Elevations in Markers of Liver Injury and Risk of Type 2 Diabetes

The Insulin Resistance Atherosclerosis Study

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A limited number of studies have reported associations of markers of liver injury, including elevated concentrations of aspartate aminotransferase (AST) and alanine aminotransferase (ALT), with prospective risk of type 2 diabetes. However, only one study has adjusted for a detailed measure of insulin sensitivity (insulin sensitivity index [S_i]), which is important given associations of obesity and S_i with nonalcoholic fatty liver disease (NAFLD). Our objective was to investigate the associations of elevated AST and ALT with incident type 2 diabetes among 906 participants in the Insulin Resistance Atherosclerosis Study who were nondiabetic at baseline. S_i and acute insulin response (AIR) were measured directly from the frequently sampled intravenous glucose tolerance test among black, Hispanic, and non-Hispanic white participants aged 40–69 years. After 5.2 years, 148 individuals had developed type 2 diabetes. Baseline AST and ALT were positively correlated with fasting insulin ($r = 0.22$ and $r = 0.35$, respectively), waist circumference ($r = 0.18$ and $r = 0.34$), and fasting glucose ($r = 0.13$ and $r = 0.29$) and inversely with S_i ($r = -0.18$ and $r = -0.30$; all $P < 0.0001$). In separate logistic regression models adjusting for age, sex, ethnicity, clinical center, and alcohol consumption, participants in the highest quartiles (Q4) of AST and ALT were at significantly increased risk of incident type 2 diabetes compared with those in the lowest three quartiles (Q1–Q3): AST: odds ratio (OR) 1.73 (95% CI 1.17–2.57); ALT: OR 2.32 (1.36–3.75). After further adjustment for smoking, waist circumference, triglyceride, HDL, impaired glucose tolerance, S_i, and AIR, both AST and ALT remained significantly associated with incident type 2 diabetes: AST, Q4 vs. Q1–Q3: OR 1.98 (1.23–3.17); ALT, Q4 vs. Q1–Q3: OR 2.00 (1.22–3.28). There were no interactions of sex, ethnicity, obesity, impaired glucose tolerance, or S_i with AST or ALT in the prediction of type 2 diabetes. When entered into the same model with adjustment for demographic variables, both C-reactive protein and ALT independently predicted type 2 diabetes. In addition, AST and ALT were positively associated with incident type 2 diabetes after excluding former and moderate to heavy drinkers. In conclusion, AST and ALT independently predict type 2 diabetes. Baseline elevations of these markers may reflect NAFLD or related pathologies. *Diabetes* **53:2623–2632, 2004**

Over the past several decades, the prevalence rates of overweight and obesity have reached epidemic levels in the U.S. and other Westernized nations (1–4). This is a development of significant public health concern in light of the numerous and often interconnected health consequences of these conditions, including type 2 diabetes, hypertension, dyslipidemia, heart disease, and selected neoplasms. Recently, a novel hepatic sequela of obesity has been described: nonalcholic fatty liver disease (NAFLD) (5,6), which is defined as "significant lipid deposition in the hepatocytes of the liver parenchyma in a patient without a history of excessive alcohol ingestion" (5). Most individuals with NAFLD in its uncomplicated form (simple steatosis) are asymptomatic. However, a subset progresses to more severe manifestations of the NAFLD disease spectrum, including nonalcoholic steatohepatitis (NASH), fibrosis, cirrhosis, and liver failure (5,6). Indeed, NAFLD is now recognized as the most common cause of cryptogenic cirrhosis (7). The pathophysiological mechanism underlying NAFLD is currently unknown, although the close link with overweight and obesity has suggested a role for insulin resistance (IR). This hypothesis is supported by observational studies that have documented elevated prevalence rates of metabolic syndrome, IR, and/or type 2 diabetes among patients with NAFLD and NASH (8,9), as well as recent clinical trial data showing improvements in NASH and reductions in liver fat content after treatment with insulin-sensitizing agents, including the thiazolidinediones and metformin (10–13).

NAFLD and NASH are diagnosed in clinical settings

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AIR, acute insulin response; ALK, alkaline phosphatase; ALT, alanine aminotransferase; AROC, area under the receiver operating characteristic; AST, aspartate aminotransferase; CRP, C-reactive protein; FSIGTT, frequently sampled intravenous glucose tolerance test; GGT, γ-glutamyltransferase; IGT, impaired glucose tolerance; IR, insulin resistance; IRAS, Insulin Resistance Atherosclerosis Study; MetS, metabolic syndrome; NAFLD, nonalcoholic fatty liver disease; NASH, nonalcoholic steatohepatitis; NGT, normal glucose tolerance.

TABLE 1
Baseline characteristics of nondiabetic participants in IRAS, stratified by diabetes status at the follow-up examination

