Original Investigation

Maternal Midpregnancy Glucose Levels and Risk of Congenital Heart Disease in Offspring

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IMPORTANCE There is a well-described association between maternal diabetes mellitus and risk of congenital heart disease (CHD) in offspring. Although the clinical diagnoses of type 2 diabetes or gestational diabetes are strong risk factors for CHD, subclinical abnormalities of glucose and insulin metabolism are common within the general population and could also confer risk for CHD. We hypothesize that continuous measures of blood analytes related to maternal diabetes are related to odds of cardiac malformations.

OBJECTIVE To explore the potential association of 2 different CHD phenotypes in offspring with maternal midpregnancy measures of glucose and insulin.

DESIGN, SETTING, AND PARTICIPANTS Case-control study from a population-based cohort of 277 pregnant women in southern and central California carrying infants with tetralogy of Fallot (TOF) (n = 55), dextrotransposition of the great arteries (dTGA) (n = 42), or healthy infants without CHD (n = 180). Serum samples were collected from 2003 through 2007. The analysis was conducted from March through June 2015.

MAIN OUTCOMES AND MEASURES Blood analytes related to maternal glucose metabolism were measured from random nonfasting second-trimester blood samples. We measured serum insulin levels by a validated radioimmunoassay, and we measured glucose levels. Multivariable logistic regression models estimated the association between these levels and case status.

RESULTS Serum glucose values were elevated in the maternal samples for offspring with TOF (median, 97.0 mg/dL [to convert to millimoles per liter, multiply by 0.0555]) relative to controls (median, 91.5 mg/dL) (P = .01, Wilcoxon rank sum test), a phenomenon not observed in the maternal samples for offspring with dTGA (median, 90.0 mg/dL) relative to controls (P = .38, Wilcoxon rank sum test). Serum insulin levels were significantly different between controls (median, 18.8 μIU/mL [to convert to picomoles per liter, multiply by 6.945]) and maternal samples for offspring with dTGA (median, 13.1 μIU/mL; P = .048, Wilcoxon rank sum test) but not with TOF (median, 14.3 μIU/mL; P = .35, Wilcoxon rank sum test). Relative to maternal blood glucose levels of infants without cardiac malformations, we observed that maternal blood glucose levels in models including insulin were strongly associated with odds of TOF (adjusted odds ratio = 7.54; 95% CI, 2.30-24.69) but not with dTGA (adjusted odds ratio = 1.16; 95% CI, 0.28-4.79).

CONCLUSIONS AND RELEVANCE These results represent a direct correlation of glucose as a continuous variable to odds of specific cardiac malformations. The association between serum glucose and odds of TOF indicates the need for additional epidemiological and mechanistic investigations into the risk conferred by insulin signaling and glucose metabolism during early pregnancy.
Clinicians have long observed an association between maternal diabetes mellitus and risk of congenital heart disease (CHD) in offspring.1-4 Retrospective cohort studies show that diabetic mothers with well-controlled blood glucose prior to pregnancy retain an elevated risk of their offspring having CHD,5,6 suggesting that an underlying maternal risk factor is correlated with both maternal diabetes and risk of CHD. The odds of the most common form of maternal diabetes, type 2 diabetes, is itself influenced by environmental risk factors (diet and physical activity) and inherited genetic risk factors for quantitative traits related to insulin sensitivity and processing, beta-cell function, and glucose metabolism.7,8 Additionally, there is emerging experimental evidence of an age-related maternal risk factor for CHD that is modifiable by exercise,9 which could be consistent with many of the environmental, behavioral, or genetic risk factors for diabetes. The relationship between maternal type 2 diabetes and CHD in offspring is poorly described, and the molecular mechanisms by which the clinical correlates of type 2 diabetes (such as diet, exercise, glucose metabolism, and insulin sensitivity) may alter normal cardiac development are not known.

