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Serum Gamma-Glutamyltransferase and the Risk of Type 2 Diabetes in a Population-Based
Cohort Study of Older Mexican Americans

by

Lynn Blythe

Thesis

Submitted to the School of Health Sciences

Eastern Michigan University

in partial fulfillment of the requirements

for the degree of

MASTER OF SCIENCE

in

Clinical Research Administration

Thesis Committee:

Stephen Sonstein, PhD, Chair

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May 20, 2007

Ypsilanti, Michigan

Dedication

This thesis is dedicated to my parents, without whom none of this would have been possible.

I thank you for my undergraduate education and, more importantly, thank you for teaching me to work hard to achieve a goal and always reach for my dreams.

Acknowledgements

I would like to extend my sincere appreciation to many people who contributed their time and effort to assist me with this work and without whom this work would not be possible. It has been my pleasure to work with each of them.

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Abstract

Our objective was to test the hypothesis that increased GGT predicts an increased risk of type 2 diabetes (T2D) in elderly Mexican Americans.

Data from a population-based cohort study of 1789 community-dwelling Mexican American men and women, aged 60-101, in the SALSA study were used. Data for 1,203 participants without diabetes at baseline were evaluated for incident diabetes. Proportional hazard models were used to predict the probability of incident T2D by GGT level.

After adjustment for age, gender, smoking, alcohol use, and BMI, the risk of developing T2D associated with GGT was significant at 1.4 (95% CI 1.2 -1.7). However, when the model was adjusted for fasting serum glucose, the risk was attenuated by 20%, and the confidence interval included 1.0 (HR 1.1, 95% CI 0.8-1.5).

In conclusion, elevated levels of GGT may be associated with an increased risk of T2D, but additional studies need to be done in this population.

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Chapter 1: Introduction and Background

Introduction

Gamma-glutamyltransferase (GGT) is elevated in cases of hepatic injury and has been used to detect chronic alcohol abuse. (Dufour et al., 2000; Hietala, Puukka, Koivisto, Antilla, & Niemela, 2005; Seitz, 2006; Whitfield, 2001). Many recent studies have investigated GGT levels and the risk of developing diseases including metabolic syndrome, type 2 diabetes and cardiovascular disease (Bo et al., 2005; Emdin, Passino, Donato, Paolicchi, & Pompella, 2002; Emdin, Pompella, & Paolicchi, 2005; Lee et al., 2007). Serum gamma-glutamyltransferase (GGT) levels that are high but still within the normal limit have been independently associated with an increased risk for type 2 diabetes in many studies (Lee, Ha et al., 2003; Nakanishi, Suzuki, & Tataru, 2004; Wannamethee, Sharper, Lennon, & Whincup, 2005). There has only been one study of hepatic enzymes in Latinos that the authors know of (Nannipieri et al., 2005). This study investigated the relationship between liver enzymes (AST, ALT, Alkaline Phosphatase, and GGT), impaired glucose tolerance, and diabetes. Of the enzymes studied, only raised GGT was an independent predictor of impaired glucose tolerance or diabetes.

Purpose of the Study

Several researchers have reported that increased GGT is independently associated with increased risk of type 2 diabetes in Asian and Caucasian populations (André et al., 2005; Lee, Ha et al., 2003; Lee, Silventoinen, Jacobs, Jusilahti, & Tuomileto, 2004; Wannamethee et al., 2005). We postulate that a similar increase in risk of T2D associated with GGT will be found in elderly Latino participants of the Sacramento Area Study on Aging (SALSA).

The major aim of this study was to evaluate whether GGT levels measured at baseline could predict incidence of type 2 diabetes in a large cohort of elderly Mexican Americans. The authors analyzed baseline laboratory and clinical data from the Sacramento Area Latino Study on Aging (SALSA) to determine the relative risk of developing type 2 diabetes on the basis of GGT level while adjusting for confounding variables.

Significance of the Study

Predicting an early increased risk of developing type 2 diabetes (T2D) may encourage a person to make lifestyle changes or obtain treatment, leading to a decreased risk of diabetes. Additionally, his/her quality and length of life may be improved, decreasing the overall burden on healthcare systems at the same time.

