



Pregravid Liver Enzyme Levels and Risk of Gestational Diabetes Mellitus During a Subsequent Pregnancy

Sneha B. Sridhar, Fei Xu, Jeanne Darbinian, Charles P. Quesenberry, Assiamira Ferrara, and Monique M. Hedderson

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OBJECTIVE

Liver enzymes are independent predictors of type 2 diabetes. Although liver fat content correlates with features of insulin resistance, a risk factor for developing gestational diabetes mellitus (GDM), the relationship between liver enzymes and GDM is unclear. The objective of this study was to assess whether pregravid liver enzyme levels are associated with subsequent risk of GDM.

RESEARCH DESIGN AND METHODS

A nested case-control study was conducted among women who participated in the Kaiser Permanente Northern California multiphasic health checkup (1984–1996) and had a subsequent pregnancy (1984–2009). Case patients were 256 women who developed GDM. Two control subjects were selected for each case patient and matched for year of blood draw, age at examination, age at pregnancy, and number of intervening pregnancies.

RESULTS

Being in the highest quartile versus the lowest quartile of γ -glutamyl transferase (GGT) levels was associated with a twofold increased risk of subsequent GDM (odds ratio 1.97 [95% CI 1.14–3.42]), after adjusting for race/ethnicity, prepregnancy BMI, family history of diabetes, and alcohol use. This result was attenuated after adjusting for homeostasis model assessment of insulin resistance (HOMA-IR), fasting status, and rate of gestational weight gain. There was significant interaction between GGT and HOMA-IR; the association with GGT was found among women in the highest tertile of HOMA-IR. Aspartate aminotransferase and alanine aminotransferase were not associated with increased GDM risk.

CONCLUSIONS

Pregravid GGT level, but not alanine aminotransferase or aspartate aminotransferase level, predicted the subsequent risk of GDM. Markers of liver fat accumulation, such as GGT level, are present years before pregnancy and may help to identify women at increased risk for subsequent GDM.

Gestational diabetes mellitus (GDM), defined as carbohydrate intolerance with first onset or recognition in pregnancy, affects 4–7% of women in the U.S. (1–3). GDM is associated with adverse perinatal outcomes and is a risk factor for the development of type 2 diabetes in both the mother and her offspring. Strategies to prevent GDM hold great potential as a means by which to prevent or delay the onset of diabetes.

Division of Research, Kaiser Permanente Northern California, Oakland, CA

Corresponding author: Sneha B. Sridhar, sneha.x.sridhar@kp.org.

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Insulin resistance and inadequate insulin response are two known mechanisms underlying the pathophysiology of both GDM and type 2 diabetes.

Laboratory tests for γ -glutamyl transferase (GGT), alanine aminotransferase (ALT), and aspartate aminotransferase (AST) are commonly performed to assess the overall health of the liver. Liver fat content has been shown to correlate with features of insulin resistance independent of BMI and abdominal obesity. While the gold standard for measuring nonalcoholic fatty liver disease is a liver biopsy, testing of ALT, AST, and GGT seems to provide reasonable non-invasive surrogate measures for use in epidemiologic studies (4). The liver is crucial to maintaining glucose homeostasis, both during fasting and postprandial states, and thereby plays a role in the development of type 2 diabetes. Serum GGT level is also a marker of oxidative stress (5,6). Oxidative stress is the condition of increased free radical activity and high lipid oxidation, and it plays a role in the etiology of type 2 diabetes by inducing insulin resistance in the peripheral tissues and impairing insulin secretion from the pancreatic β -cells (7,8). GGT catabolizes extracellular glutathione (GSH), which has an antioxidant function; therefore, GGT levels may become elevated in order to produce more GSH in response to oxidative stress (9).

Past research suggests that during a normal pregnancy, liver enzyme levels may change in response to the increased insulin resistance induced by pregnancy (10,11); therefore, it is important to determine whether prepregnancy levels of liver enzymes are related to subsequent risk of GDM, in order to clarify the temporal sequence of the association. Although liver enzymes are known to correlate with features of insulin resistance, a risk factor for the development of GDM, the relationship between prepregnancy liver enzyme levels and GDM is unclear. Thus, the aim of this case-control study is to examine the association between prepregnancy ALT, AST, and GGT levels and the risk of the subsequent development of GDM.

