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SHORT COMMUNICATION

Cardiovascular risk factors in children exposed to maternal diabetes in utero

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Abstract
Aims/hypothesis Recent studies have provided evidence that intrauterine exposure to maternal diabetes has lifelong effects on adult offspring, including increased risks of obesity, type 2 diabetes and cardiovascular disease. The aim of this study was to assess the relationship between exposure to maternal diabetes in utero and cardiovascular risk factors in healthy children and to investigate whether these associations are independent of maternal prepregnancy BMI and offspring attained BMI.
Methods Data were from a retrospective cohort of children aged 6–13 years born during 1994–2002. Multiple linear regression was used to examine the associations between exposure and cardiovascular risk factors with adjustment for demographic factors and pubertal stage and additionally for maternal prepregnancy BMI and offspring attained BMI.
Results Ninety-nine offspring of diabetic pregnancies had significantly increased E-selectin, vascular adhesion molecule 1 (VCAM1), leptin, waist circumference, BMI and systolic blood pressure and decreased adiponectin levels compared with 422 offspring of non-diabetic pregnancies after adjustment for age, sex and race/ethnicity ($p<0.05$ for each risk factor). Additional adjustment for maternal prepregnancy BMI substantially attenuated group differences in the risk factors except for E-selectin, VCAM1 and waist circumference, which remained significantly higher in exposed children.
Conclusions/interpretation Compared with unexposed children, healthy offspring exposed to maternal diabetes in utero have a worse cardiovascular risk profile. In particular, offspring have substantially increased levels of circulating cellular adhesion molecules, which are biomarkers of adverse endothelium perturbation and may be related to the earliest preclinical stages of atherosclerosis and diabetes.

Keywords Adipokines · Adiposity · Blood pressure · Cardiovascular risk factors in children · Developmental origins of health and disease · Endothelial cell adhesion molecules · Gestational diabetes · Intrauterine exposure to maternal diabetes · Lipids · Pregnancy

Abbreviations
ICAM1 Intercellular adhesion molecule 1
VCAM1 Vascular adhesion molecule 1

Introduction

Epidemiological studies have provided substantial evidence that intrauterine exposure to maternal diabetes has lifelong effects on the offspring, including increased risk of obesity, type 2 diabetes and cardiovascular disease [1]. Risk factors for adult cardiovascular diseases can appear during childhood and track into adulthood [2]. The aim of this study was to assess the relationship between exposure to maternal diabetes in utero and cardiovascular risk factors in healthy children. We also explored whether these associations were independent of maternal prepregnancy BMI and offspring attained BMI.

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Methods

Study population Data were from a retrospective cohort of healthy children aged 6–13 in 2006–2009. They were offspring of singleton pregnancies and their biological mothers were members of the Kaiser Permanente of Colorado Health plan. Participants were children exposed to diabetes in utero (exposed group) and children not exposed to diabetes in utero and without intrauterine growth restriction (defined as birthweight for gestational age below the 10th percentile) (unexposed group) [3]. Overall, 57% of the eligible mother–child pairs participated in the study. The study was approved by the relevant institutional review boards. Written informed consent from the mothers and assent from the children were obtained.

Exposure definition Physician-diagnosed maternal diabetes status was ascertained from the health plan database. Routine screening for gestational diabetes in all non-diabetic pregnancies was performed. At 24–28 weeks, all pregnant women were offered screening for gestational diabetes with a 1 h, 50 g OGTT. Women with a value ≥7.7 mmol/l were asked to undergo a 3 h, 100 g diagnostic OGTT. Gestational diabetes was diagnosed when two or more glucose values during the diagnostic OGTT met or exceeded the criteria for a positive test [4]. Exposure to diabetes in utero was defined as maternal diabetes diagnosed during the index pregnancy or presence of pre-existing maternal diabetes.

Cardiovascular risk factor measurements Childhood height was measured to the nearest 0.1 cm by stadiometer. Weight was measured to the nearest 0.1 kg using an electronic scale. Age- and sex-specific BMI *z* scores were calculated [5]. Waist circumference, measured to the nearest 0.1 cm at the end of normal expiration just above the right iliac crest at the mid-axillary line, was measured twice and averaged. Two sitting blood pressure measurements were averaged. Blood samples were drawn after an overnight fast for measurement of lipids and glucose (measured using the Olympus AU400 advanced chemistry analyser system), insulin (measured by a radioimmunoassay method), adiponectin and leptin (measured with enzyme-linked immunosorbent assay kits), non-esterified fatty acids (measured using an enzymatic colorimetric method) and soluble cell adhesion molecules, including intercellular adhesion molecule 1 (ICAM1), vascular adhesion molecule 1 (VCAM1) and E-selectin (measured by a Luminex multiplex assay kit method, Austin, TX, USA). Laboratory analyses were performed at the University of Colorado Hospital laboratory and the Children's Hospital laboratory (Aurora, CO, USA). LDL concentration was determined by the Friedewald equation [6]. HOMA-IR, used to measure insulin resistance, was calculated as fasting glucose (mmol/l)× insulin (pmol/l)]/156.3) [7].