Chapter 2: Review of Related Literature

GGT is found in the kidneys, biliary system, pancreas, and intestine (Dufour, 2000). Briefly, GGT protein catalyzes an enzymatic action, which is the transfer of a glutamyl residue to an acceptor through the glutamate's gamma carboxylic acid to an amine or other amino acid. The most abundant natural substrate is glutathione. Glutathione is extracellular and cannot pass through the cell membrane. Glutathione can be broken down into 3 amino acids (including cysteine, which may be deficient in low-protein diets) at the cell membrane by GGT. These amino acids can be taken up in the cells by the γ -glutamyl cycle. Glutathione is then reformed in the cells, where it protects cells against oxidants that are produced during normal metabolism. An increased need for reduced glutathione occurs with oxidative stress (Lee, Blomhoff, & Jacobs, 2004; Whitfield, 2001; Zhang, Forman, & Choi, 2005).

Research by Aaseth and Støa-Birketvedt (2000) evaluated 10 overweight patients with poorly controlled type 2 diabetes and discovered that intracellular glutathione was markedly increased compared to normal controls. Glutathione is a known, powerful antioxidant that may mediate the inflammatory effect of increased glucose, possibly by decreasing cytokine production in response to spikes of hyperglycemia (Wright, Scism-Bacon, & Glass, 2006).

Nonalcoholic fatty liver disease has been linked to a higher prevalence of diabetes (Bloomgarden, 2005, p. 1519). Oxidative stress resulting from nonalcoholic fatty liver disease (NAFLD) has been suggested in the mechanisms of insulin resistance, β -cell dysfunction, poorly-controlled type 2 diabetes, and subsequent complications (Bo et al., 2005; Robertson, Harmon, Tran, & Poitout, 2004; Thamer et al., 2005; Wright, et al., 2006).

GGT was first used as a test in the evaluation of liver diseases. It reaches extremely high levels in patients with biliary obstruction and is a good marker for chronic alcohol consumption (Lee, Lim, Yang, Ha, & Jacobs, 2005; Seitz, 2006). Research by Jimenez-Alonso et al. (1983) showed that hyperglycemia itself does not increase the hepatic enzyme GGT in uncontrolled diabetics. Although most researchers report that GGT appears to be a marker for oxidative stress, there is some controversy regarding the role that GGT plays in oxidative stress. Many scientists think that GGT plays an important role in protecting against oxidative stress by maintaining an adequate supply of intracellular glutathione, which protects cells against oxidants produced by normal metabolism (Lim et al., 2004; Meisinger, Löwel, Heier, Schneider, & Thorand, 2005; Zhang et al., 2005). However, others including Lee, Blomhoff et al. (2004) have noted that increased levels of serum GGT do not seem to reduce oxidative stress, implying that increased GGT is not a protective mechanism against oxidative stress.

Numerous studies have found that GGT is not just a marker of alcohol consumption, but is an independent predictor of many diseases, including cardiovascular diseases, type 2 diabetes, inflammation, and, possibly, underlying oxidative stress (Bo et al., 2005; Emdin et al., 2002; Emdin et al., 2005; Lee, Jacobs et al., 2003; Sakuta, Suzuki, Yasuda, & Ito, 2005; Wannamethee et al., 2005; Whitfield, 2001; Yamada et al., 2006).

Meisinger et al. (2005) postulated that possible mechanisms by which GGT is a marker for increased risk of type 2 diabetes include the following: (a) elevated serum GGT could indicate excess fat deposits in the liver, which may cause hepatic insulin resistance and increase the risk of type 2 diabetes by contributing to systemic insulin resistance; (b) increased GGT is a marker for oxidative stress; and (c) increased GGT may be the

expression of inflammation. Ortega, Koska, Salbe, Tatatanni, and Bunt (2006) reported that GGT was a significant predictor of insulin resistance independently of weight, BMI, or percentage of fat in Pima Indian children.

