

High Alanine Aminotransferase Is Associated With Decreased Hepatic Insulin Sensitivity and Predicts the Development of Type 2 Diabetes

Barbora Vozarova, Norbert Stefan, Robert S. Lindsay, Aramesh Saremi, Richard E. Pratley, Clifton Bogardus, and P. Antonio Tataranni

It has been proposed that liver dysfunction may contribute to the development of type 2 diabetes. The aim of the present study was to examine whether elevated hepatic enzymes (alanine aminotransferase [ALT], aspartate aminotransferase [AST], or γ -glutamyltranspeptidase [GGT]) are associated with prospective changes in liver or whole-body insulin sensitivity and/or insulin secretion and whether these elevated enzymes predict the development of type 2 diabetes in Pima Indians. We measured ALT, AST, and GGT in 451 nondiabetic (75-g oral glucose tolerance test) Pima Indians (aged 30 ± 6 years, body fat $33 \pm 8\%$, ALT 45 ± 29 units/l, AST 34 ± 18 units/l, and GGT 56 ± 40 units/l [mean \pm SD]) who were characterized for body composition (hydrodensitometry or dual-energy X-ray absorptiometry), whole-body insulin sensitivity (M), and hepatic insulin sensitivity (hepatic glucose output [HGO] during the low-dose insulin infusion of a hyperinsulinemic clamp) and acute insulin response (AIR) (25-g intravenous glucose challenge). Sixty-three subjects developed diabetes over an average follow-up of 6.9 ± 4.9 years. In 224 subjects, who remained nondiabetic, follow-up measurements of M and AIR were available. At baseline, ALT, AST, and GGT were related to percent body fat ($r = 0.16, 0.17, \text{ and } 0.11$, respectively), M ($r = -0.32, -0.28, \text{ and } -0.24$), and HGO ($r = 0.27, 0.12, \text{ and } 0.14$; all $P < 0.01$). In a proportional hazard analysis with adjustment for age, sex, body fat, M , and AIR, higher ALT [relative hazard 90th vs. 10th centiles (95% CI): 1.9 (1.1–3.3), $P = 0.02$], but not AST or GGT, predicted diabetes. Elevated ALT at baseline was associated prospectively with an increase in HGO ($r = 0.21, P = 0.001$) but not with changes in M or AIR (both $P = 0.1$). Higher ALT concentrations were cross-sectionally associated with obesity and whole-body and hepatic insulin resistance and prospectively associated with a decline in hepatic insulin sensitivity and the development of type 2 diabetes. Our findings indicate that high ALT is a marker of risk for type 2 diabetes and suggest a poten-

tial role of the liver in the pathogenesis of type 2 diabetes. *Diabetes* 51:1889–1895, 2002

Alanine aminotransferases are considered indicators of hepatocellular health, whereas γ -glutamyltranspeptidase (GGT) also reflects biliary tract function. Alanine aminotransferase (ALT) is found primarily in the liver. Aspartate aminotransferase (AST) and GGT are also found in other tissues and are therefore less-specific markers of liver function. The liver plays an important role in maintaining normal glucose concentrations during fasting as well as postprandially. It is also a major site of insulin clearance (1,2). The loss of a direct effect of insulin to suppress hepatic glucose production and glycogenolysis in the liver causes an increase in hepatic glucose production (2).

A number of recent studies have suggested that abnormal hepatocellular function is associated with obesity, insulin resistance, and type 2 diabetes. Prospective studies have found that high levels of hepatic enzymes, including ALT (3) and GGT (4), are associated with later development of diabetes. At the same time, ultrasonographic and pathological series have shown that excess deposition of fat in liver, usually termed nonalcoholic fatty liver disease, has strong cross-sectional associations with obesity, insulin resistance, and type 2 diabetes (5,6). Nonalcoholic fatty liver disease is also associated with elevation of ALT, raising the possibility that the prospective relationship between ALT and type 2 diabetes (3) may reflect cross-sectional associations with insulin resistance or obesity. Importantly, whereas previous studies have shown that ALT was associated with type 2 diabetes independent of obesity (3), measures of insulin resistance were not examined.

We have examined the associations of plasma hepatic enzyme concentrations with the development of whole-body insulin sensitivity (M) and hepatic insulin sensitivity and/or insulin secretion and type 2 diabetes in Pima Indians, a population with marked insulin resistance and one of the highest reported prevalence rates of type 2 diabetes in the world (7). Our aim was to examine: 1) whether hepatic enzyme concentrations were associated with later development of type 2 diabetes; 2) if so, whether these associations were independent of adiposity, insulin action, and insulin secretion; and 3) whether increased hepatic enzyme concentrations at baseline were associ-

From the Clinical Diabetes and Nutrition Section, National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, Phoenix, Arizona.

Address correspondence and reprint requests to Barbora Vozarova, Clinical Diabetes and Nutrition Section, National Institutes of Health, 4212 N. 16th St., Rm. 5-41, Phoenix, AZ 85016. E-mail: bvozarov@mail.nih.gov.

Received for publication 17 December 2001 and accepted in revised form 6 March 2002.

AIR, acute insulin response; ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT, γ -glutamyltranspeptidase; HGO, hepatic glucose output; IGT, impaired glucose tolerance; M , whole-body insulin sensitivity; M-high, high-dose insulin infusion; M-low, low-dose insulin infusion; NGT, normal glucose tolerance; OGTT, oral glucose tolerance test; WBC, white blood cell count; WTR, waist-to-thigh ratio.

