Association of Intrauterine Exposure to Maternal Diabetes and Obesity with Type 2 Diabetes in Youth: The SEARCH Case-Control Study

Dana Dabelea, MD¹, Elizabeth J. Mayer-Davis, PhD², Archana P. Lamichhane, MD², Ralph B. D’Agostino Jr, MD³, Angela D. Liese, PhD², Kendra S. Vehik, MD¹, K.M. Venkat Narayan, MD⁴, Phillip Zeitler, MD⁵, Richard F. Hamman, MD¹.

1. Preventive Medicine and Biometrics, University of Colorado Denver.
2. Nutrition Department, University of North Carolina at Chapel Hill.
3. Wake Forest University School of Medicine, North Carolina.
4. The Rollins School of Public Health, Emory University, Georgia.
5. Department of Pediatrics, University of Colorado Denver.

Running Title: Intrauterine Exposures and Type 2 Diabetes in Youth

Corresponding Author: Dana Dabelea, MD, PhD
University of Colorado Denver
4200 East 9th Ave Box B119
Denver, CO 80262
Dana.Dabelea@uchsc.edu

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Objective: Limited data exist on the association between in utero exposure to maternal diabetes and obesity and type 2 diabetes (T2D) in diverse youth. These associations were explored in African American (AA), Hispanic (H), and non-Hispanic white (NHW) youth participating in the SEARCH Case-Control (SEARCH CC) study.

Design and Methods: 79 youth with T2D and 190 non-diabetic control youth age 10-22 years attended a research visit. In utero exposures to maternal diabetes and obesity were recalled by biological mothers.

Results: Youth with T2D were more likely to have been exposed to maternal diabetes or obesity in utero than were non-diabetic controls (p<0.0001 for each). After adjusting for offspring age, sex and race/ethnicity, exposure to maternal DM [OR= 5.7; 95% CI=2.4-13.4] and exposure to maternal obesity [2.8; 95% CI=1.5-5.2] were independently associated with T2D. Adjustment for other perinatal and socio-economic factors did not alter these associations. When offspring BMI was added, the OR for the association between in utero exposure to obesity and T2D was attenuated toward the null (OR=1.1; 95% CI=0.5-2.4). Overall, 47.2% (95% CI=30.9%-63.5%) of T2D in youth could be attributed to intrauterine exposure to maternal diabetes and obesity.

Conclusion: Intrauterine exposures to maternal diabetes and obesity are strongly associated with T2D in youth. Prevention efforts may need to target, in addition to childhood obesity, the increasing number of pregnancies complicated by obesity and diabetes.
Type 2 diabetes (T2D) has increasingly been reported in young adults and adolescents. The SEARCH for Diabetes in Youth Study found T2D among youth of all major racial/ethnic groups and high rates were noted among minority adolescents age 15-19 years (1).

A maternal diabetic intrauterine environment has consequences on future T2D risk in the offspring (2). Among the Pima Indians, exposure to diabetes in utero was the strongest risk factor for development of T2D in young offspring (3). This association was independent of maternal obesity, father’s diabetes, age at onset of diabetes in either parent, offspring’s birth weight and later obesity (2,3).

Recent studies have shown an association between maternal pre-pregnancy obesity and excessive neonatal growth and adiposity (4), independent of diabetes in pregnancy. There is increasing interest in the hypothesis that maternal obesity during pregnancy, even in the absence of frank diabetes, is also associated with life long metabolic abnormalities in offspring, such as presence of obesity (5) and features of the metabolic syndrome (6). However, no study has specifically explored an association between exposure to maternal pre-pregnancy obesity and T2D in youth.

In the last decade an increase in the prevalence of overweight among obstetric populations has been reported (7). There is also an increasing incidence and a younger age at onset of T2D in adults (8), and an increasing prevalence of gestational diabetes in all major racial/ethnic groups (9). In this context, the Fifth International Workshop on GDM (10) has urged for studies of in utero exposure to maternal diabetes and obesity and T2D in youth in populations other than American Indians.