RESEARCH DESIGN AND METHODS

The setting is Kaiser Permanente Northern California (KPNC), an integrated health care delivery system that provides medical care for about one-third of the underlying population in the San Francisco Bay Area.

KPNC subscribers are representative of the region (12).

The source population consisted of female KPNC members who completed a voluntary multiphasic health checkup (MHC) at the Kaiser Permanente Oakland Medical Center between 1984 and 1996. KPNC members at this facility were invited to complete a comprehensive health checkup upon study enrollment. The MHC consisted of a clinic visit for the completion of questionnaires and clinical measurements, including blood pressure, weight, and serum glucose and cholesterol levels (measured in serum obtained from a random blood draw). An extra serum sample was collected and stored at -40°C for future use. The goal of the MHC was to provide health maintenance through early diagnosis (13). BMI was calculated as kilograms per square meter; height and weight were measured using a stadiometer and a balance beam scale, respectively. Information on age, sex, race/ethnicity, education level, cigarette smoking, family history of diabetes, medical history, alcohol consumption (≥ 1 vs. < 1 drink/day), coffee consumption, use of medications, and hours since last food ingestion was collected using self-administered questionnaires (13). Serum glucose was measured in serum obtained from a random blood draw using the hexokinase method, and total cholesterol was assessed by the regional laboratory of KPNC at the time of the MHC using a Kodak Ektachem chemistry analyzer. This laboratory participates in the College of American Pathologists accreditation and monitoring program.

Among women 15–45 years of age (median age 34 years) who participated in the MHC from 1985 to 1996 ($n = 27,743$ with clinical and questionnaire data, as well as an extra serum sample), we identified 4,098 women who subsequently delivered an infant by 2010 by searching the KPNC hospitalization database and the Pregnancy Glucose Tolerance and GDM Registry (3), an active surveillance registry that annually identifies all pregnancies resulting in a livebirth or stillbirth among KPNC members. Women with recognized pregravid diabetes (14) are excluded from the GDM Registry; therefore, women in whom diabetes had been diagnosed prior to the index pregnancy were not eligible to be included in the study. The Pregnancy Glucose Tolerance and GDM

Registry captures the results of all screening and diagnostic tests for GDM from the KPNC electronic laboratory database (data available since 1994).

Study Design

This is a nested case-control study, within a cohort of 4,098 women who took part in an MHC examination, had an extra tube of serum stored for future use, and had a subsequent pregnancy, on average, 7 years after the MHC examination. All cohort members in whom GDM subsequently developed were included as case patients; two control subjects were selected for each case patient from among women not meeting the GDM case definition.

GDM Case Definition

We identified 267 women with GDM according to the KPNC electronic databases. Case patients had either 1) glucose values obtained during a standard 100-g, 3-h oral glucose tolerance test (OGTT) that met the Carpenter-Coustan plasma glucose thresholds for GDM (as outlined by the American College of Obstetricians and Gynecologists) (15) in the laboratory database ($n = 228$), or 2) a hospital discharge diagnosis of GDM in the electronic hospital discharge database for pregnancies occurring before the electronic laboratory data were available (prior to 1994; $n = 39$). Standardized medical chart review was conducted by trained abstractors to confirm that these 267 women had 100-g, 3-h OGTT results meeting the Carpenter-Coustan criteria (15) for GDM (plasma glucose thresholds: fasting 5.3 mmol/L [95 mg/dL]; 1-h 10.0 mmol/L [180 mg/dL]; 2-h 8.6 mmol/L [155 mg/dL]; 3-h 7.8 mmol/L [140 mg/dL]). Case patients were excluded if, at the time of the MHC examination, they had a random glucose levels of > 200 mg/dL ($n = 6$), no indication of GDM during the index pregnancy ($n = 4$), or they had impaired glucose tolerance with insufficient follow-up testing ($n = 1$), leaving a total of 256 confirmed cases of GDM.

