Alanine Aminotransferase and Directly Measured Insulin Sensitivity in a Multiethnic Cohort

The Insulin Resistance Atherosclerosis Study

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OBJECTIVE — The objective of the present analysis was to evaluate the association of alanine aminotransferase (ALT) with directly measured insulin sensitivity (S_i) in a large, multiethnic cohort of U.S. adults and to determine whether ALT adds to existing metabolic risk definitions in identifying subjects with insulin resistance.

RESEARCH DESIGN AND METHODS — S_i was directly measured from frequently sampled intravenous glucose tolerance tests among 999 nondiabetic African-American, Hispanic, and non-Hispanic white subjects aged 40–69 years who were participating in the Insulin Resistance Atherosclerosis Study. Subjects also received an oral glucose tolerance test, and fasting insulin, ALT, and alcohol intake were determined.

RESULTS — ALT was associated with S_i after adjustment for age, sex, ethnicity, impaired fasting glucose, triglycerides, HDL, blood pressure, and waist (clinical model) ($P < 0.0001$). The association remained significant after further adjustment for fasting insulin and impaired glucose tolerance ($P = 0.004$). In logistic regression analysis, elevated ALT (upper quartile) was associated with insulin resistance (lowest quartile of S_i) after adjustment for age, sex, and ethnicity (odds ratio 3.0 [95% CI 2.2–4.1]). Elevated ALT was independently associated with insulin resistance when included in models with waist circumference, National Cholesterol Education Program criteria for metabolic syndrome, hypertriglyceridemic waist, elevated triglyceride-to-HDL ratio, or homeostasis model assessment of insulin resistance (HOMA-IR) (all $P < 0.01$). Finally, the addition of elevated ALT improved classification of insulin resistance by area under the receiver operating characteristic curve criteria for all models except HOMA-IR.

CONCLUSIONS — ALT was associated with insulin resistance independently of conventional and more detailed metabolic measures. These findings suggest that the addition of ALT to existing clinically based metabolic risk definitions is an inexpensive way to improve the identification of subjects with insulin resistance.

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Abbreviations: ALK, alkaline phosphatase; ALT, alanine aminotransferase; AROC, area under the receiver-operating characteristic; AST, aspartate aminotransferase; CRP, C-reactive protein; CVD, cardiovascular disease; EWET, enlarged waist and elevated triglycerides; FSIVGTT, frequently sampled intravenous glucose tolerance test; GGT, γ-glutamyl aminotransferase; HOMA-IR, homeostasis model assessment of insulin resistance; IFG, impaired fasting glucose; IGT, impaired glucose tolerance; IRAS, Insulin Resistance Atherosclerosis Study; NGT, normal glucose tolerance; NAFLD, nonalcoholic fatty liver disease; NCEP, National Cholesterol Education Program, S_i, insulin sensitivity index.

A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.

Insulin resistance is a central feature in the pathogenesis of type 2 diabetes (1). It is present in subjects at high risk for diabetes (2), and it predicts the development of diabetes in prospective studies (3–5). Surrogate measures of insulin resistance have been associated with a risk of incident cardiovascular disease (CVD) in most (but not all) studies (6–9), and insulin resistance is strongly associated with several important CVD risk factors, including abdominal obesity, hypertension, and elevated triglyceride and HDL concentrations (10,11). Metabolic risk factors for CVD and diabetes are known to occur together more often than is expected by chance, a phenomenon commonly referred to as the metabolic syndrome (12). Insulin resistance may play a pivotal role in this clustering of disorders (10,11).

Detailed measurement of insulin resistance requires the use of techniques (including clamps and intravenous glucose tolerance tests) that are far too costly, time-consuming, and invasive for use in large epidemiological and clinical studies. In this context, a number of simple indexes of insulin resistance have been proposed (13–18). These indexes, which use combinations of insulin and glucose concentrations at various time points during an oral glucose tolerance test, have been validated against direct measures (13–18). The use of these indexes is problematic, however, in that insulin levels are not measured in clinical practice, and the assay for insulin determination has not been standardized (19). Alternative metabolic risk definitions that use clinically available metabolic syndrome measures, including BMI, waist circumference, and lipids have been proposed (20–24); however, these definitions explain smaller amounts of the variation in directly measured insulin resistance compared with insulin-based indexes (17,18,25,26).