TABLE 1

Physical and metabolic characteristics of the study populations in the cross-sectional, prospective, and longitudinal analyses

	Baseline population		Prospective analysis		Longitudinal analysis	
	Means \pm SD	Range (min-max)	Non-progressors	Progressors	Baseline	Follow-up
<i>n</i>	451		307	63	224	224
Age (years)	30 \pm 6	18–47	26 \pm 6	27 \pm 6	26 \pm 6	30 \pm 6
Body weight (kg)	98 \pm 26	50–257	91 \pm 23	99 \pm 22*	92 \pm 23	98 \pm 26†
Body fat (%)	33 \pm 8	9–53	30 \pm 9	35 \pm 7†	31 \pm 9	31 \pm 11
WTR	1.68 \pm 0.18	1.06–2.58	1.61 \pm 0.16	1.65 \pm 0.18	1.61 \pm 0.15	1.67 \pm 0.16†
Fasting plasma glucose (mmol/l)	4.9 \pm 0.6	2.8–7.1	4.8 \pm 0.5	5.2 \pm 0.5†	4.9 \pm 0.5	4.9 \pm 0.6
2-h plasma glucose (mmol/l)	7.1 \pm 1.8	3.1–10.9	5.9 \pm 1.1	6.6 \pm 0.8†	5.9 \pm 1.1	6.6 \pm 1.6†
Fasting plasma insulin (pmol/l)	254 \pm 115	67–677	210 \pm 102	250 \pm 102‡	210 \pm 96	234 \pm 114†
M-low (mg \cdot kg EMBS ⁻¹ \cdot min ⁻¹)	2.57 \pm 0.88	1.41–6.54	3.00 \pm 1.27	2.39 \pm 0.75†	2.98 \pm 1.33	2.72 \pm 0.98†
M-high (mg \cdot kg EMBS ⁻¹ \cdot min ⁻¹)	8.6 \pm 2.1	2.4–13.7	9.4 \pm 2.05	8.6 \pm 1.9‡	9.4 \pm 2.0	8.9 \pm 2.2†
AIR (pmol/l)	1,416 \pm 933	43–7,164	1,569 \pm 1,042	1,307 \pm 751*	1,638 \pm 1,026	1,542 \pm 1,104†
Basal endogenous glucose output (mg \cdot kg EMBS ⁻¹ \cdot min ⁻¹)	1.95 \pm 0.24	1.32–2.59	1.90 \pm 0.24	1.91 \pm 0.27	1.90 \pm 0.24	1.97 \pm 0.24
HGO (mg \cdot kg EMBS ⁻¹ \cdot min ⁻¹)	0.42 \pm 0.39	0–1.93	0.29 \pm 0.34	0.40 \pm 0.36*	0.32 \pm 0.36	0.43 \pm 0.40†
ALT (units/l)	45 \pm 29	5–144	36 \pm 25	39 \pm 29	35 \pm 26	41 \pm 30†
AST (units/l)	34 \pm 18	11–103	32 \pm 15	33 \pm 15	32 \pm 16	32 \pm 19†
GGT (units/l)	56 \pm 39	8–245	49 \pm 35	54.1 \pm 38	44 \pm 27	50 \pm 31†
ALKP (units/l)	111 \pm 42	50–279	95 \pm 42	101 \pm 59	90 \pm 45	109.1 \pm 43†
Bilirubin (μ mol/l)	11 \pm 5	2–34	12 \pm 9	11 \pm 4	7 \pm 6	6 \pm 3†
Fasting plasma albumin (g/l)	40 \pm 4	28–51	41 \pm 4	39 \pm 4‡	41 \pm 4	40 \pm 4†

Symbols indicate significant differences between progressors and nonprogressors in the prospective analysis (unpaired *t* test) and between baseline and follow-up variables (except for age) in the longitudinal analysis (paired *t* test). **P* = 0.05; †*P* < 0.001; ‡*P* < 0.01. ALKP, alkaline phosphatase; EMBS, estimated metabolic body size = fat free mass + 17.7 kg.

ated with a subsequent decrease in hepatic and whole-body insulin sensitivity and/or insulin secretion.

RESEARCH DESIGN AND METHODS

Subjects. Subjects in this study were participants in a longitudinal study of the pathogenesis of type 2 diabetes initiated in 1982 (8), and, therefore, they partially overlap with all prospective studies of type 2 diabetes in Pima Indians. All participants were Pima (or closely related Tohono O'odham) Indians from the Gila River Indian Community near Phoenix, Arizona. Subjects were invited at approximately annual intervals for repeat 75-g oral glucose tolerance tests (OGTTs) and an assessment of insulin sensitivity and insulin secretion. All subjects were between 18 and 50 years of age, nondiabetic (normal glucose tolerance [NGT] or impaired glucose tolerance [IGT]) at baseline according to OGTT (1985 World Health Organization criteria) (9), nonsmokers at the time of the study, and, except for obesity, healthy according to a physical examination and routine laboratory tests. By protocol, subjects with hepatic enzyme concentrations higher than three times the upper limit of the reference range were not studied. None of the patients had known history of hepatitis, but this was not confirmed by laboratory tests in all cases. No subject had clinical or laboratory signs of acute or chronic infection or took any medication at the time of the study. The protocol was approved by the Tribal Council of the Gila River Indian Community and by the Institutional Review Board of the National Institute of Diabetes and Digestive and Kidney Diseases. All subjects provided written informed consent before participation.

