Original Article

Liver Markers and Development of the Metabolic Syndrome

The Insulin Resistance Atherosclerosis Study

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Nonalcoholic fatty liver disease (NAFLD) is emerging as a component of the metabolic syndrome, although it is not known whether markers of NAFLD, including elevated concentrations of aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALK), predict the development of metabolic syndrome. Our objective was to investigate the associations of elevated AST, ALT, and other liver markers, including C-reactive protein (CRP), with incident National Cholesterol Education Program–defined metabolic syndrome among 633 subjects in the Insulin Resistance Atherosclerosis Study who were free of metabolic syndrome at baseline. Insulin sensitivity (S_i) and acute insulin response (AIR) were directly measured from the frequently sampled intravenous glucose tolerance test among African-American, Hispanic, and non-Hispanic white subjects aged 40–69 years. After 5.2 years, 127 individuals had developed metabolic syndrome. In separate logistic regression models adjusting for age, sex, ethnicity, clinic, and alcohol consumption, subjects in the upper quartiles of ALT, ALK, and CRP were at significantly increased risk of incident metabolic syndrome compared with those in the lowest quartile: ALT, odds ratio 2.50 (95% CI 1.38–4.51); ALK, 2.28 (1.24–4.20); and CRP, 1.33 (1.09–1.63). Subjects in the upper quartile of the AST-to-ALT ratio were at significantly reduced metabolic syndrome risk (0.40 [0.22–0.74]). After further adjustment for waist circumference, S_i, AIR, and impaired glucose tolerance, the associations of ALT and the AST-to-ALT ratio with incident metabolic syndrome remained significant (ALT, 2.12 [1.10–4.09]; the AST-to-ALT ratio, 0.48 [0.25–0.95]). These associations were not modified by ethnicity or sex, and they remained significant after exclusion of former and heavy drinkers. In conclusion, NAFLD markers ALT and the AST-to-ALT ratio predict metabolic syndrome independently of potential confounding variables, including directly measured S_i and AIR. *Diabetes* 54:3140–3147, 2005

Metabolic syndrome, characterized by a core set of disorders, including abdominal obesity, dyslipidemia, hypertension, and hyperglycemia, has been shown to be an important predictor of type 2 diabetes (1–4) and of cardiovascular disease (1,5,6). Recent research has provided compelling evidence that a wider constellation of disorders may be part of the metabolic syndrome cluster. These proposed nontraditional components of the metabolic syndrome include subclinical inflammation, microalbuminuria, and, most recently, nonalcoholic fatty liver disease (NAFLD) (7,8).

NAFLD refers to a spectrum of disorders ranging from simple hepatic steatosis to more severe manifestations, including nonalcoholic steatohepatitis (NASH), which can progress to fibrosis, cirrhosis, and liver failure (7,8). NAFLD is diagnosed in clinical settings using liver biopsy, a technique that is not applicable in large epidemiological studies. However, subjects with NAFLD and NASH typically have elevated circulating concentrations of markers of liver injury, including aspartate aminotransferase (AST), alanine aminotransferase (ALT), and γ-glutamyltransferase (7,8), and it has been suggested that NAFLD may be the most common cause of chronically elevated transaminase levels (9,10). These observations indicate that markers of liver injury may be reliable surrogate measures of NAFLD and related conditions for large studies.

Subjects with NAFLD have been reported to have high prevalence rates of metabolic syndrome and associated disorders (11,12). Furthermore, liver markers have been shown to be associated with metabolic syndrome variables in large representative samples of the general population (10,13), and a number of studies have reported that ALT and other liver markers significantly predict incident type 2 diabetes (14–22). In light of these observations, it is of interest to determine the role of NAFLD in the early stages of the etiology of metabolic syndrome. However, whether NAFLD-associated biomarkers are prospectively associated with the development of metabolic syndrome has been evaluated in only one previous study of male Japanese office workers (22). The inflammatory marker C-reactive protein (CRP) is also relevant in this context

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Received for publication 20 May 2005 and accepted in revised form 4 August 2005.

AIR, acute insulin response; ALK, alkaline phosphatase; ALT, alanine aminotransferase; AROC, area under the receiver operator characteristic; AST, aspartate aminotransferase; CRP, C-reactive protein; IGT, impaired glucose tolerance; NAFLD, nonalcoholic fatty liver disease; NASH, nonalcoholic steatohepatitis; NCEP, National Cholesterol Education Program.

given its hepatic origin, elevated concentrations in metabolic syndrome (23,24), and prospective association with the development type 2 diabetes (25,26). The specific objective of this study, therefore, was to investigate the prospective associations of liver markers, including elevated concentrations of ALT and AST, as well as CRP, with risk of incident metabolic syndrome among subjects in the Insulin Resistance Atherosclerosis Study (IRAS) who were known to be free of metabolic syndrome at baseline.

RESEARCH DESIGN AND METHODS

The IRAS is a multicenter observational epidemiological study of the relationships between insulin resistance, cardiovascular disease, and its known risk factors in different ethnic groups and varying states of glucose tolerance. The design and methods of this study have been described in detail in previous publications (27). Briefly, the study was conducted at four clinical centers. At centers in Oakland and Los Angeles, California, non-Hispanic whites and African Americans were recruited from Kaiser Permanente, a nonprofit health maintenance organization. Centers in San Antonio, Texas, and San Luis Valley, Colorado, recruited non-Hispanic whites and Hispanic Americans from two ongoing population-based studies (the San Antonio Heart Study and the San Luis Valley Diabetes Study) (27). A total of 1,625 individuals participated in the baseline IRAS examination (56% women), which occurred between October 1992 and April 1994. The IRAS protocol was approved by local institutional review committees, and all participants provided written informed consent.

