Maternal Glucose Tolerance in Pregnancy Affects Fetal Insulin Sensitivity

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OBJECTIVE — Offspring of mothers with impaired glucose tolerance are far more likely to develop type 2 diabetes. We tested the hypothesis that maternal glucose tolerance in pregnancy affects fetal insulin sensitivity or β-cell function.

RESEARCH DESIGN AND METHODS — In a prospective singleton pregnancy cohort study, we analyzed glucose, insulin, and proinsulin concentrations in maternal blood at the 50-g oral glucose tolerance test (OGTT) at 24–28 weeks of gestation and in venous cord blood (n = 248). The cord blood glucose-to-insulin ratio and proinsulin concentration were used as indicators of fetal insulin sensitivity and the proinsulin-to-insulin ratio was used as an indicator of fetal β-cell function.

RESULTS — Higher OGTT blood glucose levels were associated with significantly lower cord plasma glucose-to-insulin ratios ($r = -0.31$, $P < 0.001$) and higher proinsulin concentrations ($r = 0.31$, $P < 0.001$) but not with proinsulin-to-insulin ratios. In a comparison of gestational diabetic (n = 26) versus euglycemic pregnancy, cord blood glucose-to-insulin ratios were substantially lower (geometric mean 10.1 vs. 20.0 mg/dl/μU/ml; $P < 0.001$), whereas proinsulin concentrations were much higher (24.4 vs. 13.8 pmol/l; $P < 0.001$), despite similar cord blood glucose concentrations indicating adequate management of diabetes. The differences remained significant after controlling for prepregnancy and fetal adiposity, family history of diabetes, gestational age, and other potential confounders. Significant changes in the glucose-to-insulin ratio and proinsulin concentration were also observed in obese (n = 31) mothers, but the differences became not statistically significant after adjustment for maternal glucose tolerance and fetal adiposity.

CONCLUSIONS — Maternal glucose intolerance may impair fetal insulin sensitivity (but not β-cell function) and consequently "program" the susceptibility to type 2 diabetes.

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The metabolic syndrome and type 2 diabetes have become a worldwide epidemic of concern (1,2). The rapid rise of the epidemic over recent decades points to the predominant role of preventable "environmental" influences. The question is, what factors at what time points are critically important targets for effective interventions? There is an increasing recognition that the fetal environment may "program" susceptibility to the metabolic syndrome and related disorders (3,4). This suggests an opportunity for early interventions to halt the increasing occurrence of the metabolic syndrome if we could know more about the targets and mechanisms of metabolic programming in early life.

Maternal metabolic status affects the fetal environment and plausibly has the potential to program the metabolic function axis of the offspring during critical developmental stages through various mechanisms (e.g., epigenetic changes) (5). Indeed, independent of the type of diabetes (pregestational type 1 or type 2 or gestational), offspring of diabetic mothers are far more likely to develop metabolic syndrome and type 2 diabetes (6–10). Most cases (~90%) of diabetes in pregnancy are gestational diabetes mellitus (11). Mild gestational glucose intolerance not meeting the criteria for the diagnosis of gestational diabetes mellitus has also been associated with adverse pregnancy outcomes and elevated cord blood C-peptide levels (12). Obesity is closely associated with impaired glucose tolerance (2,13), and, recently, increased insulin resistance was observed among neonates of obese mothers (14). Taken together, these observations suggest that impaired glucose tolerance in pregnancy may program the propensity to development of the metabolic syndrome. However, there is a dearth of prospective pregnancy cohort data to demonstrate what metabolic parameters are programmed in utero. We aimed to test the hypothesis that maternal glucose tolerance in pregnancy affects fetal insulin sensitivity or β-cell function. Such a relationship may underlie the long-term predisposition to the metabolic syndrome and related disorders in offspring of diabetic mothers.

