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BRIEF REPORT

Serum γ-Glutamyl Transpeptidase Is a Determinant of Insulin Resistance Independently of Adiposity in Pima Indian Children

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Context: Elevated activities of serum enzymes, including alanine aminotransferase (ALT), aspartate aminotransferase (AST), and γ-glutamyltransferase (GGT), have been associated with obesity and insulin resistance (IR). ALT is an independent predictor of type 2 diabetes mellitus (T2DM) in adult Pima Indians, and GGT predicts T2DM in other adult populations.

Objective: Our aim was to establish whether independent relationships exist between either adiposity or IR and hepatic enzymes in a group of Pima Indian children.

Subjects and Methods: In a cross-sectional study, 44 children (22 males and 22 females; 7–11 yr old) were measured for weight (WT), height, percent body fat, and serum activities of ALT, AST, and GGT. Body mass index (kilograms per meter squared) was calculated. IR was calculated from fasting plasma concentrations of glucose and insulin using the homeostasis model assessment (HOMA-IR).

Results: Hepatic enzymes were positively associated with obesity measures, fasting insulin, and HOMA-IR. GGT was additionally associated with serum lipids and white blood cell count. GGT, but not AST or ALT, was a significant determinant of HOMA-IR independently of age, sex, and WT, body mass index, or percent body fat. The model that accounted for the largest portion of the variance in HOMA-IR included WT ($\beta = 0.004$; $P = 0.008$) and GGT ($\beta = 0.20$; $P = 0.004$; total $R^2 = 0.62$; $P < 0.0001$).

Conclusion: Significant relationships between adiposity and hepatic enzyme activities exist during childhood in Pima Indians. Whether serum GGT activity predicts the development of T2DM in these children remains to be determined in follow-up studies. ***(J Clin Endocrinol Metab* 91: 1419–1422, 2006)**

OBESITY IS A major risk factor for the development of type 2 diabetes mellitus (T2DM), mainly by its contribution to insulin resistance (IR). Insulin sensitivity and liver function are inversely related. Decreased insulin sensitivity is associated with nonalcoholic fatty liver disease (NAFLD) (1, 2). Other studies suggest that a normal-functioning liver may contribute to whole body insulin sensitivity (3).

Serum activities of hepatic enzymes have been associated with obesity in adults (4) and adolescents (5), and relationships between these markers and IR or T2DM, independently of adiposity, have been shown in several studies (6–8). Furthermore, aspartate aminotransferase (AST) (9), alanine aminotransferase (ALT) (7, 9, 10), and γ-glutamyltransferase (GGT) (10–12) are independent predictors of T2DM. In adults, hepatic IR is a late phenomenon in the natural history of T2DM. Therefore, other pathophysiological mechanisms, in addition to liver damage, might explain the association between elevated hepatic enzyme activities and the development of T2DM.

The increased prevalence of childhood obesity is a major reason for the increased rates of IR and T2DM reported in children. No studies have been conducted to clarify whether the metabolic abnormalities found in Pima Indian adults in the years preceding the development of T2DM are present in children or whether the liver plays an early role in the natural history of the disease in obese and hyperinsulinemic children.

The aim of the present cross-sectional study was to establish whether independent relationships exist between adiposity and/or IR and serum hepatic enzyme activities in a group of Pima Indian children.

Subjects and Methods

Subjects

During the summer months of 2001–2004, 44 healthy Pima Indian children (22 males and 22 females), aged 7–11 yr, were studied after admission to the Clinical Research Unit of the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK; Phoenix, AZ). Children and their mothers were part of a larger study to determine eating behavior characteristics in offspring of women who developed diabetes either before or after pregnancy. From our ongoing studies into

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Abbreviations: ALT, Alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; DHEA-S, dehydroepiandrosterone sulfate; %FAT, percent body fat; FPG, fasting plasma glucose; FPI, fasting plasma insulin; GGT, γ-glutamyltransferase; HDL, high-density lipoprotein; HOMA, homeostasis model assessment; IR, insulin resistance; NAFLD, nonalcoholic fatty liver disease; T2DM, type 2 diabetes mellitus; TG, triglycerides; WBC, white blood cell count; WT, body weight.

the etiology of diabetes in the Gila River Indian Community, we canvassed our records for the identity of all children between 7 and 11 yr of age. From that list, we were able to ascertain the diabetes status of the mothers. A list of eligible mother/child pairs was then provided to a community recruiter, who invited eligible pairs to participate. Before participation, volunteers and their parents were fully informed of the nature and purpose of the study, and written informed consent/assent was obtained. The experimental protocol was approved by the institutional review boards of the NIDDK and the Phoenix Area Intertribal Council of Arizona and the Tribal Council of the Gila River Indian Community.

