Original Article
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Obesity

Maternal Weight, Weight Gain, and Metabolism are Associated with Changes in Fetal Heart Rate and Variability

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Objective: Prepregnancy obesity and extensive weight gain can lead to diseases in the offspring later in life. The aim of this study was to evaluate the effect of anthropometric and metabolic factors on the fetal autonomic nervous system (ANS) in uncomplicated pregnancies.

Methods: A total of 184 pregnant women in the second or third trimester were included, and for 104 women, maternal insulin sensitivity (ISI) was determined. Fetal heart rate (HR) and heart rate variability (HRV) were determined by magnetic recording. Associations of maternal prepregnancy BMI, weight gain, and ISI with fetal HR and HRV were evaluated by ANCOVA, partial correlation, and mediation analysis.

Results: HR was increased and HRV decreased in fetuses of mothers with overweight or obesity in comparison to normal-weight mothers. Fetal HR was negatively correlated with maternal weight gain. Maternal prepregnancy BMI was positively correlated with fetal high frequency and was negatively correlated with low frequency and low/high frequency ratio. Maternal ISI showed a negative correlation with fetal HR.

Conclusions: The results show that the fetal ANS is sensitive to alterations of prepregnancy BMI, weight changes, and glucose metabolism. These findings highlight the importance of the intrauterine environment on the developing ANS and the possible programming of obesity.

Study Importance

What is already known?

- The number of women with prepregnancy obesity is increasing.
- Epidemiological studies provide evidence for an adverse effect on the offspring in later life.

What does this study add?

- This study used magnetocardiogram, a noninvasive assessment of fetal heart activity, which allows the extraction of fetal heart rate and variability measures.
- Increased prepregnancy weight, gestational weight gain, and maternal insulin resistance are associated with adverse effects on the development of the fetal autonomic nervous system.
- We suggest novel mechanisms predisposing humans during gestation for later health problems in relation to obesity and pregnancy.

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Introduction

Obesity has become a major health concern and continues to rise worldwide in both men and women and across all age groups (1). According to the World Health Organization, approximately 2.8 million people die each year worldwide as a result of either obesity or overweight (2). Together with the rising prevalence of obesity among women of reproductive age (3), more than 40% of women exceed the Institute of Medicine's guidelines for optimal weight gain during pregnancy in the United States (4). Prenatal exposure to maternal obesity often results in infants with higher birth weights and a greater risk for obesity in later life than those born to women without obesity (5). Indeed, meta-analyses have suggested that excessive maternal weight gain can predispose the offspring to develop obesity in later life (6).

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In general, the long-lasting effects of challenges during intrauterine development have been summarized in "developmental origins of health and disease" (7). Barker et al. initially developed the theory of fetal programming on the basis of their observation that a poor intrauterine environment can interrupt metabolic signaling and lead to dysfunction of metabolic systems controlling food intake and storage, thus resulting in obesity (8,9). Further studies broadened this concept by including additional intrauterine challenges such as stress and exposure to environmental factors and provided further evidence that a variety of metabolic, cardiovascular, and neurological diseases can be triggered (10).

Intrauterine environment, including maternal nutrition, could permanently alter and program the fetal autonomic nervous system (ANS) (11-13). The fetal ANS could be investigated by magnetic and electric recordings of the heart activity and the extraction of heart rate (HR) and heart rate variability (HRV). In healthy pregnancies, fetal HR decreases across gestation, and various HRV parameters increase (14-16). HR and HRV can been used as indicators for fetal maturation. Fetuses from mothers with gestational diabetes mellitus (GDM) showed lower postprandial fetal HRV, suggesting that the development of ANS in fetuses exposed to hyperglycemia is impaired (17). GDM and variations in maternal insulin sensitivity (ISI) are also known to affect the central nervous system (18,19).

The aim of the current study was to evaluate the influence of prepregnancy BMI, maternal weight gain, and maternal ISI on the fetal HR and HRV by determining the role of the intrauterine environment on the fetal programming of autonomic function. We examined the fetal HR and HRV in a large sample of individuals. We hypothesized that high prepregnancy BMI, excessive maternal weight gain, and maternal insulin resistance in healthy pregnancies all increase fetal HR and decrease fetal HRV, suggesting that the fetal ANS is less mature.