	Diabetes status at follow-up examination			
	All participants		Excluding former and moderate/heavy drinkers	
Baseline variable	Nondiabetic	Diabetic	Nondiabetic	Diabetic
n	758	148	615	121
Age (years)	54.4 ± 8.5	56.0 ± 7.8†	54.1 ± 8.6	56.0 ± 7.8†
BMI (kg/m^2)	27.9 ± 5.3	31.1 ± 6.3‖	27.9 ± 5.4	31.2 ± 6.5‖
Waist circumference (cm)	89.2 ± 12.4	95.5 ± 13.1‖	89.0 ± 12.2	95.0 ± 12.8‖
Fasting glucose (mg/dl)	96.1 ± 10.3	106.6 ± 12.0‖	97.0 ± 10.5	106.5 ± 11.4‖
2-h glucose (μU/ml)	119.4 ± 31.5	152.8 ± 30.8‖	118.5 ± 31.1	154.2 ± 30.8‖
Fasting insulin (pmol/l)	12 (8–17)	17 (13–24)‖	12 (8–18)	17 (13–24)‖
$S_i \times 10^{-4}$ ($min^{-1} \cdot \mu U^{-1} \cdot ml^{-1}$)	1.8 (1.0–3.1)	1.0 (0.5–1.6)‖	1.9 (1.0–3.2)	1.0 (0.5–1.6)‖
AIR ($pmol \cdot ml^{-1} \cdot min^{-1}$)	411.5 (192.3–711.2)	154.7 (58.5–404.2)‖	422.9 (194.8–720.3)	166.3 (69.6–424.5)‖
CRP (mg/l)	1.6 (0.7–3.2)	2.4 (1.3–5.9)‖	1.6 (0.7–3.1)	2.4 (1.3–6.2)‖
AST (units/l)	20 (17–25)	22 (16–29)†	20 (17–25)	22.5 (17–28)†
ALT (units/l)	16 (11–22)	18 (12–30)‡	16 (11–22)	17.5 (12.5–27.5)†
ALK (units/l)	61 (50–73)	66 (54–79)†	60 (50–72)	66 (55–79)‡
Total bilirubin (mg/dl)	0.5 (0.4–0.6)	0.5 (0.4–0.6)	0.5 (0.3–0.6)	0.5 (0.4–0.6)
Sex (% male/female)	85/83	15/17	85/82	15/18
Ethnicity (% non-Hispanic white/black/Hispanic)	85/83/83	15/17/17	86/81/83	14/19/17
Glucose tolerance (% NGT/IGT)	92/67	8/33‖	93/65	7/35‖
Alcohol consumption category*	84/85	16/15		—
High-risk CRP (% ≤/>3.0 mg/l)	73.3/56.6	26.7/43.4§	73.9/57.8	26.1/42.2‖

Data are means ± SD, medians (interquartile ranges) for skewed variables, or proportions for categorical variables. Differences were assessed using *t* tests (for continuous variables) or χ^2 tests (for categorical variables); *t* tests were performed on log transformations of the following skewed variables: fasting insulin, S_i, CRP, AST, ALT, ALK, and total bilirubin. *Alcohol categories: never/very little/<0.5/0.5 to <1/1 to <3 vs. exdrinker/3+ drinks per day. †$P < 0.05$; ‡$P < 0.01$; §$P < 0.001$; ‖$P < 0.0001$ vs. nondiabetic subjects at follow-up.

using detailed techniques, such as liver biopsy and ultrasound imaging, which are not easily applied in epidemiological studies (5,6). However, patients with NAFLD and NASH are commonly characterized by elevated circulating concentrations of markers of liver injury, including aspartate aminotransferase (AST), alanine aminotransferase (ALT), and γ-glutamyltransferase (GGT) (5,6). In fact, NAFLD and NASH have been reported to be the most common causes of chronically elevated transaminase levels (14). These observations indicate that AST, ALT, and other markers of liver injury may be useful surrogate measures of NAFLD and related conditions for large studies.

Despite compelling cross-sectional evidence suggesting that NAFLD is a feature of the metabolic syndrome (MetS), the temporal relationships of NAFLD, IR, and type 2 diabetes have not been clearly described. A limited number of prospective studies have examined the associations of baseline AST, ALT, and other liver markers with risk of type 2 diabetes (15–20), although the results have been inconsistent. In a recent article from a Pima Indian longitudinal study, it was reported that ALT was a significant predictor of diabetes over an average follow-up of 7 years, with the association remaining significant after adjustment for percentage of body fat and direct measures of insulin sensitivity and secretion (19). This has been the only published study to date that has been able to consider these relationships in the context of detailed measures of IR, an issue that may be of importance given the recently documented relationships of IR with NAFLD and NASH (8,9). In addition, existing prospective studies of liver markers and diabetes risk have not considered markers of subclinical inflammation, such as C-reactive protein (CRP), which is important because inflammation is known to predict both NAFLD progression and type 2 diabetes independent of conventional risk factors (5,6,21).

The Insulin Resistance Atherosclerosis Study (IRAS) offers a unique opportunity to extend these previous findings by assessing the associations of AST, ALT, and other liver markers with risk of diabetes in a well-characterized multiethnic cohort (22). The IRAS dataset contains information on directly measured insulin sensitivity (insulin sensitivity index [S_i]) and acute insulin response (AIR) determined during the frequently sampled intravenous glucose tolerance test (FSIGTT). In addition, IRAS includes participants from two ethnic groups (Hispanic and black) that are known to experience high prevalence rates of obesity, MetS, and associated disorders (22). The specific objective of the present article was to investigate the prospective associations of markers of liver injury, including elevated concentrations of ALT and AST, with risk of diabetes among IRAS participants who were known to be nondiabetic at baseline.

RESEARCH DESIGN AND METHODS

The IRAS is a multicenter observational epidemiologic study of the relationships between IR and cardiovascular disease and its known risk factors in different ethnic groups and various states of glucose tolerance. The design and methods of this study have been described in detail in previous publications (22). Briefly, the study was conducted at four clinical centers. At centers in Oakland and Los Angeles, CA, non-Hispanic whites and blacks were recruited from Kaiser Permanente, a nonprofit health maintenance organization. Centers in San Antonio, TX, and San Luis Valley, CO, recruited non-Hispanic whites and Hispanics from two ongoing population-based studies (the San Antonio Heart Study and the San Luis Valley Diabetes Study) (22). 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. The present report includes information on 906 individuals who were free of diabetes at baseline and for whom information was available on variables of interest (Table 1).

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. The response rate was 81%, and those who attended the follow-up examination were similar to those who did not attend in terms of ethnicity, sex, baseline glucose tolerance status (normal glucose tolerance [NGT] versus impaired glucose tolerance [IGT], and BMI (all comparisons, $P > 0.32$).