Cross-sectional analyses were carried out in 451 nondiabetic (286 NGT/165 IGT) Pima Indians, 173 women and 278 men, who had been characterized for ALT, AST, and GGT concentrations; body composition; glucose tolerance; fasting plasma insulin concentrations; insulin action; hepatic glucose output (HGO) during low-dose insulin infusion; and acute insulin response (AIR) (Table 1). Plasma alkaline phosphatase, bilirubin, and albumin concentrations were available in 267 subjects (163 women and 104 men). Total white blood cell count (WBC) was available in 111 subjects (69 women and 42 men, mean $8,288 \pm 1,883$ cells/mm³).

Prospective analyses were performed in 370 Pima Indians with NGT at baseline, who had baseline plasma measurements of ALT, AST, and GGT, body fat, insulin action, and AIR and had a follow-up OGTT. Among them, 63 subjects developed diabetes (progressors), whereas 307 remained nondiabetic (nonprogressors), with an average follow-up of 6.9 ± 4.9 years (range 1 month to 18 years).

Longitudinal analyses were performed in 224 nonprogressors with NGT at

baseline who were nondiabetic at follow-up and had a baseline and follow-up assessment of ALT, AST, GGT, body composition, fasting plasma insulin concentrations, insulin action, HGO, and AIR.

Methods. All subjects were admitted for 8–10 days to the National Institutes of Health Clinical Research Unit in Phoenix, Arizona, and were fed a weight-maintaining diet (50, 30, and 20% of daily calories provided as carbohydrate, fat, and protein, respectively) and abstained from strenuous exercise.

Plasma hepatic enzyme, albumin, and bilirubin concentrations were evaluated on admission after an overnight fast and measured by colorimetric method (DADE Behring-Dimension Clinical Chemistry System) in the hospital laboratory. WBC was measured on admission in the hospital laboratory by an automated cell counter.

Body composition was estimated by underwater weighing, with simultaneous determination of residual lung volume by helium dilution (10) or by total-body dual energy X-ray absorptiometry (DPX-L; Lunar Radiation, Madi-

TABLE 2

Relationships among hepatic enzyme and selected anthropometric and metabolic characteristics (partial correlations by Spearman)

	ALT	AST	GGT
<i>n</i>	451	451	451
age	0.10*	−0.06	0.05
body weight	0.27†	0.26†	0.20†
body fat	0.16†	0.07†	0.11*
waist	0.21†	0.21†	0.14‡
WTR	0.18†	0.17‡	0.23†
fasting glucose	0.08	0.19†	0.10
fasting insulin	0.30†	0.25†	0.20†
2-h glucose	0.07	0.15‡	0.07
M-low	−0.32†	−0.28†	−0.24†
M-high	−0.26†	−0.19†	−0.15‡
AIR (only NGT)	0.10*	0.02	0.06
Basal EGO	0.12*	0.04	−0.04
HGO	0.27†	0.11*	0.16†