Anthropometrics

Height was measured without shoes. Body weight (WT) was measured while the children were wearing a preweighed robe. Body mass index (BMI) was calculated as weight divided by height squared. The percent body fat (%FAT) was measured using dual energy x-ray absorptiometry.

Analytical procedures

After consuming a standardized diet for 2 d in the Clinical Research Unit, fasting blood samples were drawn. Fasting plasma glucose (FPG) concentrations were measured using the glucose oxidase method (Beckman Instruments, Fullerton, CA), and fasting plasma insulin (FPI) concentrations were determined with an automated RIA (ICN Biochemicals, Costa Mesa, CA). Serum hepatic enzyme activities (ALT, AST, and GGT), white blood cell count (WBC) and lipids [triglycerides (TG), high-density lipoprotein (HDL) cholesterol, and total cholesterol] were measured by a colorimetric method (DADE Behring-Dimension Clinical Chemistry System). Dehydroepiandrosterone sulfate (DHEA-S) was measured by competitive chemiluminescent enzyme immunoassay (Immulite 2000 DHEA-SO4). The homeostasis model assessment of IR (HOMA-IR) was used to calculate an index from the product of FPI (microunits per milliliter) and FPG (millimolar concentrations) divided by 22.5.

Statistical methods

All statistical analyses were performed using SAS software (SAS version 8.2, SAS Institute, Inc., Cary, NC). Data are expressed as the mean ± SD and the range. BMI was statistically normalized and expressed as the number of SD from the mean (z-score). FPI, WBC, DHEA-S, and serum hepatic enzyme activities were log transformed ($\log_{10}$) to approximate a normal distribution. Sex differences in age and anthropometric and metabolic variables were evaluated by Student's *t* test or Wilcoxon test according to variable distribution. Spearman correlation coefficients were used to quantify cross-sectional relationships between serum hepatic enzyme activities and FPG, FPI, HOMA-IR, anthropometric variables (weight, waist, BMI, and %FAT), WBC, blood lipids, and DHEA-S. Separate relationships between each serum hepatic enzyme activity and the calculated HOMA-IR were also examined after adjustment for age, sex, and each of the anthropometric variables using general linear regression models. Additional adjustment for serum levels of DHEA-S was also made. Levels of statistical significance were set at $P < 0.05$.

Results

There were no sex differences in any of the anthropometric variables (Table 1). Among the metabolic variables, only WBC and TG levels showed sex differences.

Serum activities of all three hepatic enzymes were positively correlated with WT, %FAT, BMI, FPI, and HOMA-IR (Table 2). Although ALT and GGT were correlated with WBC, only GGT was positively associated with TG and total cholesterol and was negatively associated with HDL cholesterol. No association was found between DHEA-S and any of the hepatic enzymes. DHEA-S was associated with age ($r = 0.45$; $P = 0.004$) and WT ($r = 0.37$; $P = 0.02$), but not with FPI or HOMA-IR (both $P > 0.4$).

The serum activity of GGT remained correlated with HOMA-IR after adjustment for WT ($r = 0.30$; $P < 0.05$) and BMI ($r = 0.29$; $P = 0.05$) and approached significance for %FAT ($r = 0.26$; $P = 0.08$). Neither AST nor ALT was associated with HOMA-IR after adjustment for any of the anthropometric variables ($P > 0.4$ for all correlations). In multiple regression models, GGT (but not AST or ALT) was a determinant of HOMA-IR independently of age, sex, and WT ($P = 0.004$), %FAT ($P = 0.004$), or BMI ($P = 0.008$). The age- and sex-adjusted model that accounted for the largest portion of the variance in HOMA-IR ($\log_{10}$) included WT ($\beta = 0.004$; $P = 0.008$) and GGT ($\log_{10}$; $\beta = 0.20$; $P = 0.004$; total $R^2 = 0.62$; $P < 0.0001$). This independent relationship remained significant ($\beta = 0.16$; $P = 0.04$) after additional adjustment for DHEA-S. Maternal diabetes status during pregnancy was not a determinant of HOMA-IR using either multiple regression analysis or testing for interaction terms between maternal status and hepatic enzyme activities or obesity measures in the relationship with HOMA-IR (data not shown).

