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

by

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Thesis

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Thesis Committee: Stephen Sonstein, PhD, Chair

Mary N. Haan, MPH, DrPH

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.

I would like to express my sincere gratitude to Dr. Mary Haan for allowing me to develop the concept for this thesis using years of data from the SALSA study. Dr. Haan graciously extended tremendous support in numerous ways including assistance with study design, data analyses, reviewing the manuscript and overall general support for my educational goals.

I would like to express my gratitude to Dr. Stephen Sonstein, my thesis chair, for his guidance in this process and especially for mentoring throughout the Clinical Research Administration program.

I would like to express my gratitude to Kari Moore, Biostatistician and Data Manager extraordinaire, for all of her assistance with the statistical analysis methods used in the thesis.

I would like to thank my family for their support during the time that I was taking classes and preparing this thesis. Thank you to my husband Rick for his patience and encouragement along the way. Thank you to my children, Rob, Chris and David for their support and understanding.
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 participants, aged 60-101, in the SALSA study was used. Follow-up 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 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. For example, alcoholics can have GGT levels up to three times the normal laboratory limit of 0-45 U/L. Many recent studies have investigated GGT levels and the risk of developing diseases including metabolic syndrome, type 2 diabetes and cardiovascular disease. Serum gamma-glutamyl transferase (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. 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 or Caucasian populations (Wannamethee, Sharper, Lennon, & Whincup, 2005; Lee et al., 2003a; Lee et al., 2004a). We postulate that a similar increase in GGT will be found in elderly Latino participants of the Sacramento Area Study on Aging (SALSA) study and it will be associated with an increased risk of incidence of type 2 diabetes.

The major aim of this study was to evaluate whether GGT levels measured at baseline can 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 based on GGT level, while adjusting for confounding variables.

**Significance of the Study**

Predicting an early increased risk of developing Type 2 diabetes (T2D) prior to developing the disease may encourage a person to make lifestyle changes or obtain treatment leading to a decreased incidence of diabetes. Additionally, their quality and length of life may be improved, while decreasing overall burden on public health 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 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. Increased need for reduced glutathione occurs with oxidative stress (Whitfield, 2001, Lee, Blomhoff, & Jacobs, 2004b, Zhang 2005).

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

Non alcoholic fatty liver disease has been linked to a higher prevalence of diabetes (Bloomgarden, 2005, p. 1519). Oxidative stress resulting from non alcoholic fatty liver disease (NAFLD) has been suggested in the mechanisms of insulin resistance, β-cell dysfunction, poorly-controlled type 2 diabetes, and subsequent complications (Robertson, Harmon, Tran, & Poitout, 2004, Wright, Scism-Bacon, & Glass, 2006; Bo et al., 2005, Thamer et al., 2005).
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, 2005a; Seitz, 2006). Research by Jimenez-Alonso, et al. (1983) showed that hyperglycemia itself does not increase the hepatic enzyme GGT in uncontrolled diabetics. While 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 2004; Meisinger, Löwel, Heier, Schneider, & Thorand, 2005; Zhang, Forman, & Choi, 2005). However, others including Lee, et al., (2004b) note 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 (Emdin, Passino, Donato, Paolicchi, & Pompella, 2002; Emdin, Pompella, & Paolicchi, 2005; Bo et al., 2005; Whitfield, 2001; Lee et al., 2003b; Yamada et al., 2005; Wannamethee et al., 2005; Sakuta, Suzuki, Yasuda, & Ito, 2005).

Meisinger et al. (2005) postulated that possible mechanisms by which GGT is a marker for increased risk of type 2 diabetes include 1) 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; 2) increased GGT is a
marker for oxidative stress or 3) increased GGT may be the expression of inflammation. Ortega et al. (2006) reported that GGT was a significant predictor of insulin resistance independently of weight, BMI or %fat in Pima Indian children.