After an average of 5.2 years (range 4.5–6.6 years), follow-up examinations of this cohort were conducted using the baseline protocol (28,29). The present report includes information on 633 individuals who were free of diabetes and National Cholesterol Education Program (NCEP) metabolic syndrome at the baseline examination and for whom information was available on follow-up NCEP metabolic syndrome status and other variables of interest. The follow-up examination response rate was 83.2% in the subgroup of subjects free of metabolic syndrome at baseline, and those who attended follow-up were similar to those who did not in terms of ethnicity, sex, baseline ALT, and BMI (all $P > 0.10$).

The IRAS protocol required two visits, 1 week apart, of ~4 h each. Subjects were asked before each visit to fast for 12 h, to abstain from heavy exercise and alcohol for 24 h, and to refrain from smoking the morning of the examination. During the first visit, a 75-g oral glucose tolerance test was administered, with glucose tolerance status determined using World Health Organization criteria (30). During the second visit, insulin sensitivity (S_i) and insulin secretion were determined using a frequently sampled intravenous glucose tolerance test, the methods for which have been described in detail previously (20,27,29). Height and weight were measured to the nearest 0.5 cm and 0.1 kg, respectively. BMI was calculated as weight in kilograms divided by the square of height in meters (kg/m^2) and was used as an estimate of overall adiposity. Waist circumference, a validated estimate of visceral adiposity (31), was measured to the nearest 0.5 cm using a steel tape (27). Duplicate measures of anthropometry were made using a standardized protocol, and averages were used in the analysis. Ethnicity, alcohol intake, and prevalent cardiovascular disease (defined as previous heart attack, stroke, coronary artery bypass surgery, angioplasty, or endarterectomy) were assessed by self-report (27). The alcohol intake questionnaire has been described in detail previously (32). In brief, using a 10-item instrument, individuals were asked to report their current usual alcohol intake over the previous month, including beverage type. Alcohol intake was categorized as never, ex-drinker, very little, ≤0.5 drinks/day, 0.5 to <1 drink/day, 1 to <3 drinks/day, and ≥3 drinks/day.

Glucose concentration was determined using standard methods as described previously (27). Insulin levels were measured using the dextran-charcoal radioimmunoassay (33), which has a 19% external coefficient of variation (CV). This assay displays a high degree of cross-reactivity with proinsulin. Plasma lipid and lipoprotein concentrations were determined from fasting plasma samples at the central IRAS laboratory (Medlantic Research Institute, Washington, DC), using the Lipid Research Clinics methodology. AST, ALT, alkaline phosphatase (ALK), and total bilirubin were measured using standard clinical methods at the central IRAS laboratory with a Paramax PLA instrument (Baxter). CRP was measured using an in-house ultrasensitive competitive immunoassay (antibodies and antigens from Calbiochem, La Jolla, CA), with intra-assay and combined CVs of 3.1–4.0 and 3.5–4.6%, respectively (28).

Definition of the metabolic syndrome. Metabolic syndrome was defined at baseline and follow-up according to NCEP–Adult Treatment Panel III criteria as the presence of three or more of the following risk factors: abdominal obesity (waist circumference >102 cm in men or >88 cm in women), triglycerides ≥150 mg/dl, HDL cholesterol <40 mg/dl (men) or <50 mg/dl (women), blood pressure ≥130/≥85 mmHg, and fasting glucose ≥110 mg/dl (34). Subjects taking antihypertensive medication were considered to have hypertension.

Statistical analyses. The distributions of continuous variables were evaluated, and transformations were used in the analysis as required, with back-transformed values presented in the tables. For cross-sectional comparisons (Table 1), concentrations of liver markers between those with and without metabolic syndrome and its metabolic components at baseline were compared using ANCOVA adjusted for age, sex, ethnicity, and clinic.

For prospective analyses, baseline means (with SDs), medians (with interquartile range), or proportions were calculated for subjects by follow-up NCEP metabolic syndrome, with t tests, Wilcoxon tests, or χ^2 tests used to assess the statistical significance of differences. Associations of liver markers with anthropometric and metabolic variables in those free of metabolic syndrome at baseline were determined using Spearman correlation analysis. Separate multivariate logistic regression models were used to assess associations of liver markers with risk of incident metabolic syndrome, taking account of potential confounders. Each liver marker was modeled as either a continuous variable (with risk expressed per SD increase in the natural log of the marker) or as a categorical variable, comparing risk among those in the 4th quartile versus those in the 1st quartile. Three models were constructed for each liver marker: in model A, adjustments were made for age, sex, ethnicity, clinical center, and alcohol consumption; model B included additional adjustments for waist circumference and impaired glucose tolerance (IGT); model C included further adjustments for S_i and AIR. It has been pointed out that the AST-to-ALT ratio may be informative in differentiating alcoholic versus nonalcoholic liver disease (subjects with NAFLD having a ratio below 1) (5,6), and thus this ratio was considered as an additional exposure variable. In light of the well-known association between heavy drinking and liver injury, we repeated the analyses excluding subjects who had reported that they were either ex-drinkers or consumers of three or more drinks per day ("moderate/heavy drinkers"). To investigate the possibility of nonlinear associations, we also assessed risk of incident metabolic syndrome among those in the 2nd, 3rd, and 4th quartiles of the various liver markers, with subjects in quartile 1 serving as the reference category. We assessed the possibility that sex or ethnicity had modified the association between liver markers and risk of NCEP metabolic syndrome by including interaction terms in separate logistic regression models (with exposures modeled per SD increase, adjusted for demographic variables) and by plotting the odds ratios (ORs) and 95% CIs for each level of the interaction variable under consideration. Finally, we evaluated the degree to which liver markers predicted each of the individual components of the metabolic syndrome at follow-up using logistic regression.