RESEARCH DESIGN AND METHODS

— We conducted a prospective pregnancy cohort study. Patients were recruited from three obstetric care centers in Montreal: Sainte-Justine, Jewish General, and Saint Mary's Hospital. The study was approved by the research ethics committees of the participating hospitals. Pregnant women bearing a singleton fetus were recruited at 24–28 weeks of gestation upon signing an informed consent form. Exclusion criteria were *1*) multiple pregnancy; *2*) illicit drug use; *3*) maternal age <18 or >45 years; *4*) severe preexisting illnesses including pre-

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gestational diabetes mellitus, chronic hypertension, renal failure, active or chronic liver diseases, epilepsy, collagen disorders, serious pulmonary disease, serious hematological disorders, cancer, heart disease, or other life-threatening conditions; 5) endocrine disorders including growth hormone deficiency, hyperthyroidism, hypothyroidism, or other known endocrine disorders; and 6) known congenital anomalies or chromosomal abnormalities in the fetus. A total of 339 patients (25%) of 1,333 eligible pregnant women approached for recruitment accepted to participate between August 2006 and December 2008, and 248 mother-infant pairs (73% patients) with complete data on all studied biomarkers in maternal and cord blood specimens constituted the final study cohort. There were no significant differences in maternal characteristics between patients included versus those excluded in the present study. There were 11 preterm births, all mild preterm (33–36 weeks). Excluding such preterm births did not affect any primary results; thus, they were retained in the study.

In all the three participating hospitals, pregnant women were routinely tested for a random blood glucose level in the first trimester (6–13 weeks) of pregnancy to screen for undiagnosed pregestational diabetes mellitus. A random blood glucose value ≥7.8 mmol/l (140 mg/dl) was considered abnormal for further screening tests. All recruited patients had values less than this cutoff.

Data and specimen collection

Data and specimens were collected at 24–28 and 32–35 weeks of gestation and at delivery. Trained research nurses and assistants collected data on maternal, pregnancy, and birth characteristics using structured study questionnaires through face-to-face interviews and medical chart reviews. Maternal prepregnancy weight (kilograms) was based on self-report. Maternal height was measured to the nearest centimeter using the routinely available stadiometer and weight before delivery to the nearest 0.1 kg using the routinely available weighing device in each hospital. Prepregnancy BMI (weight in kilograms divided by the square of height in meters) was calculated as an indicator of maternal adiposity. Birth weight was measured to the nearest gram using the routinely available electronic weighing device in each delivery unit. Birth length was measured to the nearest 0.1 cm using the 447 Infantronic Digital Infantometer (QuickMedical, Seattle, WA) purchased for all participating hospitals. Ponderal index (kilograms per cubic meter) was calculated as an indicator of fetal adiposity.

Maternal blood specimens were collected at 24–28 weeks of gestation at the time of the routine prenatal 50-g 1-h oral glucose tolerance test (OGTT) to screen for gestational diabetes mellitus and at 32–35 weeks of gestation (random blood). Venous cord blood specimens were collected immediately after the delivery of the baby but before the expulsion of the placenta. For cesarean section deliveries, venous cord blood samples were taken immediately after removal of the placenta. Research assistants were available 24-h on-call for timely collection and processing of cord blood specimens. All specimens collected were kept on ice and stored temporarily in a 4°C refrigerator and centrifuged within 30 min after specimen collection. The separated plasma samples were stored in multiple aliquots in a freezer at −80°C until assays.

Impaired glucose tolerance and gestational diabetes mellitus

Impaired glucose tolerance ($n = 31$) was defined as blood glucose concentration ≥7.8 mmol/l (140 mg/dl) in the 1-h 50-g OGTT at 24–28 weeks of gestation but did not meet the criteria for the diagnosis of gestational diabetes mellitus. If the 50-g OGTT blood glucose concentration was between 7.8 and 11.1 mmol/l, the woman underwent the diagnostic 2-h 75-g OGTT. Gestational diabetes mellitus was diagnosed if the woman had two of three values exceeding the following cutoffs for fasting glucose: 5.3 mmol/l, 1-h 10.0 mmol/l, or 2-h 8.6 mmol/l (American Diabetes Association criteria) (15). If the 50-g OGTT blood glucose concentration was ≥11.1 mmol/l (200 mg/dl), a diagnosis of gestational diabetes mellitus was made without further tests. Gestational diabetes mellitus ($n = 26$) was well managed in participating hospitals by dietary and lifestyle interventions and insulin treatment if required to achieve euglycemia. Patients who had insulin treatment were not excluded because insulin does not pass the placenta barriers and the treatment did not affect primary outcome indicators among our patients with gestational diabetes mellitus.