Clinical measurements and procedures. The IRAS protocol required two visits, 1 week apart, of ~4 h each. Participants 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 (23). During the second visit, insulin sensitivity and insulin secretion were determined using an FSIGTT, with two modifications to the original protocol (24). First, an injection of regular insulin, rather than tolbutamide, was used to ensure adequate plasma insulin levels for the accurate computation of insulin sensitivity across a broad range of glucose tolerance (25). Second, a reduced sampling protocol (with 12 rather than 30 samples) was employed for efficiency given the large number of participants (26). Insulin sensitivity, expressed as the S_i, and AIR were calculated using mathematical modeling methods (MINMOD version 3.0) (27). The repeatability of S_i and AIR have been demonstrated in a subsample of the IRAS cohort (28), and the estimate of S_i from this modified protocol has been validated against gold standard measures of IR from the hyperinsulinemic-euglycemic clamp technique (r = 0.6 in nondiabetic subjects) (29). AIR has been validated by others using gold standard measures of insulin secretion from the hyperglycemic clamp technique (30).

Height and weight were measured to the nearest 0.5 cm and 0.1 kg, respectively. BMI was calculated as weight/height^2 (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 (22). Duplicate measures of anthropometry were made following a standardized protocol, and averages were used in the analysis. Ethnicity, smoking status, and alcohol intake were assessed by self-report (22). 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. In the current analysis, alcohol intake was treated as a seven-item categorical variable: "never," "ex-drinker," "very little," "≤0.5 drinks per day," "0.5–<1 drink per day," "1–<3 drinks/day," "3 or more drinks per day."

Laboratory procedures. Glucose concentration was determined using standard methods as described previously (22). Insulin levels were measured using the dextran-charcoal radioimmunoassay (33), which has a 19% external coefficient of variation. 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 method. CRP was measured using an in-house ultrasensitive competitive immunoassay (antibodies and antigens from Calbiochem, La Jolla, CA), with an interassay coefficient of variation of 8.9% (34). 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).

Statistical analyses. Baseline means (±SD), medians (with interquartile range), or proportions were calculated for participants by follow-up diabetes status, with t tests or χ^2 tests used to assess the statistical significance of differences. The distributions of continuous variables were evaluated, and transformations were used in the analysis as required. Given that some participants had $S_i = 0$, we used the natural log transformation of ($S_i + 1$). Baseline associations of liver markers with anthropometric and metabolic variables were determined using Spearman correlation analysis. Associations of liver markers with risk of incident diabetes were assessed in separate logistic regression models, with diabetes status at the follow-up examination as the outcome variable. Risk associated with each liver marker was expressed per SD increase of its natural log and adjusted for demographic variables (age, sex, ethnicity, and clinical center) as well as alcohol intake. To investigate the possibility of nonlinear associations, we also assessed risk of incident type 2 diabetes among those in the second, third, and fourth quartiles of the various liver markers, with participants in quartile 1 (Q1) serving as the reference category. In light of the well-known association between heavy drinking and liver injury, we repeated the analyses described above excluding participants who had reported that they were either ex-drinkers or consumers of 3 or more drinks per day ("moderate/heavy drinkers").

We assessed the possibility that demographic and/or metabolic factors had modified the association between liver markers and risk of type 2 diabetes by including interaction terms in separate logistic regression models (with exposures modeled per SD increase, adjusted for demographic variables and alcohol intake) and by plotting the odds ratios (ORs) and 95% CIs for each strata of the interaction variable under consideration, including sex, ethnicity, obesity (BMI < vs. ≥27.4 kg/m^2, representing the median split), glucose tolerance (NGT versus IGT), and insulin sensitivity (S_i < vs. ≥1.61, representing the median split).

Multivariate logistic regression was used to model associations of liver markers with risk of type 2 diabetes, taking account of potential confounders. Each liver marker was modeled as either a continuous variable (with risk expressed per SD change in the natural log of the marker) or a categorical variable using two approaches: risk among those in the fourth quartile (Q4) of the marker was compared with those in the lower three quartiles, and risk among those above the upper limit of normal for the marker was compared with those at or below this cutoff. 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 smoking, waist circumference, triglyceride, HDL, and IGT; model C included further adjustments for S_i and AIR. It has been pointed out that the AST/ALT ratio may be informative in differentiating alcoholic versus nonalcoholic liver disease (individuals with NAFLD having a ratio <1) (5,6); thus, this ratio was considered as an additional exposure variable. Finally, high-sensitivity CRP, a well-described marker of subclinical inflammation, has been shown to enhance the prognostic value of conventional diabetes risk models, including the MetS (21). We therefore compared predictive ability of individual liver markers with CRP (≤3 vs. >3, a categorization to indicate high risk recently recommended by an American Heart Association/Centers for Disease Control and Prevention consensus statement [35]), as well as analyzing improvement in the prediction of liver markers when used in combination with CRP. To this end, the areas under the receiver operating characteristic (AROC) curves for each model were calculated. The AROC curve is a measure of how well a variable is able to predict the outcome of interest. Finally, the AROC curves for each model were formally compared using the DeLong algorithm (36).

RESULTS

Baseline characteristics of the study population stratified by diabetes status at the follow-up examination are presented in Table 1. As reported in previous publications (37,38), participants who developed type 2 diabetes were older ($P < 0.05$); had higher BMI and waist circumference and higher concentrations of fasting and 2-h glucose, fasting insulin, and CRP; had lower S_i and AIR; and were more likely to have IGT at baseline compared with those who remained free of diabetes (all $P < 0.0001$). Those who developed type 2 diabetes also had higher concentrations of AST ($P < 0.05$), ALT ($P < 0.01$), and ALK ($P < 0.05$). These patterns were similar when we excluded ex-drinkers and those who reported consumption of three or more drinks per day (Table 1).