Many studies have reported an increased risk of type 2 diabetes with increased levels of GGT. Lee et al. (2003a) 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, Suzuki, & Tatara (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 et al. (2004a) 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 the increased risk of type 2 diabetes. The CARDIA study (Lee et al., 2003b) recruited 18-30 year old black and white Americans in 1985-86 and followed them for 15 years. They reported the risk of type 2 diabetes was strongly increased with higher normal levels of GGT. In addition, they postulate that this may be related to oxidative stress. Perry, Wannamethee, & Shaper (1998) examined the association between GGT levels and 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) men and women 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 was 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 women 33 to 68 years old and found that only GGT was strongly associated with increased risk of T2D in both sexes over 3 years. Meisinger et al. (2005) studied 1851 male and 1836 female 25-64 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 was a marker of T2D.

Lee et al. (2007) followed up 3,451 middle aged men and women 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 and Jacobs, 2005b) showed that all levels of GGT were 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 causes oxidative stress and inflammation, leading to type 2 diabetes.
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 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 from 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.

Analysis of covariance was done using SAS Proc GLM programming. Cox proportional hazards regression models were used to examine the association of baseline GGT level and incidence of type 2 diabetes. Clinical measures from participants without diabetes at study baseline were analyzed to determine the development of type 2 diabetes over five years of follow-up using a modification of the American Diabetes Association (ADA) diabetes diagnosis criteria. The ADA criteria (serum fasting glucose ≥ 126 or = 126 or taking oral diabetes medication or insulin) was modified by adding the additional criteria of participant report of physician diagnosis of diabetes.

Laboratory Methods

In the SALSA study a fasting blood sample was drawn from the participant into SST and EDTA tubes during their baseline home visit. One SST tube was transported to the UC-
Davis Medical Center (UCDMC) Clinical Laboratory, where it was centrifuged, separated and the resulting serum assayed for glucose, GGT and other chemistry tests 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 and the serum was frozen at -80°C before shipment and testing for fasting insulin with a radioimmunoassay method (ARUP Laboratories, Salt Lake City Utah). High sensitivity CRP testing was done at a later date with stored serum samples frozen at -70°C until testing and 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 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 is tested with a glucose oxidase 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, a direct assay for LDL was performed using LDL Direct Liquid Select from Equal diagnostics with the Cobas Mira. Fasting insulin was analyzed with double-antibody radioimmunoassay using a 125I-Human insulin tracer (Linco Research). The assay for hs-CRP was done with a latex-enhanced turbidimetric method automated for the Cobas Mira Chemistry Analyzer from Roche Diagnostics Corporation using the Genzyme Diagnostics (formerly Equal Diagnostics) High Sensitivity CRP kit.

Other covariates. The Waist-Hip Ratio (WH) was calculated as WH = (Waist, inch / Hip measure, inch). Body Mass Index (BMI) was calculated using weight in pounds and
height in inches according to the following formula: \( \text{BMI} = \frac{\text{Weight in Pounds}}{(\text{Height in inches})} \times 703 \). To obtain an estimate of insulin resistance, we used a modification of the HOMA-IR model, in which insulin resistance is calculated using a single fasting glucose and insulin result. HOMA-IR = \( \frac{(\text{Fasting insulin, mU/ml} \times \text{Fasting Glucose, mg/dl})}{405} \). Modification of the published formula allowed us to use glucose values in mg/dL rather than SI units. (Bonora et al., 2000, Wallace, Levy, & Matthews, 2004; JAMA author instructions, RCMAR website, n.d.)

**Statistical Analysis**

Analyses were done using SAS 9.1 statistical software (SAS Institute Inc., Cary, NC, USA). In multivariate models, we adjusted for diabetes risk factors. Variables with non-normal distribution 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 as compared to lower levels in a method similar to 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 is 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 (MD diabetes report, fasting glucose >125mg/dl or start date of glucose lowering medication.) or date of last follow-up exam. Participants were censored after their last examination. Cox proportional hazard models were used to calculate the multivariate adjusted hazard ratios using PHREG programming in the SAS 9.1 statistical package (SAS Institute Inc., Cary, NC, USA).
Chapter 4: Results

Table 1 shows the characteristics of study population. 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 a smoker, and more likely to have self reported kidney problems. There were no differences between diabetics and non-diabetics for sex, education level, alcohol use (beer, wine or hard liquor were tested separately), self reported liver or gallbladder problems, and nativity.