Many studies have reported an increased risk of type 2 diabetes with increased levels of GGT. Lee, Ha, et al. (2003) prospectively studied a group of 4,088 healthy, male Korean workers and found a strong dose response relationship between serum GGT levels at baseline and incident type 2 diabetes after 4 years of follow up. This relationship was observed even in nondrinkers. Nakanishi et al. (2004) found that increased serum GGT increased the risk of incidence of metabolic syndrome and type 2 diabetes in 3,000 middle-aged Japanese male office workers. Lee, Silventoinen et al. (2004) evaluated 20,158 Finnish subjects of both genders, aged 25-64, in a prospective cohort study and found that higher serum GGT was directly associated with an increased risk of type 2 diabetes. The CARDIA study (Lee, Jacobs, et al., 2003) recruited 18- to 30-year-old Black and White Americans in 1985-86 and followed them for 15 years. They reported that the risk of type 2 diabetes was strongly increased with higher normal levels of GGT. In addition, they postulated that this may be related to oxidative stress. Perry, Wannamethee, & Shaper (1998) examined the association between GGT levels and the risk of NIDDM in about 7,500 British men (aged 40-59). Their findings suggested that a raised serum GGT level is an independent risk factor for NIDDM. Wannamethee et al. (2005) conducted a prospective study of 3,500 of the surviving men from the previous study (now aged 60-79). They reported that both ALT and GGT were independent predictors of type 2 diabetes in older men and could be useful in predicting those at high risk of diabetes. Nannipieri et al. (2005) evaluated liver enzymes (AST, ALT, ALP, and GGT) in 1441 middle-aged (35-64 yrs) male and female participants

in the population-based Mexico City Diabetes Study at baseline and at 7-year follow-up to determine the incidence of impaired glucose tolerance (IGT) or type 2 diabetes. They reported that only increased GGT is an independent predictor of decreased glucose tolerance or type 2 diabetes. They theorized that GGT elevation may reflect increased hepatic insulin resistance or oxidative stress. André et al. (2005) studied ALT, AST, and GGT in 2071 French men and 2130 French women 33 to 68 years old and found that only GGT is strongly associated with increased risk of T2D at 3-year follow-up for both sexes. Meisinger et al. (2005) studied 1851 male and 1836 female 25 to 64-year-old participants in a population-based study in Germany (MONICA) and concluded that GGT is an important predictor of incident type 2 diabetes in both men and women in the general population. Vozarova et al. (2002) evaluated ALT, AST, and GGT in 451 Pima Indian adults and found that only ALT is a marker of T2D.

Lee et al. (2007) followed up 3,451 middle-aged male and female participants of the Framingham Heart Study over a 19-year period and found that increased serum GGT predicts incidence of metabolic syndrome, cardiovascular disease, and death.

Results from a recent study using data from the third NHANES study (Lee & Jacobs, 2005) showed that all levels of GGT are strongly associated with C-reactive protein (CRP). CRP is widely recognized as a marker of chronic inflammation, and the association was present in all populations tested in that study, including Mexican Americans. These results strongly suggest that GGT is involved in the inflammatory pathway. Figure 1 shows suspected pathways relating GGT to type 2 diabetes. It is likely that insulin resistance leads to increased fat deposits in the liver, which cause oxidative stress and inflammation, leading to type 2 diabetes.

