Original Paper

Higher Levels of Alanine Aminotransferase Within the Reference Range Predict Unhealthy Metabolic Phenotypes of Obesity in Normoglycemic First-Degree Relatives of Patients With Type 2 Diabetes Mellitus

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Obesity is a heterogeneous disorder with metabolically healthy and unhealthy phenotypes and varying degrees of cardiometabolic complications. To evaluate whether alanine aminotransferase (ALT) could be used for identification of obese phenotypes, the authors measured ALT, adiponectin, leptin, leptin receptor, free leptin index, high-sensitivity C-reactive protein, fasting insulin, glucose, and full lipid profile in 486 (176 men and 310 women) normoglycemic first-degree relatives of patients with type 2 diabetes mellitus with negative medication history and hepatitis screen. Patients were classified into obesity phenotypes on the basis of the degree of adiposity and the International Diabetes Federation criteria for the metabolic syndrome. One hundred and thirty-seven (28%) patients were positive for the metabolic syndrome, 32 (7%) had normal weight but metabolically unhealthy phenotype, and 201 (41%) were obese but metabolically healthy. ALT showed significant positive correlations with body mass index, waist circumference, beta-cell function, insulin, homeostasis model assessment for insulin resistance, high-sensitivity C-reactive protein, total cholesterol, and triglycerides and increased with increasing number of metabolic syndrome components. Binary logistic regression analyses showed that higher ALT levels within the normal range were significantly associated with the metabolic syndrome. ALT could be used for identification of the metabolically obese phenotype. Lowering the ALT upper normal reference limit will facilitate earlier detection of risky phenotypes of obesity. J Clin Hypertens (Greenwich). *2010;12:301–308.* *©2009 Wiley Periodicals, Inc.*

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Lifestyle modernization and rapid economic growth have led to a dramatic worldwide increase in the prevalence of obesity.[1] Closely linked to the increased prevalence of obesity is increased prevalence of obesity-related disorders such as insulin resistance, glucose intolerance, dyslipidemia, and hypertension that cluster to

form the metabolic syndrome (MS). However, due to differences in environmental influences and genetic susceptibility, the clinical and metabolic phenotypes of obesity vary widely between populations. Some obese persons have the metabolically healthy obese phenotype,[2–4] and these individuals do not have the expected metabolic profiles that are characteristic of obesity. The exact mechanisms involved in the protection against metabolic abnormalities have not been elucidated, but several metabolic and obesity-related biomarkers have been proposed as being useful for the identification of individuals with the phenotype.[5]

Concurrent with the worldwide epidemic of obesity is the emerging importance of nonalcoholic fatty liver disease (NAFLD), which has become the most common cause of unexplained abnormal liver function tests. NAFLD, which encompasses a spectrum of liver conditions that range from fatty liver to nonalcoholic steatohepatitis to cirrhosis, has emerged as the hepatic manifestation of the MS.[6,7] Obesity-associated NAFLD is identified by asymptomatic elevations in serum aminotransferases, which are strongly associated with features of the MS.[6,7] Indeed, some studies have shown that high serum levels of aspartate aminotransferase and alanine aminotransferase (ALT) are associated with the development of type 2 diabetes, end-stage liver disease, and increased risk of mortality from cardiovascular disease.[8] Therefore, asymptomatic increases in ALT can no longer be regarded as mild or easily reversible, and there is need for early identification of individuals at risk.[9] Identification of individuals at risk is not easy, however, since aminotransferase levels are affected by several factors such as sex and ethnicity.[10] Browning and colleagues[10] showed that the lower frequency of obesity-associated hepatic steatosis in some ethnic groups was not explained by differences in body mass index (BMI) and insulin resistance. We postulate that the obesity phenotype, based on whether patients have metabolically healthy or unhealthy phenotype, could have a profound impact on circulating ALT levels, and those with higher ALT levels, within the normal reference range, would have the metabolically unhealthy phenotype and increased susceptibility to obesity-related liver disease. Therefore, the aim of this study is to evaluate the metabolic characteristics of persons with ALT within the normal range to determine the ALT cutoff that could be used for identification of the metabolically healthy and unhealthy obese phenotypes.