Although maternal diagnoses of type 2 diabetes or gestational diabetes are strong risk factors for carrying a fetus with CHD, a binary diagnostic classification does not capture the wide spectrum of abnormal glucose metabolism within individuals who do not display overt disease.10 Rather than using a binary diagnosis of diabetes, we explored the potential relationship of odds of CHD with measures of glucose metabolism. We assembled a cohort comparing midpregnancy measures of glucose and insulin in 97 mothers carrying fetuses with CHD vs 180 women carrying healthy fetuses without CHD or other malformations.

**Methods**

**Population Sample**

The study population and collection methods have been previously described.11 Briefly, serum samples were collected during the 15th through 18th weeks of pregnancy from a multiethnic population-based sample of women in southern and central California counties irrespective of fasting status or time of day. Samples were collected from such pregnancies between January 20, 2003, and June 15, 2007. The analysis was conducted from March through June 2015. Delivery outcome information and offspring phenotype were linked to serum specimens by the California Birth Defects Monitoring Program. The monitoring program abstracted case information from hospital reports and medical records. We excluded infants with documented chromosomal abnormalities or single-gene disorders. We further selected infants with tetralogy of Fallot (TOF) (conotruncal category) or dextrotransposition of the great arteries (dTGA) (nonconotruncal category). As described previously, each case was ascertained by reviewing echocardiographic, catheterization, surgical, or autopsy reports. These samples included 111 mothers carrying a fetus with dTGA or TOF. We also randomly selected 223 age- and ethnicity-matched women who contributed midpregnancy specimens that were collected during the same period and delivered healthy infants without CHD or other malformations as controls as determined by the California Birth Defects Monitoring Program. All samples were obtained from the California Biobank with ethics board approval from the California Health and Welfare Agency Committee for the Protection of Human Subjects, which approved this study. Requirement for informed consent was waived because the study used deidentified data from the California Birth Defects Monitoring Program.

**Sample Collection and Storage**

This study used deidentified specimen data from the California Biobank (https://www.cdph.ca.gov/programs/GDSP/Pages/California%20Biobank%20Program.aspx), a large and unique midpregnancy serum specimen bank of pregnancies in California. Serum specimens in this bank derive from approximately 70% of all California women and were obtained during the 15th through 18th weeks of pregnancy. These serum specimens were collected from women who resided in selected regions of California (Orange, San Diego, and Central Valley counties) as part of the California prenatal screening program that offers 3 types of screening tests to pregnant women to identify individuals who are at increased risk for carrying a fetus with a specific birth defect. The collection and processing of specimens had the following steps: (1) samples were taken at draw stations using BD Vacutainer 3.5-mL serum separator tubes with no anticoagulants or preservatives and centrifuged within 30 minutes; (2) samples were received by designated clinical laboratories from draw stations at room temperature, on average 3.0 days after draw; (3) prenatal screening assays were run on samples usually on the day received; (4) samples were refrigerated up to 7 days if further testing was necessary; (5) samples were sent on cold packs via overnight mail to the California Biobank for storage; and (6) samples were aliquoted, labeled with bar codes, and frozen at −70°C within an average of 3.5 days of receipt at the California Biobank.

**Insulin and Glucose Measurements**

To measure 2 analytes, serum samples were diluted with phosphate-buffered saline. Insulin was measured from serum samples by radioimmunoassay using a kit for human insulin.
Glucose was analyzed using the hexokinase method on the cobas® c501 using kits from Roche Diagnostics at the Diabetes Research Center, Washington University, St Louis, Missouri. The typically observed coefficient of variation for the assays is less than 8.0% for insulin radioimmunoassay and less than 1.2% for glucose. After testing 334 total samples (223 controls, 64 TOF, and 47 dTGA), we excluded from analysis 57 samples with glucose measurements lower than 63 mg/dL (to convert to millimoles per liter, multiply by 0.0555) (43 controls, 9 TOF, and 5 dTGA), a value incompatible with normal cognition at the time of the blood draw, which likely reflects a delay in sample processing. Insulin values less than the limit of detection (6.4 μIU/mL [to convert to picomoles per liter, multiply by 6.945]) were assigned a value of 6.4 μIU/mL.