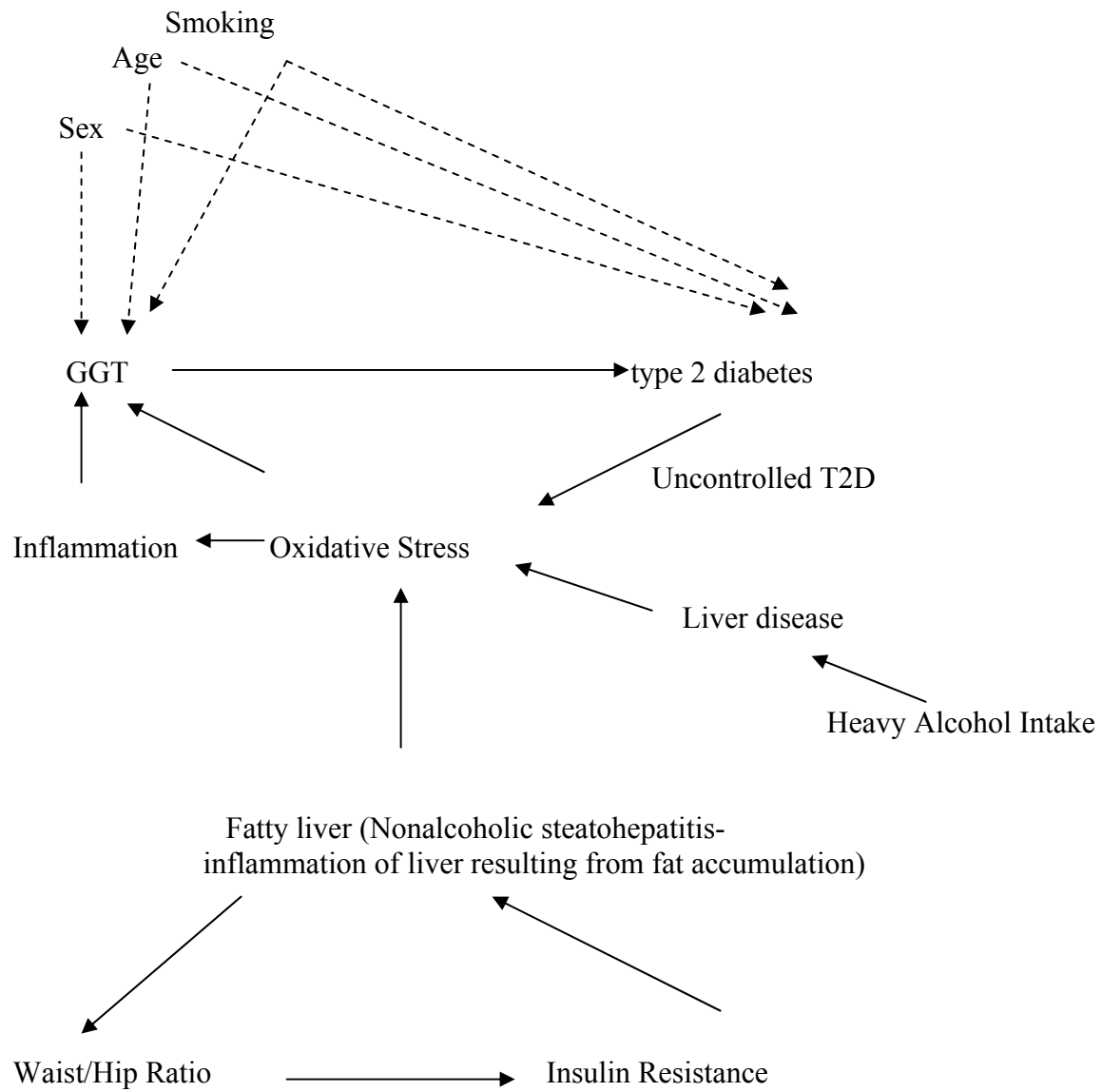


Figure 1. Diagram of known and theoretical pathways.

Chapter 3: Research Design and Methods

This study is an evaluation of data obtained from participants of a large, ongoing cohort study, The Sacramento Area Latino Study on Aging (SALSA). Participants are Mexican Americans (85%) and other Latinos (primarily Central American) who were aged 60-100 in 1998-99 and resided in a three-county area including Sacramento, California. IRB approval and written consent was obtained from the participants for interviews, cognitive testing, and clinical measures including a blood draw and analysis of the samples for markers relating to diabetes and related diseases at The University of California, Davis (UCD), at baseline and also at The University of Michigan (UM) IRBMED when the Coordinating Center moved to UM in 2000. Details of the SALSA study have been discussed previously (Haan et al., 2003). SALSA participants without diabetes at baseline and with a GGT result from baseline lab testing were eligible for this study.

Type 2 diabetes was defined by using a modification of the American Diabetes Association's (ADA) diabetes diagnosis criteria (Sacks et al., 2002). We modified the ADA's criteria (fasting glucose \geq 126 mg/dL or taking oral diabetes medication or insulin) by adding the additional criterion of participant report of physician's diagnosis of diabetes.

Laboratory Methods

In the SALSA study, a fasting blood sample was drawn from the participants into SST and EDTA tubes during the baseline home visit. One SST tube was transported to the UC-Davis Medical Center (UCDMC) Clinical Laboratory, where it was centrifuged and separated, and the resulting serum was assayed for glucose, GGT, and additional tests included in a chemistry lab panel with a Sequential Multi-channel Analyzer (SMAC). This testing was usually completed within 4 hours of draw (maximum of 6 hours). Another SST

was separated into several serum tubes and frozen at -70 °C. One serum tube was shipped for fasting insulin testing with a radioimmunoassay method (ARUP Laboratories, Salt Lake City, Utah). We tested an additional frozen serum sample for high-sensitivity CRP at a later date, using the method described in the follow-up laboratory measures section.