Using data from the multi-ethnic [non-Hispanic white (NHW), African-American (AA) and Hispanic (H)] SEARCH Case-Control (SEARCH CC) study, we hypothesized that youth with T2D would be more likely to have been exposed to a diabetic and obese intrauterine environment compared to non-diabetic control youth. We also hypothesized that the association between exposure to maternal diabetes and offspring T2D will be independent of other perinatal, early life and familial socioeconomic factors; however, the association between maternal pre-pregnancy obesity and offspring T2D will be, at least in part, accounted for by offspring body mass index (BMI).

RESEARCH DESIGN AND METHODS

Study design. SEARCH CC is an ancillary study conducted at two of the six SEARCH clinical sites. SEARCH is a multi-center study conducting population-based ascertainment of diabetes in youth with onset less than 20 years of age (1).

SEARCH CC Case Inclusion. In Colorado and South Carolina, diabetes cases were identified using a network of health care providers. Type of diabetes was based on provider diagnosis and further confirmed with biochemical data, including diabetes autoantibody measurements (1). Between July 2003 and March 2006, SEARCH patients with T2D of NHW, AA and H origin aged 10 - 22 years at study visit were invited to participate in SEARCH CC. Data collection unique to SEARCH CC included a perinatal questionnaire, completed by the biological mother. Overall, 53% of those invited participated in SEARCH CC.

SEARCH CC Control Inclusion. Because all SEARCH cases arose from health care provider offices, we recruited control subjects from primary care offices in
the same geographic areas. Within clinical sites, control recruitment over-sampled youth based on the distribution of age, sex, and racial/ethnic background of cases. Overall, 49% of those invited participated in SEARCH CC. All control subjects were confirmed as non-diabetic by fasting glucose values.

Measurements Maternal diabetes during pregnancy, including both pre-gestational and gestational diabetes (GDM), was reported by the biological mother. Exposure to diabetes in utero was considered present if the mother had diabetes diagnosed prior to delivery and absent if the mother was not diagnosed with diabetes or diabetes was diagnosed after delivery (3). When mother had diabetes diagnosed prior to delivery, it was GDM in over 90% of cases (i.e., diabetes first diagnosed during pregnancy). Exposure to diabetes in utero was validated in a sample of 64 Colorado participants with birth certificate data collected after 1990 and maintained by the Colorado Department of Public Health and Environment (CDPHE). Consistent with other studies (11), self-reported exposure to diabetes in utero demonstrated good agreement with the CDPHE data for both cases (kappa=0.71) and controls (kappa= 0.79).

Self-reported maternal pre-pregnancy weight (kilograms) and height (meters) were used to compute maternal pre-pregnancy body mass index (BMI). Self-reported weight correlates well with measured weight (12) and self-reported pre-pregnancy weight was recently validated against measured weight 3 months before the last menstrual cycle in a sample of multi-ethnic women (13) . Exposure to maternal overweight/obesity was defined as pre-pregnancy BMI $\geq 25$ kg/m$^2$. The biological mother also reported maternal smoking and alcohol use during pregnancy, history of breastfeeding the offspring, paternal diabetes status, offspring birth weight and gestational age. Birth weight was validated in the Colorado sample through CDPHE birth certificates. The Spearman correlation between recalled and recorded birth weight was $r = 0.95$ for cases and $r = 0.94$ for controls. Childhood height and weight were measured using a stadiometer and an electronic scale, respectively. Age and gender specific BMI Z-scores were derived based on the Centers for Disease Control and Prevention national standards (14). The study was reviewed and approved by the local Institutional Review Boards (IRBs).