METHODS

Patients and Clinical Features

A total of 486 (176 men and 310 women) apparently healthy persons, aged 17 to 49 years, were recruited by informing patients with type 2 diabetes mellitus to invite their first-degree relatives for clinical and laboratory review. Criteria for inclusion in our study were both parents or one parent and/or a sibling with type 2 diabetes, fasting normoglycemia (defined as a fasting glucose <5.6 mmol/L), and freedom from any current illness. Alcohol consumption (consumption of alcohol in Kuwait is illegal), current ingestion of any medication (prescription and over-the-counter) known to affect liver function, and laboratory evidence of viral or autoimmune liver diseases were exclusion criteria. The study was approved by the ethics committees of the Faculty of Medicine, Kuwait University and the Ministry of Health, Kuwait, and performed in accordance with the Declaration of Helsinki. All patients gave informed voluntary consent to participate in the study.

The weight, height, waist circumference (WC), and hip circumferences were measured using standard methods and the BMI was calculated. The number of features of the MS was based on the International Diabetes Federation (IDF) criteria[11] using European cutoff values for WC (>94 cm for men or >80 cm for women) and any two of the following: triglycerides (TGs) ≥ 1.7 mmol/L; high-density lipoprotein cholesterol (HDL-C) <1.03 mmol/L for men or <1.29 mmol/L for women; blood pressure $>130/85$ mm Hg; or hypertensive and on treatment. The patients were then classified as MS positive if they met the criteria or MS negative if they did not.

Patients were classified based on BMI categories (normal weight, BMI <25 kg/m^2; overweight, BMI 25–29.9 kg/m^2; and obese, BMI ≥ 30 kg/m^2) and as metabolically healthy (MS negative) or more metabolically unhealthy (MS positive).

LABORATORY METHODS

ALT Activity

ALT was determined by an enzymatic rate method on the Beckman DXC analyzer (Beckman Corporation, Fullerton, CA). Our laboratory uses the upper limit of normal provided by the manufacturer and these are currently set at 45 U/L for men and 34 U/L for women. However we calculated the 95th percentiles for ALT levels in MS-negative patients with BMI <25 kg/m^2 and set the upper limit of normal in men and women at the 95th percentile.

Adiponectin Assay

Fasting plasma adiponectin was measured using a commercially available enzyme-linked immunoassay (ELISA) kit (Linco Research, St Charles, MO) with a sensitivity of 0.39 μg/mL. The inter-assay and intra-assay coefficients of variation on pooled plasma specimen with adiponectin concentration of 8.2 μg/mL were 4.7% and 6.8%, respectively.

Leptin Assay

Plasma leptin concentration was determined with the DSL-10-23100 ACTIIVE ELISA kit (Diagnostics Systems Laboratories, Webster, TX), which has an assay sensitivity of 0.5 ng/mL. The inter-assay and intra-assay coefficients of variation on pooled plasma specimen with leptin concentration of 23.6 ng/mL were 4.1% and 5.3%, respectively.

sOB-R Assay and Calculation of FLI

The concentration of soluble leptin receptor (sOB-R) was determined with the RD194002100 human leptin receptor double monoclonal antibody ELISA kit (BioVendor, Brno, Czech Republic). The assay has a sensitivity of 0.4 U/mL. Free leptin index (FLI) was determined by calculating the ratio between the levels of leptin and sOB-R.

hs-CRP Assay

The concentration of C-reactive protein (CRP) was determined by a high-sensitivity (hs) chemiluminescent assay on the Immulite (DPC, Los Angeles, CA) automated analyzer according to the manufacturers' instructions. The assay has a lower limit of detection of 0.1 mg/L.

Other Laboratory Methods

Fasting serum insulin was determined by an ELISA (DSL-10-1600 ACTIVE; Diagnostics Systems Laboratories). Insulin resistance was calculated using the homeostasis model assessment for insulin resistance (HOMA-IR) using a calculator downloaded from http://www.dtu.ox.ac.uk/.[12] The HOMA calculator also gives estimates of steady-state beta-cell function and insulin sensitivity. Because insulin resistance develops as a continuous trait in these patients with higher-than-normal risk of type 2 diabetes, we have not used a cutoff point for determination of insulin resistance. Therefore, we divided the entire cohort into quartiles of HOMA-IR and classified patients in the highest quartile as being more insulin resistant than those in the lowest quartile.