Control Selection and Matching Criteria

Among women without an indication of GDM, control subjects were randomly selected; two control subjects were individually matched to each case patient based on the year of MHC serum collection date (± 3 months), age at MHC serum collection (± 2 years), the number of intervening pregnancies (0, 1, ≥ 2),

and age at delivery of the index pregnancy (± 2 years). We matched for the year of serum collection to account for any potential degradation in the quality of the serum over time, thereby assuring that the sample storage time was approximately the same for case patients and control subjects. Since GDM is more common in older women, we matched on age at serum collection and age at delivery. We matched on the number of pregnancies to account for any differences in pregnancies between the initial examination and the index pregnancy. Control subjects were excluded from the analysis if they had glucose values that were diagnostic of GDM found during medical chart abstraction ($n = 5$); an abnormal screening glucose level but no follow-up diagnostic glucose test ($n = 5$); or one abnormal glucose value on the diagnostic glucose test ($n = 5$), suggestive of "mild" GDM. Of the 512 matched control subjects identified, 497 were eligible.

Exposure Variables

Serum Biomarker Assays

Serum samples were thawed, aliquoted, and transported in batches on dry ice to the laboratory of Dr. Peter Havel at the University of California, Davis, for analysis. GGT, ALT, and AST were measured on a Poly-Chem analyzer (MedTest DX, Cortlandt Manor, NY). The intra-assay and interassay coefficients of variation were 4.7% and 5.6% (GGT), 4.5% and 11.7% (ALT), and 2.7% and 9.5% (AST), respectively. Insulin was measured with a radioimmunoassay (Millipore). The intra-assay and interassay coefficients of variation were $<4.0\%$ and $<10\%$, respectively. Insulin resistance was calculated based on the homeostasis model assessment of insulin resistance (HOMA-IR) using the following equation: (fasting glucose \times fasting insulin)/22.5, where glucose was measured in millimoles per liter and insulin in milliunits per milliliter (16).

Rate of Gestational Weight Gain per Week

The rate of gestational weight gain per week was calculated as follows: (weight measured at or before the glucose screening test – prepregnancy weight)/weeks of gestation attained at the time of the weight measurement.

Statistical Analysis

Conditional logistic regression was used to obtain odds ratios (ORs) to estimate

the relative risk of GDM in relation to prepregnancy GGT, ALT, and AST levels. Women were categorized by quartile of GGT, ALT, and AST levels, as defined among control subjects. Variables evaluated for confounding included race/ethnicity, pregravid BMI (in kilograms per square meter), parity, cigarette smoking, alcohol use, maternal education, and family history of diabetes, all assessed at the time of liver enzyme measurement. To examine the effect of weight gain during pregnancy up to the time of GDM diagnosis, we added this variable to the fully adjusted conditional logistic regression model.

To assess confounding, we entered covariates into a logistic regression model, one at a time, and compared the adjusted and unadjusted estimates. We included covariates that altered unadjusted estimates by $\geq 10\%$.

To assess the potential modifying effects of the prepregnancy tertile of HOMA-IR (dichotomized as ≥ 67 th percentile vs. <67 th percentile), prepregnancy BMI, racial/ethnic group, and time since MHC examination, we included appropriate cross-product (interaction) terms in regression models. This study was approved by the human subjects committee of the Kaiser Foundation Research Institute.

RESULTS

Table 1 summarizes the demographic, anthropometric, reproductive, and metabolic characteristics of the study participants, by case/control status. Women in whom GDM developed were more likely to have <12 years of education, to be Asian or Hispanic, to have two or more children at the time of the MHC, to abstain from alcohol, and to have a family history of type 2 diabetes compared with women in whom GDM did not develop. Women in whom GDM developed also had higher levels of several cardiometabolic risk factors at the MHC examination, including BMI, serum glucose, total cholesterol, systolic and diastolic blood pressure, and serum insulin concentrations, and weight gain from the MHC examination to the index pregnancy. Mean prepregnancy GGT and ALT levels (in units per liter) were significantly higher in women in whom GDM developed, when compared with those in whom GDM did not develop (28.0 vs. 22.4 and 8.5 vs. 6.7 units/L,

respectively; P value <0.001). The mean prepregnancy AST level was also higher among women in whom GDM developed versus those in whom it did not (13.9 vs. 11.8 units/L), although the difference was not significant.