**P* < 0.05; †*P* < 0.001; ‡*P* < 0.01. EGO, endogenous glucose output.

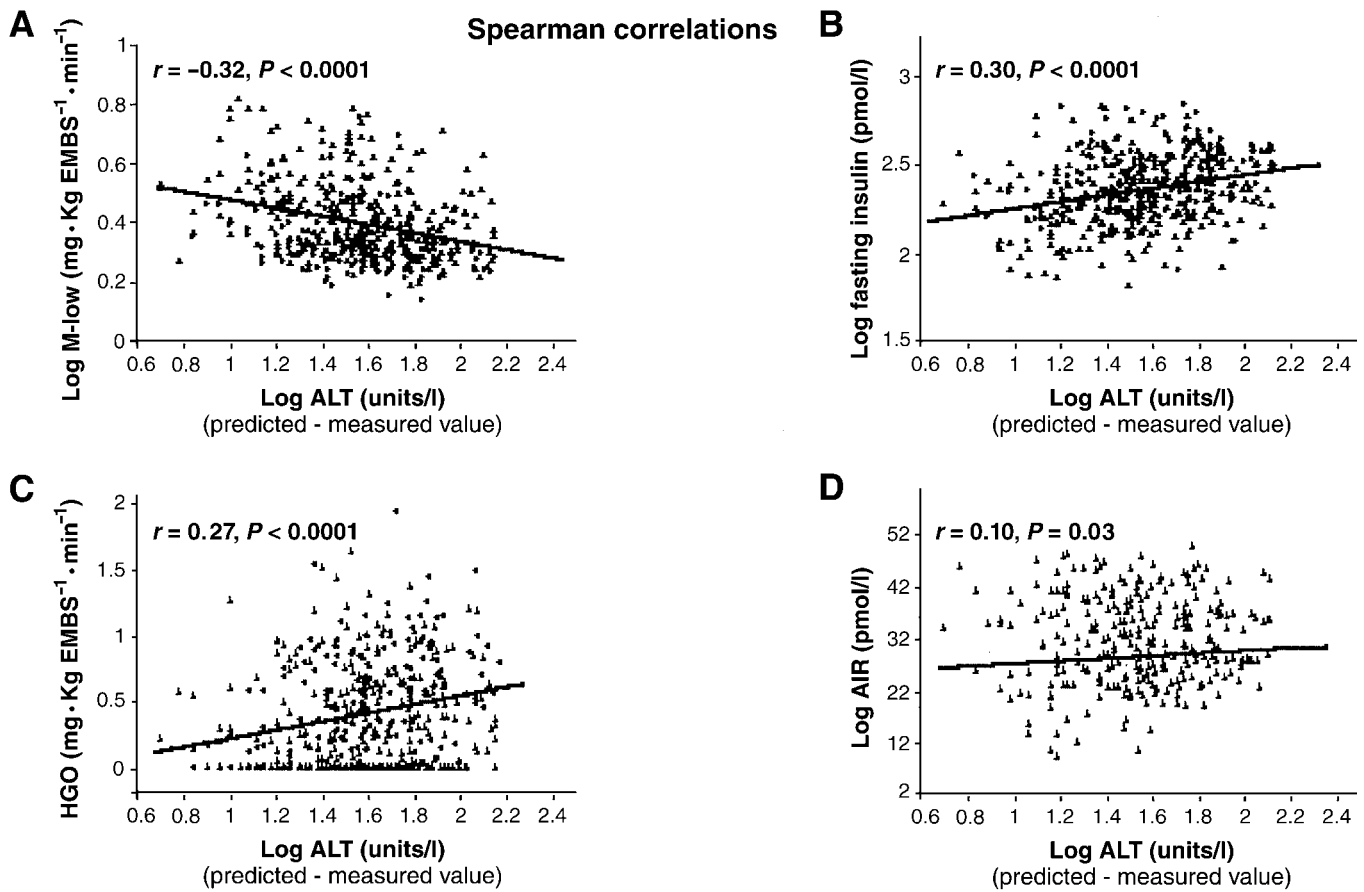


FIG. 1. Relationship between ALT and insulin action (M-low (A), fasting plasma insulin (B), HGO (C), and AIR (D) in 451 nondiabetic Pima Indians (278 NGT/165 IGT). Triangles indicate subjects with NGT, and circles indicate subjects with IGT. *D*: Because even mildly elevated glucose concentrations can secondarily affect insulin secretion, only data from subjects with NGT were included. (Lines in the graphs represent simple regression between log-transformed values, and *r* represents Spearman correlations.) EMBS, estimated metabolic body size (fat-free mass + 17.7 kg).

son, WI) with calculations of percent body fat, fat mass, and fat-free mass as previously described (11). Waist and thigh circumferences were measured at the umbilicus in the supine position and the gluteal fold in the standing position, and waist-to-thigh ratio (WTR) was calculated as an index of body fat distribution.

At least 3 days after admission and after a 12-h overnight fast, subjects underwent a 2-h, 75-g OGTT (8). Plasma glucose concentrations were determined by the glucose oxidase method (Beckman Instruments, Fullerton, CA) and plasma insulin concentrations by an automated immunoassay (Access; Beckman Instruments).

Insulin action was assessed at physiologic and supra-physiologic insulin concentrations during a two-step hyperinsulinemic-euglycemic glucose clamp as previously described (12). In brief, after an overnight fast, a primed continuous intravenous insulin infusion was administered for 100 min at a constant rate of 290 pmol (40 mU) per meter squared body surface area per minute (low dose, M-low), followed by a second 100-min infusion at a rate of 2,900 pmol (400 mU) \cdot m⁻² \cdot min⁻¹ (high dose, M-high). These infusions achieved steady-state plasma insulin concentrations of 954 ± 318 and $17,574 \pm 6,486$ pmol/l (means \pm SD), respectively. The rate of total insulin-stimulated glucose disposal was calculated for the last 40 min of the low-(M-low) and high-dose insulin infusions (M-high). The rate of endogenous glucose output was calculated before insulin infusion [BGSO, calculated from Steele's equation using a primed (30 μ Ci) continuous (0.3 μ Ci per min) 3-³H-glucose infusion] and during the last 40 min of low-dose insulin infusion (HGO). HGO was used as an estimate of hepatic insulin sensitivity. M-low was also corrected for the rate of HGO (12). *M* values were adjusted for the steady-state plasma glucose and insulin concentrations and were normalized to the estimated metabolic body size (EMBS = fat-free mass + 17.7 kg) as previously described (12).

AIR was measured in response to a 25-g intravenous glucose tolerance test and calculated as the average incremental plasma insulin concentration from 3–5 min after the glucose bolus (12). Because even mildly elevated glucose

concentrations can secondarily affect insulin secretion, only data from subjects with NGT were included in the analyses performed with AIR.

Statistical analyses. Statistical analyses were performed using the software of the SAS Institute (Cary, NC). Results are given as means \pm SD (unless indicated otherwise). The values for hepatic enzymes, fasting insulin, M-low, and AIR were logarithmically transformed before analysis to approximate normal distributions.

In the cross-sectional analyses, the relationship between hepatic enzymes and anthropometric and metabolic variables was examined by Spearman correlation coefficients. Multiple linear regression models and partial correlations were used to examine the relationships after adjusting for covariates. Differences between subjects with NGT and IGT were assessed by unpaired *t* test.

In the prospective analyses, we assessed the metabolic predictors of diabetes. Risk factors for progression from NGT to diabetes were estimated by proportional hazards analyses. Effects of hepatic enzymes, M-low, HGO, AIR, and insulin were expressed as relative hazards with 95% CIs. For the purpose of presentation, the relative hazard estimates were scaled for comparison at the 10th and 90th percentiles, i.e., the risk of developing diabetes of a hypothetical subject at the 90th percentile compared with the hazard of a hypothetical subject at the 10th percentile (or at the 10th and 90th percentiles in the case of a negatively related variable). Differences in anthropometric and metabolic variables between progressors and nonprogressors were assessed by unpaired *t* tests.