Nonalcoholic fatty liver disease (NAFLD), which refers to a spectrum of disorders ranging from simple hepatic steatosis to more severe pathological con-

Table 1—*Baseline characteristics of nondiabetic subjects, stratified by quartiles of FSIVGTT-measured insulin sensitivity (S_i): the Insulin Resistance Atherosclerosis Study*

Variable	Insulin sensitivity (S_i)			
	Quartile 1	Quartile 2	Quartile 3	Quartile 4
n	251	247	250	251
$S_i \times 10^{-4}$ ($min^{-1} \cdot UU^{-1} \cdot ml^{-1}$)	0.5 (0.2–0.7)	1.2 (1.1–1.4)	2.1 (1.8–2.5)	4.2 (3.4–5.4)
Age (years)	55.8 ± 8.2	55.0 ± 8.7	54.4 ± 8.2	54.0 ± 8.7
BMI (kg/m^2)	32.6 ± 6.6	29.1 ± 4.6	27.0 ± 4.1	25.0 ± 3.8
Waist circumference (cm)	100.6 ± 13.1	92.2 ± 10.3	87.8 ± 9.9	82.2 ± 10.1
Fasting glucose (mg/dl)	103.8 ± 11.6	99.5 ± 9.8	97.7 ± 10.7	93.7 ± 10.6
Fasting insulin (pmol/l)	21 (17–29)	15 (11–19)	11 (8–14)	8 (6–11)
Triglycerides (mg/dl)	138 (101–198)	122 (79–179)	107 (78–157)	88 (64–128)
HDL (mg/dl)	41.7 ± 12.4	45.8 ± 15.8	47.7 ± 14.0	52.9 ± 16.2
CRP (mg/l)	2.5 (1.6–6.8)	2.0 (1.1–4.5)	1.6 (0.7–3.4)	0.9 (0.5–1.8)
Systolic BP (mmHg)	127.7 ± 16.7	124.5 ± 16.8	120.9 ± 18.3	117.1 ± 14.6
Diastolic BP (mmHg)	79.4 ± 8.6	78.8 ± 9.5	77.9 ± 10.2	75.6 ± 8.4
ALT (units/l)	21 (15–29)	16 (11–23)	16 (11–22)	14 (9–19)
AST (units/l)	24 (19–29)	20 (16–25)	20 (16–26)	19 (16–24)
ALK (units/l)	65 (55–79)	62 (52–74)	61 (51–72)	55 (46–69)
Sex (% female)	24.0	26.2	24.2	25.6
Ethnicity				
% NHW	17.5	22.4	27.9	32.2
% AA	27.7	29.6	22.7	20.0
% HA	32.4	23.6	23.3	20.7
IFG (%)	44.5	23.8	18.3	13.4
IGT (%)	42.5	30.4	19.9	7.2
Alcohol consumption (% former/heavy drinker)	22.5	25.7	28.9	23.0

Data are means ± SD, medians (interquartile ranges) for skewed variables, or proportions. Subjects in quartile 1 of S_i are the least insulin sensitive (i.e., the most insulin resistant), whereas subjects in quartile 4 of S_i are the most insulin sensitive (i.e., the least insulin resistant). All $P < 0.0001$ (except age [$P = 0.10$]) for overall differences across categories for continuous variables (from ANOVA); ANOVA tests performed on log transformations of skewed variables. All $P < 0.0001$ (except sex [$P = 0.52$] and alcohol consumption [$P = 0.48$]) for overall differences across categories for categorical variables (from χ^2 tests). AA, African American; BP, blood pressure; HA, Hispanic American.

ditions, has recently been proposed as a feature of the metabolic syndrome risk cluster based on high prevalence rates of diabetes, obesity, and insulin resistance in these patients (27–29). NAFLD is characterized by chronic elevations in liver transaminase levels, including alanine aminotransferase (ALT), aspartate aminotransferase (AST), and γ-glutamyltransferase (GGT) (30–32). It has been suggested that NAFLD is the most common cause of chronically elevated transaminase levels in the general population (33). These observations indicate that ALT, AST, and other markers of liver injury may be useful surrogate measures of NAFLD for large epidemiological studies, an idea that is supported by data showing significant correlations between transaminase levels and directly measured liver fat content (34).