Statistical Analyses- Logistic regression was used to generate odds ratios (OR) and 95% confidence intervals (CI) for the association of exposure to maternal diabetes in utero and exposure to maternal obesity in utero with T2D in the offspring. An interaction term between each in utero exposure and race/ethnicity was used to evaluate whether the association differed according to the race/ethnicity. A series of multivariate logistic regression models were developed for each in utero exposure of interest: 1) model 1, adjusted for offspring age, sex and race/ethnicity in order to minimize the potential for residual confounding; 2) model 2, in addition to model 1 variables, the two in utero exposures were adjusted for each other; 3) model 3, markers of other early life exposures (maternal age and behavior during pregnancy, child birth weight, breastfeeding status) and shared familial factors (household income, maternal education) were added to model 2 variables; 4) model 4, offspring’s current weight status (BMI Z score) was added to model 2.

The risk of T2D in the children attributable to exposure to diabetes and overweight/obesity in utero was also estimated. Population attributable fraction
(PAF) is the percent of a disease in a population that is due to a specific exposure (15). While PAFs are usually derived for single risk factors, they also can be estimated for groups of simultaneous factors. In this situation, a PAF estimates the proportional amount by which disease risk would be reduced if all of the factors were to be simultaneously eliminated from the population (16). For this analysis, mutually exclusive exposure categories were derived (i.e., exposure to maternal diabetes only, to maternal overweight/obesity only, to both, and to neither) and category-specific attributable fractions were computed, as described by Miettinen (15) \( \Pi_i = [(O_R-1)/O_Ri] \), where \( \Pi_i \) = proportion of cases falling into each exposure category; and \( O_Ri \) = adjusted odds ratio (for offspring age, sex and race/ethnicity) comparing each exposed group with the unexposed category (i = 0). This formula produces internally valid estimates when confounding exists and, as a result, adjusted odds ratios must be used (15,16). By summing the category-specific fractions, a summary PAF was derived, representing the overall proportion of T2D in youth attributable to these exposures.

RESULTS
Analyses included 79 youth with T2DM and 190 non-diabetic control youth with completed data on variables of interest. As shown in Table 1, youth with T2D were older, more likely to be of AA background, had higher BMI, families with lower socio-economic indicators and more paternal history of diabetes. Of note, 30.4% of youth with T2D were exposed to maternal diabetes and 57% to maternal overweight/obesity in utero, compared to 6.3% and 27.4% , respectively, of non-diabetic control youth (p<0.0001 for each).

Figure 1 Panel A shows the percent of youth exposed to maternal diabetes in utero according to case control status and race/ethnicity. T2D youth were more likely to have been exposed to maternal diabetes in utero than control youth (OR=7.3; 95% CI, 3.2 – 16.8, p< 0.0001, adjusted for age, sex and race). A similar pattern was observed in all racial/ethnic groups [NHW: OR=5.5 (1.5-18.9); H: OR =4.7 (0.7-32.2); AA: OR=10.6 (2.1-51.5), adjusted for age and sex]. No difference in the association of exposure to diabetes in utero and case-control status was observed according to race/ethnicity (p-value for interaction =0.7).

Figure 1 Panel B shows the percent of youth exposed to maternal obesity in utero, according to case/control status and race/ethnicity. T2D youth were more likely to have been exposed to maternal obesity in utero than controls (OR=3.6; 95% CI, 1.9 – 6.4, p< 0.0001, adjusted for age, sex and race). A similar pattern was observed in all racial/ethnic groups [NHW: OR=2.2 (0.9-5.8); H: OR =13.4 (1.9-95.2); AA: OR=4.2 (1.7-10.2), adjusted for age and sex]. There was no difference in the association of exposure to obesity in utero and case-control status according to race/ethnicity (p-value for interaction =0.8).

