Original Article

Programming of infant neurodevelopment by maternal obesity: potential role of maternal inflammation and insulin resistance

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Background and Objectives: Recent studies show that maternal obesity is associated with impaired offspring neurodevelopmental outcomes. The mechanism underlying the association is unclear. However, there is evidence to suggest a role for intra-uterine exposure to inflammation and insulin resistance (IR). We aimed to determine if maternal IR and inflammation were associated to fetal neurodevelopment as indicated by fetal heart rate variability (HRV), an index of fetal cardiac autonomic nervous system development. **Method and Study Design:** A total of 44 healthy maternal-fetal pairs (maternal pre-pregnancy BMI distribution: n=20 normal weight, 8 overweight, 16 obese) were analyzed. We assessed maternal inflammation (plasma IL-6 and TNF-α) and IR (HOMA index). Fetal HRV, a proxy for fetal neurodevelopment, was assessed using fetal magnetocardiogram at the 36th week of pregnancy. The relationships between maternal inflammation and IR with fetal HRV (SD1 and SD2) were estimated individually by Pearson bivariate correlations. **Results:** No correlations were observed between the fetal HRV components with maternal HOMA-IR and maternal plasma levels of IL-6 and TNF-α (all $p<0.05$). However, the negative association between maternal TNF-α level and fetal SD2 approached significance (correlation coefficient=-0.29, 95% confidence interval=-0.62,-0.03, p=0.07). **Conclusion:** Maternal IR and inflammation during pregnancy were not associated with fetal cardiac autonomic nervous system development. Further studies with a larger sample size and more maternal inflammatory indicators are needed to explore these relationships.

Key Words: maternal obesity, inflammation, insulin resistance, fetal heart rate variability

INTRODUCTION

Maternal obesity is a strong risk factor for increased offspring adiposity and metabolic disorders such as diabetes and cardiovascular disease.[1,2] More recently, studies have suggested that maternal obesity affects offspring neurodevelopmental outcomes during childhood.[3-5] The mechanism underlying this association remains unclear; however, the role of intrauterine exposure to insulin resistance (IR) and inflammation has been reported.

Inflammation and IR are greater in obese pregnant women when compared to normal weight pregnant women and fetuses exposed to obesity are more insulin resistant.[6,7] Greater maternal inflammatory cytokine levels are associated with an increased offspring risk of schizophrenia in adulthood,[8] and children born to diabetic mothers have a higher risk of impaired brain development and neurological deficits.[4] In a rodent model, higher maternal blood inflammatory marker levels are associated with higher inflammatory cytokine levels in the offspring brain.[9] Rodent offspring exposed to maternal obesity and high fat diet have increased brain inflammation and show neurobehavioral disturbances.[10] Taken together, these studies suggest that the adverse effect of maternal obesity on offspring neurodevelopment may be mediated through IR and inflammation.

To the best of our knowledge, the association of mater-

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nal IR and inflammation with offspring neurodevelopment *in utero* has not been investigated in humans. Therefore, we investigated whether measures of maternal IR and levels of inflammation are associated with fetal heart rate variability (HRV). Fetal HRV is an index of fetal cardiac autonomic nervous system development, has been used as a proxy for fetal neurodevelopment, and predictor for developmental outcomes in early childhood.[11]

Understanding the mechanism through which maternal obesity affects offspring neurodevelopment can facilitate clinical trials for examining the corresponding causal relationship, which consequently can help in developing interventions required for optimising the offspring health.

RESEARCH DESIGN AND METHODS

Participants were a subset of maternal-fetal pairs (n=44) from two studies conducted at University of Kansas Medical Center (KUMC) in 2012–2015 whose blood analysis data were available. Participants were recruited from the KUMC Obstetrics and Gynaecology clinic at their prenatal care visits or through email advertisement within the University. Inclusion criteria were 18–35 years of age and having a singleton pregnancy. Participants were excluded if they met one or more of the following conditions: pre-pregnancy BMI <18.5 kg/m^2, gestational diabetes mellitus, or pre-existing chronic diseases that can influence fetal growth and development including chronic kidney and liver diseases. Both studies were approved by the Human Subject Committee of KUMC (HSC 13126 and HSC 0312), and every participant provided written consent. Maternal anthropometric measures and blood collection as well as fetal HRV analysis were conducted at 36 weeks of pregnancy.