Biochemical assays

Maternal blood glucose concentrations (millimoles per liter and milligrams per deciliter) in the first trimester random blood and 50-g OGTT blood samples at 24–28 weeks of gestation were taken from routine prenatal clinical test records. Blood glucose concentrations were determined by the same glucose oxidase method in the certified clinical biochemistry laboratories of all participating hospitals. Cord blood plasma glucose concentrations were determined in the biochemistry laboratory of Sainte-Justine Hospital, using the automated glucose oxidase method (Beckman-Coulter, Brea, CA). Intra- and inter-assay coefficients of variation were 2 and 3%, respectively.

Plasma insulin (picomoles per liter and microunits per milliliter) and proinsulin (picomoles per liter) concentrations were determined in maternal OGTT blood at 24–28 weeks, in maternal random blood at 32–35 weeks, and in cord blood in the same laboratory at Sainte-Justine Hospital. Plasma insulin was measured by an automated ultrasensitive chemiluminescent immunometric assay (Beckman-Coulter). The intra-assay and interassay coefficients of variation were 2 and 6%, respectively. Cross-reactivity was 0.3% with proinsulin, and none with C-peptide. Plasma proinsulin was measured by a quantitative ELISA kit (ALPCO Diagnostics, Salem, OR). The intra-assay and interassay coefficients of variation were 3 and 5%, respectively. Cross-reactivity was 0.1% with human insulin and <0.01% with C-peptide.

Primary outcomes

The cord plasma glucose (milligrams per deciliter)-to-insulin (microunits per milliliter) ratio was used as the primary indicator of fetal insulin sensitivity (16,17); other indicators include cord plasma insulin and proinsulin concentrations. Cord blood proinsulin-to-insulin ratio was used as a surrogate indicator of β-cell function (18,19). Maternal fasting was not requested for the delivery visit.

Statistical analysis

Means ± SD are presented for continuous variables. Geometric means are presented for biomarkers with skewed crude data distribution (insulin, proinsulin, glucose-to-insulin ratio, and proinsulin-to-insulin ratio). Log transformation was applied for variables with skewed data distribution in all comparisons. Pearson correlation analysis was used to assess the association

between continuous variables; partial correlation analysis was used to assess the association controlling for other continuous variables. Generalized linear regression analyses were conducted to assess the associations controlling for multiple covariables (categorical and continuous). The covariables included maternal glucose tolerance (normal, impaired, and gestational diabetes mellitus; per SD increase in the 50-g OGTT glucose concentration), prepregnancy BMI (BMI ≥30 kg/m^2 obese, 25.0–29.9 kg/m^2 overweight, and <25.0 kg/m^2 normal weight; per SD increase), maternal ethnicity (French mother tongue, the majority group in Quebec, and others), family history of diabetes (yes or no, among first-degree relatives), maternal age (<35 or ≥35 years), parity (primiparous: yes or no), smoking (yes or no), alcohol use (yes or no), gestational hypertensive complications (yes or no), bacterial vaginosis or other minor infections (yes or no) (no patients had any major infections requiring hospitalization or emergency care), glucose intravenous administration during delivery (yes or no), mode of delivery (cesarean or vaginal), infant sex, gestational age (weeks), birth weight (SD score, based on Canadian fetal growth standards [20]), and ponderal index (per SD increase). All data management and analyses were conducted using SAS (version 9.0; SAS Institute, Cary, NC). Two-tailed $P < 0.05$ was considered statistically significant.

Table 1—*Maternal, pregnancy, and neonatal characteristics and metabolic parameters in a prospective singleton pregnancy cohort study*

