selectin and ICAM-1 levels independently of HOMA-IR, adipokines, and visceral fat accumulation. Plasma concentrations of these molecules were consistently related to in-

cident cardiovascular disease in apparently healthy individuals in large prospective studies (20,21). Our findings therefore suggest that NAFLD may be an early marker of endothelial dysfunction, independently of insulin resistance and traditional risk factors (7). Mechanism(s) linking NALFD to endothelial dysfunction are unclear, but impaired lipoprotein metabolism and oxidized LDL accumulation are potential candidates (22,23,26, 27).

In nonobese, nondiabetic insulin-resistant subjects, the presence of NAFLD may therefore indicate a host of unsuspected derangements in oxidative balance and endothelial function, not routinely assessed, that contribute to increased cardiovascular disease risk: in day-to-day clinical practice, a diagnosis of NAFLD would simply require chronically elevated liver enzyme levels, an ultrasonographic bright liver, and exclusion of viral infection and of exposure to hepatotoxins (including alcohol) by interview of patient and relatives. Early identification of such patients at higher cardiometabolic risk may trigger earlier lifestyle and pharmacological interventions. Consistently, therapeutic measures in NAFLD also ameliorate insulin sensitivity and cardiovascular risk profile, the ultimate goal of a diagnosis of metabolic syndrome (29–31).

Further studies are required to test the validity of this proposal and to overcome the limitations of our study: its cross-sectional nature, which prevents any causal inference, and the absence of obese and diabetic subjects. However, the prevalence of NAFLD and insulin resistance is even higher in obesity and diabetes, so the association between NAFLD and insulin resistance could be further strengthened. Consistent with previous findings, lower sex-specific ALT cutoff levels identified insulin-resistant subjects more accurately (16,17). However, because liver fat content was not directly measured, some control subjects might have fatty liver despite normal ultrasound and liver enzyme levels; even so, this definition of NAFLD proved to be useful in clinical practice. Furthermore, misclassification of NAFLD would attenuate the magnitude of the difference in oxidative stress and endothelial dysfunction observed toward the null hypothesis, making our results a conservative estimate of the relationship between NAFLD, oxidative stress, and endothelial dysfunction.

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