A number of studies have reported that ALT, AST, and/or GGT levels independently predict incident type 2 diabetes, metabolic syndrome, and CVD (reviewed in ref. 31). In addition, these markers have been shown to be associated with indirect measures of insulin resistance including fasting insulin levels and the homeostasis model assessment of insulin resistance (HOMA-IR) (35,36). Very little information is available, however, about the association of these markers with more detailed measures of insulin sensitivity, including clamps or intravenous glucose tolerance tests (37–39). This issue is of interest because liver markers, which are inexpensive and routinely collected in clinical settings, may provide a simple and accurate enhancement to models currently used to identify subjects with insulin resistance. The objective of the present analysis, therefore, was to evaluate the association of liver markers with a direct measure of insulin sensitivity in a large, multiethnic cohort of U.S. adults and to determine whether liver markers add to existing metabolic risk definitions in identifying subjects with insulin resistance.

RESEARCH DESIGN AND METHODS

— The Insulin Resistance Atherosclerosis Study (IRAS) is a multicenter observational epidemiological study of the relationships between insulin resistance and CVD 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 (40). A total of 1,625 individuals (56% women) participated in the baseline IRAS examination (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 999 individuals who were free of diabetes at baseline and for whom information was available on variables of interest, including insulin

sensitivity, transaminase levels, and covariates (Table 1).

Clinical measurements and procedures

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 on 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. During the second visit, insulin sensitivity and insulin secretion were determined using a frequently sampled intravenous glucose tolerance test (FSIVGTT), with two modifications to the original protocol (41). 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 (42). Second, a reduced sampling protocol (with 12 rather than 30 samples) was used for efficiency, given the large number of participants (43). Insulin sensitivity, expressed as the insulin sensitivity index (S_i), was calculated using mathematical modeling methods (Minmod version 3.0, 1994) (44). The repeatability and validity of S_i from this modified protocol have been reported previously (45,46).

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) and was used as an estimate of overall adiposity. We determined minimum waist circumference, measured to the nearest 0.5 cm using a steel tape, at the natural indentation or at a level midway between the iliac crest and the lower edge of the rib cage if no natural indentation was present (40,47).

Duplicate measures of anthropometry were made following a standardized protocol, and averages were used in the analysis. Ethnicity and alcohol intake were assessed by self-report (40). The alcohol intake questionnaire has been described in detail previously (48). 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, drinking was categorized as nondrinker or moderate drinker (defined as never drinking or drinking "very little," "≤0.5 drinks per day," "0.5–<1 drink per day," or "1–<3 drinks/day") versus former or heavy drinkers (defined as "ex-drinker" or drinking "≥3 drinks per day"). Former and heavy drinkers were combined because former drinkers were most likely to have stopped for health reasons.

Laboratory procedures

Glucose concentration was determined using standard methods as described previously (40). Insulin levels were measured using the dextran-charcoal radioimmunoassay (49), 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. C-reactive protein (CRP) was measured using an in-house ultrasensitive competitive immunoassay (antibodies and antigens from Calbiochem, La Jolla, CA), with an interassay CV of 8.9% (50). AST, ALT, and alkaline phosphatase (ALK) were measured in plasma after one freeze-thaw cycle using standard clinical methods (and including pyridoxal phosphate for the measurement of ALT) at the central IRAS laboratory with a Paramax PLA instrument (Baxter).

Metabolic risk definitions

Metabolic syndrome was defined using criteria proposed by the National Cholesterol Education Program (NCEP) Adult Treatment Panel III (23). The HOMA-IR, a widely used, validated simple measure of insulin resistance, was calculated from fasting insulin and glucose concentrations, as described (13). Elevated CRP was defined as >3.0 mg/l according to joint recommendations from the Centers for Disease Control and Prevention and the American Heart Association (51). Hypertriglyceridemic waist was defined as described by Despres and colleagues (20), elevated triglyceride-to-HDL ratio (≥3) according to Reaven and colleagues (21), and enlarged waist and elevated triglycerides (EWET) according to Kahn and Valdez (22).

Statistical analyses

Means ± SD for normally distributed continuous variables, medians (with interquartile ranges) for skewed continuous variables, or proportions for categorical variables were calculated according to quartiles of S_i, with overall differences across the quartiles assessed using ANOVA or χ^2 tests. The distributions of continuous variables were evaluated, and the natural log transformations of skewed variables were used in the analysis. Given that some subjects had $S_i = 0$, we used the natural log transformation of $(S_i + 1)$. Spearman correlation coefficients were used to assess crude and adjusted (for age, sex, ethnicity, and alcohol intake) associations of transaminase levels with anthropometric and metabolic variables.