	All subjects	Normal glucose tolerance	Impaired glucose tolerance and gestational diabetes mellitus
n	248	191	57
Mother			
Ethnicity: French Canadians	112 (45.1)	88 (46.1)	24 (42.1)
Age (years)	31 ± 4.7	30.8 ± 4.7	31.7 ± 4.7
≥35	57 (23.0)	43 (22.5)	14 (24.6)
Primiparous	100 (40.3)	84 (44.0)	16 (28.1)§
Tobacco smoking (yes/no) (%)	18 (7.3)	15 (7.9)	3 (5.3)
Drinking alcohol (yes/no) (%)	34 (13.7)	19 (10.0)	15 (26.3)‖
Family history of diabetes (%)	47 (19.0)	31 (16.2)	16 (28.1)§
Height (cm)	164.1 ± 6.4	164.9 ± 6.3	162.8 ± 6.4§
Pregnancy			
Prepregnancy weight (kg)	64.5 ± 14.1	63.8 ± 13.2	67.2 ± 16.6
Prepregnancy BMI (kg/m^2)	23.9 ± 5.0	23.4 ± 4.6	25.3 ± 5.9§
Gestational age at recruitment (weeks)	25.7 ± 1.1	25.8 ± 1.1	25.6 ± 1.1
Weight gain in pregnancy (kg)	15.7 ± 5.7	16.0 ± 5.5	14.9 ± 6.2
Gestational hypertension	14 (5.7)	13 (6.8)	1 (1.8)
Vaginosis or other minor infections*	39 (15.7)	30 (15.7)	9 (15.8)
Glucose intravenously at delivery†	119 (48.4)	91 (47.9)	28 (50.0)
Cesarean section	70 (28.5)	47 (24.7)	23 (41.1)§
Baby			
Sex (boy)	133 (53.9)	102 (53.7)	31 (54.4)
Gestational age (weeks)	39.0 ± 1.5	39.1 ± 1.4	38.5 ± 1.7§
Birth weight (g)	3,444 ± 445	3,447 ± 432	3,438 ± 489
Birth length (cm)	50.6 ± 2.1	50.7 ± 2.1	50.3 ± 2.1
Ponderal index (kg/m^3)	26.6 ± 2.8	26.4 ± 2.8	27.0 ± 3.0
Birth weight *z* score‡	0.1 ± 0.9	0.1 ± 0.9	0.3 ± 1.1
Preterm birth (<37 weeks)	11 (4.4)	6 (3.1)	5 (8.8)

Data are means ± SD for continuous variables and *n* (%) for frequency variables. $n = 248$. *Minor infections refer to any treatment for infections during pregnancy. No patients had any "major" infections requiring hospitalizations or emergency care. †Patients who were given glucose intravenously during the delivery (5% dextrose in all such cases). ‡Based on sex- and gestational age–specific Canadian fetal growth standards. §$P <$ 0.05; ‖$P < 0.01$ in tests for differences comparing women with impaired glucose intolerance and gestational diabetes mellitus to those with normal glucose tolerance.

RESULTS

Maternal and pregnancy characteristics

Comparing patients with impaired glucose tolerance and gestational diabetes mellitus with those with a euglycemic pregnancy, we found no significant differences in maternal age, ethnicity, smoking, prepregnancy weight, weight gain in pregnancy, and rates of gestational hypertensive complications, bacterial vaginosis and other minor infections, and intravenous glucose administration during delivery (Table 1). Patients with impaired glucose tolerance and gestational diabetes mellitus were less likely to be primiparous (28 vs. 44%), were shorter in height (mean 162.8 vs. 164.9 cm), had a higher prepregnancy BMI (25.3 vs. 23.4 kg/m^2), and were more likely to have taken alcohol during pregnancy (26 vs. 10%) or have a family history of diabetes (28 vs. 16%). Their infants were born slightly earlier (mean gestational age 38.5 vs. 39.1 weeks) and had similar birth weight, birth length, and ponderal index. About half of the women were given intravenous glucose during delivery; they were retained in all analyses because the glucose administration did not affect the primary outcomes, cord blood glucose-to-insulin ratio ($P = 0.2$), proinsulin-to-insulin ratio, and proinsulin concentration (all $P = 0.9$).

Correlations between maternal and fetal metabolic parameters

Higher 50-g OGTT glucose concentrations (lower glucose tolerance) were strongly correlated with higher cord plasma insulin ($r = 0.30$; $P < 0.001$) and proinsulin ($r = 0.31$; $P < 0.001$) concentrations and lower glucose-to-insulin ratios ($r = -0.31$; $P < 0.001$) but were not correlated with proinsulin-to-insulin ratios in cord blood. First trimester random blood glucose levels were positively correlated with cord blood proinsulin concentrations ($r = 0.17$; $P < 0.01$). Cord plasma proinsulin concentrations were strongly positively correlated with maternal plasma proinsulin concentrations at both 24–28 ($r = 0.37$; $P < 0.001$) and 32–25 weeks ($r = 0.32$; $P < 0.001$) of gestation.