Covariates Of the 521 mothers in this study, 456 had prepregnancy weight and height recorded in the medical records; self-reported prepregnancy weight and measured height for the remaining 65 mothers were obtained during the study visit. The correlation between recorded weight and self-reported weight and between prepregnancy recorded height and height measured at the study visit was 0.93 and 0.93 respectively. Race/ethnicity was self-reported as Hispanic (any race), non-Hispanic white, non-Hispanic black or mixed race/other. Self-reported Tanner staging was used to categorise pubertal development with a diagrammatic representation of staging using scales ranging from 1 (prepubertal) to 5 (adult). Young people were categorised as Tanner <2 (prepubertal) and ≥2 (pubertal). Maternal level of education and total household income were self-reported during the study visit.

Statistical analysis Non-normally distributed variables were log transformed for statistical testing. The χ^2 and *t* tests were used to compare characteristics between exposed and unexposed offspring. Multiple linear regression was used to examine the associations between exposure and cardiovascular risk factors with adjustment for demographic factors, pubertal stage, maternal prepregnancy BMI and offspring attained BMI. Statistical analyses were performed using PC-SAS (version 9.2).

Results

Of the 99 exposed children, eight were exposed to pregestational type 1 diabetes and 91 were exposed to gestational diabetes. Among the mothers with gestational diabetes, 23% were treated with insulin during their pregnancy. Table 1 shows that offspring of diabetic pregnancies were slightly younger, more likely to be prepubertal and more likely to be non-Hispanic white than offspring of non-diabetic pregnancies. Mothers of exposed offspring were more likely to have been overweight or obese prior to pregnancy.

Table 2 shows the mean levels of selected cardiovascular risk factors by exposure status, adjusted for age, sex, race/ethnicity, Tanner stage, maternal education and total household income (model 1), additionally adjusted for maternal prepregnancy BMI (model 2) and further adjusted for offspring attained BMI (model 3). In model 1, offspring of diabetic pregnancies had significantly higher levels of E-selectin, VCAM1 and leptin, larger waist circumference, higher BMI *z* scores and systolic blood pressure, and lower

Table 1 Characteristics of offspring and mothers by maternal diabetes status

Characteristic	Diabetes during pregnancy	No diabetes during pregnancy	*p* value
Number of mother/offspring pairs	99	422	–
Child's age (years), mean±SD	9.5±1.7	10.6±1.4	<0.0001
Female children	46 (46.5)	215 (51.0)	0.4
Tanner stage			<0.0001
<2	71 (71.7)	210 (49.9)	
≥2	28 (28.3)	211 (50.1)	
Race/ethnicity of children			0.05
Non-Hispanic black	5 (5.1)	36 (8.5)	
Hispanic	30 (30.3)	172 (40.8)	
Non-Hispanic white	60 (60.6)	191 (45.3)	
Mixed race/other	4 (4.0)	23 (5.4)	
Maternal education			0.5
<High school	4 (4.0)	3 (0.7)	
High school	9 (9.1)	53 (12.6)	
>High school	86 (86.9)	366 (86.7)	
Total household income (US$)			0.4
<50,000/year	17 (17.2)	88 (20.9)	
≥50,000/year	82 (82.8)	333 (79.1)	
Maternal prepregnancy BMI category			0.0002
Normal: <25.0 kg/m^2	37 (38.1)	247 (60.2)	
Overweight: 25.0–29.9 kg/m^2	31 (32.0)	96 (23.4)	
Obese: ≥30.0 kg/m^2	29 (29.9)	67 (16.3)	

Data are *n* (%) except for number of mother/offspring pairs and child's age

adiponectin levels. Significant differences according to exposure status in E-selectin, VCAM1 and waist circumference remained after adjustment for maternal prepregnancy BMI and for offspring attained BMI. Differences between the other cardiovascular factors were also observed, with higher triacylglycerol, LDL-cholesterol, diastolic blood pressure, non-esterified fatty acids, HOMA-IR and ICAM1 levels and lower HDL-cholesterol in offspring exposed to maternal diabetes; however, these differences did not reach statistical significance ($p>0.10$ for each; data not shown).

Independently of maternal diabetes status, there was a positive association between maternal prepregnancy BMI and offspring waist circumference ($p<0.0001$), BMI *z* score ($p<0.0001$), triacylglycerol ($p=0.04$), LDL-cholesterol ($p=0.03$), HOMA-IR ($p=0.001$), systolic blood pressure ($p=0.003$), diastolic blood pressure ($p=0.01$), leptin ($p<0.0001$) and E-selectin ($p=0.03$) (data not shown). Each of these associations was attenuated after adjustment for child attained BMI ($p>0.10$ for each).