Maternal pre-pregnancy BMI and gestational weight gain

Pre-pregnancy weight was self-reported and body height was measured using a wall stadiometer to the nearest centimetre. Maternal pre-pregnancy BMI was calculated by dividing the pre-pregnancy body weight in kilograms by the square of body height in metres. Gestational weight gain (GWG) was calculated by subtracting the self-reported or medically recorded highest body weight during pregnancy from the self-reported pre-pregnancy body weight. Pre-pregnancy BMI was classified as normal weight (18.5–24.9 kg/m^2), overweight (25.0–29.9 kg/m^2), or obese (>30.0 kg/m^2). GWG was classified on the basis of the 2009 IOM recommendations.[12]

Blood assay

Maternal blood was drawn after an overnight fast from the peripheral vein. Plasma was obtained through centrifugation and stored at −80 °C in a liquid nitrogen tube until analysis for glucose, insulin, TNF-α, and IL-6 levels. The plasma glucose analysis was conducted using an enzymatic colorimetric method (Cayman®). Moreover, the plasma insulin level was determined using the ELISA method (Alpco®). The plasma TNF-α and IL-6 levels were determined using the ELISA method (Quantikine, R&D® and Ebioscience®, respectively). Insulin resistance was determined using the homeostasis model assessment (HOMA-IR) with the following mathematical formula:[13]

HOMA-IR = [fasting blood insulin (μIU/mL) × fasting blood glucose]/22.5

Fetal HRV assessment

Subjects were seated in a reclining chair in front of an 83 channel CTF systems dedicated fetal biomagnetometer that covered the gravid maternal abdomen. Recordings lasted 18 minutes. The data were recorded using a 300 Hz sampling rate with a 0-75 Hz filter. Data were processed by filtering at 1-40 Hz and subjected to independent component analysis to extract maternal and fetal MCG signals. Individual components were used to reconstruct the fetal MCG. The MCG recordings were then presented to the Kubios HRV Analysis 2.1 software program (University of Kuopio, Kuopio, Finland). Fetal short-term and overall HRV were calculated from the Poincare analysis using the metrics SD1 (short-term) and SD2 (overall HRV).

Statistical analysis

Values are presented as the mean±SD or the median (quartile 1−quartile 3). Categorical variables are presented as n (%). The relationships between maternal factors (HOMA-IR index, TNF-α, and IL-6 levels) and fetal HRV (SD1 and SD2) were individually estimated using Pearson bivariate correlation. Factors with significant correlations were further analysed in a multiple regression model with each fetal HRV measure adjusted as a potential confounder. The level of significance was set at α=0.05. Statistical analyses were performed using IBM statistics, SPSS, Version 20.0.

RESULTS

Descriptive statistics for maternal and offspring variables are listed in Table 1. Most women (88.6%) were Caucasian. The average pre-pregnancy BMI was 27.6 kg/m^2. Before pregnancy, 45.5% of the women were in the normal BMI category, whereas 18.2% and 36.4% were overweight and obese, respectively. The average GWG was 15.8 kg. According to the 2009 IOM GWG recommendations, 56.8% and 38.6% of the women gained excessively and adequately, respectively.

Plasma TNF-α levels were not detectable in three participants; therefore, these participants were excluded from the analysis. Bivariate correlations between fetal HRV and maternal factors are listed in Table 2. We observed no correlations between fetal HRV and maternal HOMA-IR, IL-6, and TNF-α levels. Therefore, the associations were not further investigated. However, we found a marginal trend towards significance between maternal TNF-α level and fetal SD2 (correlation coefficient=-0.29; p=0.07).

DISCUSSION

Maternal overweight and obesity are correlated with IR and inflammation in both the mother and fetus.[6,14-16] In addition to their role in predisposing the fetus to obesity and metabolic disorders,[17-19] recent studies suggest that maternal IR and inflammation play crucial roles in the mechanism through which maternal adiposity affects fetal neurodevelopment.[9,18-21] Insulin resistance, as in the case of maternal diabetes, is with an increased risk of cognitive and developmental problems in children.[21,23]