Partial correlation analyses revealed that maternal glucose tolerance (OGTT glucose level), prepregnancy adiposity (BMI), and fetal adiposity (ponderal index) were all negatively associated with

Table 2—*Maternal and fetal metabolic parameters comparing women with normal glucose tolerance, impaired glucose tolerance, and gestational diabetes mellitus*

	Normal glucose tolerance	Impaired glucose tolerance	Gestational diabetes mellitus	*P* value for difference trend		Adjusted *P* value*
n	191	31	26			
Mother in pregnancy						
First trimester, random blood						
Glucose (mg/dl)	83.4 ± 14.1	85.2 ± 12.5	90.4 ± 15.9	0.13	0.05	0.48
Glucose (mmol/l)	4.6 ± 0.8	4.7 ± 0.7	5.0 ± 0.9			
24–28 weeks, 50 g OGTT						
Glucose (mg/dl)	105.6 ± 17.9	152.8 ± 12.7‡	170.4 ± 28.8§	<0.0001	<0.0001	<0.0001
Glucose (mmol/l)	5.9 ± 1.0	8.5 ± 0.7	9.5 ± 1.6			
Insulin (mU/ml)	39.4 ± 53.6	69.3 ± 69.0‡	93.0 ± 76.6§	<0.0001	<0.0001	<0.0001
Insulin (pmol/l)	236.5 ± 321.5	415.6 ± 414.0	557.9 ± 459.5§			
Proinsulin (pmol/l)	17.5 ± 10.9	21.3 ± 15.6	27.3 ± 21.3‡	<0.0001	<0.001	0.003
Proinsulin-to-insulin ratio‖	0.07 ± 0.08	0.05 ± 0.04	0.05 ± 0.05	0.20	0.12	0.11
32–35 weeks, random blood						
Proinsulin (pmol/l)	16.6 ± 13.4	20.3 ± 18.5	17.8 ± 23.4	0.30	0.28	0.47
Fetus (cord blood)						
Glucose (mg/dl)	84.7 ± 17.4	78.7 ± 11.1	81.1 ± 16.7	0.12	0.13	0.12
Glucose (mmol/l)	4.6 ± 1.0	4.4 ± 0.6	4.5 ± 0.9			
Insulin (mU/ml)	4.1 ± 4.8	4.3 ± 3.2	7.8 ± 8.2†	<0.001	0.001	0.02
Insulin (pmol/l)	24.8 ± 28.6	25.7 ± 19.1	47.0 ± 49.2†			
Glucose (mg/dl)-to-insulin (μU/ml) ratio	20.0 ± 24.2	18.2 ± 14.5	10.1 ± 10.0†	<0.001	<0.001	0.03
Proinsulin (pmol/l)	13.8 ± 9.4	14.9 ± 8.7	24.4 ± 15.8§	<0.0001	<0.0001	<0.0001
Proinsulin-to-insulin ratio‖	0.56 ± 0.59	0.58 ± 0.44	0.52 ± 0.42	0.84	0.76	0.40

Data are means ± SD unless otherwise indicated. Geometric means are presented for biomarkers (insulin, proinsulin, glucose-to-insulin ratio, and proinsulin-to-insulin ratio) with highly skewed crude data distributions. Pseudo-SDs (in regular scale) are presented for geometric means by calculating the average SDs within the 95% CIs (1 SD change is not a constant in back-transformation from the log to regular scale, larger in the upper tail). 50 g OGTT = 50 g, 1-h OGTT. *Adjusted *P* values for the differences across the three study groups. For maternal measurements, the comparisons were adjusted for ethnicity, age, parity, smoking, alcohol use, and prepregnancy BMI (SD score); for cord blood measurements, the comparisons were further adjusted for family history of diabetes, weight gain in pregnancy (SD score), gestational hypertension, bacterial vaginosis and other minor infections (no major infections reported), mode of delivery, maternal glucose (5% dextrose, in all such cases) infusion during delivery, infant sex, gestational age, birth weight (SD score), and ponderal index (SD score). †$P < 0.05$; ‡$P < 0.01$; §$P < 0.001$, for comparisons to the reference group (normal glucose tolerance) after the adjustments; similar differences were observed in boys and girls (data not shown). ‖The same unit (picomoles per liter) was used for both insulin and proinsulin in calculating the proinsulin-to-insulin ratio.