Multivariate associations of liver markers (ALT, AST, and ALK) with directly measured insulin sensitivity were assessed in separate multiple linear regression models, with $\log(S_i + 1)$ as the dependent variable. Two multivariate models were constructed for each liver marker (the primary independent variables). In model A, covariates included measures conventionally available in clinical settings, including age, sex, ethnicity, impaired fasting glucose (IFG), triglycerides, HDL, blood pressure, and waist circumference. Model B involved the addition of more complex, research-oriented measures not usually available in clinical settings, including impaired glucose tolerance (IGT) diagnosed with a 2-h 75-g oral glucose tolerance test (which replaced IFG in these models) and fasting insulin concentration. Using the same approach, we also tested models using the AST-to-/ALT ratio, low values of which are thought to reflect NAFLD (30,31).

As a complementary approach, we tested the association of liver markers with insulin resistance using logistic regression models. Under this approach, the dependant variable was insulin resistance defined as the lowest quartile of S_i based on the distribution among subjects with normal glucose tolerance ($S_i \leq 1.23\ \text{min}^{-1} \cdot \text{UU}^{-1} \cdot \text{ml}^{-1}$), whereas the main independent variables, ALT, AST and ALK, defined as the upper quartiles of these variables, were elevated. These models were adjusted for age, sex, and ethnicity. We assessed the possibility that demographic and/or metabolic factors had modified the association between liver markers and insulin resistance by including interaction terms in separate logistic regression models and by plotting the ORs and 95% CI for each strata of the interaction variable under consideration, including sex, ethnicity, obesity (BMI < versus ≥27.4 kg/m^2, representing the median split), glucose tolerance (NGT

Table 2—*Multiple linear regression analysis of association of ALT with directly measured insulin sensitivity (S_i) from the FSIVGTT: the Insulin Resistance Atherosclerosis Study*

	Model A (n = 985)			Model B (n = 985)		
Variable	β	t	P	β	t	P
Intercept	3.59	11.82	<0.0001	3.32	12.07	<0.0001
Sex	−0.25	−8.33	<0.0001	−0.11	−3.85	0.0001
Age (years)	−0.005	−2.96	0.003	−0.004	−2.37	0.02
African American	−0.15	−4.44	<0.0001	−0.09	−2.86	0.004
Hispanic	−0.10	−3.32	0.009	−0.06	−2.30	0.02
IFG	−0.08	−2.61	0.009	—*	—	—
IGT	—	—	—	−0.20	−7.32	<0.0001
Log triglyceride (mg/dl)	−0.10	−3.74	0.0002	−0.03	−1.17	0.2
Log HDL (mg/dl)	0.26	4.95	<0.0001	0.18	3.74	0.0002
Systolic blood pressure (mmHg)	−0.002	−2.67	0.008	−0.002	−2.36	0.02
Waist circumference (cm)	−0.02	−15.08	<0.0001	−0.01	−8.87	<0.0001
Fasting insulin (pmol/l)	—	—	—	−0.32	−13.29	<0.0001
Log ALT	−0.13	−5.78	<0.0001	−0.06	−2.87	0.004
R^2	—	0.41	—	—	0.52	—

*Variable not included in the model.

versus IGT), and alcohol consumption (nondrinkers or moderate drinkers versus former or heavy drinkers).

Next, using the same logistic regression approach, we formally compared liver markers with other metabolic syndrome components as well as surrogate definitions of insulin resistance and metabolic risk (including HOMA-IR, NCEP metabolic syndrome, hypertriglyceridemic waist, and elevated triglyceride-to-HDL ratio) in terms of their ability to predict insulin resistance. 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. The AROC curves for each model were formally compared using the DeLong algorithm (52). We determined whether adding ALT to selected other metabolic syndrome components and surrogate definitions of insulin resistance and metabolic risk improved the ability of these classifications to predict insulin resistance. Finally, the results were unchanged when the analyses described above were *1*) adjusted for medications that might increase liver enzyme levels and *2*) repeated excluding subjects with CRP >10 mg/l (data not shown).

RESULTS — Characteristics of study subjects, stratified by quartiles of S_i, are presented in Table 1. Subjects in quartile 1 of S_i have the lowest insulin sensitivity (i.e., are the most insulin resistant). Measures of body mass as well as glucose, insulin, triglycerides, blood pressure, and CRP declined in a stepwise manner across quartiles of increasing insulin sensitivity, whereas HDL increased across S_i quartiles (all $P < 0.0001$ from ANOVA). A higher proportion of African-American and Hispanic-American subjects (compared with non-Hispanic white subjects), as well as subjects with IFG or IGT (compared with subjects with NGT), were represented in the lowest quartile of S_i (all $P < 0.0001$ from ANOVA). ALT, AST, and ALK declined steadily across quartiles of increasing insulin sensitivity (all $P < 0.0001$ from ANOVA), with the most insulin-sensitive subjects clearly having the lowest concentrations of each liver marker.