Results of clinical measures obtained at baseline examination are shown in Table 2. There were no differences in height or waist to hip ratio between diabetics and nondiabetics. Compared to nondiabetics, diabetics had a larger hip and waist circumference, higher BMI and greater weight.

Laboratory test results from baseline are shown in Table 3. 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.

A total of 6,187 person years of follow up were analyzed. Table 4 shows that the incidence density of T2D in the study population was 28.77 per 1000 person years. The highest crude rates were among men aged 70-79 (43.9) and women aged 70-79 (30.1). The oldest participants (80+ years) had the lowest incidence of diabetes with 15 incident cases per 1000 person years for males and 11 cases per 1000 person years for females.
Table 5 shows the association of baseline GGT and risk for incident diabetes. In an unadjusted model, increased GGT was associated with a 40% increased risk of developing type 2 diabetes. After adjustment for age and gender, the RR was still significant.

Adjustment for covariates in model 3 or model 4 did not affect the association between GGT and T2D. Adjustment for each significantly correlated 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.
Chapter 5: Conclusions, Limitations, and Recommendations for Further Research

Glucose was so highly predictive in this study that it overpowered the predictive power of GGT. Since the insulin resistance test measure (HOMA-IR) is calculated with the glucose result, it was also highly predictive of T2D in this study.

There were several limitations to this study. One limitation of this study is that we only had fasting serum samples available instead of plasma sample as recommended by the ADA. Fasting plasma samples results would have yielded a more stable measure due to the time lag between blood draw and processing. While serum has been reported to have glucose concentrations five percent higher than plasma, if the cells stay in contact with the serum as in our study, the rate of glucose disappearance is higher in serum and can be affected by other factors (Ladenson, Tsai, Michael, Kessler, & Joist, 1974). Another limitation is there was only one GGT measure done at baseline to analyze. It is possible that fluctuation of GGT levels due to alcohol use may affect the predictive value of this test. GGT is found in the pancreas, liver, and kidney. Adjustment for self reported kidney or liver problems did not change the results. It is possible that the significant relationship that we saw between GGT and fasting serum glucose is due to increased concentration of GGT in the pancreas in this population. Since the study population only included elderly Mexican Americans, these results may indicate a population based bias. 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. The last limitation was our assumption that every
incident case of diabetes was type 2 diabetes; however this is a likely assumption due to the age of the study population.

Since there was no interaction between glucose and GGT level, but the glucose level was so highly predictive of T2D, 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. Figure 2 shows the suspected pathway between oxidative stress and type 2 diabetes, increased glucose and increased GGT.

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 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 into this relationship.
References


appropriate specimen [Electronic version]? American Journal of Clinical Pathology, 62, 545-552.


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, (S-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 of GGT, which is commonly used to refer to this enzyme in order to avoid the use of the Greek letter. (Whitfield, 2001)
Appendix A. IRB Approval
Table 1. Characteristics of Study Population at Baseline