Figure 2 shows the association of offspring T2D status with exposure to maternal diabetes in utero (Panel A) and exposure to maternal overweight/obesity in utero (Panel B), in sequentially adjusted models. Model 1 presents the OR and 95% CI for the associations of interest, when adjustment is made for offspring age, sex and race/ethnicity. Model 2 each intrauterine exposure was adjusted for the other, resulting in some attenuation of the OR of interest; however, both exposures remained independently associated with offspring
T2D status. In Model 3, additional adjustment for other perinatal exposures and markers of shared socio-economic factors had no substantial influence. Finally, the addition of the subjects’ current BMI-z score in Model 4 made no difference to the association between maternal diabetes and offspring T2D status (Panel A), but substantially attenuated towards the null [1.1 (95% CI, 0.5-2.3)] the OR for the association between maternal obesity and offspring T2DM (Panel B). The association between maternal pre-pregnancy BMI and offspring T2D was graded across categories of maternal overweight (BMI 25-29; OR=2.6, CI = 1.2-5.5, adjusted for age, sex and race) and obesity (BMI ≥ 30, OR = 4.6, CI = 2.2-9.5), when compared with normal pre-pregnancy BMI (< 25). On adjustment for offspring BMI Z, these associations became non-significant. Similar results were obtained when maternal pre-pregnancy BMI was modeled as a continuous variable.

Table 2 shows the proportion of cases and controls exposed to maternal diabetes only, maternal overweight/obesity only (BMI ≥ 25), and both; the ORs for the association between each exposure and T2D status, adjusted for offspring age, sex and race/ethnicity; and the proportion of T2D in youth attributable to each exposure. Exposure to maternal diabetes in utero in the absence of obesity was infrequent and, although associated with T2D in the offspring [OR=3.9; 95% CI=1.1-14.5], resulted in an attributable risk of only 4.7%. Exposure to maternal overweight/obesity in utero in the absence of diabetes was frequent and, given an OR for T2D of 2.5 (95% CI=1.3-5.0), contributed to an additional 19.7%. Finally, exposure to both maternal diabetes and maternal overweight/obesity in utero was frequent in cases (24.1%) and rare in controls (2.6%) but most strongly associated with T2D [OR=19.2; 95% CI= 6.1-60.8] and therefore contributed to an additional 22.8% of T2D in the offspring. Overall, 47.2% (95% CI=30.9%-63.5%) of early onset T2D could be attributed to intrauterine exposure to maternal diabetes and maternal obesity.

DISCUSSION
We found that intrauterine exposure to maternal diabetes and overweight/obesity are strongly associated with T2D in youth. Our study provides novel evidence these exposures are important determinants of T2D in youth of racial/ethnic groups other than American Indians, together contributing to 47% of T2D in the offspring.

The association between exposure to maternal diabetes in utero and T2D in youth of NHW, H and AA race/ethnicity is of similar magnitude (OR=7.3; CI= 3.2-16.8) to that reported in Pima Indians [OR=10.4; CI=4.4-25.1] (3). Several mechanisms that are not mutually exclusive may explain this association. They include genetic predisposition, shared familial factors, as well as specific intrauterine effects. Work from the Pima Indians (17) and other populations (18,19) strongly suggests that the effect of exposure to maternal diabetes in utero on offspring T2D risk is in addition to genetic susceptibility. Within the same Pima family, siblings born after mother’s diagnosis of diabetes have a 3-fold higher risk of developing T2D at an early age than siblings born before (17).Our findings that the association is independent of exposure to maternal obesity, other pre-natal, early life and familial factors, support the previous evidence. Moreover, in our sample of 65 youth with maternal history of diabetes, the odds for T2D was 2.5-fold higher (95% CI=0.9-7.3) when the mother was diagnosed before versus after pregnancy. This suggests that, even in the selected group of offspring at high genetic
risk, exposure to diabetes *in utero* is associated with further increase in T2D risk. We found that the association between exposure to maternal diabetes *in utero* and T2D in youth is not accounted for by childhood BMI. This is consistent with animal (20) and human (21,22) data suggesting that the effect of exposure to diabetes *in utero* on offspring’s future risk for T2D is not completely explained through development of obesity, but is also mediated through subsequent beta-cell dysfunction in the offspring.

Our study provides novel evidence that exposure to maternal obesity *in utero* is associated with T2D in youth independent of diabetes during pregnancy. However, adjustment for childhood BMI attenuates the association towards the null. This is consistent with a causal pathway in which exposure to maternal obesity increases the risk for childhood overweight, which may increase the risk for T2D. The pathway is supported by other studies suggesting that the risk that a child would be overweight increases with maternal pre-pregnancy BMI (5), and that adolescents exposed to maternal obesity are at increased risk of developing the metabolic syndrome (6).