Fasting plasma glucose, total cholesterol (TC), TGs, and HDL-C were analyzed on an automated analyzer (Beckman DXC, Beckman Corporation, Brea, CA). Low-density lipoprotein cholesterol (LDL-C) was calculated using the Friedewald formula.[13]

Statistical Methods

SPSS 16.0 software (SPSS Inc, Chicago, IL) was used for statistical analysis and $P<.05$ was considered statistically significant for all analyses. Data are presented as mean ± standard deviation (SD) unless otherwise specified. The Kolmogorov–Smirnov goodness-of-fit test was used to test for normality of the data. Several variables (ALT, insulin, TGs, hs-CRP, leptin, and FLI) that diverged significantly from normal distribution were log-transformed when parametric tests were used. Comparisons between two groups were performed with the Mann–Whitney *U* test, and the Kruskal–Wallis analysis of variance was used to compare between more than two groups. Spearman correlation coefficient was used to describe the association between serum ALT and other continuous variables of interest; the partial correlation coefficient was used to control for the effects of WC and sex. Multiple logistic regression analysis with ALT as the dependent variable and other variables as independent variables was used to identify significant determinants of ALT levels in men and women. Binary logistic regression analysis was used to ascertain the association of ALT with the MS as a dependent variable with and without adjustment for the confounding effects of age, WC, LDL-C, and HOMA-IR.

RESULTS

Characteristics of the Study Population

Table I summarizes the characteristics of the patients grouped by sex, BMI, and MS status. One hundred and thirty-seven (28%) of the patients were MS positive and, of these, only 32 (7%) had normal weight but metabolically unhealthy phenotype. Two hundred and one (41%) were obese but metabolically healthy. In general, the patients who were MS positive had higher WC, higher ALT, more atherogenic lipid profile, were more insulin resistant, and had lower adiponectin levels than patients who were MS negative.

Correlations and Determinants of ALT

The Figure shows that ALT increased significantly ($P<.0001$) with increasing WC. Table II shows the correlations of ALT with other variables. ALT showed significant positive correlations with BMI, WC, beta-cell function, insulin, HOMA-IR, TC, TGs, and LDL-C and inverse correlations with

Table I. Anthropometric and Biochemical Characteristics of the Study Patients Grouped by BMI and Metabolic Syndrome Status