Table 2 shows the ORs and 95% CIs for risk of GDM determined by pregravid liver enzyme levels. The first model adjusted for race/ethnicity, BMI, family history of diabetes, and alcohol use at the time of the MHC. For GGT, there was a trend of increasing risk of GDM as the quartile increased. Being in the fourth versus the first quartile of GGT resulted in a twofold increase in the odds of the development of GDM, and the association was significant (OR 1.97 [95% CI 1.14–3.42]) (Table 2). After further adjusting for HOMA-IR (in tertiles), fasting status (≥ 6 h since the last food at the time of MHC examination), and rate of gestational weight gain (in tertiles) among the full cohort, the association was no longer significant (OR 1.57 [95% CI 0.84–2.93]). Similar results were found when restricted to women who were fasting for ≥ 6 h. We also conducted a sensitivity analysis excluding women who drank one or more alcoholic drinks per day at the time of the MHC examination, and the results were similar.

HOMA-IR was calculated both in women who had fasted for at least 6 h ($n = 419$) and in those who had not fasted ($n = 306$). There were no significant differences by fasting status in correlations between HOMA-IR and BMI, GGT, ALT, AST, and glucose levels. Among women who were fasting, the correlations between HOMA-IR and the liver enzymes were as follows: GGT $r = 0.32$, $P < 0.0001$; AST $r = 0.10$, $P = 0.05$; and ALT $r = 0.22$, $P < 0.0001$. Among women who were not fasting, the correlations between HOMA-IR and the liver enzymes were as follows: GGT $r = 0.16$, $P < 0.01$; AST $r = 0.09$, $P = 0.12$; and ALT $r = 0.18$, $P < 0.01$. While women who were not fasting for ≥ 6 h had higher HOMA-IR levels, there remained a significant difference between case patients and control subjects ([mean \pm SD] women fasting <6 h: case patients 9.0 ± 11.5 ; control subjects 4.5 ± 3.8 ; $P < 0.001$; women fasting ≥ 6 h: case patients 4.1 ± 3.5 ; control subjects 2.9 ± 2.9 ; $P < 0.001$); therefore, we chose to include everyone in the analytic cohort regardless of fasting status.

Table 1—Characteristics of case patients and control subjects

| Characteristics | GDM case patients (n = 256) | Control subjects (n = 497) | P value |
|---|--------------------------------|----------------------------------|---------------------|
| Age at MHC examination | 28.2 ± 5.5 | 28.4 ± 5.2 | 0.78 ^a |
| Age at delivery (years) | 35.4 ± 5.1 | 35.1 ± 4.9 | 0.43 ^b |
| <30 | 39 (15.2) | 80 (16.1) | |
| 30–34 | 73 (28.5) | 145 (29.2) | |
| 35–39 | 102 (39.8) | 183 (36.8) | |
| ≥40 | 42 (16.4) | 89 (17.9) | |
| Time between examination and delivery | 7.1 ± 4.4 | 6.7 ± 4.4 | 0.21 ^a |
| Education (years) | | | 0.24 ^b |
| ≤12 | 74 (28.9) | 119 (23.9) | |
| 13–15 | 85 (33.2) | 157 (31.6) | |
| ≥16 | 92 (35.9) | 214 (43.1) | |
| Unknown | 5 (2.0) | 7 (1.4) | |
| Race/ethnicity | | | <0.001 ^b |
| Non-Hispanic White | 50 (19.5) | 186 (37.4) | |
| African American | 91 (35.5) | 184 (37.0) | |
| Asian/Pacific Islander | 80 (31.3) | 84 (16.9) | |
| Hispanic | 35 (13.7) | 43 (8.7) | |
| Parity | | | <0.001 ^b |
| 0 | 142 (55.5) | 278 (55.9) | |
| 1 | 47 (18.4) | 106 (21.3) | |
| ≥2 | 44 (17.2) | 70 (14.1) | |
| Unknown | 23 (9.0) | 43 (8.7) | |
| Gestational age at birth (weeks) | | | 0.01 ^b |
| ≥37 | 218 (84.8) | 460 (90.7) | |
| <37 | 39 (15.2) | 39 (7.7) | |
| Large-for-gestational age at birth ^c | | | <0.01 ^b |
| No | 198 (81.1) | 427 (89.5) | |
| Yes | 46 (18.9) | 50 (10.5) | |
| Alcohol use | | | <0.001 ^b |
| None | 74 (28.9) | 81 (16.3) | |
| Occasional or more drinks/day | 149 (58.2) | 346 (69.6) | |
| Unknown | 33 (12.9) | 70 (14.1) | |
| Smoking | | | 0.40 ^b |
| Never | 150 (58.6) | 277 (55.7) | |
| Former | 37 (14.5) | 92 (18.5) | |
| Current | 38 (14.8) | 61 (12.3) | |
| Unknown | 31 (12.1) | 67 (13.5) | |
| Hypertension status at index pregnancy | | | <0.001 ^b |
| No hypertension | 138 (53.9) | 326 (65.5) | |
| Preexisting hypertension ^d | 28 (10.9) | 18 (3.6) | |
| Gestational hypertension | 33 (12.9) | 68 (13.7) | |
| Preeclampsia | 42 (16.4) | 37 (7.4) | |
| Family history of diabetes | 151 (59.0) | 192 (38.6) | <0.001 ^b |
| BMI (kg/m ²) | 26.0 ± 6.5 | 23.7 ± 4.6 | <0.001 ^a |
| Weight change from MHC to pregnancy (kg) | 8.9 ± 9.9 | 4.4 ± 8.2 | <0.001 ^a |
| Rate of gestational weight gain (kg/week) ^e | 0.3 ± 0.2 | 0.4 ± 0.2 | <0.07 ^a |
| Serum glucose (mg/dL) | 89.6 ± 13.5 | 83.6 ± 8.3 | <0.001 ^a |
| Serum cholesterol (mg/dL) | 182.9 ± 33.2 | 176 ± 32.6 | <0.01 ^a |
| Systolic blood pressure (mmHg) | 115.6 ± 14.7 | 113.3 ± 13.4 | <0.05 ^a |
| Diastolic blood pressure (mmHg) | 69.9 ± 10.4 | 68.3 ± 9.0 | <0.05 ^a |
| White blood cell count (1,000 cells per mm ³) | 6.9 ± 1.9 | 6.5 ± 1.9 | <0.01 ^a |
| GGT (units/L) | 28.0 ± 21.7 | 22.4 ± 16.6 | <0.001 ^f |
| ALT (units/L) | 8.5 ± 9.5 | 6.7 ± 3.8 | <0.001 ^f |
| AST (units/L) | 13.9 ± 25.3 | 11.8 ± 6.6 | 0.18 ^f |
| HOMA-IR index | 4.1 ± 3.5 | 2.9 ± 2.9 | <0.001 ^a |
| Insulin (μU/mL) | 25.8 ± 28.6 | 17.5 ± 16.7 | <0.001 ^f |