RESULTS

The prevalence of cardiovascular disease was 2.5 and 1.8% in those with and without metabolic syndrome, respectively, at baseline ($P = 0.6$). Subjects with NCEP metabolic syndrome at baseline had significantly elevated concentrations of AST, ALT, ALK, and CRP and significantly reduced the AST-to-ALT ratio after adjustment for demographic variables (all $P < 0.01$, Table 1, *top*). Similarly, metabolic syndrome disorders, when assessed individually, were characterized by significant differences in liver markers, with ALT, the AST-to-ALT ratio, and CRP showing the strongest and most consistent results across the disorders (Table 1, *top*). These findings were essentially unchanged when the analysis was repeated excluding former and heavy drinkers (Table 1, *bottom*).

Baseline characteristics of subjects without metabolic syndrome, stratified by metabolic syndrome status at the follow-up examination, are presented in Table 2. As reported in a previous publication (35), subjects who developed metabolic syndrome at follow-up were older ($P < 0.05$); had higher BMI and waist circumference and higher concentrations of fasting and 2 h glucose, fasting insulin, and lower S_i; and were more likely to have IGT at baseline compared with those who remained free of metabolic syndrome (all $P < 0.0001$). Those who developed metabolic syndrome also had higher concentrations of ALT and ALK and lower levels of the AST-to-ALT ratio (all $P <$

TABLE 1
Concentrations of liver markers in nondiabetic subjects with and without NCEP-defined metabolic syndrome and its components at the baseline IRAS examination

Variable		AST (units/l)	ALT (units/l)	AST-to-ALT ratio (units/l)	ALK (units/l)	Bilirubin (mg/dl)	CRP (mg/l)
All subjects (*n*)		864	864	864	859	845	865
NCEP metabolic syndrome	No	20.98	15.55	1.34	60.56	0.48	1.46
	Yes	22.84*	19.60†	1.17†	63.64‡	0.51	2.78†
Fasting hyperglycemia	No	21.22	15.80	1.34	61.01	0.48	1.63
	Yes	22.64	20.47†	1.10†	62.99	0.47	2.27*
Abdominal obesity	No	20.91	15.44	1.35	59.98	0.48	1.36
	Yes	22.81*	19.44†	1.17†	64.77§	0.49	3.05†
Elevated triglyceride	No	21.12	16.09	1.31	61.34	0.47	1.58
	Yes	22.25	17.65‡	1.26	61.44	0.51‡	2.15†
Reduced HDL	No	21.52	15.69	1.37	59.44	0.47	1.50
	Yes	21.38	17.43*	1.22†	63.33*	0.50‡	1.98§
Hypertension	No	20.72	15.84	1.30	60.60	0.47	1.43
	Yes	22.37*	17.37‡	1.29	62.24	0.50‡	2.14†
Excluding former and heavy drinkers (*n*)		701	701	701	696	682	702
NCEP metabolic syndrome	No	20.67	15.49	1.33	59.67	0.48	1.41
	Yes	23.57†	20.57†	1.15†	63.86*	0.52‡	2.79†
Fasting hyperglycemia	No	21.17	15.89	1.33	60.36	0.49	1.60
	Yes	22.56	21.07†	1.07†	62.53	0.48	2.22*
Abdominal obesity	No	20.69	15.39	1.34	59.45	0.49	1.32
	Yes	23.32§	20.51†	1.14†	64.07*	0.50	3.15†
Elevated triglyceride	No	20.94	16.16	1.29	60.62	0.47	1.53
	Yes	22.52‡	18.06‡	1.25	61.34	0.52‡	2.12§
Reduced HDL	No	21.12	15.72	1.34	57.88	0.47	1.45
	Yes	21.70	17.74*	1.22*	63.58†	0.51‡	1.97§
Hypertension	No	20.53	15.77	1.30	60.30	0.47	1.40
	Yes	22.53*	17.91*	1.26	61.28	0.50	2.09†

Adjusted for age, sex, ethnicity, clinic. *$P < 0.01$, †$P < 0.0001$, ‡$P < 0.05$, §$P < 0.001$.

0.05). These patterns were similar when we excluded ex-drinkers and those reporting consumption of three or more drinks per day (Table 2).

Associations of liver markers with anthropometric and metabolic variables in those free of metabolic syndrome at baseline are presented in Table 3 (*top*). ALT, ALK, bilirubin, and CRP showed positive correlations, and the AST-to-ALT ratio inverse correlations, with anthropometric measures, fasting glucose, insulin, and triglyceride and inverse correlations with S_i and HDL. The magnitude of these associations was strongest for ALT, especially those for waist circumference, fasting glucose, and fasting insulin ($r = 0.34$, 0.25, and 0.26, respectively, all $P < 0.0001$). ALT was positively correlated, and the AST-to-ALT ratio and ALK were inversely correlated, with alcohol consumption. These correlation results were very similar when we repeated the analyses after excluding ex-drinkers and those consuming three or more drinks per day (Table 3, *bottom*).