Follow-up laboratory measures. No blood samples were taken in the second year of follow-up. Blood samples taken at the third-year follow-up visit and later (2002-2006) were tested at the Chemistry Lab of the Michigan Diabetes Research and Training Center (MDRTC). Glucose and lipid assays were performed on a Cobas Mira Chemistry Analyzer from Roche Diagnostics Corporation, Indianapolis, IN, USA. Glucose was tested with a hexokinase method. Lipid testing included Total Cholesterol, HDL Cholesterol, and Triglycerides. The LDL result was calculated, but if the Triglyceride level was greater than 400mg/dl, the lab performed a direct assay for LDL with the LDL Direct Liquid Select reagent from Equal Diagnostics and the Cobas Mira instrument. The laboratory determined fasting insulin levels on serum samples by using a double-antibody radioimmunoassay with a 125I-Human insulin tracer (Linco Research, Billerica, MA) and hs-CRP level by using a latex-enhanced, immunoturbidimetric method and the High Sensitivity CRP kit (Genzyme Diagnostics, Cambridge, MA, formerly Equal Diagnostics) that is automated for use on the Cobas Mira Chemistry Analyzer (Roche Diagnostics Corporation).

Other covariates. The Waist-Hip Ratio (WH) was calculated as $WH = (\text{Waist, inch} / \text{Hip measure, inch})$. Body Mass Index (BMI) was calculated as weight in pounds and height in inches according to the following formula: $BMI = (\text{Weight in Pounds} / (\text{Height in inches}) \times (\text{Height in inches})) \times 703$. We calculated an estimate of insulin resistance with a modification of the HOMA-IR model that uses a single fasting glucose and insulin result.

We modified the standard formula to allow us to use glucose values in mg/dL rather than SI units. This modification yielded the following formula: $\text{HOMA-IR} = (\text{Fasting insulin, mU/ml} * \text{Fasting Glucose, mg/dl}) / 405$. (Bonora et al., 2000; Wallace, Levy, & Matthews, 2004; JAMA, 2001; RCMAR website, 2006.)

Statistical Analysis

All analyses were done with SAS 9.1 statistical software (SAS Institute Inc., Cary, NC, USA). In multivariate models, we adjusted for diabetes risk factors. Variables with non-normal distributions were log transformed. GGT as a continuous variable was used in the reported analysis. GGT quintiles were also examined to determine the significance between high normal levels of GGT and lower levels in a method similar to that used in many published studies, but there was no significant difference in results.

Variable data are presented as means. The means, medians, and proportions of participant characteristics, risk factors, and lab results were calculated from baseline data. All analyses were performed separately on men and women because some studies have shown that GGT is only predictive of T2D in males. There was no difference when the hazard models were analyzed by gender, so the data are not shown. Additionally, self-reported kidney problems were significantly related to T2D but did not alter the results when the Proportional Hazard model was adjusted for them (data not shown).

The relationship between GGT and other variables was analyzed with a Spearman correlation matrix. Significant covariates ($\alpha = 0.05$) that were tested in the incidence models included height, weight, waist circumference, HDL, LDL, Triglycerides, fasting glucose, fasting insulin, HOMA-IR (Insulin Resistance), hs-CRP, AST, Alkaline Phosphatase, and Total Bilirubin.

To calculate incidence rates, we used the incidence density approach. The length of follow-up was calculated as days from baseline exam to diabetes diagnosis or date of last follow-up exam. The date of diabetes diagnosis was the date that any of the following occurred: (a) participant reported that a physician diagnosed them with diabetes; (b) participant's fasting glucose result was ≥ 126 mg/dL; and (c) participant reported starting to take a glucose-lowering medication. Participants were censored after their last examinations. We used the PHREG procedure in the SAS 9.1 statistical package (SAS Institute Inc., Cary, NC, USA) to generate Cox proportional hazard models and calculate the multivariate-adjusted hazard ratios.

Chapter 4: Results

Table 1 shows characteristics of the study population at baseline. There was a statistically significant difference in the means between the group of participants who developed incident diabetes and the group that never developed diabetes for the variables of age, smoking status, and self-reported kidney problems. The participants who developed diabetes were younger at age of enrollment, less likely to be smokers, and more likely to have self-reported kidney problems. There were no differences between diabetics and nondiabetics for sex, education level, alcohol use (beer, wine, or hard liquor were tested separately), self-reported liver or gallbladder problems, or nativity.