Data are mean ± SD or N (%), unless otherwise indicated. ^at Test to compare differences in mean values of continuous variables except as noted below for Wilcoxon test. ^bχ² Test for categorical variables. ^cSubset of women with singleton births; large-for-gestational age >90th percentile based on race and gestational age-specific quantiles. ^dIncludes women who experienced preeclampsia superimposed on preexisting hypertension. ^eWeight change in kilograms per week from beginning of index pregnancy until screening glucose (measurement obtained 1 h after the 50-g oral challenge). Data were available for 235 case patients and 446 control subjects.

^fWilcoxon test for differences in median values.

Neither ALT nor AST was associated with an increased risk of GDM, and no clear trend was observed. The interaction between BMI and race/ethnicity did not reach statistical significance.

Figure 1 displays the ORs for GDM stratified by prepregnancy tertile of HOMA-IR, dichotomized (≥67th percentile vs. <67th percentile). There was significant interaction by prepregnancy HOMA-IR ($P = 0.082$), and the associations with being in the top three quartiles of GGT level were stronger for women in the highest tertile of HOMA-IR (≥67th percentile) before pregnancy (Quartile 2 OR 2.71 [95% CI 1.05–7.01]; Quartile 3 3.78 [1.42–10.08]; and Quartile 4 4.93 [1.93–12.60] vs. Quartile 1 1.00). No significant associations were observed among women in the lower two tertiles of HOMA-IR.

Figure 2 displays the ORs for GDM stratified by prepregnancy BMI, dichotomized (BMI ≥25 vs. <25 kg/m²). There was increasing risk of GDM as the tertile of GGT increased, and being in the highest tertile of GGT with a prepregnancy BMI of <25 kg/m² resulted in the greatest risk of GDM (OR 2.44 [95% CI 1.23–4.83]).

CONCLUSIONS

In this case-control study, pregravid GGT, but not ALT or AST, was found to be associated with an increased risk of the development of GDM. The association appeared to be moderated by increased insulin resistance, and in the stratified analysis, it was present only among women who were in the top tertile of HOMA-IR before pregnancy.