TABLE 1. Anthropometric and metabolic characteristics of the subjects

	Boys (n = 22)	Girls (n = 22)	P
Age (yr)	9.2 ± 1.4 (7.3–11)	9.6 ± 1.3 (7.3–11.6)	0.2
Body weight (kg)	51.1 ± 18.29 (21–84)	48.4 ± 18.03 (19–94)	0.6
%FAT	39.2 ± 10.6 (18–54)	40.4 ± 9.5 (20–57)	0.7
BMI (kg/m^2)	25.3 ± 6.8 (14.5–38)	24.3 ± 6.6 (14.5–43)	0.5
Fasting glucose (mg/dl)	90 ± 6 (78–104)	87 ± 7 (75–106)	0.2
Fasting insulin (μU/ml)	33.9 ± 12.2 (21–63)	35.6 ± 11.2 (21–68)	0.4
HOMA-IR	7.5 ± 2.7 (4–12.6)	7.7 ± 2.7 (4.5–15.6)	0.8
TG (mg/dl)	78.7 ± 32 (35–158)	101 ± 34.6 (42–168)	0.02
Total cholesterol (mg/dl)	136.8 ± 28.1 (68–192)	145.1 ± 21.2 (120–198)	0.3
HDL cholesterol (mg/dl)	45.5 ± 10 (30–71)	44.1 ± 8.1 (27–58)	0.6
GGT (5–85 U/liter)[a]	24.5 ± 13.6 (10–55)	22.8 ± 19.7 (5–92)	0.3
AST (5–85 U/liter)[a]	28.1 ± 7.8 (17–49)	24.8 ± 10.1 (13–58)	0.1
ALT (5–85 U/liter)[a]	51.4 ± 18.2 (27–92)	45.1 ± 20.3 (20–103)	0.2
WBC (cell/mm^3)	8.4 ± 1.9 (6–13)	7 ± 1.3 (4.5–9.5)	0.01
DHEA-S (μg/dl)	46.2 ± 25.6 (16–109)	44.1 ± 37.6 (13–115)	0.7

Data are the mean ± SD (minimum-maximum).

[a] Range of normality. GGT, AST, ALT, and WBC were $\log_{10}$ transformed before statistical analysis.

TABLE 2. Correlations between serum hepatic enzyme activities and anthropometric and metabolic characteristics of the subjects

	ALT	AST	GGT	HOMA-IR
Age (yr)	0.08	−0.03	0.16	0.44[a]
Body weight (kg)	0.48[b]	0.34[c]	0.65[b]	0.77[b]
%FAT	0.48[b]	0.39[a]	0.64[b]	0.64[b]
BMI (SD score)	0.54[b]	0.44[b]	0.71[b]	0.73[b]
Fasting glucose (mg/dl)	0.16	0.11	0.20	0.38[c]
Fasting insulin (μU/ml)	0.37[c]	0.28[d]	0.61[b]	0.97[b]
HOMA-IR	0.41[a]	0.31[c]	0.65[b]	
TG (mg/dl)[e]	0.06	0.18	0.54[b]	0.65[b]
Total cholesterol (mg/dl)	0.01	0.06	0.29[d]	0.22
HDL cholesterol (mg/dl)	−0.19	−0.20	−0.43[a]	−0.58[b]
WBC (cell/mm^3)[e]	0.41[a]	0.24	0.49[b]	0.50[b]
DHEA-S (μg/dl)	0.08	0.02	0.11	0.10

GGT, AST, ALT, and WBC were $\log_{10}$ transformed before statistical analysis. Variables without superscripts were not significantly related.

[a] $P < 0.01$.

[b] $P \leq 0.001$.

[c] $P < 0.05$.

[d] $P = 0.06$.

[e] WBC and TG were adjusted by gender.