	Metabolic Syndrome Negative			Metabolic Syndrome Positive		
	BMI <25	BMI 25–29.9	BMI >30	BMI <25	BMI 25–29.9	BMI >30 (12)
Male patients, No.	34	20	72	12	13	25
Age, y	24.9 (8.2)	25.8 (8.9)	26.7 (9.0)	44 (8.2)[a]	32.8 (8.5)[a]	35.6 (7.1)[a]
BMI, kg/m^2	22.0 (2.5)	26 (5.9)	34.4 (3.7)[a]	23 (1.4)	27.3 (2.4)[a]	34.3 (4.4)[a]
Waist circumference, cm	85.1 (7.5)	95.0 (14.5)	112.8 (10.7)[a]	98.4 (8.9)[a]	109.8 (11.3)[a]	115.4 (11.6)[a]
Systolic blood pressure, mm Hg	108 (9)	112 (9)	115 (7)	130 (4)[a]	120 (15)[a]	121 (15)[a]
Diastolic blood pressure, mm Hg	69 (6)	72 (7)	75 (7)	68 (6)	75 (10)	76 (9)
Total cholesterol, mmol/L	4.3 (0.8)	4.6 (0.9)	5.0 (1.0)	4.3 (0.5)	5.7 (1.7)[a]	6.1 (2.1)[a]
HDL cholesterol, mmol/L	1.06 (0.26)	1.1 (0.2)	1.1 (0.2)	0.8 (0.3)[a]	0.9 (0.2)[a]	0.9 (0.2)[a]
Triglycerides, mmol/L	0.8 (0.4)	1.0 (0.5)	1.2 (0.4)	1.7 (0.3)[a]	2.2 (1.6)[a]	2.3 (1.8)[a]
LDL cholesterol, mmol/L	2.8 (0.8)	3.0 (0.9)	3.3 (1.0)	3.2 (0.2)	3.7 (1.3)	4.0 (1.5)
Fasting glucose, mmol/L	4.9 (0.3)	4.9 (0.4)	5.0 (0.4)	5.4 (0.5)	5.0 (0.5)	5.1 (0.6)
Fasting insulin, µIU/mL	5.3 (4.3)	7.7 (9.1)	7.1 (6.0)	8.2 (3.1)[a]	9.5 (5.5)[a]	10.1 (4.9)[a]
Insulin sensitivity	140.0 (57.0)	126.0 (61.6)	125.7 (75.3)	94.0 (36.4)[a]	89.5 (43.8)[a]	86.5 (46.4)[a]
Beta-cell function	85.0 (37.0)	100.8 (61.2)	110.0 (90.4)	108 (41.3)[a]	115.1 (42.3)[a]	114.0 (42.2)[a]
HOMA-IR	0.9 (0.6)	1.1 (0.9)	1.2 (0.8)	1.3 (0.5)[a]	1.4 (0.6)[a]	1.6 (0.3)[a]
ALT, U/L	22.0 (9.6)	26.0 (11.6)	27.6 (11.0)	29.9 (8.1)[a]	32.8 (10.4)[a]	37.5 (9.9)[a]
Adiponectin, µg/mL	8.3 (3.4)	7.6 (2.8)	6.9 (2.0)	3.9 (0.8)[a]	5.1 (1.9)[a]	4.5 (1.4)[a]
Leptin, ng/mL	6.6 (7.5)	14.5 (16.3)	28.3 (22.0)[a]	23.4 (9.1)[a]	19.1 (10.5)[a]	20.0 (10.3)[a]
Leptin receptor, U/L	24.1 (6.9)	21.6 (7.2)	17.3 (2.8)[a]	19.2 (3.2)[a]	18.3 (3.5)[a]	14.4 (2.8)[a]
Free leptin index, ng/U	0.08 (0.06)	0.33 (0.35)	0.57 (0.38)[a]	0.46 (0.19)[a]	0.54 (0.23)[a]	0.92 (0.21)[a]
hs-CRP, mg/L	0.15 (0.18)	0.55 (1.10)[a]	0.68 (0.42)[a]	0.38 (0.11)[a]	1.0 (1.8)[a]	1.98 (2.36)[a]
Female patients, No.	60	34	129	20	23	44
Age, y	27.2 (8.4)	28.1 (7.4)	30.2 (7.4)	34.4 (5.8)[a]	31.1 (14.4)	37.2 (8.9)[a]
BMI, kg/m^2	21.8 (2.4)	27.3 (7.1)	35.5 (4.9)[a]	22.1 (2.8)	27.9 (1.2)[a]	35.0 (4.3)[a]
Waist circumference, cm	82.4 (9.2)	92.0 (8.8)[a]	105.5 (12.9)[a]	91.2 (6.9)[a]	93.9 (8.9)[a]	107.0 (13.0)[a]
Systolic blood pressure, mm Hg	110 (16)	108 (9)	111 (10)	120 (17)[a]	129 (19)[a]	126 (19)[a]
Diastolic blood pressure, mm Hg	69 (8)	69 (6)	71 (6)	75 (8)	80 (13)	77 (7)
Total cholesterol, mmol/L	4.5 (0.8)	4.9 (0.8)	4.9 (0.9)	5.1 (1.7)	5.0 (1.3)	5.0 (0.7)
HDL cholesterol, mmol/L	1.4 (0.3)	1.4 (0.3)	1.3 (0.3)	0.94 (0.3)[a]	1.1 (0.2)[a]	1.0 (0.2)[a]
Triglycerides, mmol/L	0.7 (0.6)	0.8 (0.5)	0.9 (0.4)	1.7 (0.4)[a]	1.3 (0.3)[a]	1.9 (1.1)[a]
LDL cholesterol, mmol/L	2.7 (0.6)	2.9 (0.7)	3.2 (0.7)	3.0 (0.9)	3.1 (1.3)	3.0 (0.7)
Fasting glucose, mol/L	4.8 (0.5)	4.9 (0.4)	4.8 (0.4)	4.1 (0.3)	5.3 (0.5)	5.2 (0.6)
Fasting insulin, µIU/mL	4.8 (2.7)	4.6 (5.1)	5.9.0 (5.0)	6.2 (1.9)[a]	7.4 (4.0)[a]	11.8 (9.2)[a]
Insulin sensitivity	153.0 (59.2)	131.9 (51.8)	128.1 (46.8)	103.9 (33.8)[a]	109.2 (63.2)[a]	91.7 (47.2)[a]
Beta-cell function	85.9 (28.7)	108.7 (87.6)	111.4 (38.8)	95.8 (26.9)[a]	81.4 (30.0)[a]	112.7 (45.7)[a]
HOMA-IR	0.8 (0.3)	1.0 (0.9)	1.2 (0.6)	1.6 (0.5)[a]	1.6 (0.4)[a]	1.7 (1.2)[a]
ALT, U/L	15.9 (7)	18.0 (7.3)	18.7 (6.5)	23.7 (5.6)[a]	23.0 (7.7)[a]	25.0 (8.1)[a]
Adiponectin, µg/mL	11.3 (4.2)	12.6 (7.0)	9.7 (3.4)	5.9 (0.5)[a]	4.3 (0.2)[a]	5.5 (2.0)[a]
Leptin, ng/mL	17.7 (9.6)	33.6 (17.2)	58.4 (29.3)[a]	25.7 (11.3)[a]	27.7 (5.6)[a]	41.7 (21.6)[a]
Leptin receptor, U/L	23.2 (10.5)	21.0 (8.7)	14.5 (7.5)[a]	17.9 (6.2)[a]	18.5 (2.9)[a]	17.9 (4.5)[a]
Free leptin index, ng/U	0.5 (0.5)	0.8 (0.7)	2.1 (1.7)[a]	1.6 (0.8)[a]	1.6 (1.9)[a]	2.1 (0.2)[a]
hs-CRP, mg/L	0.26 (0.39)	0.29 (0.31)	1.5 (1.0)[a]	1.1 (0.4)[a]	1.45 (0.56)[a]	2.94 (0.76)[a]