<table>
<thead>
<tr>
<th></th>
<th>Overall (n=1203)</th>
<th>Incident DM (n=178)</th>
<th>DM (n=1025)</th>
<th>P value (difference of the means)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age at Enrollment (yrs)</td>
<td>70.8 (SD = 7.2, n = 1203)</td>
<td>69.5 (SD = 6.3, n = 178)</td>
<td>71.0 (SD = 7.3, n = 1025)</td>
<td>&lt;0.0035 ‡</td>
</tr>
<tr>
<td>Sex (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>40.7% (n = 490)</td>
<td>46.6% (n = 83)</td>
<td>39.7% (n = 407)</td>
<td>0.0827 †</td>
</tr>
<tr>
<td>Female</td>
<td>59.3% (n = 713)</td>
<td>53.4% (n = 95)</td>
<td>60.3% (n = 618)</td>
<td></td>
</tr>
<tr>
<td>Education yrs (mean)</td>
<td>7.3 (SD = 5.3, n = 1193)</td>
<td>7.39 (SD = 5.6, n = 175)</td>
<td>7.29 (SD = 5.5, n = 1018)</td>
<td>0.8155 ‡</td>
</tr>
<tr>
<td>Smoking Status</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- Current (%)</td>
<td>12.3% (n = 146)</td>
<td>7.4% (n = 13)</td>
<td>13.1% (n = 133)</td>
<td>0.0015 †</td>
</tr>
<tr>
<td>- Past (%)</td>
<td>40.8% (n = 485)</td>
<td>52.6% (n = 92)</td>
<td>38.7% (n = 393)</td>
<td></td>
</tr>
<tr>
<td>- Never (%)</td>
<td>47.0% (n = 559)</td>
<td>40.0% (n = 70)</td>
<td>48.2% (n = 489)</td>
<td></td>
</tr>
<tr>
<td>Current Beer Use (%)</td>
<td>46.6% (n = 555)</td>
<td>46.9% (n = 82)</td>
<td>46.6% (n = 473)</td>
<td>0.941 †</td>
</tr>
<tr>
<td>Current Wine use (%)</td>
<td>40.5% (n = 482)</td>
<td>41.2% (n = 72)</td>
<td>40.4% (n = 410)</td>
<td>0.8522 †</td>
</tr>
<tr>
<td>Current Hard Liquor Use (%)</td>
<td>30.9% (n = 368)</td>
<td>31.4% (n = 55)</td>
<td>30.8% (n = 313)</td>
<td>0.8758 †</td>
</tr>
<tr>
<td>Liver problems (%)</td>
<td>2.81% (n = 33)</td>
<td>4.65% (n = 8)</td>
<td>2.50% (n = 25)</td>
<td>0.1146 †</td>
</tr>
<tr>
<td>Kidney problems (%)</td>
<td>6.18% (n = 72)</td>
<td>9.94% (n = 17)</td>
<td>5.53% (n = 55)</td>
<td>0.027 †</td>
</tr>
<tr>
<td>Gallbladder problems (%)</td>
<td>17.87% (n = 210)</td>
<td>22.41% (n = 39)</td>
<td>17.08 (n = 171)</td>
<td>0.0902 †</td>
</tr>
<tr>
<td>Nativity (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- Born in USA</td>
<td>45.9% (n = 547)</td>
<td>46.9% (n = 82)</td>
<td>45.8% (n = 466)</td>
<td>0.2013 †</td>
</tr>
<tr>
<td>- Born in Mexico</td>
<td>47.7% (n = 569)</td>
<td>49.7% (n = 87)</td>
<td>47.3% (n = 481)</td>
<td></td>
</tr>
<tr>
<td>- Born in Other Country</td>
<td>6.45% (n = 77)</td>
<td>3.43% (n = 6)</td>
<td>6.97% (n = 71)</td>
<td></td>
</tr>
</tbody>
</table>

* Chi sq method
‡ T-test method
## Table 2. Baseline Physical Measurements

<table>
<thead>
<tr>
<th></th>
<th>Whole Group (n=1203)</th>
<th>Incident DM (n=178)</th>
<th>No Diabetes (DM) (n=1025)</th>
<th>P value (difference of the means)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Waist to hip ratio (in)</td>
<td>0.9 (SD = 0.09, n = 1082)</td>
<td>0.91 (SD = 0.09, n = 166)</td>
<td>0.9 (SD = 0.09, n = 916)</td>
<td>0.0713 ‡</td>
</tr>
<tr>
<td>Hip circumference (in)</td>
<td>41.7 (SD = 4.7, n = 1083)</td>
<td>42.9 (SD = 5.4, n = 167)</td>
<td>41.5 (SD = 4.6, n = 916)</td>
<td>&lt;0.0019 ‡</td>
</tr>
<tr>
<td>Waist circumference (in)</td>
<td>37.38 (SD = 5.2, n = 1088)</td>
<td>38.86 (SD = 5.6, n = 168)</td>
<td>37.11 (SD = 5.1, n = 920)</td>
<td>&lt;0.0001 ‡</td>
</tr>
<tr>
<td>Height (ft)</td>
<td>5.22 (SD = 0.33, n = 1091)</td>
<td>5.24 (SD = 0.33, n = 169)</td>
<td>5.22 (SD = 0.33, n = 922)</td>
<td>0.5913 ‡</td>
</tr>
<tr>
<td>Weight (lbs)</td>
<td>163 (SD = 34, n = 1086)</td>
<td>173 (SD = 34, n = 169)</td>
<td>160 (SD = 33, n = 917)</td>
<td>&lt;0.0001 ‡</td>
</tr>
<tr>
<td>BMI (weight in lbs / (height in inches x 12) x 703)</td>
<td>29.1 (SD = 5.6, n = 1084)</td>
<td>30.7 (SD = 5.3, n = 169)</td>
<td>28.7 (SD = 5.6, n = 915)</td>
<td>&lt;0.0001 ‡</td>
</tr>
</tbody>
</table>