Baseline univariate associations of liver function markers with anthropometric and metabolic variables are presented in Table 2. AST, ALT, and ALK showed moderate statistically significant positive correlations with BMI, waist circumference, fasting and 2-h glucose, and fasting insulin and significant inverse correlations with S_i and HDL. The magnitude of these associations was generally strongest for ALT. Total bilirubin was positively correlated with waist circumference and fasting glucose and inversely correlated with HDL and CRP. Alcohol consumption was inconsistently related with liver markers, although there were positive associations with AST, ALT, and bilirubin and an inverse association with ALK. These correlation results were very similar when we repeated the

TABLE 2
Spearman correlation analysis of associations of markers of liver injury with baseline metabolic and anthropometric variables in nondiabetic IRAS participants

Variable	AST	ALT	ALK	Bilirubin
All participants				
Age	0.02	−0.09	0.08	−0.01
BMI	0.12	0.20	0.15	−0.02
Waist circumference	0.18	0.34	0.15	0.15
Fasting glucose	0.13	0.29	0.08	0.11
2-h glucose	0.06	0.10	0.14	−0.05
Fasting insulin	0.22	0.35	0.19	0.07
S_i	−0.18	−0.30	−0.21	−0.03
AIR*	0.06	0.02	0.04	0.03
Systolic blood pressure	0.12	0.14	0.09	0.04
Diastolic blood pressure	0.06	0.10	0.00	0.12
HDL	−0.07	−0.18	−0.16	−0.23
Triglyceride	0.10	0.14	0.14	0.10
CRP	0.04	0.02	0.23	−0.17
Alcohol consumption	0.09	0.17	−0.15	0.13
Excluding former and moderate/heavy drinkers				
Age	0.01	−0.08	0.08	−0.04
BMI	0.15	0.22	0.15	−0.01
Waist circumference	0.20	0.36	0.15	0.15
Fasting glucose	0.13	0.30	0.07	0.11
2-h glucose	0.08	0.13	0.14	−0.04
Fasting insulin	0.25	0.37	0.19	0.07
S_i	−0.19	−0.28	−0.21	−0.02
AIR*	0.09	0.03	0.05	0.01
Systolic blood pressure	0.11	0.15	0.06	0.01
Diastolic blood pressure	0.07	0.12	0.01	0.14
HDL	−0.12	−0.23	−0.19	−0.25
Triglyceride	0.11	0.17	0.15	0.09
CRP	0.04	0.04	0.22	−0.19
Alcohol consumption	0.06	0.15	−0.17	0.12

See Table 1 for units. *Correlations for AIR adjusted for S_i. Analyses with all participants: $P < 0.0001$ for $r > 0.12$; $P < 0.001$ for $r > 0.10$; $P < 0.01$ for r>0.08; $P < 0.05$ for $r > 0.05$. Analyses excluding former and moderate/heavy drinkers: $P < 0.0001$ for $r > 0.14$; $P < 0.001$ for $r > 0.12$; $P < 0.01$ for $r > 0.10$; $P < 0.05$ for $r > 0.06$.

analyses after excluding ex-drinkers and those who consumed three or more drinks per day (Table 2).

After adjustment for age, sex, clinical center, ethnicity, and alcohol intake, AST and ALT were significantly associated with 5-year risk of incident diabetes (OR 1.25 [95% CI 1.06–1.48]; OR 1.38 [1.14–1.67], respectively, per 1-SD increase in the natural log of the independent variable; Table 3). These results were essentially unchanged when we repeated the analyses after excluding ex-drinkers and those who consume three or more drinks per day, although the magnitude of the association for ALK was slightly stronger (Table 3). Given the general absence of an association of bilirubin with risk of diabetes, this variable was excluded from subsequent analyses.

TABLE 3
Logistic regression analysis of markers of liver injury with risk of incident diabetes in nondiabetic IRAS participants

Independent variable	Unit*	OR†	95% CI
All participants			
AST	0.40	1.25§	1.06–1.48
ALT	0.63	1.38‖	1.14–1.67
ALK	0.30	1.18	0.98–1.43
Total bilirubin	0.46	1.21	0.98–1.48
Excluding former and moderate/heavy drinkers			
AST	0.38	1.25‡	1.04–1.51
ALT	0.61	1.42‖	1.15–1.76
ALK	0.29	1.30‡	1.05–1.61
Total bilirubin	0.46	1.24	0.99–1.55

*ORs refer to risk of incident type 2 diabetes associated with a 1-SD difference in the natural log of the independent variable. †Adjusted for age, sex, clinical center, ethnicity, and alcohol intake. ‡$P < 0.05$; §$P < 0.001$; ‖$P < 0.0001$.

Risk of incident type 2 diabetes among those in the second, third, and fourth quartiles (Q2, Q3, and Q4, respectively) of the various liver markers, compared with those in (Q1) (the reference category) is presented in Fig. 1. After adjustment for age, sex, clinical center, ethnicity, and alcohol intake, trends across quartiles of AST, ALT, and ALK were statistically significant (all $P < 0.05$), and participants in Q4 of ALT and ALK were at significantly elevated diabetes risk. Associations across quartiles of AST and ALT seemed to be nonlinear, with ORs either below or approximating the null value for participants in Q2 and Q3 of these variables. Q4 of AST and ALT had specificities of 77 and 79%, respectively, and sensitivities of 34% for classifying participants as having diabetes at follow-up. Consistent with results from Tables 2 and 3, these findings were unchanged when we repeated the