After adjustment for age, sex, clinical center, ethnicity, and alcohol intake, the natural logs of ALT, the AST-to-ALT ratio, ALK, and CRP were significantly associated with 5-year risk of incident metabolic syndrome (ALT, OR 1.43 [95% CI 1.15–1.77]; the AST-to-ALT ratio, 0.72 [0.57–0.90]; ALK, 1.31 [1.06–1.61]; and CRP, 1.33 [1.09–1.63] per 1-SD increase in the natural log of the independent variable) (Table 4, *top*). These results were similar when we repeated the analyses after excluding ex-drinkers and those consuming three or more drinks per day (Table 4, *bottom*). Given the general absence of an association of AST and bilirubin with risk of metabolic syndrome, these variables were excluded from subsequent analyses. Furthermore, in subsequent analyses, we included all subjects in the cohort in light of the highly consistent associations of liver markers with risk of metabolic syndrome when former and heavy drinkers were excluded.

Risk of incident metabolic syndrome among those in the 2nd, 3rd, and 4th quartiles of ALT, the AST-to-ALT ratio, ALK, and CRP compared with those in quartile 1 (the reference category) are presented in Fig. 1. After adjustment for age, sex, clinical center, ethnicity, and alcohol intake, trends across quartiles of these variables were statistically significant (all $P < 0.05$). Subjects in the 4th quartile of ALT and CRP and the 2nd, 3rd, and 4th quartiles of ALK were at significantly elevated metabolic syndrome risk, whereas those in the 4th quartile of the AST-to-ALT ratio were at significantly reduced risk (all $P < 0.01$). We also assessed risk of metabolic syndrome development among those with CRP ≥3 mg/l, a recently recommended risk definition (36), as well as those in quartile 4 of ALT (vs. all other subjects). Although these definitions identified similar proportions of subjects (~24%), quartile 4 of ALT had a stronger magnitude of association and a larger area under the receiver operator characteristic (AROC) curve compared with CRP ≥3 mg/l (OR 2.08 [95% CI 1.31–3.30], AROC curve 0.60 vs. 1.53 [0.98–2.39], AROC curve 0.58). There were no significant interactions by sex or ethnicity on associations of ALT, the AST-to-ALT ratio (Fig. 2), ALK, or CRP (data not shown) with risk of incident metabolic syndrome (all interaction term *P* values ≥0.13). Although associations between the liver markers and metabolic syndrome risk were not

TABLE 2
Baseline characteristics of IRAS subjects free of NCEP metabolic syndrome and diabetes at baseline, stratified by NCEP metabolic syndrome status at the follow-up examination

	NCEP metabolic syndrome status at follow-up examination			
	All subjects		Excluding former and heavy drinkers	
Variable	No metabolic syndrome	Metabolic syndrome	No metabolic syndrome	Metabolic syndrome
n	506	127	412	100
Age (years)	52.4 ± 8.6	53.2 ± 8.1	54.0 ± 8.8	53.0 ± 8.0
BMI (kg/m^2)	26.3 ± 4.0	29.0 ± 4.8*	26.3 ± 4.0	28.9 ± 4.8*
Waist circumference (cm)	85.5 ± 10.4	91.8 ± 10.6*	85.1 ± 10.2	91.4 ± 10.5*
Fasting glucose (mg/dl)	95.1 ± 9.5	100.0 ± 10.7*	95.1 ± 9.5	99.3 ± 10.5*
2-h glucose (mg/dl)	114.9 ± 31.2	129.0 ± 32.4*	113.8 ± 30.9	129.1 ± 32.7*
Fasting insulin (pmol/l)	10 (7–15)	14 (10–18)	10 (8–15)	14 (10–18)*
HDL (mg/dl)	51.3 ± 15.8	44.4 ± 12.2*	51.5 ± 16.6	44.5 ± 12.8*
Triglyceride (mg/dl)	93 (69–128.5)	117 (88–154)	94 (69–130)	119 (90–154)*
Systolic blood pressure (mmHg)	122.8 ± 18.0	130.6 ± 18.2*	122.2 ± 17.3	129.6 ± 17.7*
Diastolic blood pressure (mmHg)	76.7 ± 9.4	80.0 ± 9.4†	76.4 ± 9.1	79.6 ± 9.3‡
$S_i \times 10^{-4}$ ($min^{-1} \cdot UU^{-1} \cdot ml^{-1}$)	2.2 (1.3–3.6)	1.5 (1.0–2.3)*	2.2 (1.3–3.6)	1.5 (0.9–2.2)*
AIR ($pmol \cdot ml^{-1} \cdot min^{-1}$)	393.1 (181.0–670.6)	367.3 (143.5–664.6)	395.5 (181.4–677.5)	401.1 (166.7–720.4)
CRP (mg/l)	1.3 (0.6–2.4)	1.8 (0.9–4.2)‡	1.3 (0.6–2.4)	1.7 (0.8–4.5)§
AST (units/l)	20 (16–25)	21 (16–27)	20 (16–25)	20.5 (16–27)
ALT (units/l)	15 (10–21)	17 (11–25)§	15 (10–20)	17 (11–25)
AST-to-ALT ratio	1.3 (1.0–1.8)	1.2 (0.9–1.6)§	1.4 (1.0–1.8)	1.2 (0.9–1.6)§
ALK (units/l)	59 (48–71)	62 (52–75)§	58 (48.5–71)	62 (52–75)
Total bilirubin (mg/dl)	0.5 (0.3–0.6)	0.5 (0.4–0.7)	0.5 (0.3–0.6)	0.5 (0.4–0.6)
Sex (% male/female)	82.4/78.0	17.6/22.0	83.2/78.3	16.8/21.8
Ethnicity (% NHW/AA/HA)	78.7/81.4/80.0	21.3/18.6/20.0	78.2/83.3/80.4	21.8/16.7/19.7
Glucose tolerance (% IGT)	84.7/65.4	15.3/34.6*	85.3/64.8	14.8/35.2*
Alcohol consumption category¶	80.3/78.1	19.7/21.9	—	—