Statistical Analysis
Glucose and insulin levels were compared between cases and controls using standard descriptive statistics. Both glucose and insulin were analyzed in log base 2 scale, which was normally distributed. Multivariable logistic regression models were used to estimate the association between glucose and insulin levels and the odds of TOF and dTGA; results are presented as adjusted odds ratios (AORs) with 95% confidence intervals. We evaluated the potential for nonlinear effects using nonlinear terms and splines. We did not observe statistical evidence of nonlinearity. Glucose homeostasis depends on secretion of insulin; therefore, we constructed a logistic regression model that included both log glucose and log insulin values, along with an interaction term for their combination. No significant interaction between glucose and insulin values was observed. All models adjusted for maternal age (years) and race/ethnicity (Hispanic; white, non-Hispanic), which were obtained from intake forms associated with the screening program. Additionally, we conducted a sensitivity analysis with the identical analytical approach, excluding 5 participants with a serum glucose level greater than 200 mg/dL (no controls, 4 TOF, and 1 dTGA), which is an accepted demarcation indicating type 2 diabetes. All analyses were performed using SAS version 9.4 statistical software (SAS Institute, Inc.).

Results
We analyzed 42 samples from mothers carrying a fetus with dTGA, 55 maternal samples with a fetus with TOF, and 180 control samples (Table 1). Serum insulin levels were significantly different between controls (median, 18.8 μIU/mL) and maternal samples for offspring with dTGA (median, 13.1 μIU/mL; P = .048, Wilcoxon rank sum test) but not with TOF (median, 14.3 μIU/mL; P = .35, Wilcoxon rank sum test) (Table 1).

Serum glucose values were elevated in the TOF maternal samples (median, 97.0 mg/dL) relative to controls (median, 91.5 mg/dL) (P = .01, Wilcoxon rank sum test), a phenomenon not observed in the dTGA maternal samples (median, 90.0 mg/dL) relative to controls (P = .18, Wilcoxon rank sum test) (Table 1). In logistic regression models using transformed glucose and insulin values adjusting for ethnicity and maternal age at sample collection, we observed that glucose values maintained a strong relationship with odds of TOF (AOR = 4.54; 95% CI, 1.71-12.05) that was not found in the dTGA group (AOR = 0.69; 95% CI, 0.19-2.44) (Table 2, model 1).

Homeostatic regulation of serum glucose is dependent on insulin secretion; thus, the 2 values are strongly interdependent. Therefore, we constructed a logistic regression model including both log glucose and log insulin values. Accounting for insulin values in the adjusted model, log glucose values displayed an even stronger association with odds of TOF (AOR = 7.54; 95% CI, 2.30-24.69) than in the univariable model not including insulin. This relationship was not observed between log glucose and dTGA (AOR = 1.16; 95% CI, 0.28-4.79) (Table 2, model 2).

Examination of analyte values revealed that 5 individuals (4 from the TOF group, 1 from the dTGA group) showed random glucose values greater than 200 mg/dL, an accepted criterion for the clinical diagnosis of diabetes. To exclude the possibility that the observed association between glucose level and odds of TOF was driven by this small number of potentially overtly diabetic participants, we performed a sensitiv-
The effect of maternal serum insulin on risk for CHD has not been previously assessed, except indirectly in population-based studies including women treated with insulin. Even diabetic mothers treated with insulin with well-controlled glucose levels retain an increased risk of CHD in offspring, which could be related to either an underlying insulin resistance or, conceivably, treatment-related hyperinsulinemia. Nondiabetic mothers with insulin resistance and compensatory hyperglycemia have been extensively studied. However, insulin resistance cannot be directly assessed without a fasting sample or glucose tolerance protocol. The question therefore remains whether elevated blood glucose or a variety of correlated but independent traits such as beta-cell function, exercise, or insulin resistance is behind the observed association.