Values are expressed as mean (standard deviation). Abbreviations: ALT, alanine aminotransferase; BMI, body mass index; HDL, high-density lipoprotein; HOMA-IR, homeostasis model assessment of insulin resistance; hs-CRP, high-sensitivity C-reactive protein; LDL, low-density lipoprotein. [a]Significantly different ($P<.05$) compared (Mann–Whitney U test) with lean patients (BMI <25 kg/m^2) and those without the metabolic syndrome.

insulin sensitivity, HDL-C, and adiponectin. Except for TC, HDL-C, and TG, these variables retained their correlations with ALT after correction for WC. However, after correction for sex and WC, insulin, beta-cell function, insulin sensitivity, and LDL-C were the only parameters that remained

significantly correlated with ALT. Leptin, leptin receptor, and FLI were not significantly associated with ALT (Table II). Table III shows that the significant determinants of ALT in both sexes are obesity indices, insulin resistance parameters, and metabolic variables that are components of the MS. Adiponectin was the only adipokine that emerged as a significant determinant of ALT.

ALT and Obesity Phenotypes

In all categories of obesity, ALT was significantly ($P<.05$) higher in MS-positive patients compared with MS-negative patients (Table I). We analyzed the characteristics of patients with normal and high-normal ALT levels (Figure). The observed upper 95% ALT levels in MS-negative patients with BMI <25 kg/m^2 were 18.2 U/L for women and 28.1 for men. For practical purposes, however, we used cutoff levels of 18 U/L for women and 28 U/L for men to define normal (<18 U/L in women and <28 U/L in men) and high-normal (>18 U/L in women and >28 U/L in men) patients. The Figure shows that patients with high-normal ALT levels had significantly higher WC and significantly lower adiponectin levels. Mean (SD) hs-CRP was significantly ($P<.05$) higher in patients with high-normal ALT (men, 0.84 [0.28]; women, 1.62 [0.35] mg/L) compared with patients with normal ALT (men, 0.43 [0.04]; women, 0.77 [0.21] mg/L). Similarly, patients in the highest quartile of HOMA-IR had significantly ($P<.05$) higher mean (SD) ALT (men, 29.47 [1.28]; women, 19.46 [1.78] U/L) compared with patients in the first quartile (men, 26.38 [2.46]; women, 15.53 [0.85]) U/L).

ALT activity >18 U/L in women was significantly associated with the MS (odds ratio [OR], 4.7; 95% confidence interval [CI], 4.0–5.6; P=.002). Similar association was observed in men with ALT >28 (OR, 2.6; 95% CI, 1.8–4.1; P=.02). In men (OR, 1.25; 95% CI, 1.19–1.31; P=.01) and women (OR, 2.1; 95% CI, 1.3–2.9; P=.02), the associations remained significant after controlling for the confounding effects of age, WC, LDL-C, and HOMA-IR.

DISCUSSION

The most important finding of this study is that ALT, even within the normal laboratory reference range, is significantly associated with obesity phenotypes, metabolic variables, and the MS. Therefore, ALT could be used to distinguish metabolically healthy patients from metabolically unhealthy patients whether they are lean or obese. Although