ALT, AST, and ALK were significantly inversely associated with S_i (all $P < 0.0001$), with ALT showing the strongest inverse correlation ($r = -0.28$, $P < 0.0001$) (as shown in Table 1 of the online appendix available at http://dx.doi.org/10.2337/dc07-0086). Liver markers were significantly associated with fasting insulin and waist circumference, again with notably stronger correlations with ALT ($r = 0.35$ and $r = 0.34$, respectively, both $P < 0.0001$) compared with AST and ALK. These univariate correlations were not appreciably altered after adjustment for age, sex, ethnicity, and alcohol intake (see Table 1 of the online appendix).

Multiple linear regression analysis indicated that the natural log of ALT was significantly associated with insulin sensitivity after adjustment for age, sex, and ethnicity, as well as the conventionally measured clinical variables IFG, triglycerides, HDL, blood pressure, and waist circumference ($t = -5.78$, $P < 0.0001$) (model A in Table 2). The association between ALT and insulin sensitivity remained significant in a more complex model, which also adjusted for fasting insulin and IGT ($t = -2.87$, $P = 0.004$) (model B in Table 2). Further adjustment for AST did not appreciably attenuate the associations of ALT with the S_i in either the clinical or more complex models nor did additional adjustment for former/heavy drinking (data not shown). In models using the AST/ALT ratio in place of ALT and AST, this variable was also significantly associated with the S_i in both model A ($t = 4.0$, $P < 0.0001$) and model B ($t = 2.1$, $P = 0.04$). Although both AST and ALK were significantly associated with the S_i in clinical models, neither was significant in more complex models adjusting for insulin and IGT (see Table 2 of the online appendix). In light of these findings, subsequent analyses in the article are restricted to ALT.

In logistic regression analysis, elevated ALT (upper quartile) was significantly associated with insulin resistance (lowest quartile of the S_i) after adjustment for age, sex, and ethnicity (OR 3.0 [95% CI 2.2–4.1]) (Fig. 1). This association was highly consistent across subgroups of sex, ethnicity, glucose intolerance, obesity, and former/heavy drinking ($P > 0.36$ for all interactions) (Fig. 1). In addition, compared with CRP and all individual metabolic syndrome components (except elevated waist circumference), elevated ALT was more strongly associated with

Table 3—*Effect of adding CRP or ALT to models testing the association of metabolic risk definitions with directly measured insulin sensitivity*