Discussion

In Pima Indian children, serum activities of the hepatic enzymes AST, ALT, and GGT were found to be within normal limits. Despite this normalcy, hepatic enzyme activities were correlated with HOMA-IR, independently of age and sex. However, only serum GGT activity persisted as a significant determinant of HOMA-IR after additional adjustment for anthropometric variables (WT, BMI, or %FAT). Although a previous study in adults has shown similar independent relationships between GGT and HOMA-IR (8), to our knowledge, this independent relationship between GGT and HOMA-IR in children has not previously been described.

In this study, it is not possible to resolve whether GGT is a good marker of the effect of insulin on the liver, because we do not have direct measurements of hepatic insulin sensitivity. However, we propose three explanations for why serum GGT activity could be a marker of whole body insulin sensitivity.

First, GGT could be a marker of hepatocyte damage or hepatic dysfunction. Although the etiological role of the liver in later development of T2DM is debatable, animal models support a role of hepatic IR leading to severe glucose intolerance (3). Furthermore, NAFLD, an emerging obesity-related (13) liver disease in children, is a condition associated with elevated concentrations of GGT (14, 15). IR is the main pathogenic factor in the etiology of NAFLD in adults (14, 16) and in children (17).

Secondly, GGT could be a marker of oxidative stress. Upregulation of GGT activity might reflect increased consumption of glutathione, the most abundant intracellular antioxidant found throughout the body, and may thus be a marker of oxidative stress. Therefore, increased GGT activity may reflect not only hepatic oxidative stress (mediated by fat accumulation inside hepatocytes) (15), but also systemic oxidative stress. Oxidative stress has been associated with IR in several studies (18, 19). Reactive oxygen species can activate transcription factors, such as nuclear factor-κB and activating protein-1 (20), participants in important inflammatory signaling pathways recently implicated in the pathogenesis of IR.

Thirdly, GGT activity could be a marker of chronic inflammation. Serum GGT activity might be a reflection of the chronic inflammation associated with low levels of antiinflammatory hormones present in obesity (*e.g.* adiponectin) or with the reduced effectiveness of insulin as a modulator of cytokine action (21). Indeed, adiponectin has been found to be inversely related to GGT and predicts GGT activity independently of IR (22). In the present study, serum GGT activity was positively associated with WBC and TG and was negatively associated with HDL cholesterol levels. Low HDL levels and increased TG levels are typical features not only in T2DM, but also in inflammatory diseases. Serum GGT activity has been shown to predict concentrations of inflammatory markers such as C-reactive protein and fibrinogen (23) and is strongly associated with C-reactive protein independently of sex, obesity, and alcohol and smoking habits (24).

Dietary factors, such as fruit or protein intake (25) and alcohol (25) or coffee consumption (26), are less likely to be factors given the age and food choices of these children. Puberty, which is normally associated with a decline in insulin sensitivity, could be a confounding factor. However, the measured DHEA-S concentrations, the first hormone that increases in both sexes before the onset of gonadal maturation (27), were in the prepubertal range (10–60 μg/dl) in most children (75%), and the remainder had only mild elevations (60–115 μg/dl). Furthermore, no association was found between this hormone and HOMA-IR or hepatic enzymes.

In contrast to results in adult Pima Indians (7), we found that serum GGT activity, rather than ALT activity, was the hepatic enzyme related to IR independently of adiposity in children. There are two possible explanations for this discrepancy. First, in adults, the influence of alcohol might have confounded the relationship between GGT and IR. Secondly, in this study, we used an estimate of insulin sensitivity, whereas in adult Pima Indians we used the euglycemic hyperinsulinemic clamp. Although HOMA-IR is more practical and less invasive (especially for younger children) than the euglycemic clamp, it is based on fasting values and, therefore, does not accurately assess glucose uptake during insulin stimulation. However, in large validation studies in children (28, 29), HOMA-IR as well as indices derived from the oral glucose tolerance test (29) were well correlated with insulin sensitivity measured by the euglycemic hyperinsulinemic clamp technique.

In conclusion, in a population of children at high risk for the development of T2DM, serum GGT activity showed a significant relationship with HOMA-IR independently of weight or adiposity. Whether serum GGT activity can predict the development of T2DM in these children remains to be determined in follow-up studies.

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