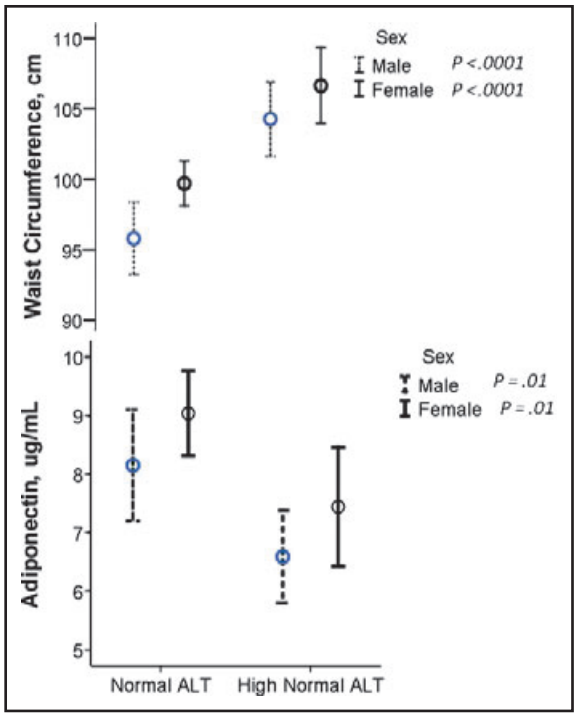

Figure. Mean (circles) and 95% confidence interval (bars) of waist circumference (upper panel) and adiponectin (lower panel) in relation to alanine aminotransferase (ALT) status.

the exact pathogenesis of raised ALT in obese patients remains unclear, the mechanisms of higher ALT in the normal range in our cohort is probably similar to the continuum of progressive increases that occurs in the stages of NAFLD.

In this study, ALT showed independent associations with insulin and insulin resistance (Table II). The mechanism of higher ALT in the metabolically unhealthy patients is most likely related to the higher HOMA-IR, which probably leads to increased free fatty acid flux, increased hepatic TG synthesis, and progressive hepatic TG accumulation that leads to hepatocellular damage and increased ALT.[14,15] However, as hepatic TG accumulation does not always lead to hepatocellular injury and higher ALT, other factors are probably involved in a "second hit."[16] Although the second hit is currently believed to be due to increased oxidative stress within the hepatocytes,[16] other metabolic abnormalities present in those with high-normal ALT may also play a role in those who progress from normal to higher and, with time, abnormal ALT. Adipokines such as adiponectin and leptin have been proposed to be contributors to this progression.[17]

We have shown in this study that obese patients with adiponectin levels higher than lean patients

Table II. Correlations (Spearman Rank) and Partial Correlations of Alanine Aminotransferase After Corrections for Waist Circumference and Sex

Variable	Spearman's Correlation (*P* Value)	Partial Correlation: Correction for Waist Circumference (*P* Value)	Partial Correlation: Correction for Waist and Sex (*P* Value)
Age, y	0.11 (NS)	−0.04 (NS)	0.14 (NS)
Waist, cm	0.34 (<.0001)	–	–
BMI, kg/m^2	0.31 (<.001)	−0.13 (NS)	−0.03 (NS)
Systolic blood pressure, mm Hg	0.08 (NS)	0.01 (NS)	−0.038 (NS)
Diastolic blood pressure, mm Hg	0.05 (NS)	−0.03 (NS)	−0.097 (NS)
hs-CRP, mg/L	0.22 (.016)	−0.05 (NS)	−0.04 (NS)
Total cholesterol, mmol/L	0.16 (.001)	0.22 (NS)	0.23 (NS)
Triglycerides, mmol/L	0.24 (<.0001)	−0.13 (NS)	−0.08 (NS)
HDL cholesterol, mmol/L	−0.27 (<.0001)	−0.13 (NS)	0.08 (NS)
LDL cholesterol, mmol/L	0.19 (<.0001)	0.35 (.029)	0.27 (.016)
Glucose, mmol/L	0.035 (NS)	−0.09 (NS)	−0.020 (NS)
Insulin, μIU/mL	0.16 (.003)	0.39 (.015)_	0.43 (.008)
HOMA-IR	0.24 (<.0001)	0.39 (.016)	0.42 (.010)
Leptin, ng/mL	−0.09 (NS)	−0.18 (NS)	−0.03 (NS)
Leptin receptor, U/L	0.02 (NS)	0.09 (NS)	0.05 (NS)
Free leptin index, ng/U	−0.04 (NS)	−0.06 (NS)	−0.04 (NS)
Adiponectin, μg/mL	−0.30 (<.0001)	−0.18 (.027)	−0.06 (NS)
Insulin sensitivity	−0.24 (<.0001)	−0.18 (.03)	−0.20 (.02)
Beta-cell function	0.15 (.01)	0.39 (.016)	0.47 (.003)

Abbreviations: BMI, body mass index; HDL, high-density lipoprotein; HOMA-IR, homeostasis model assessment of insulin resistance; hs-CRP, high-sensitivity C-reactive protein; LDL, low-density lipoprotein; NS, not significant (*P*>.05).