Model	Independent variable	Prevalence (%)	OR (95% CI)	*P* value	AROC
Individual components					
1	Elevated waist circumference	31.1	6.13 (4.44–8.45)	<0.0001	0.74
2	Elevated triglyceride	30.6	1.68 (1.23–2.25)	0.0004	0.61
3	Low HDL	50.5	2.60 (1.97–3.44)	<0.0001	0.66
4	Elevated blood pressure	46.5	2.37 (1.78–3.14)	<0.0001	0.65
5	IFG	15.0	2.23 (1.69–2.94)	<0.0001	0.64
6	CRP >3 mg/l	29.8	2.49 (1.84–3.36)	<0.0001	0.65
7	ALT (upper quartile)	26.5	3.00 (2.19–4.12)	<0.0001	0.66
Waist and ALT					
8	Elevated waist circumference		5.73 (4.14–7.95)	<0.0001	
	ALT (upper quartile)		2.64 (1.88–3.70)	<0.0001	0.76*
Hypertriglyceridemic waist models					
9	Hypertriglyceridemic waist	17.9	2.73 (1.94–3.84)	<0.0001	0.64
10	Hypertriglyceridemic waist		2.56 (1.80–3.63)	<0.0001	
	CRP >3 mg/l		2.37 (1.74–3.21)	<0.0001	0.68†
11	Hypertriglyceridemic waist		2.55 (1.79–3.62)	<0.0001	
	ALT (upper quartile)		2.88 (2.08–3.97)	<0.0001	0.68††
12	Hypertriglyceridemic waist		2.37 (1.65–3.39)	<0.0001	
	CRP >3 mg/l		2.42 (1.77–3.31)	<0.0001	
	ALT (upper quartile)		2.94 (2.12–4.09)	<0.0001	0.72†††
EWET models					
13	EWET	23.1	4.61 (3.32–6.41)	<0.0001	0.69
14	EWET		4.15 (2.97–5.80)	<0.0001	
	CRP >3 mg/l		2.09 (1.52–2.86)	<0.0001	0.72‡
15	EWET		4.27 (3.05–5.97)	<0.0001	
	ALT (upper quartile)		2.71 (1.95–3.78)	<0.0001	0.72‡‡
16	EWET		2.15 (1.56–2.96)	<0.0001	
	CRP >3 mg/l		2.77 (1.98–3.88)	<0.0001	
	ALT (upper quartile)		3.80 (2.70–5.35)	<0.0001	0.74‡‡‡
TG-to-HDL ratio models					
17	TG/HDL (≥3)	40.7	2.44 (1.84–3.23)	<0.0001	0.65
18	TG/HDL (≥3)		2.37 (1.78–3.16)	<0.0001	
	CRP >3 mg/l		2.45 (1.80–3.33)	<0.0001	0.69§
19	TG/HDL (≥3)		2.29 (1.72–3.05)	<0.0001	
	ALT (upper quartile)		2.83 (2.05–3.91)	<0.0001	0.69§§
20	TG/HDL (≥3)		2.22 (1.66–2.98)	<0.0001	
	CRP >3 mg/l		2.51 (1.84–3.44)	<0.0001	
	ALT (upper quartile)		2.90 (2.08–4.03)	<0.0001	0.72§§§
HOMA-IR models					
21	HOMA-IR (upper quartile)	32.7	11.72 (8.45–16.23)	<0.0001	0.79
22	HOMA-IR (upper quartile)		11.01 (7.92–15.30)	<0.0001	
	CRP >3 mg/l		1.99 (1.40–2.82)	0.0001	0.81¶
23	HOMA-IR (upper quartile)		10.46 (7.51–14.58)	<0.0001	
	ALT (upper quartile)		1.84 (1.27–2.66)	0.0013	0.79¶¶
24	HOMA-IR (upper quartile)		9.75 (6.98–13.63)	<0.0001	
	CRP >3 mg/l		2.04 (1.44–2.90)	<0.0001	
	ALT (upper quartile)		1.90 (1.31–2.77)	0.0008	0.81¶¶¶
Metabolic syndrome models					
25	NCEP metabolic syndrome	26.8	4.44 (3.25–6.07)	<0.0001	0.70
26	NCEP metabolic syndrome		3.99 (2.90–5.48)	<0.0001	
	CRP >3 mg/l		2.04 (1.48–2.79)	<0.0001	0.72#
27	NCEP metabolic syndrome		4.01 (2.91–5.51)	<0.0001	
	ALT (upper quartile)		2.55 (1.83–3.56)	<0.0001	0.73##
28	NCEP metabolic syndrome		3.56 (2.57–4.93)	<0.0001	
	CRP >3 mg/l		2.11 (1.53–2.91)	<0.0001	
	ALT (upper quartile)		2.62 (1.87–3.67)	<0.0001	0.75###
29	NCEP metabolic syndrome		2.01 (1.38–2.91)	0.0002	

Table 3—*Continued.*

Model	Independent variable	Prevalence (%)	OR (95% CI)	*P* value	AROC
	CRP >3 mg/l		1.87 (1.31–2.67)	0.0006	
	HOMA-IR (upper quartile)		8.17 (5.78–11.54)	<0.0001	
	ALT (upper quartile)		1.80 (1.23–2.64)	0.0024	0.82####