Data are means ± SD or, for skewed variables or proportions, median (interquartile range). Differences assessed using *t* test or Wilcoxon tests (for continuous variables) or χ^2 tests (for categorical variables). *$P < 0.0001$, †$P < 0.001$, ‡$P < 0.01$, §$P < 0.05$ vs. no metabolic syndrome at follow-up. ¶Alcohol categories: never, very little, ≤0.5 drinks/day, 0.5 to <1 drink/day, and 1 to <3 drinks/day vs. ex-drinker and ≥3 drinks/day. AA, African Americans; HA, Hispanic American; NHW, non-Hispanic white.

uniformly significant for all individual subgroups, the associations were entirely in the same direction as those in the pooled analysis.

ALT, the AST-to-ALT ratio, ALK, and CRP were significantly associated with risk of incident metabolic syndrome after adjustment for demographic variables and alcohol consumption (Table 5, model A). ORs for ALT and the AST-to-ALT ratio were attenuated slightly but remained significant after additional adjustment for waist circumference and IGT, although associations of ALK and CRP were no longer significant (Table 5, *model B*). Additional adjustment for S_i and AIR had little impact on the associations of ALT and the AST-to-ALT ratio with risk of metabolic syndrome. These associations remained significant when the liver markers were modeled as either continuous (risk per SD increase for ALT: OR 1.31 [95% CI 1.04–1.66]; risk per SD increase for the AST-to-ALT ratio: 0.76 [0.60–0.98]) or categorical variables (quartile 4 vs. quartile 1 for ALT: 2.12 [1.10–4.09]; quartile 4 vs. quartile 1 for AST/ALK: 0.48 [0.25–0.95]).

Results of analyses of liver markers and prediction of individual metabolic syndrome components are presented in Table 6. After adjustment for demographic variables and alcohol consumption, ALT, the AST-to-ALT ratio, ALK, and CRP were significantly associated with incident fasting hyperglycemia. In addition, CRP was associated with risk of developing abdominal obesity and hypertension, and ALK was associated with the development of high triglycerides and low HDL. These associations were significant regardless of whether the exposures were modeled as continuous (risk per SD increase) or categorical variables (quartile 4 vs. quartile 1), except for the association of ALK with low HDL, for which only the continuous variable analysis was significant. Finally, after demographic and alcohol adjustment, baseline liver markers were not associated with increases in 2-h glucose concentration at follow-up in this cohort (data not shown).

DISCUSSION

In the present study we documented, among nondiabetic subjects, significant cross-sectional associations of liver markers, including ALT, the AST-to-ALT ratio, ALK, and CRP, with metabolic syndrome and its component disorders, most notably fasting hyperglycemia and abdominal obesity. Furthermore, in subjects free of metabolic syndrome at baseline, these liver markers were significantly correlated with metabolic syndrome variables including S_i, obesity measures, glucose, and lipids. Most importantly, we demonstrated that concentrations of ALT and the AST-to-ALT ratio were prospectively associated with risk of developing metabolic syndrome after adjustment for covariates including demographic variables, alcohol consumption, abdominal obesity, IGT, and directly measured S_i and AIR. These associations were not modified by sex or ethnicity and were unchanged with the exclusion of former and heavy drinkers from the analysis. Finally, liver markers were significantly associated with the development of individual components of the metabolic syndrome,

TABLE 3
Spearman correlation analysis of baseline associations of liver markers with metabolic and anthropometric variables in IRAS subjects free of diabetes and NCEP metabolic syndrome

Variable	AST	ALT	AST-to-ALT ratio	ALK	Bilirubin	CRP
All subjects (n = 632)						
Age	0.05	−0.05	0.12	0.11	−0.02	0.05
BMI	0.11	0.15	−0.10	0.09	0.03	0.31
Waist	0.19	0.34	−0.28	0.13	0.22	0.19
Fasting glucose	0.07	0.25	−0.25	0.11	0.10	0.03
Fasting insulin	0.19	0.26	−0.18	0.14	0.09	0.25
S_i	−0.15	−0.21	0.14	−0.16	−0.05	−0.30
AIR*	0.06	0.02	0.01	0.07	0.00	0.06
Systolic blood pressure	0.12	0.10	−0.03	0.05	0.05	0.14
Diastolic blood pressure	0.15	0.15	−0.05	0.07	0.11	0.08
HDL	−0.05	−0.18	0.19	−0.18	−0.25	0.03
Triglyceride	0.09	0.12	−0.09	0.11	0.12	0.14
Alcohol consumption	0.04	0.15	−0.16	−0.14	0.10	−0.11
Excluding former and heavy drinkers (n = 512)						
Age	0.05	−0.06	0.11	0.11	−0.06	0.03
BMI	0.12	0.15	−0.09	0.10	0.04	0.31
Waist	0.12	0.34	−0.28	0.11	0.22	0.20
Fasting glucose	0.05	0.25	−0.27	−0.01	0.09	0.00
Fasting insulin	0.20	0.26	−0.17	0.12	0.07	0.27
S_i	−0.17	−0.21	0.12	−0.15	−0.03	−0.32
AIR*	0.11	0.04	0.01	0.09	−0.01	0.08
Systolic blood pressure	0.11	0.11	−0.04	0.01	0.02	0.10
Diastolic blood pressure	0.14	0.15	−0.06	0.04	0.10	0.04
HDL	−0.07	−0.20	0.18	−0.20	−0.25	0.03
Triglyceride	0.05	0.11	−0.09	0.07	0.08	0.14
Alcohol consumption	0.07	0.16	−0.17	−0.15	0.10	−0.15