cord blood glucose-to-insulin ratio. After the mutual adjustments, the correlation coefficients changed little for maternal glucose tolerance (crude $r = -0.31$, partial $r = -0.29$, $P < 0.001$), but decreased substantially for prepregnancy adiposity (crude $r = -0.21$, partial $r = -0.16$, $P = 0.01$) and decreased for fetal adiposity (crude $r = -0.21$, partial $r = -0.19$, $P = 0.003$). Similarly, a stronger correlation with fetal proinsulin concentration was observed for maternal glucose tolerance (crude $r = 0.31$, partial $r = 0.30$, $P < 0.001$) than for prepregnancy adiposity (crude $r = 0.19$, partial $r = 0.12$, $P = 0.05$) or fetal adiposity (crude $r = 0.24$, partial $r = 0.23$, $P < 0.001$). Similar correlations were observed in boys and girls (data not shown).

Differences in fetal metabolic parameters by maternal glucose tolerance

As expected, glucose, insulin, and proinsulin concentrations at the OGTT were substantially higher in patients with impaired glucose tolerance and even more so in those with gestational diabetes compared with euglycemic pregnancy (Table 2). However, there were no significant differences in cord blood glucose concentrations and proinsulin-to-insulin ratios among the three study groups. From euglycemia to impaired glucose tolerance to gestational diabetes mellitus, first trimester random blood glucose levels trended marginally higher ($P = 0.05$), whereas cord blood glucose-to-insulin ratios trended significantly lower, and insulin and proinsulin concentrations trended significantly higher ($P < 0.001$). Marked changes in cord blood metabolic biomarkers were observed in patients with gestational diabetes mellitus, whereas the changes were much milder in patients with impaired glucose tolerance not meeting the criteria for the diagnosis of gestational diabetes mellitus. The marked changes in cord blood metabolic parameters (insulin, proinsulin, and glucose-to-insulin ratio) among patients with gestational diabetes mellitus remained significant after adjustment for maternal smoking, alcohol use, prepregnancy adiposity (BMI), family history of diabetes, gestational age, ponderal index, and other potential confounders. The differences were similar and remained significant in subgroups with ($n = 119$) and without ($n = 129$) maternal intravenous glucose administration during delivery (data not shown). Among patients with gestational diabetes mellitus ($n = 26$), insulin treatment ($n = 17$) did not affect the primary outcomes, cord blood glucose-to-insulin ratio ($P = 0.4$), proinsulin-to-insulin ratio ($P = 0.6$), and proinsulin concentration ($P = 0.5$). Therefore, the observed differences in the primary outcomes comparing gestational diabetic versus euglycemic pregnancies should be attributable to diabetes rather than to the insulin treatment.

Table 3—*Regression coefficients (95% CI) for the associations of maternal glucose tolerance (glucose concentration at the OGTT), prepregnancy adiposity (BMI), and fetal adiposity (ponderal index) with cord blood glucose-to-insulin ratio and proinsulin concentration*

	Cord blood glucose-to-insulin ratio	*P* value	Cord blood proinsulin (pmol/l)	*P* value
OGTT glucose, per SD (30 mg/dl) increase				
Crude	−6.1 (−8.8 to −3.5)	<0.0001	3.6 (1.9 to 5.3)	<0.0001
Adjusting for prepregnancy BMI	−5.5 (−8.1 to −2.9)	<0.0001	3.4 (1.7 to 5.1)	<0.0001
Further adjusting for fetal PI	−5.3 (−8.0 to −2.6)	0.0002	3.0 (1.3 to 4.7)	0.0005
Full adjustment*	−5.3 (−8.3 to −2.3)	0.0006	4.1 (2.3 to 6.0)	<0.0001
Prepregnancy BMI, per SD (5 units) increase				
Crude	−4.7 (−7.4 to −2.1)	0.02	2.0 (0.3 to 3.7)	0.02
Adjusting for OGTT glucose	−3.8 (−6.5 to −1.2)	0.005	1.5 (−0.2 to 3.1)	0.09
Further adjusting for fetal PI	−3.3 (−5.9 to −0.7)	0.01	1.2 (−0.5 to 2.8)	0.16
Full adjustment*	−3.2 (−6.1 to −0.3)	0.03	1.3 (−0.4 to 3.1)	0.14
Infant PI, per SD (2.8 units) increase				
Crude	−5.6 (−8.2 to −2.9)	0.0002	3.2 (1.5 to 4.9)	0.0002
Adjusting for OGTT glucose	−5.2 (−7.8 to −2.7)	<0.0001	3.0 (1.4 to 4.6)	0.0004
Further adjusting for prepregnancy BMI	−4.9 (−7.5 to −2.4)	0.0002	2.9 (1.3 to 4.5)	0.0005
Full adjustment*	−5.0 (−7.8 to −2.3)	0.0004	2.7 (0.9 to 4.4)	0.003