It is important to note that cardiac development is largely complete by the second trimester, when these blood samples were drawn. There are reasonable clinical data suggesting that second-trimester glucose metabolism is well correlated with maternal physiology in both the first trimester and the preconception period. However, measurements of glucose, insulin, insulin resistance, and clinical correlates from the period encompassing preconception and the first trimester in mothers carrying infants with CHD are necessary to confirm the observed associations. Although it would be inconsistent with the current understanding of maternal-fetal glucose homeostasis, we cannot exclude the possibility that carrying a fetus with TOF affects maternal glucose metabolism.

**Discussion**

In this case-control study from a large California cohort, we observed that a random maternal glucose measurement taken during the second trimester was strongly associated with odds of delivering infants with TOF compared with women who delivered infants without structural malformations. This association persisted after controlling for age and ethnicity, adjusting for insulin measures, and even excluding mothers with glucose values indicative of overt diabetes. These data are consistent with the long-recognized association between maternal diabetes and congenital heart disease and suggest that there may be additional unmeasured risk within the general population that includes prediabetic individuals not diagnosed as having diabetes.

Blood glucose levels are influenced by a variety of factors such as diet, exercise, beta-cell function, and insulin resistance; thus, glucose levels may simply be a marker of risk conferred by another physiological process. Although we directly measured insulin levels in these women, insulin resistance cannot be directly assessed without a fasting sample or glucose tolerance protocol. The question therefore remains whether elevated blood glucose or a variety of correlated but independent traits such as beta-cell function, exercise, or insulin resistance is behind the observed association.

It is important to note that cardiac development is largely complete by the second trimester, when these blood samples were drawn. There are reasonable clinical data suggesting that second-trimester glucose metabolism is well correlated with maternal physiology in both the first trimester and the preconception period. However, measurements of glucose, insulin, insulin resistance, and clinical correlates from the period encompassing preconception and the first trimester in mothers carrying infants with CHD are necessary to confirm the observed associations. Although it would be inconsistent with the current understanding of maternal-fetal glucose homeostasis, we cannot exclude the possibility that carrying a fetus with TOF affects maternal glucose metabolism.

**Table 2. Logistic Regression Models of Maternal Insulin and Glucose Levels in Subcategories of Congenital Heart Disease for All Participants**

<table>
<thead>
<tr>
<th>Analyte</th>
<th>OR (95% CI)*</th>
<th>Model 1</th>
<th>Adjusted*</th>
<th>Model 2</th>
<th>Adjusted*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Crude</td>
<td>Adjusted*</td>
<td>Crude</td>
<td>Adjusted*</td>
<td></td>
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<tr>
<td>TOF vs controls</td>
<td></td>
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</tr>
<tr>
<td>Log2(glucose)</td>
<td>4.83 (1.89-12.40)</td>
<td>4.54 (1.71-12.05)</td>
<td>8.60 (2.72-27.24)</td>
<td>7.54 (2.30-24.69)</td>
<td></td>
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<tr>
<td>Log2(insulin)</td>
<td>0.95 (0.75-1.19)</td>
<td>0.97 (0.76-1.23)</td>
<td>0.74 (0.57-0.98)</td>
<td>0.78 (0.59-1.02)</td>
<td></td>
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<tr>
<td>dTGA vs controls</td>
<td></td>
<td></td>
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<tr>
<td>Log2(glucose)</td>
<td>0.68 (0.19-2.38)</td>
<td>0.69 (0.19-2.44)</td>
<td>1.24 (0.30-5.07)</td>
<td>1.16 (0.28-4.79)</td>
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<tr>
<td>Log2(insulin)</td>
<td>0.76 (0.57-1.02)</td>
<td>0.78 (0.58-1.04)</td>
<td>0.75 (0.54-1.03)</td>
<td>0.77 (0.56-1.06)</td>
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**Table 3. Sensitivity Analysis by Logistic Regression of Maternal Insulin and Glucose Levels in Subcategories of Congenital Heart Disease for Participants With Random Glucose Levels Less Than 200 mg/dL**