Adjusted for age, sex, ethnicity, and clinic. All subjects combined: $P < 0.05$ for $|r| > 0.08$, $P < 0.01$ for $|r| > 0.12$, $P < 0.001$ for $|r| > 0.14$, $P < 0.0001$ for $|r| > 0.16$. Excluding former and heavy drinkers: $P < 0.05$ for $|r| > 0.09$, $P < 0.01$ for $|r| > 0.13$, $P < 0.001$ for $|r| > 0.15$, $P < 0.0001$ for $|r| > 0.17$. *Also adjusted for S_i.

including fasting hyperglycemia, elevated triglycerides, and low HDL.

A limited number of previous studies have assessed prospective risk factors for the development of metabolic syndrome. Significant variables identified to date include physical inactivity (37), elevated proinsulin, and reduced adiponectin concentrations (35,38) and components of the syndrome itself, including elevated waist circumference and triglyceride levels (35). Han et al. (39) reported that CRP was associated with metabolic syndrome development in women but not men in the Mexico City Diabetes Study. We found that CRP predicted metabolic syndrome after adjustment for demographic variables and alcohol adjustment, with no significant interactions of sex or ethnicity. However, the association was eliminated with

TABLE 4
Logistic regression analysis of markers of liver injury with risk of incident NCEP metabolic syndrome in IRAS subjects

Independent variable	Unit	OR (95% CI)
All subjects (n = 632)		
Log AST	0.39	1.17 (0.97–1.41)
Log ALT	0.62	1.43 (1.15–1.77)*
Log AST-to-ALT ratio	0.48	0.72 (0.57–0.90)*
Log ALK	0.30	1.31 (1.06–1.61)†
Log total bilirubin	0.46	1.12 (0.90–1.40)
Log CRP	1.08	1.33 (1.09–1.63)*
Excluding former and heavy drinkers (n = 512)		
Log AST	0.37	1.15 (0.92–1.43)
Log ALT	0.59	1.41 (1.10–1.79)*
Log AST-to-ALT ratio	0.47	0.73 (0.57–0.95)†
Log ALK	0.28	1.29 (1.02–1.64)†
Log total bilirubin	0.46	1.09 (0.85–1.40)
Log CRP	1.09	1.29 (1.03–1.61)†

Units reflect SDs of the baseline independent variable. OR (adjusted for age, sex, clinical center, ethnicity, and alcohol intake) refers to risk of incident metabolic syndrome associated with a 1-SD difference in the independent variable. *$P < 0.01$, †$P < 0.05$.

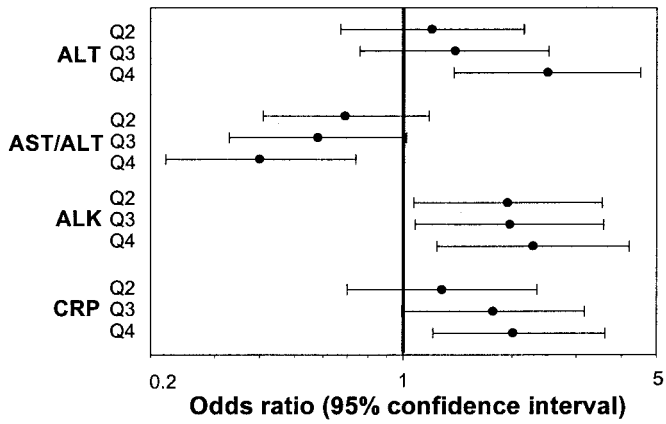

FIG. 1. Risk of incident metabolic syndrome among those in the 2nd, 3rd, and 4th quartiles of ALT, AST-to-ALT ratio, ALK, and CRP compared with those in quartile 1 (the reference category). ORs are adjusted for age, sex, clinical center, ethnicity, and alcohol intake. Trends across quartiles of ALT, AST-to-ALT ratio, ALK, and CRP were statistically significant (all $P < 0.05$).