have the metabolically healthy phenotype and lower ALT levels (Table I). Adiponectin is an anti-inflammatory adipokine that improves hepatic and peripheral insulin sensitivity.[18,19] Lower levels of this protective hormone are associated with metabolic perturbations that could lead to higher circulating ALT levels. In the liver, adiponectin has been shown to decrease intrahepatic production of free fatty acids and increase oxidation of free fatty acids, thereby resulting in decreased intrahepatic TG accumulation.[18,19] While there is clearly a role for adiponectin as a determinant of ALT (Table II and Table III), the relationship between leptin and ALT is less clear. Although leptin is thought to contribute to both hits of NAFLD development,[20,21] in agreement with studies from other populations,[22] we have not found an association between leptin and ALT. The higher leptin found in association with the MS or with increasing BMI in MS-negative patients (Table I) may be a reflection of the associations of leptin resistance with the development of certain obesity phenotypes rather than of a pathogenetic role in the development of obesity-related increases in ALT.

What are the clinical implications of our results? The first thing that comes to mind is the need to lower the upper reference limits for ALT despite the well-known concerns that this could lead to physicians being faced with a large number of asymptomatic patients with abnormal ALT results. As we have shown that ALT could be used to delineate metabolic phenotypes, we suggest that patients with high-normal or mild increases in ALT should be specific targets for intervention to prevent progression to hepatic steatosis, NAFLD, and other metabolic complications. The correlations of ALT with cardiometabolic risk factors (Table II and Table III) also suggest that ALT would be a useful adjunct for cardiovascular disease risk assessment. Lessons from longitudinal epidemiologic studies of type 2 diabetes mellitus and cardiovascular disease risks have taught us to lower the upper acceptable limits for fasting blood glucose, LDL-C, and TGs so that there could be early therapeutic intervention in persons at risk.[11] We suggest that the same approach be used for ALT, whose significant associations with cardiometabolic risk factors suggest the need to think beyond its traditional use as a liver function test.

STRENGTHS AND LIMITATIONS

The main strength of this study is that we have evaluated metabolic associations of ALT in a group of normoglycemic-, alcohol-, and medication-free

Table III. Multiple Linear Regression Analysis of Alanine Aminotransferase Level in Relation to Other Variables

Variable	Males β (*P* Value)	Females β (*P* Value)
Age, y	0.108 (NS)	0.016 (NS)
Waist, cm	0.399 (<.0001)	0.170 (.006)
BMI, kg/m^2	0.408 (<.0001)	0.252 (.001)
Systolic blood pressure, mm Hg	0.126 (NS)	−0.011 (NS)
Diastolic blood pressure, mm Hg	−0.108 (NS)	0.016 (NS)
hs-CRP, mg/L	0.188 (.001)	0.307 (.011)
Total cholesterol, mmol/L	0.212 (.01)	0.207 (.001)
Triglycerides, mmol/L	0.184 (.04)	0.175 (.03)
HDL cholesterol, mmol/L	−0.187 (.03)	−0.179 (.04)
LDL cholesterol, mmol/L	0.190 (.024)	0.213 (.001)
Insulin, μIU/mL	0.189 (.03)	0.225 (.001)
HOMA-IR	0.163 (.002)	0.290 (<.0001)
Leptin, ng/mL	0.079 (NS)	0.005 (NS)
Leptin receptor, U/L	−0.033 (NS)	0.056 (NS)
Free leptin index, ng/U	0.005 (NS)	−0.043 (NS)
Adiponectin, μg/mL	−0.261 (.015)	−0.189 (.03)

Values are expressed as standardized regression coefficients (β). Abbreviations: BMI, body mass index; HDL, high-density lipoprotein; HOMA-IR, homeostasis model assessment of insulin resistance; hs-CRP, high-sensitivity C-reactive protein; LDL, low-density lipoprotein; NS, not significant ($P>.05$).

patients with high risk of developing type 2 diabetes mellitus. Although the lack of liver biopsy to confirm the absence of obesity-related hepatic pathology could be construed as a major weakness, this procedure would have been ethically difficult to justify in the presence of normal ALT levels. The cross-sectional design of the study is also a potential limitation, and we caution that ALT levels need to be repeated and monitored over time since repeat testing, especially in asymptomatic individuals with elevated levels, may show normal results.[23] However, it should be noted that our study is on individuals with higher ALT within the "normal" reference range.