The above associations may be due to specific intrauterine effects (the “fuel-mediated teratogenesis”). For example, excess maternal pre-gestational obesity may increase lipid availability, modulate delivery of lipid substrates to the fetus, and have programming consequences (23). Data in rats (24) demonstrate that preconception obesity brought about by overfeeding leads to obesity, metabolic alterations and increased adipose tissue cellularity in the offspring. Importantly, in rats, this process is in addition to maternal and paternal genetic influences (24). However, these associations may also be due in part to increased genetic susceptibility to obesity, coupled with postnatal availability of excess calories. More research is needed in this area since, distinguishing between specific intrauterine mechanisms and general familial (genetic and non-genetic) factors are important for the development of randomized trials aimed at testing effective interventions.

Our study has several limitations. Recall bias is of potential concern. However, exposure to diabetes *in utero* was validated in a sample of participants, with very good agreement coefficients. Another concern is the potential for selection bias. However, the prevalence of intrauterine exposures within race/ethnic control groups (Figure 1) are similar to those reported from the general population (7,9). We had limited data on paternal diabetes and were not able to explore how timing of exposure to paternal diabetes and obesity may be associated with an increased risk of T2D in the offspring. In order to derive PAFs, we used ORs as measures of risk associations. When the outcome or exposure of interest is common, the adjusted ORs may exaggerate a risk association. However, even after correcting the ORs to better represent the true relative risks (25), the overall PAF was still 42.7%, well within the estimated 95% confidence limits (30.9% to 63.5%).

In conclusion, intrauterine exposures to maternal diabetes and obesity are strongly associated with T2D in youth. In our multi-ethnic population, 47% of T2D in youth could be attributed to the combined effect of these exposures. Our data suggest that prevention of T2D in youth may need to take a life course approach, targeting, in addition to childhood obesity, the increasing number of pregnancies complicated by obesity and diabetes.
ACKNOWLEDGEMENTS

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Reference List


Table 1. Characteristics of youth with type 2 diabetes and non-diabetic controls.

<table>
<thead>
<tr>
<th>Variables</th>
<th>T2D (N=79)</th>
<th>Controls (N=190)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (Years), Mean (SD)</td>
<td>15.7 (2.8)</td>
<td>14.4 (2.8)</td>
<td>0.003</td>
</tr>
<tr>
<td>Gender (% Male)</td>
<td>29.1</td>
<td>38.9</td>
<td>0.1</td>
</tr>
<tr>
<td>Race/ Ethnicity (%)</td>
<td></td>
<td></td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Non-Hispanic white</td>
<td>27.9</td>
<td>55.8</td>
<td></td>
</tr>
<tr>
<td>African-American</td>
<td>54.4</td>
<td>27.9</td>
<td></td>
</tr>
<tr>
<td>Hispanic</td>
<td>17.7</td>
<td>16.3</td>
<td></td>
</tr>
<tr>
<td>BMI, Mean (SD)</td>
<td>34.7 (7.5)</td>
<td>24.0 (6.8)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>BMI Z-score, Mean (SD)</td>
<td>2.1 (0.7)</td>
<td>0.8 (1.1)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Exposure to maternal DM in utero (% Yes)</td>
<td>30.4</td>
<td>6.3</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Exposure to maternal obesity (BMI ≥25) in utero (% Yes)</td>
<td>57.0</td>
<td>27.4</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Maternal Education (%)</td>
<td>0.009</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Less than high school</td>
<td>13.9</td>
<td>4.7</td>
<td></td>
</tr>
<tr>
<td>High school and more</td>
<td>86.1</td>
<td>94.2</td>
<td></td>
</tr>
<tr>
<td>Household income (%)</td>
<td></td>
<td></td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Less than $25,000</td>
<td>49.4</td>
<td>21.6</td>
<td></td>
</tr>
<tr>
<td>More than $25,000</td>
<td>50.6</td>
<td>77.9</td>
<td></td>
</tr>
<tr>
<td>Maternal smoking during pregnancy (% Yes)</td>
<td>11.4</td>
<td>11.1</td>
<td>0.9</td>
</tr>
<tr>
<td>Maternal alcohol consumption during pregnancy (% Yes)</td>
<td>1.27</td>
<td>14.7</td>
<td>0.001</td>
</tr>
<tr>
<td>Birth Weight (grams), Mean (SD)</td>
<td>3218 (654)</td>
<td>3288 (620)</td>
<td>0.4</td>
</tr>
<tr>
<td>Breastfeeding (% Yes)</td>
<td>30.4</td>
<td>65.8</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Paternal history of diabetes (% Yes)</td>
<td>29.1</td>
<td>6.3</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>
Table 2. Proportion of Type 2 Diabetes in Youth Attributable to Intrauterine Exposure to Maternal Diabetes and Overweight/Obesity