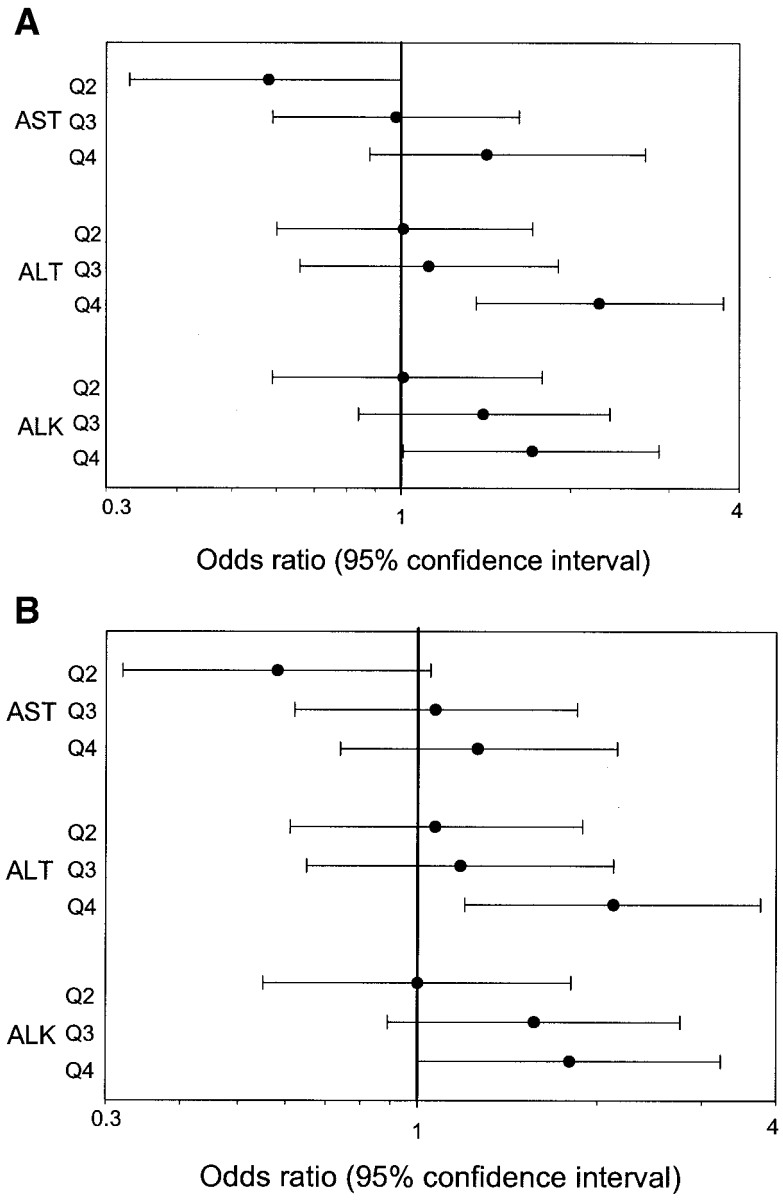

FIG. 1. Logistic regression analysis of quartiles of liver injury markers with risk of incident diabetes in nondiabetic IRAS participants. ORs and 95% CIs for individuals in Q2, Q3, and Q4 of each marker are presented, with individuals in the lowest quartile serving as the reference category. ORs were adjusted for age, sex, clinical center, ethnicity, and alcohol intake. Quartile ranges: AST 6–16, 17–20, 21–25, and >25 units/l; ALT 0–11, 12–16, 17–23, and >23 units/l; ALK, 23–50, 51–61, 62–74, and >74 units/l. Quartile averages: AST Q1 = 14.0, Q2 = 18.5, Q3 = 22.9, and Q4 = 41.2; ALT Q1 = 8.1, Q2 = 14.0, Q3 = 19.5, and Q4 = 44.7; ALK Q1 = 42.6, Q2 = 56.0, Q3 = 67.5, and Q4 = 93.2. *A*: Analyses with all participants combined. *B*: Analyses excluding participants who were either former drinkers or moderate/heavy drinkers. All *P* values <0.05 for trend.

analyses after excluding ex-drinkers and those who consume three or more drinks per day. In light of these consistent findings when ex- and moderate/heavy drinkers were excluded, we present the remainder of our results with all participants included in the analysis.

Associations of AST and ALT with risk of type 2 diabetes within subgroups of sex, ethnicity, obesity (BMI $<$ vs. $\geq$27.4 kg/m^2), glucose tolerance (NGT versus IGT), and insulin sensitivity (S_i $<$ vs. $\geq$1.61) are presented in Fig. 2. There were no significant interactions of any of these variables on the associations between the liver variables and diabetes risk (all interaction term $P > 0.14$). Although associations between the liver markers and diabetes risk were not uniformly significant for all individual subgroups, the directions of the associations were almost entirely positive.

After adjustment for demographic variables and alcohol consumption, AST and ALT were significantly associated with incident type 2 diabetes (Table 4, model A). These associations were significant regardless of whether the exposures were modeled as continuous (risk per SD increase) or categorical variables. In the categorical analyses, we compared risk among those in Q4 with those in Q1, Q2, and Q3, because our previous analysis in Fig. 1 indicated that risk seemed to be restricted to this former group. We also conducted analyses comparing participants in Q4 of liver markers with those in Q1: the results for ALT were unchanged, whereas those for AST were slightly attenuated (data not shown). Furthermore, participants with AST or ALT concentrations above the upper limit of normal had a significantly elevated risk of diabetes development (Table 4, model A). After additional adjustment for a wide range of potential confounders, including smoking, waist circumference, triglyceride, HDL, and IGT, AST continued to be associated with diabetes risk in both continuous and categorical models, whereas ALT was associated with diabetes in the categorical models (Table 4, model B). Further adjustment for S_i and AIR increased the magnitude of the associations slightly, with AST being significantly associated with diabetes in continuous (OR 1.28 [95% CI 1.05–1.56]) and categorical models (Q4 vs. Q1–Q3: OR 1.98 [1.23–3.17]; upper limit of normal: OR 2.51