Logistic regression models were adjusted for age, sex, and ethnicity; rows 1–5 are NCEP-defined traits. The dependent variable is the lowest quartile of S_I measured by an FSIVGTT. Models 8, 10–12, 14–16, 18–20, 22–24, and 26–29 show multivariate results with ORs for risk definitions adjusted for other variables in the model. Independent variables are elevated waist (waist circumference >102 cm [men] or >88 cm [women]), elevated triglyceride level (triglycerides ≥150 mg/dl), low HDL (HDL cholesterol <40 mg/dl [men] or <50 mg/dl [women]), elevated blood pressure (blood pressure ≥130/≥85 mmHg), IFG (fasting glucose ≥110 mg/dl), ALT (upper quartile [≥23 units/l]), hypertriglyceridemic waist (waist circumference ≥90 cm [men] or ≥80 cm [women] and triglycerides ≥175 mg/dl), EWET, (waist circumference ≥95 cm [men] or ≥88 cm [women] and triglycerides ≥128 mg/dl); HOMA [(insulin × glucose [millimoles per liter])/22.5, with elevated HOMA ≥4.02], and NCEP metabolic syndrome (≥3 of the disorders listed in the first 5 rows of this table). *P* values for AROC comparisons: *Adding ALT to waist circumference, $P = 0.01$; †Adding CRP to hypertriglyceridemic waist, $P = 0.001$; ††Adding ALT to hypertriglyceridemic waist, $P = 0.003$; †††Adding CRP and ALT to hypertriglyceridemic waist, $P < 0.0001$; ‡Adding CRP to EWET, $P = 0.002$; ‡‡Adding ALT to EWET, $P = 0.01$; ‡‡‡Adding CRP and ALT to EWET, $P < 0.0001$; §Adding CRP to elevated triglycerides-to-HDL (Tg/HDL) ratio, $P = 0.004$; §§Adding ALT to elevated triglycerides-to-HDL ratio, $P = 0.005$; §§§Adding CRP and ALT to elevated triglycerides-to-HDL ratio, $P < 0.0001$; ¶Adding CRP to HOMA-IR, $P = 0.02$; ¶¶Adding ALT to HOMA-IR, $P = 0.21$; ¶¶¶Adding CRP and ALT to HOMA-IR, $P = 0.004$; #Adding CRP to NCEP metabolic syndrome, $P = 0.03$; ##Adding ALT to NCEP metabolic syndrome, $P = 0.005$; ###Adding CRP and ALT to NCEP metabolic syndrome, $P < 0.0001$; ####Adding CRP, ALT, and HOMA-IR to NCEP metabolic syndrome, $P < 0.0001$.

insulin resistance and was as good as or better in classifying nondiabetic subjects with insulin resistance based on AROC curve criteria (Table 3, models 1–7). Further, elevated ALT performed better than hypertriglyceridemic waist and elevated triglyceride-to-HDL ratio in classifying nondiabetic subjects with insulin resistance, although this was not the case for EWET, NCEP metabolic syndrome, or HOMA-IR (models 9, 13, 17, 21, and 25 in Table 3). Finally, elevated ALT was independently associated with insulin resistance when included in models with waist circumference, NCEP metabolic syndrome, EWET, hypertriglyceridemic waist, elevated triglyceride-to-HDL ratio, and HOMA-IR or NCEP metabolic syndrome and HOMA-IR (with or without elevated CRP in the models), although the addition of elevated ALT improved the classification of insulin resistance by the AROC curve criteria only for models not including HOMA-IR (models 8, 10–12, 14–16, 18–20, 22–24, and 26–29 in Table 3).

CONCLUSIONS — In this article, we report that ALT was significantly associated with insulin resistance, independently of both traditional clinical metabolic syndrome risk factors as well as more detailed metabolic variables including fasting insulin and IGT. Further, we found that elevated ALT was independently associated with insulin resistance when included in models with established metabolic risk definitions and, moreover, that elevated ALT improved the ability of many of these models to classify subjects with insulin resistance. In addition to being a marker of NAFLD, these data therefore suggest that ALT may also be a useful indicator of insulin resistance for application in clinical settings and large epidemiological studies. The importance of this observation is highlighted by the fact that the majority of existing surrogate measures of insulin resistance involve the measurement of insulin concentrations (13–18), which are not used in clinical practice. Further, laboratory assays for the assessment of insulin are expensive and have not undergone standardization (19). Although other metabolic risk definitions that use clinically available measures (including the triglyceride-to-HDL ratio and various definitions of the metabolic syndrome) are available (20–24), these definitions explain relatively modest amounts of the variation in insulin sensitivity compared with indexes that include fasting insulin (25). That ALT, an inexpensive and routinely measured clinical variable, improves the classification/identification of insulin resistance represents an important advance.