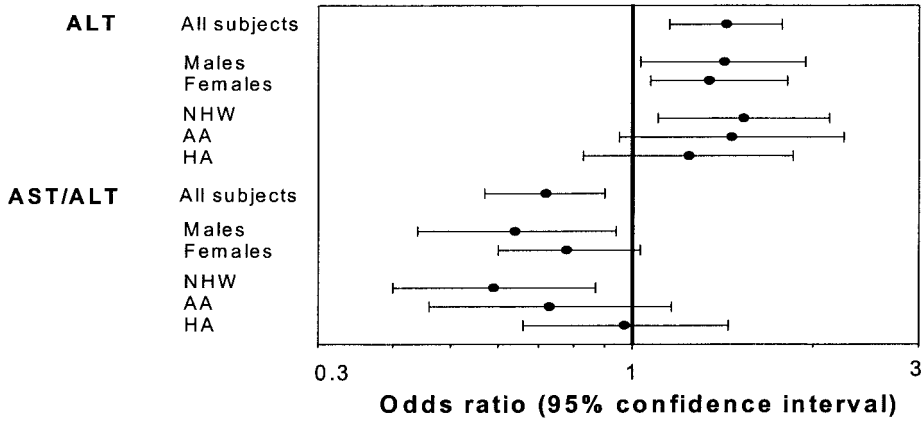

FIG. 2. Associations of ALT and AST-to-ALT ratio (per SD) with risk of incident metabolic syndrome within subgroups of sex and ethnicity. ORs are adjusted for age, clinical center, alcohol intake, sex (in ethnicity models), and ethnicity (in sex models). Interaction term *P* values: sex, ALT *P* = 0.61, AST-to-ALT ratio *P* = 0.13; ethnicity, ALT *P* = 0.62, AST-to-ALT ratio *P* = 0.13.

further adjustment for waist circumference and IGT. Only one previous study has evaluated liver markers and prediction of metabolic syndrome. In a study of male Japanese office workers, Nakanishi et al. (22) reported that γ-glutamyltransferase and ALT were significantly associated with 7-year risk of metabolic syndrome after multivariate adjustment.

A growing body of evidence supports the notion that NAFLD is a feature of the metabolic syndrome. Previous cross-sectional studies have reported high prevalence rates of metabolic syndrome and its components in subjects with clinically documented NAFLD (11,12). Similarly, large, population-based studies have reported elevated concentrations of NAFLD markers in subjects with features of the metabolic syndrome, including obesity and diabetes (10,13), as well as metabolic syndrome formally defined using NCEP criteria (40). Finally, a number of recent studies have found that elevations in liver markers, particularly ALT, are prospectively associated with incident type 2 diabetes, an important sequela of metabolic syndrome (14–22). The unique contribution of the present study is the demonstration that ALT and the AST-to-ALT ratio significantly predicted incident metabolic syndrome in a well-characterized multiethnic cohort, associations that were robust to adjustment for a large number of risk factors for diabetes and metabolic syndrome, including direct measures of S_i and insulin secretion. This novel finding raises the possibility that NAFLD may play an early role in the etiology of metabolic syndrome. Although moderate and heavy alcohol consumption increases transaminase levels (10), our results were largely unchanged after the exclusion of ex-drinkers and moderate/heavy drinkers. In addition, because the majority of subjects in the upper quartiles of ALT had concentrations below upper limit of normal for this test, the observation may highlight the importance of variation within what are thought to be "normal ranges" for liver function markers (41).

There are a number of possible mechanisms to explain the associations of ALT and the AST-to-ALT ratio with incident metabolic syndrome. First, these markers are known to be significantly correlated with increased hepatic fat content (42), a disorder that has detrimental effects on components of the metabolic syndrome. It has been demonstrated that hepatic fat content, directly measured using proton spectroscopy, is associated with several features of insulin resistance independent of body weight (43). In particular, hepatic fat was associated with defects in the suppression by insulin of endogenous glucose production (43), which is notable in the context of our results showing that ALT and AST-to-ALT ratio predicted the onset of fasting hyperglycemia (Table 5). It is also possible that these associations are reflective of more generalized insulin resistance. In a recent study of nonobese NAFLD subjects and control subjects closely matched on age and body composition, Bugianesi et al. (44) documented a pattern of metabolic defects in NAFLD subjects consistent with insulin resistance involving the liver and peripheral tissues including adipose tissue and skeletal muscle.

Our study is limited by the unavailability of gold standard measures of NAFLD such as liver biopsy. Although

TABLE 5
Multivariate logistic regression analysis of markers of liver injury with risk of incident NCEP metabolic syndrome in IRAS subjects

Independent variable	Unit	Model A	Model B	Model C
ALT	SD of log	1.43 (1.15–1.77)*	1.29 (1.03–1.62)†	1.31 (1.04–1.66)†
	Q4 vs. Q1	2.50 (1.38–4.51)*	2.04 (1.08–3.84)†	2.12 (1.10–4.09)†
AST-to-ALT ratio	SD of log	0.72 (0.57–0.90)*	0.79 (0.62–0.99)†	0.76 (0.60–0.98)†
	Q4 vs. Q1	0.40 (0.22–0.74)*	0.49 (0.26–0.95)†	0.48 (0.25–0.95)†
ALK	SD of log	1.31 (1.06–1.61)†	1.22 (0.97–1.52)	1.22 (0.97–1.53)
	Q4 vs. Q1	2.28 (1.24–4.20)*	1.74 (0.92–3.31)	1.84 (0.95–3.60)
CRP	SD of log	1.33 (1.09–1.63)*	1.03 (0.81–1.29)	0.97 (0.76–1.23)
	Q4 vs. Q1	2.01 (1.12–3.60)†	0.90 (0.47–1.73)	0.78 (0.39–1.54)

Data are OR (95% CI) from separate models. Model A adjusted for age, sex, ethnicity, clinical center, and alcohol intake; model B adjusted for age, sex, ethnicity, clinical center, alcohol intake, waist circumference, and IGT; model C adjusted for age, sex, ethnicity, clinical center, alcohol intake, waist circumference, IGT, S_i, and AIR. ORs in rows indicated by SD refer to risk of incident metabolic syndrome associated with a 1-SD difference in the independent variable (see Table 3); ORs in rows indicated by Q4 vs. Q1 refer to risk of incident metabolic syndrome among those in the 4th quartile of the independent variable of interest compared with those in the lowest quartile. *$P < 0.01$, †$P < 0.05$.