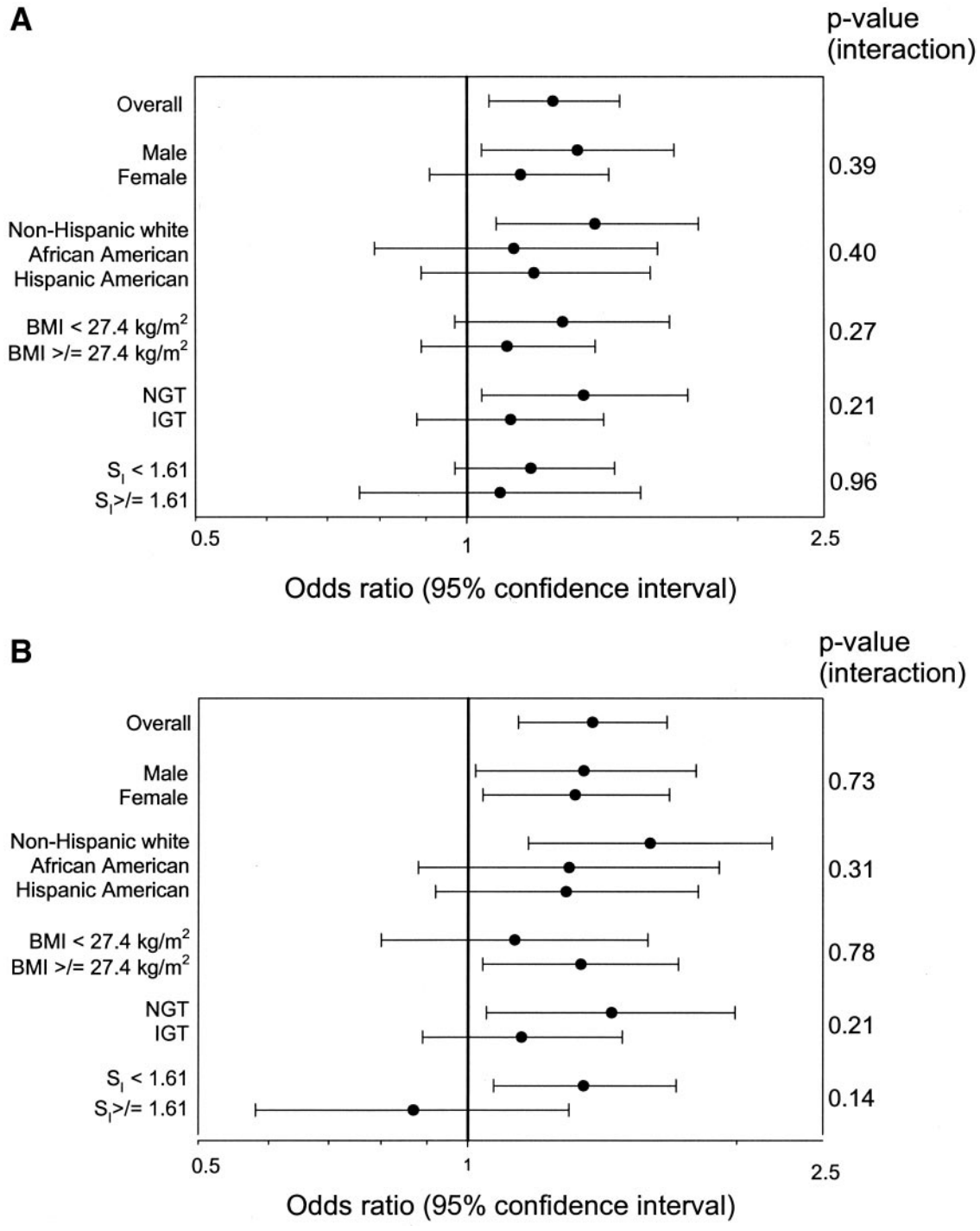

FIG. 2. Logistic regression analysis of markers of liver injury with risk of incident diabetes in nondiabetic IRAS participants by subgroups of sex, ethnicity, obesity, glucose tolerance, and insulin sensitivity. ORs refer to risk of incident type 2 diabetes associated with a 1-SD increase in the natural log of the independent variable (see Table 3) and were adjusted for age, sex, clinical center, ethnicity, and alcohol intake. *A*: Log AST. *B*: Log ALT.

[1.26–4.99]). ALT was significantly associated with risk of diabetes only in the categorical models (Q4 vs. Q1–Q3: OR 2.00 [1.22–3.28]; upper limit of normal: OR 4.07 [1.31–12.62]; Table 4, model C). In fully adjusted models, participants in Q4 of AST and ALT had 1.5- and 1.7-fold increased risks of diabetes, respectively, compared with those in Q1, although these ORs were not statistically significant as a result of reduced statistical power. In addition, we assessed the risk of diabetes associated with the AST/ALT ratio. In a model adjusted for demographic variables and alcohol intake, the AST/ALT ratio was associated with significantly reduced risk of diabetes (OR 0.81 [0.66–0.99] per SD increase), although the association was attenuated and no longer significant after adjustment for covariates in model C described above (OR 1.00 [0.78–1.27]). Finally, we assessed the independence of individual liver markers by including AST and ALT in the same model with adjustment for demographic variables and alcohol

TABLE 4
Multivariate logistic regression analysis of markers of liver injury with risk of incident diabetes in nondiabetic IRAS participants

Independent variable	Unit	Model A*		Model B†		Model C‡	
		OR	95% CI	OR	95% CI	OR	95% CI
AST	SD	1.25¶	1.06–1.48	1.22‖	1.01–1.47	1.28‖	1.05–1.56
	Q4 vs. Q1–Q3	1.73¶	1.17–2.57	1.76‖	1.14–2.71	1.98¶	1.23–3.17
	Above ULN	2.17¶	1.21–3.90	2.18‖	1.14–4.18	2.51¶	1.26–4.99
ALT	SD	1.38#	1.14–1.67	1.17	0.95–1.44	1.22	0.97–1.54
	Q4 vs. Q1–Q3	2.32**	1.53–3.52	1.69‖	1.07–2.66	2.00¶	1.22–3.28
	Above ULN	3.24‖	1.24–8.45	3.14‖	1.07–9.19	4.07‖	1.31–12.62
ALK	SD	1.18	0.98–1.43	1.03	0.83–1.27	1.02	0.81–1.29
	Q4 vs. Q1–Q3	1.52	1.00–2.29	1.20	0.76–1.88	1.16	0.70–1.95
	Above ULN§	—	—	—	—	—	—