n = 248 mother-infant pairs. OGTT = 50 g, 1-h OGTT at 24–28 weeks of gestational age; PI, ponderal index (kilograms per cubic meter). *Variables in the full adjustment models included OGTT glucose concentration, prepregnancy BMI, maternal ethnicity, age, parity, smoking, alcohol use, family history of diabetes, weight gain in pregnancy (SD score), gestational hypertensive complications, bacterial vaginosis and other minor infections, mode of delivery, maternal glucose (5% dextrose) intravenously during delivery, infant sex, gestational age (weeks), birth weight (SD score), and ponderal index (SD score). The adjusted results were similar in subgroups with or without maternal glucose intravenous infusion during delivery and among boys and girls (data not shown).

Maternal obesity

Infants of obese (n = 31) mothers had lower cord plasma glucose-to-insulin ratios (geometric mean 13.0 vs. 20.5 for normal-weight mothers; crude P = 0.004, adjusted P = 0.20) and higher insulin (6.1 vs. 4.1 μU/ml; crude P = 0.007, adjusted P = 0.12) and proinsulin (18.7 vs. 13.9 pmol/l, crude P = 0.008, adjusted P = 0.16) concentrations, but those differences became not statistically significant after adjustment for maternal glucose tolerance and fetal ponderal index alone. Both crude and adjusted differences in these biomarkers were not statistically significant, comparing infants of overweight (n = 48) versus normal-weight mothers.

Effects per SD change

The effect estimates per SD change in multivariate regression analyses showed that maternal glucose tolerance had the greatest impact on cord plasma glucose-to-insulin ratio and proinsulin concentration (Table 3). The effect size for maternal glucose tolerance on the fetal glucose-to-insulin ratio decreased by ~10% after adjustment for prepregnancy adiposity but was hardly affected by other adjustments. The effect of maternal glucose tolerance on fetal proinsulin concentration decreased by ~10% after adjustment for prepregnancy adiposity or fetal adiposity but increased in the full adjustment model. In contrast, the effects of prepregnancy adiposity on both the fetal proinsulin concentration and glucose-to-insulin ratio decreased by ~20% after adjustment for maternal glucose tolerance alone and by ~30 and 40% for the glucose-to-insulin ratio and proinsulin concentration, respectively, after adjustment for maternal glucose tolerance and fetal adiposity; the association became not statistically significant for fetal proinsulin. The effect of fetal adiposity on cord plasma glucose-to-insulin ratio and proinsulin decreased by ~10% after adjustment for maternal glucose tolerance and prepregnancy adiposity but were hardly affected by other adjustments.

CONCLUSIONS

Major findings

To the best of our knowledge, this is the first prospective pregnancy cohort study to demonstrate that maternal glucose tolerance in pregnancy may affect fetal insulin sensitivity (as indicated by the glucose-to-insulin ratio and proinsulin concentration) but not β-cell function (as indicated by the proinsulin-to-insulin ratio).