Table 1. Characteristics of subjects

Characteristics	Values
Mother	
Race	
Caucasian	39 (88.6)
African	3 (6.82)
Hispanic	1 (2.27)
Asian	1 (2.27)
Age (year)	28.9±3.3
GA of delivery (week)	39.4±0.9
Pre-pregnancy BMI	27.6±6.4
Normal weight	20 (45.5)
Overweight	8 (18.2)
Obese	16 (36.4)
GWG (kg)	15.8±6.2
GWG category (IOM 2009)	
Inadequate	2 (4.5)
Adequate	17 (38.6)
Excessive	25 (56.8)
Blood biomarkers	
Glucose level (mg/dL)	93.6±22.6
Insulin level (μIU/mL)	6.00 (3.43–8.10)
HOMA-IR index	1.84 (0.74–1.93)
IL-6 level	1.18 (0.95–1.49)
TNF-α level (pg/mL), n=41	1.31 (0.56–2.75)
Fetal HRV	
36 wks, n=44	
SD1 (msec)	4.16 (3.21–4.97)
SD2 (msec)	34.5 (25.8– 39.8)

GA: gestational age; GWG: gestational weight gain; IOM: Institute of Medicine; HOMA-IR: homeostatic model assessment of insulin resistance; IL-6: interleukin 6; TNF-α: tumor necrosis factor α; HRV: heart rate variability; SD1: standard deviation 1 (short term variability); SD2: standard deviation 2 (long term variability).
Values are n (%), mean±SD, or median (Q1-Q3).

We did not observe a relationship between maternal HOMA-IR levels and fetal HRV. Insulin is one of the key regulators of neurodevelopment, and the disruption of insulin regulation in the hippocampus by maternal diabetes was suggested to underlie the cognitive and memory deficit in children born to diabetic mothers.[22]

We did not observe a relationship between maternal levels of inflammatory cytokines (IL-6 and TNF-α) and fetal HRV. In animal models, maternal obesity induced both maternal and fetal inflammation, which resulted in neurodevelopmental disturbances in early childhood and later in life.[10,20] Increased levels of inflammatory cytokines in fetal circulation and amniotic fluid induced fetal brain inflammation and consequently impaired neurocognitive and development function.[24] However, Aye et al reported that an increase in maternal inflammation is not always followed by an increase in fetal inflammation.[14] In a study involving 60 women who had a caesarean section, maternal and fetal cytokines (IL-1β, IL-6, IL-8, MCP-1, and TNF-α) as well as inflammatory pathway activation in the placenta were investigated. The study determined that maternal pre-pregnancy BMI was associated with increased maternal cytokine levels and the activation of some placental inflammatory pathways; nevertheless, the fetal inflammatory profile was not affected.

Since we did not observe an association between maternal inflammatory cytokine levels and fetal HRV, our results suggest that maternal obesity may affect offspring neurodevelopment through mechanisms other than intrauterine exposure to maternal inflammation. Other studies have shown fetal HRV and cardiac-somatic integration to be predictive of infant neurobehavior.[11,25] A recent report comparing fetal HRV, motor activity and cardiac-somatic coupling in several hundred pregnant women found reduced fetal HRV only in the obese group, at the 36 week GA time point.[26] Additionally, the lack of association in our study might be due to the fact that our participants were relatively healthy, since we did not observe a significant association between maternal inflammatory cytokine levels and pre-pregnancy BMI or GWG (data not shown).

Our study is limited by a relatively small sample size. In addition, we analysed only the maternal levels of IL-6 and TNF-α among many cytokines to indicate the inflammation status; however, the true mechanism might involve other cytokines. Further studies with a larger sample size that includes a wide range of inflammatory markers and controlling for possible confounding factors are required. Finally, more advanced analytical procedures that consider the development, coupling and integration of other fetal oscillators (breathing, body, eye movements) may improve sensitivity needed to provide comprehensive knowledge on how maternal factors during pregnancy affect fetal neurodevelopment.

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Table 2. Pearson correlations between maternal adiposity, inflammatory cytokines, and HOMA-IR, and fetal HRV

Maternal predictors	n	Fetal HRV			
		SD1		SD2	
		r (CI)	*p*	r (CI)	*p*
HOMA-IR	44	-0.26 (-0.57,0.06)	0.18	-0.19 (-0.50,0.13)	0.71
IL-6	44	0.17 (-0.14,0.49)	0.27	0.09 (-0.22,0.40)	0.57
TNF-α	41	-0.03 (-0.38,0.31)	0.84	-0.29 (-0.62,0.03)	0.07

HOMA-IR=Homeostatic model assessment of insulin resistance; IL-6: interleukin 6; TNF-α: tumor necrosis factor α; HRV: heart rate variability; SD1: standard deviation 1 (short term variability); SD2: standard deviation 2 (long term variability); r=correlation coefficient; CI: 95% confidence interval.

AUTHOR DISCLOSURES

All authors have no conflicts of interest to disclose.

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