In the longitudinal analyses, the predictive effect of baseline hepatic enzyme concentrations on change (follow-up adjusted for baseline) in body fat, fasting plasma insulin concentrations, M-low, AIR, and HGO were evaluated using multiple linear regression models. Models were adjusted for sex, follow-up age, and the time of follow-up. Change in fasting insulin concentrations, M-low, and HGO were additionally adjusted for change in body fat, and change in AIR was adjusted for change in body fat and M-low. Differences

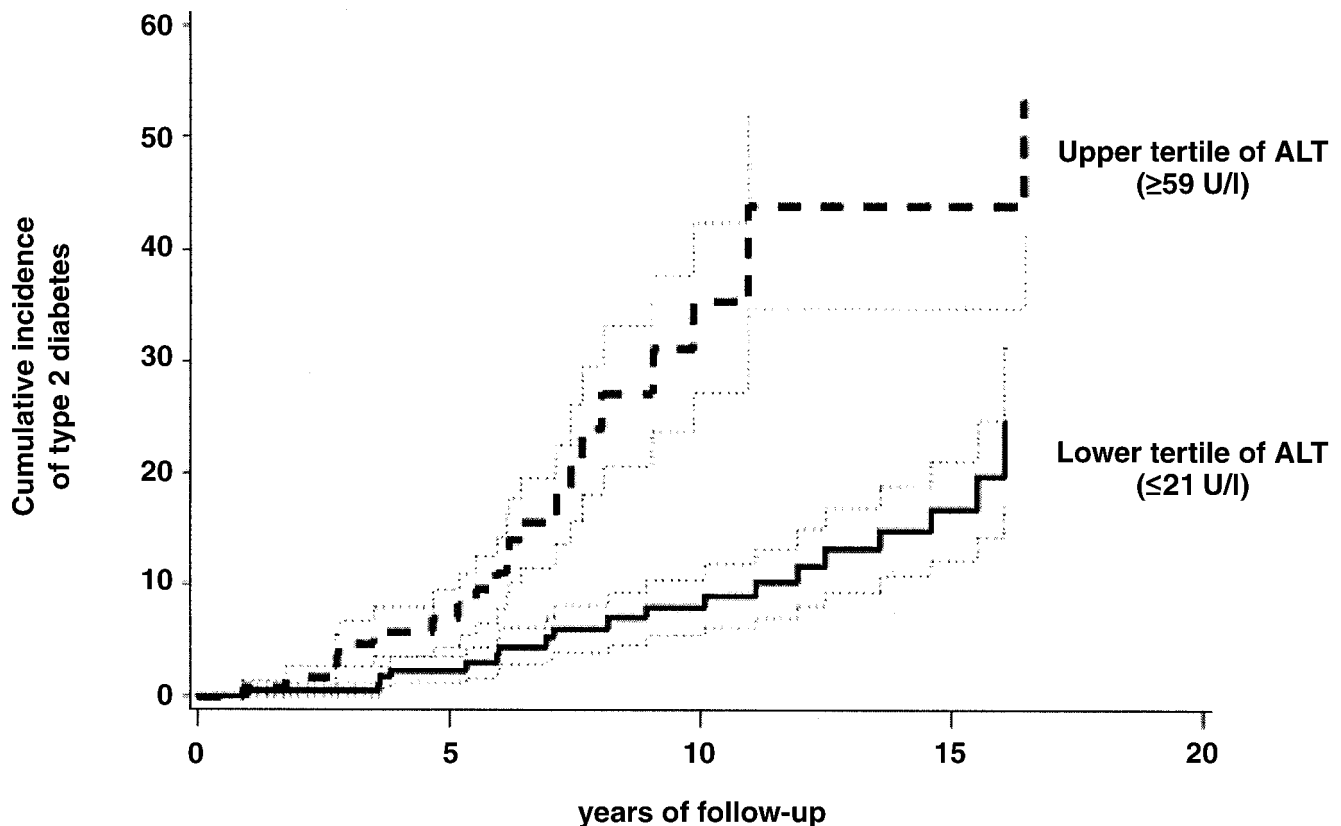


FIG. 2. Kaplan-Meier curve for cumulative incidence of type 2 diabetes (cumulative incidence \pm 1 SE) in 370 subjects with NGT with ALT in the upper and lower tertiles after adjustment for age, sex, percent body fat, insulin sensitivity, and AIR. There were 24 events in the upper tertile, 19 in the middle tertile, and 20 in the lower tertile. For clarity, only the curves for the upper and lower tertiles are shown. The curve for the middle tertile (not shown) lies between the other two.

between anthropometric and metabolic variables at baseline and follow-up were assessed by paired *t* tests.

RESULTS

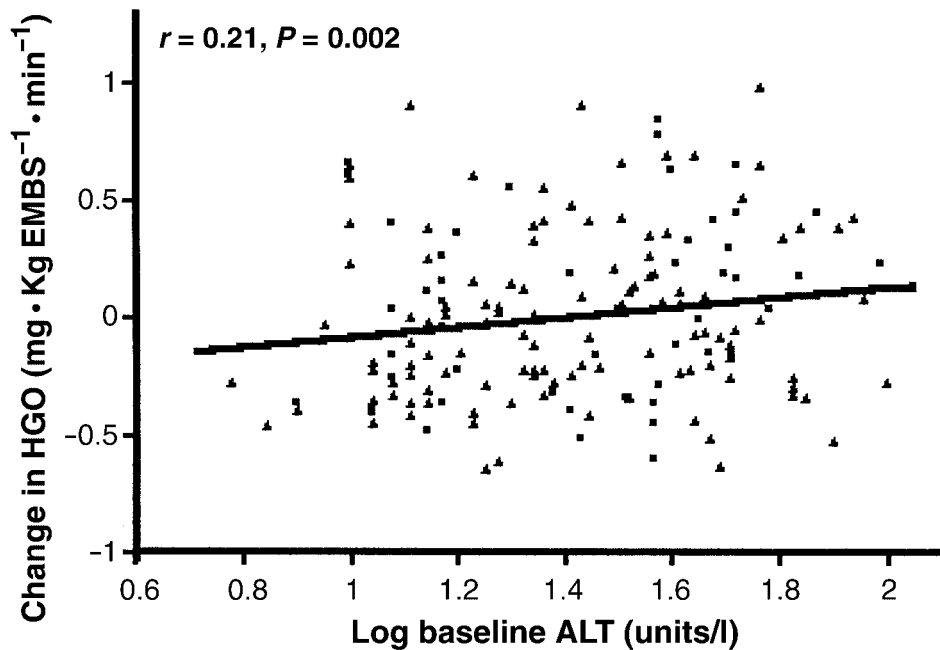
The anthropometric and metabolic characteristics of the study populations for the cross-sectional, prospective, and longitudinal analyses are summarized in Table 1.