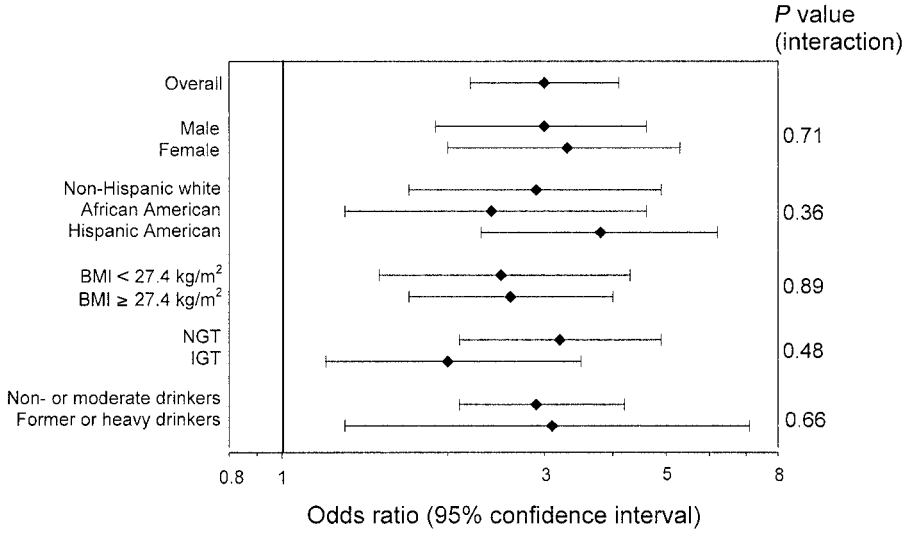

Figure 1—*Logistic regression analysis of association of ALT (upper quartile) with insulin resistance (lowest quartile of FSIVGTT-measured insulin sensitivity) among nondiabetic IRAS subjects overall and by subgroups of sex, ethnicity, obesity, glucose tolerance, and alcohol consumption (nondrinkers or moderate drinkers versus former or heavy drinkers). ORs were adjusted for age, sex, and ethnicity.*

The majority of previous studies have shown significant positive associations of baseline liver markers (especially ALT and GGT) with risk of incident type 2 diabetes, metabolic syndrome, and CVD (32). In this regard, we have previously reported significant associations of ALT with incident diabetes and metabolic syndrome in this cohort (53,54). In addition,

liver markers have been associated with several abnormalities of the metabolic syndrome, including obesity, hyperglycemia, and dyslipidemia, as well as surrogate measures of insulin resistance (35,36). Relatively few studies, however, have examined the relationship between ALT and direct measures of insulin resistance. Vozarova at al. (37) reported a significant association of ALT with clamp-derived whole-body insulin sensitivity in Pima Indians, independent of age, sex, and adiposity. Similarly, Schindhelm et al. reported that ALT was independently associated with clamp-derived whole-body insulin sensitivity in a sample of Dutch subjects with type 2 diabetes (38). Further, GGT was associated with clamp-derived insulin sensitivity in 245 nondiabetic subjects in the Tübingen family study (39). Our findings extend these observations with FSIVGTT data from a large multiethnic ethnic sample of adults at various stages of glucose tolerance (NGT and IGT).

ALT was more strongly related to insulin resistance compared with AST or ALK, and the significant association of ALT with insulin resistance was maintained even after adjustment for insulin levels and IGT. This observation may be related to a number of characteristics of ALT, including its greater specificity to hepatic tissue and its stronger correlation with directly measured liver fat content relative to that of other liver markers (32,34). Significant, positive correlations between liver fat content, measured directly using proton magnetic resonance spectroscopy, and hepatic insulin resistance have been documented (34), and Vozarova et al. (37) reported that ALT measured at baseline was associated with worsening hepatic glucose output (a measure of hepatic insulin resistance) after 7 years of follow-up. In light of this body of evidence, we hypothesize that increased ALT may be specifically reflecting hepatic insulin resistance. This concept is supported by the observation that ALT was associated with the S_i even after multiple adjustment for other metabolic syndrome variables, which themselves are probably markers of either generalized whole-body insulin resistance or insulin resistance in specific tissues, such as adipose tissue or muscle.

In addition to the availability of a detailed measure of insulin sensitivity, the major strengths of our study include the use of data from a large, well-characterized cohort of U.S. adults, which included members of two ethnic groups (African Americans and Hispanics) that are known to have a high prevalence of insulin resistance and have an increased risk of type 2 diabetes and CVD. Further, the cohort included a large number of subjects with IGT. However, our study is limited by a lack of direct measures of liver fat and visceral adipose tissue, as well as by an absence of information on hepatitis B and C serology. In addition, we did not measure GGT, which has been shown in other studies to predict metabolic syndrome, type 2 diabetes, and CVD and to be associated with insulin sensitivity (55–57).

In summary, ALT was associated with insulin sensitivity independently of conventional and more detailed metabolic measures. These findings suggest that ALT, with proper laboratory standardization, might be added to existing metabolic risk definitions based on clinical measures to improve the identification of subjects with insulin resistance.

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