ORs in rows indicated by SD refer to risk of incident type 2 diabetes associated with a 1-SD difference in the natural log of the independent variable (see Table 3); ORs in rows indicated by Q4 vs. Q1–Q3 refer to risk of incident type 2 diabetes among those in Q4 of the independent variable of interest compared with those in the lower three quartiles. ORs in rows indicated by ULN (upper limit of normal) refer to risk of incident type 2 diabetes among those above the ULN for the independent variable of interest (AST = 35 units/l, ALT = 56 units/l, and ALK = 125 units/l). *Adjusted for age, sex, ethnicity, clinical center, and alcohol intake. †Adjusted for age, sex, ethnicity, clinical center, alcohol intake, smoking, waist circumference, triglyceride, HDL, and IGT. ‡Adjusted for age, sex, ethnicity, clinical center, alcohol intake, smoking, waist circumference, triglyceride, HDL, IGT, S_i , and AIR. §Not estimable because none of the incident cases of diabetes had ALK concentrations above the upper limit of normal. ‖$P < 0.05$; ¶$P < 0.01$; #$P < 0.001$; **$P < 0.0001$.

intake. Whereas ALT was significantly associated with diabetes at follow-up in this model, AST was no longer significant (data not shown).

Participants with a CRP concentration >3.0 mg/l had a significant twofold increased risk of diabetes development after adjustment for demographic variables and alcohol intake, a magnitude of association that was similar to those for AST and ALT in which the variables were modeled comparing participants in Q4 with those in Q1–Q3 (Table 5). Furthermore, the AROC curve of the CRP model was 62%, which did not differ significantly from those of the liver marker models (AROC curve = 60 and 64% for AST and ALT, respectively; all $P > 0.43$ vs. the AROC curve for CRP model). When modeled together with individual liver markers in similarly adjusted models, CRP and each of the liver markers were independently associated with diabetes risk, with very little attenuation in the magnitude of the ORs (Table 5). Adding ALT (Q4 versus Q1–Q3) to the CRP model significantly improved the prediction of type 2 diabetes (AROC curve = 66 vs. 62% for the CRP model alone; $P = 0.02$). When the analyses above were repeated adjusting for covariates in model C, each of the liver markers continued to be significantly associated with diabetes incidence, although the associations between CRP and diabetes were no longer significant (data available in an online appendix at http://diabetes.diabetesjournal.org). When these analyses were repeated using SD changes in liver markers and CRP in fully adjusted models, the findings were generally similar, with no significant association for CRP, a borderline significant association with ALT, and a significant association with AST (data available in online appendix).

DISCUSSION

The public health implications of the emerging pandemic of obesity are dire in light of the growing list of associated metabolic consequences. For some time, this list has included dyslipidemia, hypertension, type 2 diabetes, and cardiovascular disease (39). More recently, obesity has been implicated in other pathophysiological pathways, including cancer at selected sites (40) and liver disease (5,6). In the present article, we documented significant cross-sectional associations of several markers of liver injury with MetS variables, including insulin sensitivity, obesity measures, glucose, and lipids among nondiabetic individuals. In addition, we demonstrated that concentra-

TABLE 5
Multivariate logistic regression analysis of liver injury markers with risk of incident diabetes in nondiabetic IRAS participants

Independent variable	Unit	OR*	95% CI	AROC	*P*
Liver markers vs. CRP					
CRP	>3.0 mg/l	2.00§	1.37–2.93	0.62	—
AST	Q4 vs. Q1–Q3	1.73‡	1.17–2.57	0.60	0.55¶
ALT	Q4 vs. Q1–Q3	2.32‖	1.53–3.52	0.64	0.44¶
Adding liver markers to CRP					
AST	Q4 vs. Q1–Q3	1.66†	1.12–2.47	—	—
CRP	>3.0 mg/l	1.95§	1.32–2.86	0.63	0.21#
ALT	Q4 vs. Q1–Q3	2.29‖	1.50–3.48	—	—
CRP	>3.0 mg/l	1.98§	1.34–2.91	0.66	0.02#

Comparisons of individual liver markers with CRP and analysis of improvement of prediction of liver markers in combination with CRP. *Model adjusted for age, sex, ethnicity, clinical center, and alcohol intake. †$P < 0.05$; ‡$P < 0.01$; §$P < 0.001$; ‖$P < 0.0001$. ¶Versus area under the receiver operator characteristic curve for CRP model; #versus area under the receiver operator characteristic curve for CRP model without liver markers.

tions of AST and ALT were prospectively associated with risk of type 2 diabetes after adjustment for covariates, including MetS variables, directly measured insulin sensitivity and AIR, and CRP (a marker of subclinical inflammation). Furthermore, sex, ethnicity, BMI, glucose tolerance status, or insulin sensitivity did not modify the associations of liver markers with diabetes risk. The unique contributions of this article include the availability of data on directly measured S_i and AIR determined during the FSIGTT, as well as the inclusion of participants from two ethnic groups (Hispanic and black) that are known to experience high prevalence rates of obesity, MetS, and associated disorders. Although the associations of liver markers with diabetes risk were not statistically significant in separate analyses of these two ethnic groups, CIs were wide and there was considerable overlap between them. These observations are the result of reductions in statistical power in the stratified analyses, and thus further studies among blacks and Hispanics are warranted.