Discussion

In this multi-ethnic cohort of healthy children aged 6–13 years, offspring exposed to maternal diabetes in utero had significantly increased E-selectin, VCAM1, leptin, waist circumference, BMI and systolic blood pressure and decreased adiponectin levels compared with non-exposed children. Differences in E-selectin, VCAM1 and waist circumference

Table 2 Mean levels of cardiovascular factors in offspring exposed and not exposed to maternal diabetes in utero

Cardiovascular characteristic	Offspring of diabetic pregnancy	Offspring of non-diabetic pregnancy	*p* value
E-selectin (ng/ml)[a]			
Model 1	1.8	1.4	<0.0001
Model 2	1.7	1.4	<0.0001
Model 3	1.7	1.4	0.0001
VCAM1 (ng/ml)			
Model 1	53.8	48.4	0.005
Model 2	54.0	49.2	0.02
Model 3	54.4	49.2	0.008
BMI *z* score			
Model 1	0.70	0.35	0.01
Model 2	0.49	0.33	0.26
Model 3	–	–	–
Waist circumference (cm)			
Model 1	68.5	64.2	0.0003
Model 2	66.8	64.2	0.02
Model 3	65.1	64.1	0.02
Systolic blood pressure (mmHg)			
Model 1	106	104	0.05
Model 2	105	104	0.17
Model 3	105	104	0.38
Adiponectin (μg/ml)[a]			
Model 1	9.2	10.5	0.02
Model 2	9.3	10.3	0.06
Model 3	9.4	10.3	0.12
Leptin (ng/ml)[a]			
Model 1	7.1	5.3	0.004
Model 2	6.2	5.2	0.09
Model 3	5.5	5.1	0.35

Model 1: adjusted for age, sex, race/ethnicity, Tanner stage, household income and maternal education

Model 2: model 1 plus adjustment for maternal prepregnancy BMI

Model 3: model 2 plus adjustment for offspring attained BMI

[a] Geometric means reported

were independent of maternal prepregnancy BMI and persisted after adjustment for offspring attained BMI.

Levels of the endothelial markers E-selectin and VCAM1 in exposed offspring were 29% and 11% higher, respectively, than those of unexposed offspring, suggesting significant upregulation of endothelial cell adhesion molecules in early life after exposure to maternal diabetes during pregnancy. Our results also show an association between offspring E-selectin levels and maternal prepregnancy BMI, independently of maternal diabetes exposure, indicating that exposure to maternal diabetes and pregravid adiposity are both contributors to increased levels of E-selectin in children. Blood levels of E-selectin and VCAM1 are markers of endothelial activation [8]. Under the influence of adhesion molecules, monocytes and lymphocytes attach to endothelial cells, initiating the atherosclerotic process [9]. This preclinical phase of cardiovascular disease lasts many decades, suggesting that upregulation of endothelial cell adhesion molecules in early life may be a predictor of increased risk of cardiovascular disease in later life.

Our study demonstrated that exposure to maternal diabetes in utero is a strong predictor of increased waist circumference in the offspring. This result remained significant after controlling for maternal prepregnancy BMI and for attained child BMI. Our results indicate that maternal pregravid BMI, independently of maternal diabetes, is a strong predictor of childhood BMI.

Exposure to maternal diabetes was moderately associated with increased systolic blood pressure in the children. This association appears to be at least partly mediated by increased offspring attained adiposity. We demonstrated that both systolic and diastolic blood pressure were significantly associated with prepregnancy maternal BMI, independently of maternal diabetes status.

Leptin levels were 34% higher and adiponectin levels were 12% lower in exposed offspring compared with non-exposed children. These differences were attenuated by adjustment for offspring attained BMI, indicating that the leptin and adiponectin levels found in the exposed offspring reflect their higher BMI.

Although important trends of higher triacylglycerol, LDL-cholesterol and non-esterified fatty acids and lower HDL-cholesterol levels were observed in exposed offspring, we found no significant associations in this age group. We observed a positive association between maternal prepregnancy BMI and offspring triacylglycerol and LDL-cholesterol, regardless of maternal diabetes status.

The strengths of this study include the clinical diagnosis of maternal diabetes during pregnancy using a standard protocol, the direct measurement of maternal prepregnancy BMI, the measurement of several traditional and novel cardiovascular factors and the racial/ethnic diversity of the study participants.

This study also has limitations. The relatively small sample size of exposed offspring prevented the examination of possible differences in the effect of maternal diabetes exposure across different racial/ethnic groups. However, the associations were consistent among racial/ethnic groups. Because differences in some cardiovascular risk factors have been shown to strengthen during and after puberty [10], the differences we observed between the groups may be underestimated as the exposed children were significantly younger and more likely to be prepubertal than the unexposed children.

In summary, when compared with offspring not exposed to maternal diabetes, exposed offspring have a worse cardiovascular risk profile. In particular, offspring have substantially increased levels of circulating cellular adhesion molecules, which are biomarkers of adverse endothelium perturbation and are related to the earliest preclinical stages of atherosclerosis and diabetes. These adverse effects of maternal diabetes on offspring can be observed early in life.

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Duality of interest The authors declare that there is no duality of interest associated with this manuscript.

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