CONCLUSIONS

Obesity is a heterogeneous disorder with different phenotypes and varying degrees of cardiometabolic complications. We propose that ALT would be a useful adjunct for the identification of the metabolically unhealthy phenotypes that will benefit from early intervention.

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REFERENCES

1 World Health Organization. *Obesity Preventing and Managing the Global Epidemic*. WHO Consultation on Obesity. WHO Technical report 894. Geneva: World Health Organization; 2000.
2 Ruderman NB, Schneider SH, Berchtold P. The "metabolically-obese," normal-weight individual. *Am J Clin Nutr*. 1981;34:1617–1621.
3 Ruderman NB, Berchtold P, Schneider S. Obesity-associated disorders in normal-weight individuals: some speculations. *Int J Obes*. 1982;6:151–157.
4 Ruderman N, Chisholm D, Pi-Sunyer X, et al. The metabolically obese, normal-weight individual revisited. *Diabetes*. 1998;47:699–713.
5 Karelis AD, St. Pierre DH, Conus F, et al. Metabolic and body composition factors in subgroups of obesity: what do we know? *J Clin Endocrinol Metab*. 2004;89:2569–2575.
6 Marchesini G, Bugianesi E, Forlani G, et al. Nonalcoholic fatty liver, steatohepatitis, and the metabolic syndrome. *Hepatology*. 2003;37:917–923.
7 Angelico F, Del Ben M, Conti R, et al. Insulin resistance, the metabolic syndrome, and non alcoholic fatty liver disease. *J Clin Endocrinol Metab*. 2005;90:1578–1582.
8 Ekstedt M, Franzen LE, Mathiesen UL, et al. Long-term follow-up of patients with NAFLD and elevated liver enzymes. *Hepatology*. 2006;44:865–873.
9 Ratziu V, Poynard T. Assessing the outcome of nonalcoholic steatohepatitis? It's time to get serious. *Hepatology*. 2006;44:802–805.
10 Browning JD, Szczepaniak LS, Dobbins R, et al. Prevalence of hepatic steatosis in an urban population in the United States: impact of ethnicity. *Hepatology*. 2004; 40:1387–1395.
11 Alberti KG, Zimmet P, Shaw J, et al. The metabolic syndrome – a new worldwide definition. *Lancet*. 2005; 366:1059–1062.
12 Diabetes Trials Unit, Oxford, URL. HOMA Calculator v2.2: http://www.dtu.ox.ac.uk/. Accessed January–March 2009.
13 Friedewald WT, Levy RT, Fredrickson DS. Estimation of the concentration of low density lipoprotein cholesterol in plasma without use of the ultracentrifuge. *Clin Chem*. 1972;18:449–502.
14 Browning JD, Horton JD. Molecular mediators of hepatic steatosis and liver injury. *J Clin Invest*. 2004;114:147–152.
15 Samuel VT, Liu ZX, Qu X, et al. Mechanism of hepatic insulin resistance in non-alcoholic fatty liver disease. *J Biol Chem*. 2004;279:32345–32353.
16 Angulo P. Non alcoholic fatty liver disease. *N Engl J Med*. 2002;346:1221–1231.
17 Tsochatzis EA, Papatheodoridis GV, Archimandritis AJ. Adipokines in nonalcoholic steatohepatitis: from pathogenesis to implications in diagnosis and therapy. *Mediators Inflamm*. 2009; 2009: 831670. doi: 10.1155/2009/831670.
18 You M, Considine RV, Leone TC, et al. Role of adiponectin in the protective action of dietary saturated fat against alcoholic fatty liver in mice. *Hepatology*. 2005; 42:568–577.

19 Xu A, Wang Y, Keshaw H, et al. The fat-derived hormone adiponectin alleviates alcoholic and nonalcoholic fatty liver diseases in mice. *J Clin Invest*. 2003;112:91–100.
20 Kaplan LM. Leptin, obesity, and liver disease. *Gastroenterology*. 1998;115:997–1001.
21 Chitturi S, Farrell G, Frost L, et al. Serum leptin in NASH correlates with hepatic steatosis but not fibrosis: a manifestation of lipotoxicity? *Hepatology*. 2002;36:403–409.
22 Liangpunsakul S, Chalasani N. Relationship between unexplained elevations in alanine aminotransferase and serum leptin in U.S. adults: results from the Third National Health and Nutrition Examination Survey (NHANES III). *J Clin Gastroenterol*. 2004;38:891–897.
23 Friedman LS, Dienstag JL, Watkins E, et al. Evaluation of blood donors with elevated serum alanine aminotransferase levels. *Ann Intern Med*. 1987;107:137–144.