TABLE 6
Multivariate logistic regression analysis of markers of liver injury and risk of incident components of NCEP metabolic syndrome in IRAS subjects

Independent variable	Unit	Fast hyperglycemia (112/742)*	Abdominal obesity (96/619)*	High triglyceride (98/611)*	Low HDL (72/442)*	Hypertension (142/466)*
ALT	SD of log	1.46 (1.16–1.82)†	1.18 (0.93–1.50)	1.24 (0.99–1.55)	1.26 (0.97–1.63)	1.09 (0.87–1.36)
	Q4 vs. Q1	3.12 (1.65–5.91)‡	1.29 (0.66–2.53)	1.73 (0.92–3.26)	1.61 (0.76–3.40)	0.91 (0.48–1.71)
AST-to-ALT ratio	SD of log	0.67 (0.52–0.86)†	0.87 (0.68–1.12)	0.86 (0.68–1.09)	0.89 (0.67–1.17)	1.01 (0.81–1.26)
	Q4 vs. Q1	0.48 (0.25–0.91)§	1.06 (0.53–2.12)	0.71 (0.37–1.35)	0.69 (0.33–1.45)	0.99 (0.52–1.87)
ALK	SD of log	1.28 (1.03–1.60)§	1.15 (0.91–1.46)	1.28 (1.01–1.61)§	1.44 (1.11–1.88)†	1.05 (0.84–1.30)
	Q4 vs. Q1	2.10 (1.11–3.98)§	1.43 (0.71–2.86)	2.41 (1.17–4.97)§	1.89 (0.88–4.06)	1.01 (0.55–1.84)
CRP	SD of log	1.40 (1.13–1.73)†	1.49 (1.19–1.86)‡	1.15 (0.91–1.42)	1.16 (0.89–1.51)	1.44 (1.16–1.78)‡
	Q4 vs. Q1	2.38 (1.27–4.46)†	3.25 (1.59–6.66)†	1.23 (0.65–2.32)	1.61 (0.77–3.38)	2.47 (1.33–4.56)†

Data are OR (95% CI) from separate models. Subjects were free of diabetes and the outcome of interest at baseline but not necessarily free of metabolic syndrome. Sample sizes therefore vary in different analyses. OR adjusted for age, sex, ethnicity, clinical center, and alcohol intake; ORs in rows indicated by SD refer to risk of incident metabolic syndrome associated with a 1-SD difference in the independent variable (see Table 3). ORs in rows indicated by Q4 vs. Q1 refer to the rate of incident metabolic syndrome among those in the 4th quartile of the independent variable compared with those in the lowest quartile. *Events/total at risk. †$P < 0.01$, ‡$P < 0.001$, §$P < 0.05$.

serum markers of liver damage, including ALT and AST, may be reasonable noninvasive surrogate measures for use in epidemiological studies (9,10), some nondifferential misclassification of NAFLD based on transaminase concentrations is likely, and it has been reported that the repeatability of elevated ALT is poor (45). These limitations would serve to attenuate the magnitude of our effect measures toward the null, and thus our results can probably be considered as conservative. It should be noted that absolute differences in liver marker concentrations between those who did and did not develop metabolic syndrome were small, although these differences remained significant after adjustment for a wide range of well-characterized metabolic risk factors. This observation is important in contributing to our understanding of the etiology of the metabolic syndrome.

Our results have a number of clinical and public health implications. Taken together with studies reporting frequent metabolic syndrome and diabetes in subjects with NASH (11,12), our results indicate that subjects who are likely to have NAFLD based on chronically elevated transaminase levels, low reported alcohol intake, and no exposure to hepatotoxic chemical or biological agents should be considered at high risk for the development of metabolic syndrome and its sequelae. Our results suggest that this risk is approximately twofold for those in the upper quartile for ALT in fully adjusted models. Currently, it is not known whether interventions among subjects who have elevated liver transaminase levels ultimately prevent the development of metabolic syndrome or its associated consequences, including type 2 diabetes. However, it is notable that approaches that are known to be effective in preventing diabetes, including weight loss and treatment with insulin sensitizing oral antidiabetic agents (thiazolidinediones or metformin), also improve NAFLD and NASH and reduce liver fat content (46–49).

In conclusion, we found that markers of liver injury, including ALT and the AST-to-ALT ratio were significantly associated with risk of incident metabolic syndrome, even among light alcohol consumers and after adjustment for a broad spectrum of metabolic risk factors including directly measured S_i and insulin secretion. These findings suggest that pathologies related to elevated transaminase levels may be associated with the development of metabolic syndrome.

ACKNOWLEDGMENTS

This study was supported by National Heart, Lung, and Blood Institute contracts U01-HL47887, U01-HL47889, U01-HL47892, U01-HL47902, DK-29867, and RO1-58329 and by National Center for Research Resources/National Institutes of Health Grant M01-RR-43.

A.J.G.H. is a Scholar of the Canadian Diabetes Association and a New Investigator of the University of Toronto Banting and Best Diabetes Centre.

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