Cross-sectional analysis. ALT, AST, and GGT concentrations did not differ between individuals with NGT and IGT (all $P > 0.05$). ALT was positively correlated with AST ($r = 0.64$, $P = 0.0001$) and GGT ($r = 0.58$, $P = 0.0001$), and AST was correlated with GGT ($r = 0.56$, $P = 0.0001$).

In general, after adjusting for age and sex, hepatic enzyme concentrations were positively correlated with measures of adiposity, fasting plasma insulin, and HGO and negatively correlated with the rate of insulin-stimulated glucose disposal (Table 2 and Fig. 1). Hepatic enzymes were not related to measures of central adiposity (waist and WTR, all $P > 0.1$) independent of body fat. After adjustment for age, sex, and adiposity, ALT, AST, and GGT were associated with M-low (all $P < 0.0001$). When M-low, fasting insulin concentrations, and HGO were included in the same model, all three were independent determinants of ALT concentrations (all $P < 0.01$). This was in contrast to AST or GGT concentrations. Only ALT was associated with M-high ($P = 0.0001$) after adjustment for age, sex, and body fat. ALT, AST, and GGT were positively related to WBC, before and after adjustment for body fat and insulin action, in a partial correlation ($r = 0.25$, 0.26 , and 0.20 , respectively, all $P < 0.01$).

Prospective analysis. Hepatic enzymes at baseline did not differ between progressors and nonprogressors (Table 1). In a proportional hazards analyses, with adjustment for age and sex, a high ALT predicted the progression from NGT to diabetes [relative hazard 90th vs. 10th percentiles (95% CI): 2.3 (1.4–3.6), $P = 0.0004$]. Ninetieth and tenth percentiles of ALT were 70 and 12 mU/l, respectively. The predictive effect of a high ALT persisted after additional adjustment for percent body fat, AIR, and M-low [1.9 (1.1–3.3), $P = 0.02$] and/or HGO [1.9 (1.1–3.3), $P = 0.03$]. In the model that included age, sex, percent body fat, M-low, AIR, and ALT in addition to ALT, M-low [5.2 (1.9–14.4), $P = 0.003$] and AIR [4.4 (1.9–9.8), $P = 0.0001$] were both predictive of type 2 diabetes. When subjects with IGT at baseline were included in the analysis together with subjects with NGT, ALT (adjusted for age, sex, percent body fat, M-low, and AIR) was predictive of future diabetes [2.5 (1.7–3.7), $P = 0.0001$]. HGO was higher in progressors compared with nonprogressors (Table 1) but was not predictive of diabetes independently of M-low and AIR [1.6 (0.95–2.8), $P = 0.07$]. When divided into tertiles, subjects in the upper tertile of relative ALT (≥ 59 mU/l, adjusted for age, sex, body fat, M-low, and AIR) had a higher cumulative incidence at all time points of follow-up compared with subjects in the lower tertile (≤ 21 mU/l) (Fig. 2). AST, GGT, alkaline phosphatase, bilirubin, and albumin concentrations as well as AST/ALT ratio did not predict development of type 2 diabetes (data not shown).

Longitudinal analysis. A high ALT at baseline was



HGO at follow-up	Determinant	Estimate	Standard error	p-value
	Intercept	-0.47	0.19	0.02
	Age follow-up	0.005	0.004	0.18
	Sex	-0.19	0.06	0.003
	Body fat at follow-up	0.03	0.006	0.0001
	Body fat at baseline	-0.008	0.006	0.15
	HGO at baseline	0.28	0.07	0.0001
	ALT at baseline	0.17	0.08	0.001
	Time of follow-up	0.00004	0.00002	0.06

FIG. 3. Relationship between ALT at baseline and the change (follow-up adjusted for baseline) in HGO (adjusted for sex, follow-up age, change in body fat, and time of follow-up).

associated with an increase in HGO after adjustment for time of follow-up, follow-up age, sex, and change in body fat ($P = 0.001$) (Fig. 3). ALT was not associated with decline in M-low ($P = 0.3$). Plasma AST and GGT at baseline were not associated with change in M-low or HGO (data not shown). Plasma ALT, AST, and GGT concentrations at baseline were not associated with the subsequent change in AIR, fasting plasma insulin concentrations, or percent body fat (all $P > 0.1$).

DISCUSSION

In the present study, we found that among Pima Indians with NGT, high ALT concentrations predicted the development of type 2 diabetes. Moreover, we demonstrated that a high ALT at baseline was associated with a decline in hepatic insulin sensitivity.

The cross-sectional relationships between hepatic enzymes and adiposity confirm previous findings in other populations (4–6). In accordance with two previous studies, we found that the relationship between hepatic enzyme concentrations and insulin sensitivity was independent of the degree of adiposity (5,6).