Maternal glucose tolerance in pregnancy "programs" fetal insulin sensitivity

Most studies on insulin sensitivity of newborns have been focused on the association with fetal growth. Studies of metabolic biomarkers in neonatal blood at 2–3 postnatal days and in cord blood presented conflicting evidence concerning the association between insulin sensitivity and fetal growth (16,17,21,22). Very few data are available on the effects of maternal metabolic parameters on fetal insulin sensitivity. In a study of patients who underwent elective cesarean section, Catalano et al. (14) reported a strong positive correlation (r = 0.35) between maternal insulin resistance at delivery and fetal insulin resistance. In contrast, we observed a similarly strong association, but between maternal glucose tolerance in middle gestation and fetal insulin sensitivity (r = −0.31 for glucose-to-insulin ratio, r = 0.31 for proinsulin). The cord blood glucose-to-insulin ratio was substantially lower, and the proinsulin concentration was much higher for infants of mothers with gestational diabetes mellitus, despite similar cord blood glucose levels, indicating adequate management of diabetes. More importantly, we observed markedly impaired fetal insulin sensitivity in gestational diabetes mellitus independent of maternal and fetal adiposity, family history of diabetes, gestational age, and other potential confounders. In addition, the observed moderate positive correlation between first trimester random blood glucose and cord blood proinsulin concentrations suggests that

maternal metabolic function even in early pregnancy may influence fetal metabolic function. Taken together, our data suggest a mother-baby metabolic programming cycle: maternal glucose intolerance impairs fetal insulin sensitivity and hence programs the vulnerability to the metabolic syndrome; when these female offspring become mothers, they are more likely to be glucose intolerant and in turn program more babies to be susceptible to the metabolic syndrome. To break such a metabolic programming cycle, it would be important to improve maternal glucose tolerance during early and middle pregnancy periods.

For patients with impaired glucose tolerance not meeting the criteria for the diagnosis of gestational diabetes mellitus, we observed mild negative alterations (not statistically significant) in fetal metabolic parameters. This finding may be explained by the limited power of our study to detect small differences. In the large pregnancy cohort study of patients without overt diabetes in pregnancy (n = 23,316), the Hyperglycemia and Pregnancy Outcomes (HAPO) study group observed a continuous association between maternal glucose tolerance at 24–32 weeks of gestation and cord blood C-peptide levels (12), indicating that mild glucose intolerance may affect fetal metabolic function. The study did not evaluate fetal insulin sensitivity. Gestational glucose tolerance even within normal ranges has been associated with the risk of type 2 diabetes in young Pima Indian offspring (23).

Maternal glucose tolerance and maternal and fetal adiposity

Our data confirm the recent finding of Catalano et al. (14) that maternal adiposity may affect fetal insulin sensitivity. In addition, we found that the effects of maternal obesity could be largely mediated by maternal glucose tolerance and fetal adiposity. The effects of maternal glucose tolerance on fetal insulin sensitivity were much stronger than those of maternal adiposity and independent of a genetic influence (as indicated by family history of diabetes) and other potential confounders. To a lesser extent, the effects of maternal glucose tolerance could be partly explained by fetal adiposity.

Strengths, weaknesses, and future research directions

Our prospective cohort design and timely collection and processing of cord blood specimens provide relatively robust data on the relationships between maternal and fetal metabolic parameters. Common standard protocols for assessing insulin sensitivity (e.g., euglycemic insulin clamp) are difficult or impractical to implement and have not been validated for newborns. To avoid interference with routine patient care, we took convenient cord blood samples. The newborns were not in a uniform metabolic state; therefore, we did not use the homeostasis model assessment of fetal insulin sensitivity and β-cell function. The nonuniform metabolic state would have increased the noise variations in our "crude" surrogate insulin sensitivity and β-cell function indicators and hence attenuated the observed associations. However, fasting may be not a reliable option for assessing insulin sensitivity in newborns as it is in adults, because neonates of diabetic mothers tend to develop neonatal hypoglycemia after the delivery. Furthermore, insulin sensitivity primarily reflects peripheral insulin resistance in newborns in contrast to hepatic insulin resistance in adults (24). There is a need for studies on the validity of various insulin sensitivity and β-cell function indicators for newborns. Future studies may examine differences in other metabolic biomarkers and follow up on whether these fetal metabolic changes persist in postnatal life or predict components of the metabolic syndrome in childhood, adolescence, and adulthood.

In summary, our data provide some evidence of metabolic programming in utero by maternal glucose tolerance in pregnancy. Further research to better understand the role of the prenatal metabolic environment in developmental programming may pave the way for early interventions to halt the increasing epidemic of the metabolic syndrome and type 2 diabetes.

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