<table>
<thead>
<tr>
<th>Exposure Category</th>
<th>Cases (%)</th>
<th>Controls (%)</th>
<th>OR (95% CI)*</th>
<th>PAF (%)**</th>
</tr>
</thead>
<tbody>
<tr>
<td>Not exposed to either maternal DM or maternal obesity</td>
<td>36.7%</td>
<td>68.9%</td>
<td>1</td>
<td>Unexposed</td>
</tr>
<tr>
<td>Exposed to maternal DM only</td>
<td>6.3%</td>
<td>3.7%</td>
<td>3.9 (1.1-14.5)</td>
<td>4.7%</td>
</tr>
<tr>
<td>Exposed to maternal obesity only</td>
<td>32.9%</td>
<td>24.7%</td>
<td>2.5 (1.3-5.0)</td>
<td>19.7%</td>
</tr>
<tr>
<td>Exposed to both maternal DM and maternal obesity</td>
<td>24.1%</td>
<td>2.6%</td>
<td>19.2 (6.1-60.8)</td>
<td>22.8%</td>
</tr>
<tr>
<td>Overall proportion of T2DM in youth attributable to in utero exposure to maternal DM and obesity</td>
<td></td>
<td></td>
<td></td>
<td>47.2 (30.9% 63.5%) ***</td>
</tr>
</tbody>
</table>

* OR=Odds ratios for the association between mutually exclusive exposure categories and case/control status, additionally adjusted for age, sex and race/ethnicity

**PAF=Population Attributable Fractions, calculated using the formula: \( P_i \left[ \frac{(OR_i - 1)}{OR_i} \right] \), where \( P \) = proportion of cases in each exposure category; and \( OR_i \) = adjusted odds ratios comparing each exposed group with the unexposed reference category (i=0).

*** Calculated using the formula: \( \Sigma \sqrt{P_i \left[ \frac{(OR_i - 1)}{OR_i} \right]} \)
Figure 1. Percent of youth exposed in utero to maternal diabetes (Panel A) and maternal overweight/obesity (Panel B) by case/control status and race/ethnicity. Cases: white bars; Controls: black bars; Panel A: NHW p=0.01; H p=0.02; AA p=0.02; Panel B: NHW p=0.1; H p=0.04; AA p=0.001
**Figure 2.** The association between offspring Type 2 diabetes status with exposure to maternal diabetes in utero (Panel A) and exposure to maternal overweight/obesity in utero (Panel B) in sequentially adjusted multiple logistic regression models

Model 1: Adjusted for offspring age, sex, race/ethnicity
Model 2: Model 1 + maternal obesity *in utero* (Panel A) or maternal diabetes *in utero* (Panel B)
Model 3: Model 2 + maternal alcohol and smoking in pregnancy, maternal current age, parity, offspring’s birth weight, breastfeeding, maternal education, household income
Model 4: Model 2 + current offspring BMI Z