Our findings with GGT are consistent with previous research examining liver enzymes and type 2 diabetes. Several cohort studies have found that higher GGT levels predict the development of type 2 diabetes (17–22). The association between GGT and type 2 diabetes has been found to have a dose-response relationship (19,21) and to be independent of known risk factors for diabetes (17,20). One study (23) found that GGT concentrations were independently associated with the risk of prediabetes and were positively associated with insulin resistance. Few studies have examined liver enzyme levels during pregnancy in relation to GDM risk, and findings have been inconsistent. In a study of 2,610

Table 2—Association of GDM with prepregnancy ALT, AST, and GGT levels, from conditional logistic regression models

| Pregpregnancy risk factor | Case patients (n = 256) | Control subjects (n = 497) | Conditional logistic regression models | | |
|---------------------------|----------------------------|-------------------------------|--|-------------------------------------|-------------------------------------|
| | | | Crude | Multivariable-adjusted ¹ | Multivariable-adjusted ² |
| ALT (units/L) | | | | | |
| Quartile 1 (0.5–3.9) | 44 (17.2) | 124 (25.0) | 1.00 | 1.00 | 1.00 |
| Quartile 2 (4.0–5.9) | 61 (23.8) | 124 (25.0) | 0.90 (0.55–1.47) | 0.79 (0.45–1.39) | 0.80 (0.43–1.50) |
| Quartile 3 (6.0–8.4) | 70 (27.3) | 124 (25.0) | 1.25 (0.76–2.04) | 1.04 (0.59–1.82) | 1.18 (0.62–2.23) |
| Quartile 4 (8.5–35.0) | 81 (31.6) | 125 (25.0) | 1.55 (0.92–2.59) | 1.10 (0.61–1.99) | 1.12 (0.57–2.23) |
| AST (units/L) | | | | | |
| Quartile 1 (2.0–7.9) | 73 (28.5) | 124 (25.0) | 1.00 | 1.00 | 1.00 |
| Quartile 2 (8.0–10.4) | 53 (20.7) | 124 (25.0) | 0.63 (0.40–0.97) | 0.47 (0.28–0.79) | 0.38 (0.21–0.68) |
| Quartile 3 (10.5–14.4) | 58 (22.7) | 124 (25.0) | 0.73 (0.45–1.18) | 0.62 (0.36–1.07) | 0.51 (0.27–0.96) |
| Quartile 4 (14.5–92.0) | 72 (28.1) | 125 (25.0) | 0.84 (0.48–1.45) | 0.59 (0.31–1.11) | 0.53 (0.25–1.13) |
| GGT (units/L) | | | | | |
| Quartile 1 (8.0–13.4) | 37 (14.5) | 124 (25.0) | 1.00 | 1.00 | 1.00 |
| Quartile 2 (13.5–17.9) | 47 (18.4) | 124 (25.0) | 1.41 (0.84–2.36) | 1.28 (0.73–2.25) | 1.20 (0.63–2.26) |
| Quartile 3 (18.0–24.9) | 74 (28.9) | 124 (25.0) | 2.03 (1.23–3.37) | 1.72 (0.99–2.97) | 1.55 (0.84–2.87) |
| Quartile 4 (25.0–173.0) | 98 (38.3) | 125 (25.0) | 2.91 (1.77–4.77) | 1.97 (1.14–3.42) | 1.57 (0.84–2.93) |

Data are n (%) or OR (95% CI). ¹Adjusted for race/ethnicity, prepregnancy BMI, family history of diabetes, and alcohol use at time of the MHC examination (one or more vs. less than one drink/day). ²Further adjusted for HOMA-IR (in tertiles), fasting status (defined as ≥ 6 h since ingestion of the last food at the time of the MHC examination), and rate of gestational weight gain up to screening test (in tertiles).

women in Malaysia in 2012 (24), GGT, ALT, and AST levels were measured at the time of the 50-g glucose challenge test, prior to the 2-h, 75-g OGTT. The study found no association between the levels of pregnancy liver enzymes and the risk of GDM. Another study (25) assessed GGT levels at the time of the OGTT and found that they were positively correlated with the 2-h glucose level. Additionally, increased GGT level was significantly associated with GDM risk in the multivariable logistic regression analysis. The authors of the two aforementioned studies justified the use of liver enzymes levels during pregnancy (on average, up to 2 weeks before the OGTT) for the purpose of predicting the development of GDM, given that elevated transaminase levels are a chronic reflection of diabetes risk, “predictive of events sometimes years ahead” (24). To our knowledge, no study other than the current study has examined the association between prepregnancy liver enzyme levels and GDM. Liver enzyme levels are relatively inexpensive to measure and can be used to identify women who are at risk for the development of GDM.