† Chi sq method
‡ T-test method
### Table 3. Baseline Laboratory Test Results

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

† Chi sq method  
‡ T-test method  
* used transformed data for T-test
<table>
<thead>
<tr>
<th>Gender and Age at Diagnosis</th>
<th>Number of incident cases</th>
<th>Person-Years</th>
<th>Crude rate per 1,000 PY</th>
</tr>
</thead>
<tbody>
<tr>
<td>Men</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;60-69</td>
<td>28</td>
<td>894.75</td>
<td>31.29</td>
</tr>
<tr>
<td>70-79</td>
<td>48</td>
<td>1092.39</td>
<td>43.94</td>
</tr>
<tr>
<td>80+</td>
<td>7</td>
<td>464.87</td>
<td>15.06</td>
</tr>
<tr>
<td><strong>Total Men</strong></td>
<td><strong>83</strong></td>
<td><strong>2452.01</strong></td>
<td><strong>33.85</strong></td>
</tr>
<tr>
<td>Women</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 60-69</td>
<td>33</td>
<td>1292.43</td>
<td>25.53</td>
</tr>
<tr>
<td>70-79</td>
<td>55</td>
<td>1828.92</td>
<td>30.07</td>
</tr>
<tr>
<td>80+</td>
<td>7</td>
<td>613.46</td>
<td>11.41</td>
</tr>
<tr>
<td><strong>Total Women</strong></td>
<td><strong>95</strong></td>
<td><strong>3734.81</strong></td>
<td><strong>25.44</strong></td>
</tr>
<tr>
<td>All</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 60-69</td>
<td>61</td>
<td>2187.18</td>
<td>27.89</td>
</tr>
<tr>
<td>70-79</td>
<td>103</td>
<td>2921.31</td>
<td>35.26</td>
</tr>
<tr>
<td>80+</td>
<td>14</td>
<td>1078.33</td>
<td>12.98</td>
</tr>
<tr>
<td><strong>Total All</strong></td>
<td><strong>178</strong></td>
<td><strong>6186.82</strong></td>
<td><strong>28.77</strong></td>
</tr>
</tbody>
</table>
### Table 5. GGT and Adjusted Relative Risk of Type 2 Diabetes Incidence

<table>
<thead>
<tr>
<th>GGT Result</th>
<th>Hazard Ratio</th>
<th>95% Confidence Limits</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Model 1</td>
<td>1.41</td>
<td>1.15 - 1.74</td>
<td>0.001</td>
</tr>
<tr>
<td>Model 2</td>
<td>1.38</td>
<td>1.11 - 1.71</td>
<td>0.004</td>
</tr>
<tr>
<td>Model 3</td>
<td>1.42</td>
<td>1.14 - 1.77</td>
<td>0.002</td>
</tr>
<tr>
<td>Model 4</td>
<td>1.33</td>
<td>1.0 - 1.75</td>
<td>0.04</td>
</tr>
<tr>
<td>Model 5</td>
<td>1.13</td>
<td>0.84 - 1.51</td>
<td>0.426</td>
</tr>
</tbody>
</table>

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
Figure 1. Diagram of known and theoretical pathways
Fatty liver disease

**OR**

Oxidative stress $\rightarrow$ increased Glucose $\rightarrow$ increased GGT $\rightarrow$ T2D

Insulin Resistance

*Figure 2. Pathway Showing Possible GGT Relationship to Glucose*