<table>
<thead>
<tr>
<th>Analyte</th>
<th>OR (95% CI)*</th>
<th>Model 1</th>
<th>Adjusted*</th>
<th>Model 2</th>
<th>Adjusted*</th>
</tr>
</thead>
<tbody>
<tr>
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<tr>
<td>TOF vs controls</td>
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</tr>
<tr>
<td>Log2(glucose)</td>
<td>2.93 (0.96-8.96)</td>
<td>2.76 (0.87-8.74)</td>
<td>5.45 (1.41-21.08)</td>
<td>4.61 (1.14-18.67)</td>
<td></td>
</tr>
<tr>
<td>Log2(insulin)</td>
<td>0.93 (0.73-1.18)</td>
<td>0.95 (0.75-1.22)</td>
<td>0.78 (0.59-1.03)</td>
<td>0.82 (0.61-1.09)</td>
<td></td>
</tr>
<tr>
<td>dTGA vs controls</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Log2(glucose)</td>
<td>0.41 (0.10-1.60)</td>
<td>0.41 (0.10-1.66)</td>
<td>0.72 (0.16-3.33)</td>
<td>0.68 (0.15-3.12)</td>
<td></td>
</tr>
<tr>
<td>Log2(insulin)</td>
<td>0.74 (0.55-0.99)</td>
<td>0.75 (0.56-1.01)</td>
<td>0.76 (0.55-1.05)</td>
<td>0.78 (0.56-1.08)</td>
<td></td>
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</tbody>
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Abbreviations: dTGA, dextrotransposition of the great arteries; OR, odds ratio; TOF, tetralogy of Fallot.

a Model 1 shows results for glucose or insulin alone. Model 2 shows results with both analytes simultaneously evaluated.

b Adjusting for maternal race/ethnicity (Hispanic; white, non-Hispanic) and maternal age at sample collection (continuous, in years).
tory hyperinsulinemia may represent a parallel situation. Although exogenously administered insulin falls in US Food and Drug Administration pregnancy category B, insulin interacts with the insulinlike growth factor 1 pathway and may affect fetal growth and development when crossing the placenta. Additionally, insulin signaling in other tissues is involved in a variety of human diseases. However, in our study, random insulin levels during pregnancy were not directly associated with risk of CHD. Although risk of CHD has not been conclusively related to the ethnicities included in the study population, there are well-described ethnic differences in glucose metabolism within women of childbearing age accounted for in the adjusted model.

Conclusions

We observed an association of maternal glucose levels with the risk of TOF in offspring. If confirmed in larger studies conducted earlier in pregnancy, these observations could have important public health implications for identifying women at risk for carrying infants with CHD and targeting interventions to improve glucose homeostasis (such as exercise and maintaining a healthy weight). Additional epidemiological and experimental work is necessary to describe the causal risk factor related to both serum glucose levels and cardiac development and to understand the mechanism by which risk is conferred from mother to child.

ARTICLE INFORMATION

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Author Contributions: Dr Priest had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. Study concept and design: Priest, Reaven, Knowles, Shaw. Acquisition, analysis, or interpretation of data: Priest, Yang, Knowles, Shaw. Drafting of the manuscript: Priest, Yang, Knowles, Shaw. Critical revision of the manuscript for important intellectual content: Priest, Reaven, Knowles, Shaw. Statistical analysis: Priest, Yang, Shaw. Obtained funding: Priest, Knowles, Shaw. Administrative, technical, or material support: Reaven, Knowles, Shaw. Study supervision: Knowles, Shaw.

Conflict of Interest Disclosures: None reported.

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REFERENCES