A limited number of prospective studies have examined the associations of AST, ALT, and other liver markers with risk of subsequent diabetes. In two articles, it was reported that there was no association between ALK concentrations and diabetes risk (16,19), an observation that may be related to the lack of specificity of ALK in indicating liver disease. Five studies have assessed the diabetes risk associated with elevated GGT (15–19), and in four of these studies, the association with diabetes was statistically significant after adjustment for potential confounders (15–18). There have also been five studies of AST and/or ALT and diabetes risk (16–20). In the late 1980s, Ohlson et al. (20) reported that ALT significantly predicted diabetes after covariate adjustment in a cohort of Swedish men who were born in 1913. Participants in the upper quintile of AST were at significantly increased risk of diabetes in the CARDIA (Coronary Artery Risk Development in Adults) study after multivariate adjustment (17), and both ALT and AST were independently associated with diabetes in a cohort of male Korean workers (18). In contrast, in a study of male Japanese office workers, neither ALT nor AST was associated with diabetes risk (16). In each of the studies described above, IR either was not assessed or was estimated by way of indirect measures, including fasting insulin concentration, a limitation that may be important given the documented relationships of IR with NAFLD and NASH (5,6,8,9). In a recent article from a Pima Indian longitudinal study, Vozarova et al. (19) reported that ALT was a significant predictor of diabetes over an average follow-up of 7 years. This association remained significant after adjustment for percentage of body fat and direct measures of insulin sensitivity and secretion, a finding that is similar to that from the present study. In the Pima study, the association with AST was not significant after adjustment for covariates. Theirs is the only other published study to date to have been able to consider these relationships in the context of detailed measures of IR.

Three general mechanisms could explain the association between liver markers and risk of diabetes. As mentioned earlier, elevations in AST and ALT likely reflect NAFLD, a condition that is now known to be characterized by IR and high diabetes prevalence (5,6,8,9). Although it is possible that liver marker elevations reflect generalized IR, our multivariate analysis showed that associations between these markers and diabetes risk were independent of directly measured insulin sensitivity. It is also conceivable that the mechanism could operate through IR that is localized to the liver and that raised liver markers in this context reflect hepatic IR. It has been reported, for example, that chronic hyperinsulinemia in animal models of type 2 diabetes downregulates insulin receptor substrate-2, resulting in excessive gluconeogenesis (41).

NAFLD is also characterized by excessive lipid deposition in the hepatocyes. Ectopic deposition of fat in the liver (reflected here by elevated liver marker concentrations) may also be indicative of more generalized susceptibility to fatty infiltration in other organs, such as the skeletal muscle, the myocardium, and the pancreas. Through lipotoxic processes that were described in a recent review by Unger (42), fat deposition in these organs (particularly the muscle and pancreas) clearly predispose individuals to diabetes. It has been demonstrated that hepatic fat content, directly measured using proton spectroscopy, is associated with several features of IR independent of body weight (43). Most notable in the context of the present study, hepatic fat was associated with defects in the suppression by insulin of endogenous glucose production (43).

Finally, chronic subclinical inflammation represents a third possible mechanism linking liver function and diabetes risk. Inflammation, reflected by elevations in markers such as CRP, is a consistent predictor of type 2 diabetes (21,44) and is thought to be a factor in the natural history of NAFLD and NASH (5,6). CRP is an acute-phase reactant of hepatic origin; thus, we hypothesized that there would be cross-sectional associations between CRP and liver markers. Although we were able to confirm this association for ALK, this was not the case for AST and ALT. Furthermore, we found that, in minimally but not fully adjusted models, CRP and liver markers predicted diabetes independent of each other and that the associations were of similar magnitude. We previously reported significant associations between insulin sensitivity and CRP in this cohort (34) and, furthermore, that CRP predicts diabetes after adjustment for fasting insulin but not directly measured insulin sensitivity (37). However, it is unlikely that previous studies reporting associations between CRP and diabetes risk reflected underlying NAFLD because other markers of chronic subclinical inflammation (including interleukin-6 and plasminogen activator inhibitor 1) are significantly associated with diabetes incidence independent of other risk factors (37,44).

Our study is limited by the unavailability of gold standard measures of NAFLD such as liver biopsy. However, serum markers of liver damage, including ALT and AST, may be reasonable noninvasive surrogate measures for use in epidemiological studies. Individuals with NAFLD are known to have elevated transaminase concentrations, and, on the basis of analyses of data from the Third National Health and Nutrition Examination Survey (1988–1994), Clark et al. (7,14) suggested that NAFLD may be the most common cause of chronic elevations of these markers. However, some nondifferential misclassification of NAFLD on the basis of 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; thus, our results can probably be considered as conservative estimates of the relationship between NAFLD and diabetes risk. Although moderate and heavy alcohol consumption increases transaminase levels (14), our results were largely unchanged after the exclusion of ex- and moderate/heavy drinkers.

These findings have important clinical and public health implications. Our results support the implication of obesity as a toxic state for yet another organ system, highlighting the already strong rationale for aggressive intervention on obesity at the population level. Taken together with studies reporting frequent MetS and diabetes in individuals with NASH (8,9), our results indicate that individuals with chronically elevated transaminase levels, low reported alcohol intake, and no exposure to hepatotoxic chemical or biological agents should be considered at high risk for glucose intolerance (our results indicate that this risk is ~2-fold for those in the upper quartile and ~2.5- to 4-fold for those above the upper limit of normal for these markers in fully adjusted models). Currently, it is not known whether improving NAFLD will ultimately prevent the development of diabetes. However, it is notable that interventions 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 (10–13).

Although the absolute differences in liver marker concentrations between those who did and did not develop diabetes were small, these differences remained significant after adjustment for a wide range of well-characterized metabolic risk factors for diabetes. This observation is important in contributing to our understanding of the cause of the MetS and type 2 diabetes. In addition, because the majority of individuals in the upper quartiles of AST and ALT had concentrations below upper limits of normal for these tests, the observation may highlight the importance of variation within what are thought to be "normal ranges" for liver function markers.

In conclusion, we found that markers of liver injury, including AST and ALT, were significantly associated with risk of incident type 2 diabetes, even among light alcohol consumers and after adjustment for a broad spectrum of type 2 diabetes risk factors, including directly measured insulin sensitivity and secretion. These findings suggest that NAFLD or related pathologies may predispose to type 2 diabetes.

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