Results of clinical measures obtained at baseline examination are shown in Table 1 on the next page. There were no differences in height or waist-to-hip ratio between diabetics and nondiabetics. Compared to nondiabetics, diabetics had larger hip and waist circumferences, higher BMIs, and greater weight.

Table 1

Characteristics of the Study Population at Baseline

Characteristic	Whole group (n = 1203)	Incident DM (n = 178)	DM (n = 1025)	P value (difference of the means)
Age at enrollment, yrs (mean)	70.8 (SD = 7.2, n = 1203)	69.5 (SD = 6.3, n = 178)	71.0 (SD = 7.3, n = 1025)	< 0.0035 ‡
Sex (%)				
-Male	40.7% (n = 490)	46.6% (n = 83)	39.7% (n = 407)	0.0827 †
-Female	59.3% (n = 713)	53.4% (n = 95)	60.3% (n = 618)	
Education in yrs (mean)	7.3 (SD = 5.3, n = 1193)	7.39 (SD = 5.6, n = 175)	7.29 (SD = 5.5, n = 1018)	0.8155 ‡
Smoking status				
- Current (%)	12.3% (n = 146)	7.4% (n = 13)	13.1% (n = 133)	0.0015 †
- Past (%)	40.8% (n = 485)	52.6% (n = 92)	38.7% (n = 393)	
- Never (%)	47.0% (n = 559)	40.0% (n = 70)	48.2% (n = 489)	
Current beer use (%)	46.6% (n = 555)	46.9% (n = 82)	46.6% (n = 473)	0.941 †
Current wine use (%)	40.5% (n = 482)	41.2% (n = 72)	40.4% (n = 410)	0.8522 †
Current hard liquor use (%)	30.9% (n = 368)	31.4% (n = 55)	30.8% (n = 313)	0.8758 †
Liver problems (%)	2.81% (n = 33)	4.65% (n = 8)	2.50% (n = 25)	0.1146 †
Kidney problems (%)	6.18% (n = 72)	9.94% (n = 17)	5.53% (n = 55)	0.027 †
Gallbladder problems (%)	17.87% (n = 210)	22.41% (n = 39)	17.08% (n = 171)	0.0902 †
Nativity (%)				
- Born in USA	45.9% (n = 547)	46.9% (n = 82)	45.8% (n = 466)	0.2013†
- Born in Mexico	47.7% (n = 569)	49.7% (n = 87)	47.3% (n = 481)	
-Born in Other Country	6.45% (n = 77)	3.43% (n = 6)	6.97% (n = 71)	
Waist-to-hip ratio mean (in)	0.9 (SD = 0.09, n = 1082)	0.91 (SD = 0.09, n = 166)	0.9 (SD = 0.09, n=916)	0.0713 ‡
Hip circumference, inches (mean)	41.7 (SD = 4.7, n = 1083)	42.9 (SD= 5.4, n = 167)	41.5 (SD = 4.6, n = 916)	< 0.0019 ‡
Waist circumference, inches (mean)	37.38 (SD = 5.2, n = 1088)	38.86 (SD = 5.6, n = 168)	37.11 (SD = 5.1, n = 920)	< 0.0001 ‡
Height, ft (mean)	5.22 (SD = 0.33, n = 1091)	5.24 (SD = 0.33, n = 169)	5.22 (SD = 0.33, n = 922)	0.5913 ‡
Weight, lbs (mean)	163 (SD = 34, n=1086)	173 (SD = 34, n = 169)	160 (SD = 33, n = 917)	< 0.0001 ‡
BMI (mean)	29.1 (SD = 5.6, n = 1084)	30.7 (SD = 5.3, n = 169)	28.7 (SD = 5.6, n = 915)	< 0.0001 ‡

† Chi-squared test

‡ t test

SD = standard deviation

n = total number of observations

Baseline laboratory test results are shown in Table 2. With the exception of HDL, the diabetic group was higher on every measure, including glucose, insulin, insulin resistance, hs-CRP, lipid measures, and liver enzymes. HDL was lower in diabetics than in nondiabetics.