Prospective analyses revealed that higher ALT concentrations predict type 2 diabetes in subjects with NGT. We have also shown that the predictive effect of ALT was independent of the degree of adiposity. Our results are in agreement with the finding of Ohlsson et al. (3), who described a higher ALT as a risk factor for type 2 diabetes independent of obesity, body fat distribution, plasma glucose, lipid, AST, bilirubin concentrations, and family history of diabetes in nondiabetic Swedish men. Recently, a report by Perry et al. (4) described an association between elevated GGT and risk of developing type 2 diabetes independent of obesity and alcohol intake.

Why might ALT be associated with later development of type 2 diabetes? We further examined this by assessing whether ALT was associated with deterioration of risk factors for type 2 diabetes (obesity, M , and AIR) that have been established in the Pima population. Importantly, ALT was not associated with a decline in insulin sensitivity or insulin secretion. We have made the important and novel observation that ALT appears to have associations with both hepatic insulin resistance and later decline in hepatic insulin sensitivity. In contrast, AST and GGT concentra-

tions at baseline were not related to a decrease in hepatic or whole-body insulin action or AIR. The etiologic role of the liver in later development of type 2 diabetes in humans is debated. The potential for isolated hepatic insulin resistance to lead to more generalized abnormalities of glucose tolerance is supported by animal models in which disruption of insulin signaling targeted to the liver causes diabetes (2).

In cross-sectional studies, high ALT has also been associated with fatty liver disease (5), and fatty liver disease has been associated with insulin resistance (13,14). The most obvious explanation for our findings is that raised ALT reflects fatty change in the liver and that this in turn reflects pathophysiological changes predating the development of type 2 diabetes. In animal models, chronic hyperinsulinemia is found to predispose the liver to both relative resistance to insulin action on suppression of gluconeogenesis, characterized by failure to increase insulin receptor substrate-2 and an increase in lipogenesis due to upregulation of sterol regulatory element-binding protein 1c (15). By this mechanism, it is proposed that hyperinsulinemia might directly lead to hepatic insulin resistance with associated fatty changes in the liver. Moreover, because insulin suppresses genes encoding gluconeogenic enzymes (16), and ALT is a gluconeogenic enzyme, it is also possible that ALT is an indicator of impaired insulin signaling not necessarily associated with liver injury. Methodological limitations in estimating liver glucose output using radiotracer techniques may explain why ALT, but not more direct measures of hepatic insulin sensitivity (such as HGO), predicts future type 2 diabetes in Pima Indians.

A second pathophysiological mechanism is also possible. We and others have been interested in the association of markers of chronic inflammation and later type 2 diabetes (17,18). Fatty change is a characteristic response of liver to the proinflammatory cytokine tumor necrosis factor- α (19). Fatty change and concomitant raised ALT may therefore reflect inflammation, which may impair insulin signaling both in the liver and systemically. Our observation that a high ALT is associated with a high WBC independent of obesity and insulin resistance is consistent with this hypothesis.

We do not know if alcohol consumption and/or hepatitis C may have contributed to the variability of ALT in this group of Pimas. However, alcohol consumption appears to be an unlikely confounder of our results, since studies have demonstrated higher, lower, and no differences in the prevalence of type 2 diabetes in alcohol consumers (20–22). Moreover, AST-to-ALT ratio and GGT, which are thought to be better indicators of alcoholic liver disease than ALT (23,24), were not predictive of type 2 diabetes in our study, and there is no relationship between self-reported alcohol consumption and type 2 diabetes in Pima Indians (25). Hepatitis C is a known independent predictor of type 2 diabetes (26–28), even in patients without cirrhosis (26). However, all of the recruited subjects were without a known history of hepatitis B and C and without clinical and laboratory signs of acute or chronic infection. Moreover, hepatitis C has only 2% prevalence among Pima Indian population (C. Wilson, unpublished observations).

In summary, high ALT concentrations are cross-section-

ally associated with obesity, whole-body and hepatic insulin resistance and prospectively associated with a decline in hepatic insulin sensitivity and risk of type 2 diabetes. Our findings indicate that higher ALT is a risk factor for type 2 diabetes and indicate a potential role of increased hepatic gluconeogenesis and/or inflammation in the pathogenesis of type 2 diabetes.

ACKNOWLEDGMENTS

The authors wish to thank members of the Gila River Indian Community for their participation. We also gratefully acknowledge Mike Milner, the nurses of the Clinical Research Unit, and the staff of the metabolic kitchen for their care of the patients in these studies.