Among patients without hepatitis, increased levels of GGT indicate deposition of excess fat in the liver (26), which is known to be characterized by insulin resistance (23). Some researchers speculate that elevations in liver enzyme levels may reflect an underlying insulin resistance that is localized to

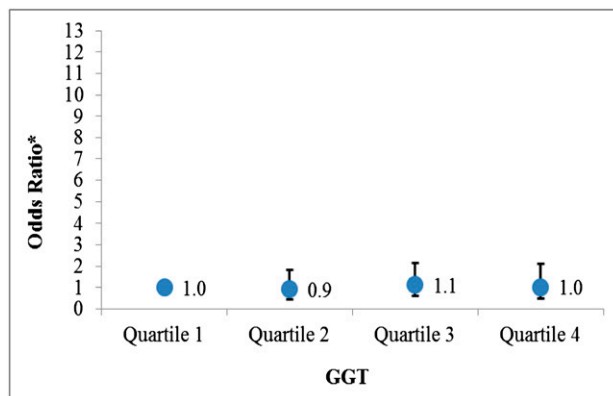
the liver, independent of peripheral insulin sensitivity or resistance (23). A 2011 study by Bonnet et al. (27) found that increased plasma GGT level, even within the normal reference range, was a biomarker of both systemic and hepatic insulin resistance, as well as increased insulin secretion and decreased hepatic insulin clearance among both healthy men and women in a nonpregnant, nondiabetic state. The authors concluded that even a minimal increase in GGT level could serve as an indirect marker of enhanced hepatic insulin resistance and impaired glucose disposal in skeletal muscles (27). Our finding that elevated pregravid GGT levels resulted in an increased risk of GDM, to a greater degree among women in the highest tertile of HOMA-IR, lends support to the hypothesis that insulin resistance underlies this association. Additionally, elevated GGT level could be a response to oxidative stress, which plays a critical role in the pathogenesis of diabetes by impairing insulin secretion. Elevations in GGT, thought to be produced in part as a result of oxidative stress, result in increased transport of the tripeptide GSH into cells, where it can protect the cells from oxidative damage (28). It is thought that GGT level may also be a marker of exposure to certain environmental pollutants. Lee et al. (29) hypothesize that persistent organic pollutants may reside in adipose tissue and act as endocrine disruptors, and the persistent organic pollutants may further interact

with obesity to impact diabetes risk. GGT activity could reflect the formation of GSH conjugates during xenobiotic metabolism. The authors recommend prospective studies and toxicological studies to test their hypotheses.

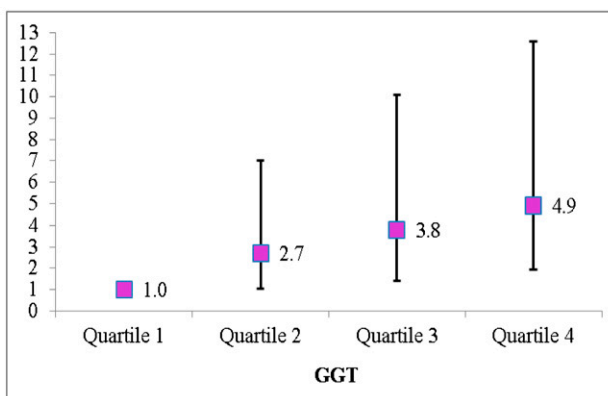
In the current study, neither ALT nor AST was associated with GDM. AST is present throughout the body and levels can be elevated in a multitude of clinical disorders (30); it has also not been consistently associated with diabetes (24). ALT is found primarily in the liver and is thought to be a marker of liver fat accumulation (31). The lack of association between ALT level and GDM in the current study is surprising, given that ALT has been considered a marker of risk for the development of type 2 diabetes (24); however, several studies have found no association between ALT and type 2 diabetes (32,33). Additionally, a 2013 meta-analysis (34) concluded that associations of liver aminotransferase levels and type 2 diabetes risk appear to have been overestimated in previous studies. It is possible that the lack of association is due to the relatively younger age of our study participants, as older age may be a risk factor for increased susceptibility to certain liver diseases. Alternatively, the lack of association may be due to a different mechanistic effect of ALT in the development of GDM and type 2 diabetes. Further studies are needed.