Table 2

Baseline Laboratory Test Results

Lab test	Whole group mean (n = 1193)	Incident DM mean (n = 189)	DM mean (n = 1004)	P value ‡ (difference of the means)
Fasting glucose, mg/dL	93.70 (n = 1083)	103.42 (n = 167)	91.93 (n = 916)	< 0.0001
Fasting insulin, mU/ml	10.74 (n = 1074)	12.57 (n = 165)	10.40 (n = 909)	< 0.0001
CRP, mg/L (High sensitivity)	5.25 (n = 1041)	5.46 (n = 162)	5.21 (n = 879)	< 0.0001*
Insulin resistance (HOMA-IR)	2.439 (n = 1070)	3.285 (n = 165)	2.285 (n = 905)	< 0.0001*
AST, U/L	23.68 (n = 1082)	24.57 (n = 167)	23.52 (n = 915)	< 0.0001*
Alkaline phosphatase, U/L	80.26 (n = 1082)	82.53 (n = 167)	79.85 (n = 915)	< 0.0001*
GGT, U/L	28.80 (n = 1069)	33.84 (n = 167)	27.86 (n = 902)	< 0.0001*
Total bilirubin, mg/dL	0.779 (n = 1080)	0.801 (n = 167)	0.775 (n = 913)	< 0.0001*
Total cholesterol, mg/dL	214.2 (n = 1085)	206.5 (n = 167)	215.6 (n = 918)	< 0.0001
HDL, mg/dL	53.65 (n = 1085)	50.50 (n = 167)	54.23 (n = 918)	< 0.0001*
LDL, mg/dL	125.8 (n = 1084)	120.0 (n = 167)	126.9 (n = 917)	< 0.0001
Triglycerides, mg/dL	173.7 (n = 1084)	180.1 (n = 167)	172.5 (n = 917)	< 0.0001*

† Chi-squared test

‡ t test

* used transformed data for t test

n = total number of observations

A total of 6,187 person years of follow-up were analyzed. Table 3 below shows the incidence rates of type 2 diabetes in the study population overall and by gender and age. The highest crude rates were among men and women aged 70-79. The oldest participants (80+ years) had the lowest incidence of diabetes.

Table 3

Incidence Rate of Type 2 Diabetes by Gender and Age at Diagnosis

Gender and age at diagnosis	Number of incident cases	Person-years	Crude rate per 1,000 PY
Men			
< 60-69	28	894.75	31.29
70-79	48	1092.39	43.94
80 +	7	464.87	15.06
Total men	83	2452.01	33.85
Women			
< 60-69	33	1292.43	25.53
70-79	55	1828.92	30.07
80 +	7	613.46	11.41
Total women	95	3734.81	25.44
All			
< 60-69	61	2187.18	27.89
70-79	103	2921.31	35.26
80 +	14	1078.33	12.98
Total all	178	6186.82	28.77

Table 4 shows the association of baseline GGT and risk for incident diabetes in a series of Cox models with progressive adjustment for covariates. In model 1, increased GGT was associated with a 40% increased risk of developing type 2 diabetes. After adjustment for age and gender (model 2), the RR was still significant. Adjustment for covariates in model 3 and model 4 did not affect the association between GGT and T2D. Adjustment for each covariate except fasting glucose, HOMA-IR, and/or fasting insulin was done, and none reduced the significance. When fasting serum glucose, HOMA-IR, or fasting serum insulin

was introduced into the model (Model 5), the association was attenuated by nearly 20% and was no longer significant. A test for interaction between GGT and glucose was not significant ($p = 0.25$).

Table 4

GGT and Adjusted Relative Risk of Type 2 Diabetes Incidence

GGT result	Hazard ratio	95% Confidence limits		P value
		lower	upper	
Model 1	1.41	1.15	1.74	0.001
Model 2	1.38	1.11	1.71	0.004
Model 3	1.42	1.14	1.77	0.002
Model 4	1.33	1	1.75	0.04
Model 5	1.13	0.84	1.51	0.426

Notes.