REFERENCES

1. Duckworth WC, Hamel FG, Peavy DE: Hepatic metabolism of insulin. *Am J Med* 85:71–76, 1988
2. Michael MD, Kulkarni RN, Postic C, Previs SF, Shulman GI, Magnuson MA, Kahn CR: Loss of insulin signaling in hepatocytes leads to severe insulin resistance and progressive hepatic dysfunction. *Mol Cell* 6:87–97, 2000
3. Ohlson LO, Larsson B, Bjorntorp P, Eriksson H, Svardudd K, Welin L, Tibblin G, Wilhelmsen L: Risk factors for type 2 (non-insulin-dependent) diabetes mellitus: thirteen and one-half years of follow-up of the participants in a study of Swedish men born in 1913. *Diabetologia* 31:798–805, 1988
4. Perry LJ, Wannamethee SG, Shaper AG: Prospective study of serum gamma-glutamyltransferase and risk of NIDDM. *Diabetes Care* 21:732–737, 1998
5. Marchesini G, Brizi M, Bianchi G, Tomassetti S, Bugianesi E, Lenzi M, McCullough AJ, Natale S, Forlani G, Melchionda N: Nonalcoholic fatty liver disease: a feature of the metabolic syndrome. *Diabetes* 50:1844–1850, 2001
6. Dixon JB, Bhathal PS, O'Brien PE: Nonalcoholic fatty liver disease: predictors of nonalcoholic steatohepatitis and liver fibrosis in the severely obese. *Gastroenterology* 121:91–100, 2001
7. Knowler WC, Pettitt DJ, Saad MF, Bennett PH: Diabetes mellitus in the Pima Indians: incidence, risk factors and pathogenesis. *Diabetes Metab Rev* 6:1–27, 1990
8. Lillioja S, Mott DM, Spraul M, Ferraro R, Foley JE, Ravussin E, Knowler WC, Bennett PH, Bogardus C: Insulin resistance and insulin secretory dysfunction as precursors of non-insulin-dependent diabetes mellitus: prospective studies of Pima Indians. *N Engl J Med* 329:1988–1992, 1993
9. World Health Organization: *Diabetes Mellitus: Report of a WHO Study Group*. Geneva, World Health Org., 1985 (Tech. Rep. Ser., no. 727)
10. Siri WE: Body composition from fluid spaces and density: analysis of methods. In *In Techniques for Measuring Body Composition*. Brozek JHA, Ed. Washington, DC, National Academy of Sciences, 1961, p. 223–244
11. Tataranni PA, Ravussin E: Use of dual-energy X-ray absorptiometry in obese individuals. *Am J Clin Nutr* 62:730–734, 1995
12. Lillioja S, Mott DM, Howard BV, Bennett PH, Yki-Jarvinen H, Freymond D, Nyomba BL, Zurlo F, Swinburn B, Bogardus C: Impaired glucose tolerance as a disorder of insulin action: longitudinal and cross-sectional studies in Pima Indians. *N Engl J Med* 318:1217–1225, 1988
13. Goto T, Onuma T, Takebe K, Kral JG: The influence of fatty liver on insulin clearance and insulin resistance in non-diabetic Japanese subjects. *Int J Obes Relat Metab Disord* 19:841–845, 1995
14. Banerji MA, Buckley MC, Chaiken RL, Gordon D, Lebovitz HE, Kral JG: Liver fat, serum triglycerides and visceral adipose tissue in insulin-sensitive and insulin-resistant black men with NIDDM. *Int J Obes Relat Metab Disord* 19:846–850, 1995
15. Shimomura I, Matsuda M, Hammer RE, Bashmakov Y, Brown MS, Goldstein JL: Decreased IRS-2 and increased SREBP-1c lead to mixed insulin resistance and sensitivity in livers of lipodystrophic and ob/ob mice. *Mol Cell* 6:77–86, 2000
16. O'Brien RM, Grammer DK: Regulation of gene expression by insulin. *Biochem J* 278:609–619, 1991
17. Vozarova B, Weyer C, Lindsay RS, Pratley RE, Bogardus C, Tataranni PA: High white blood cell count is associated with a worsening of insulin sensitivity and predicts the development of type 2 diabetes. *Diabetes* 51:455–461, 2002

18. Lindsay RS, Krakoff J, Hanson RL, Bennett PH, Knowler WC: Gamma globulin levels predict type 2 diabetes in the Pima Indian population. *Diabetes* 50:1598–1603, 2001
19. Yin M, Wheeler MD, Kono H, Bradford BU, Gallucci RM, Luster MI, Thurman RG: Essential role of tumor necrosis factor alpha in alcohol-induced liver injury in mice. *Gastroenterology* 117:942–952, 1999
20. Holbrook TL, Barrett-Connor E, Wingard DL: A prospective population-based study of alcohol use and non-insulin-dependent diabetes mellitus. *Am J Epidemiol* 132:902–909, 1990
21. Ajani, UA, Hennekens CH, Spelsberg A, Manson JE: Alcohol consumption and risk of type 2 diabetes mellitus among US male physicians. *Arch Intern Med* 160:1025–1030, 2000
22. Monterrosa AE, Haffner SM, Stern MP, Hazuda HP: Sex difference in lifestyle factors predictive of diabetes in Mexican-Americans. *Diabetes Care* 18:448–456, 1995
23. Cohen JA, Kaplan MM: The SGOT/SGPT ratio: an indicator of alcoholic liver disease. *Dig Dis Sci* 24:835–838, 1979
24. Fauci A, Braunwald E, Isselbacher K, Wilson J, Martin J, Kasper D, Hauser S, Longo D, Eds: *Harrison's Principles of Internal Medicine*. New York, McGraw-Hill, 1997
25. Saremi A, Hanson R, Knowler W: Diabetes and alcohol dependence in an American Indian Population (Abstract). *Diabetes* 50 (Suppl. 2):A395, 2001
26. Knobler H, Schihmanter R, Zifroni A, Fenakel G, Schattner A: Increased risk of type 2 diabetes in noncirrhotic patients with chronic hepatitis C virus infection. *Mayo Clin Proc* 75:355–359, 2000
27. Mason AL, Lau JY, Hoang N, Qian K, Alexander GJ, Xu L, Guo L, Jacob S, Regenstein FG, Zimmerman R, Everhart JE, Wasserfall C, Maclaren NK, Perrillo RP: Association of diabetes mellitus and chronic hepatitis C virus infection. *Hepatology* 29:328–333, 1999
28. Ryu JK, Lee SB, Hong SJ, Lee S: Association of chronic hepatitis C virus infection and diabetes mellitus in Korean patients. *Korean J Intern Med* 16:18–23, 2001