The strengths of this study include our ability to exclude women with

HOMA-IR < 67th Percentile



HOMA-IR ≥ 67th Percentile



*Adjusted for race/ethnicity, pre-pregnancy BMI, family history of diabetes, alcohol use, fasting status (defined as six or more hours since last food at time of the MHC exam), maternal age at delivery, difference in age between MHC exam and delivery, and year of MHC exam.

Figure 1—ORs for association between pregravid liver enzyme levels and HOMA-IR and the risk of GDM.

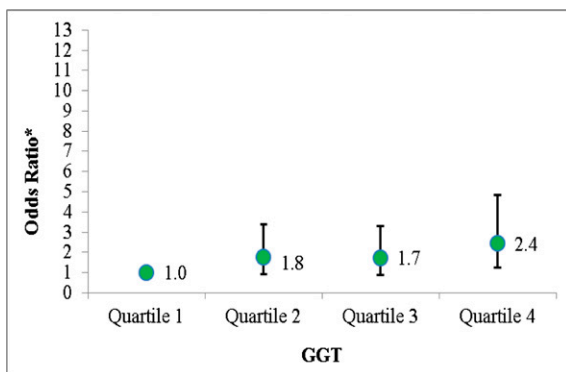
glucose values indicative of recognized pregestational diabetes. We had the unique ability to look at liver enzyme levels measured, on average, 7 years before pregnancy in a large number of GDM case patients and matched control subjects in a diverse cohort. Assessing the liver enzyme concentrations before pregnancy is vital to establishing the temporal sequence of the association between liver enzymes and GDM, particularly given the fact that liver enzyme concentrations may change during pregnancy, in part due to increases in sex steroid levels, which affect metabolic and hepatic functions (10). While strong evidence links elevated liver enzyme concentrations to insulin resistance, it is

unclear which one is the antecedent, and this represents an avenue on which future studies should focus. The study was limited by the lack of data on hepatic insulin resistance, liver fat content, and body fat mass and distribution; the latter might have provided insight as to whether the association between GGT and GDM was mediated by visceral fat. Past research indicates that fatty liver is associated with insulin resistance and inflammation in women with a history of GDM, and higher levels of biomarkers (as measured by a validated fatty liver index) estimating excess liver fat in these women was associated with an increased risk of the development of type 2 diabetes

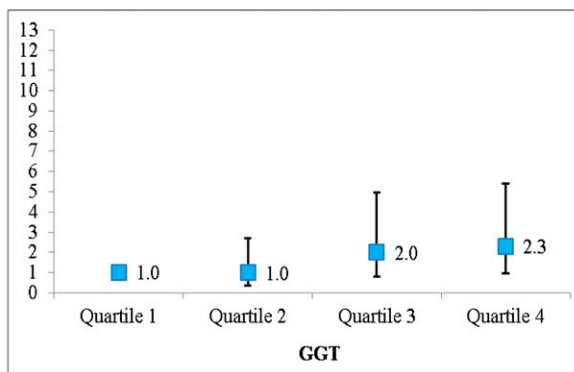
within 10 years of follow-up (35). This supports the hypothesis that excess liver fat is one potential biologic mechanism explaining the association between GGT and GDM.

Based on our findings, the liver enzyme GGT, which is known to be associated with an increased risk of the development of type 2 diabetes, appears to also be associated with subsequent GDM, which is characterized by decreased insulin sensitivity and increased insulin resistance. This study, which is, to our knowledge, the first of its kind, demonstrates that elevations in GGT levels may be present many years before a pregnancy characterized by GDM. Monitoring GGT levels before

BMI < 25 kg/m²



BMI ≥ 25 kg/m²



*Adjusted for race/ethnicity, family history of diabetes, alcohol use, maternal age at delivery, difference in age between MHC exam and delivery, and year of MHC exam.

Figure 2—ORs for association between pregravid liver enzyme levels and BMI and the risk of GDM.

pregnancy may help to identify women who are at increased risk for the subsequent development of GDM.

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