Model 1 unadjusted

Model 2 Model 1 + adj for age and sex

Model 3 Model 2 + adj for smoking and alcohol

Model 4 Model 3 + adj for BMI, HDL, LDL, triglycerides, AST, alkaline phosphatase, hs-CRP, and, total bilirubin

Model 5 Model 4 + adj for glucose

Chapter 5: Conclusions, Limitations, and Recommendations for Further Research

Glucose was highly predictive of type 2 diabetes in this study, and it reduced the association between GGT and type 2 diabetes. The insulin resistance test measure (HOMA-IR) is calculated with the glucose result, so it reduced the association as well. GGT is highly correlated with glucose, insulin, and insulin resistance, so it is likely that the effect of GGT on T2D is through the insulin resistance mechanism.

There were several limitations to this study. One limitation of this study is that we only had fasting serum samples available rather than plasma samples as recommended by the ADA. Fasting plasma sample results would have yielded a more stable measure because there was a time lag between blood draw and processing. Although serum has been reported to have glucose concentrations 5% higher than those of plasma, if the cells stay in contact with the serum, as in our study, the rate of glucose disappearance will be higher in serum and can be affected by other factors (Ladenson, Tsai, Michael, Kessler, & Joist, 1974). Insulin resistance could affect the breakdown rate of glucose between the groups, causing the glucose level in the incident diabetic group to remain higher and appear highly predictive of T2D. It is possible that the significant relationship that we saw between GGT and fasting serum glucose resulted from increased concentrations of GGT in the pancreases of this population. Adjustment for self-reported kidney or liver problems did not change the results. The study population only included elderly Mexican Americans, so these results may indicate that GGT levels are lower in Mexican Americans or the elderly. The follow-up period used for one of the positive studies (Perry et al., 1998) discussed earlier was more than double the length of our follow-up period of 5 years.

Although there was no interaction between glucose and GGT level, the glucose level was so highly predictive of T2D that we postulate that GGT may be on the pathway between glucose and T2D, potentially beginning with fatty liver disease or insulin resistance, causing oxidative stress and leading to increased insulin resistance, more fatty liver deposits, increased glucose, and ultimately, T2D. Figure 2 below shows the suspected pathway between oxidative stress, increased glucose, increased GGT, and type 2 diabetes.

In conclusion, GGT appears to be a predictor of risk of developing type 2 diabetes, but adjustment for fasting glucose attenuated the risk in our study population. This may be due to differences in amounts of GGT in the organs or GGT function and metabolism in our study population or, possibly, study limitations as described previously. It would be important to repeat this analysis in all ages of this population group to further characterize the relationship of GGT, oxidative stress, insulin resistance, and type 2 diabetes in Mexican Americans. Additionally, analyzing data from a lengthened follow-up period would yield more information about this relationship.

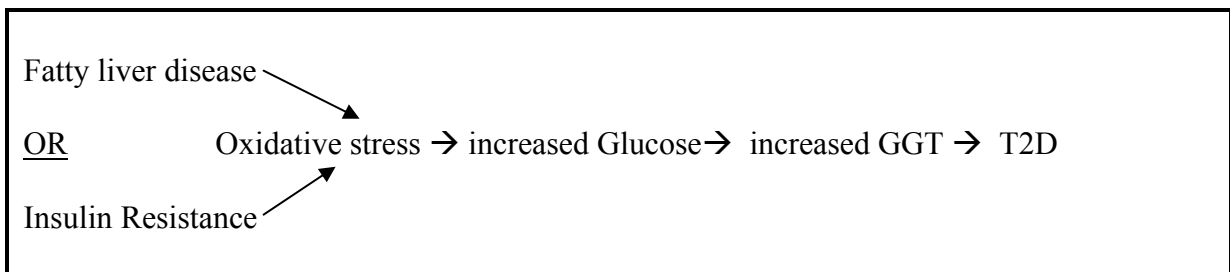


Figure 2. Pathway showing possible GGT relationship to glucose.

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NOTE: Much of the early literature regarding this enzyme is located under the name gamma-glutamyl transpeptidase, which is the older name for the enzyme, E.C.2.3.2.2, (5-L-Glutamyl)-peptide:amino-acid 5-glutamyl transferase. The International Union of Biochemistry and Molecular Biology and The Expert Panel on Enzymes of the International Federation of Clinical Chemistry prefer the name γ -glutamyltransferase, so most of the newest literature will be under the new name or the abbreviation GGT, which is commonly used to refer to this enzyme in order to avoid the use of the Greek letter (Whitfield, 2001).

Appendix: IRB Approval