Methods

Study population

A magnetocardiogram (MCG) was recorded for the duration of 15 minutes in 184 pregnant women. In six of the recordings, the MCG could not be extracted because of the low signal to noise ratio, resulting in a total number of 178 included data sets. In a subsample comprising 104 participants, an oral glucose tolerance test (OGTT) was performed. For anthropometric and metabolic characteristics of the participants, see *Results* and Table 1.

Written informed consent was received from participants prior to all measurements. The Ethics Committee of the Medical Faculty of the University of Tübingen, Germany, approved the study plan. Self-reported health information was collected, including past medical history, current health condition, maternal age, parity, gravidity, height, and body weight before pregnancy and at the time of measurement. Inclusion criteria included having uncomplicated pregnancies and normal perinatal outcome. Exclusion criteria included having hypertension, cardiovascular disease, diabetes mellitus, and GDM.

Participants were assigned to four groups, depending on their prepregnancy BMI: women with underweight (BMI < 18.5 kg/m^2; $n = 9$), normal weight (BMI 18.5-24.9; $n = 120$), overweight (BMI 25.0-29.9; $n = 32$), and obesity (BMI ≥ 30.0; $n = 17$).

TABLE 1 Maternal and birth outcomes for pregnant women with normal pregnancies

Variable	Range	Mean	SD
Study population, n = 178			
Gravidity	1 to 5	1.72	0.96
Parity	0 to 5	0.56	0.80
Age (y)	21 to 45	31.72	5.16
Height (m)	1.52 to 1.85	1.67	0.06
Gestational age (wk)	26 to 38	30.42	2.92
Gestational age at birth (wk)	37 to 42	40.21	1.27
Birth weight (g)	2,500 to 4,670	3,390.98	445.60
Birth length (cm)	44 to 56	51.28	2.32
Weight before pregnancy (kg)	44 to 115	66.09	12.36
Weight during pregnancy (kg)	53 to 117	74.29	12.02
BMI before pregnancy (kg/m^2)	16.8 to 42.4	23.53	3.98
BMI during pregnancy (kg/m^2)	18.2 to 42.9	26.470	3.88
Maternal weight gain (kg/wk)	−0.22 to 0.93	0.38	0.21
Subsample from OGTT participants, n = 104			
Fasting plasma glucose (mg/dl)	63 to 90	78.78	5.41
Fasting plasma insulin (pmol/L)	22 to 311	68.70	38.23
Insulin sensitivity (μmol · kg^{-1} · min^{-1} · pmol/L)	−0.52 to 0.12	0.07	0.03

OGTT, oral glucose tolerance test.

Maternal weight gain per week in the second and third trimester was calculated according to the following formula:

$$\text{Maternal weight gain} = \frac{\text{Weight}_{\text{during pregnancy}}\ (\text{kg}) - \text{Weight}_{\text{before pregnancy}}\ (\text{kg}) - 1.25\ \text{kg}}{\text{Gestational Age}\ (\text{weeks}) - 12\ \text{weeks}}$$

For the weight gain during the first trimester (12 weeks), we assumed that women gained the average recommended weight of 1.25 kg (20).

On the basis of the Institute of Medicine (20) recommendations for maternal weight gain in the respective prepregnancy BMI groups, participants were assigned to three maternal weight gain groups: below recommended weight gain (low; $n = 66$), within recommended weight gain (normal; $n = 53$), and above recommended weight gain (high; $n = 59$). Because these recommendations are different based on prepregnancy BMI, no absolute values for the weight gain groups are given.

Birth weight, birth length, and gestational age at birth of the newborns were collected from birth records ($n = 88$ female; $n = 90$ male).

Laboratory measurements and calculations

In a subsample, an OGTT (75-g glucose challenge) was performed. Blood samples were obtained at three time points: before glucose ingestion, after 60 minutes, and after 120 minutes. Calculations and laboratory procedures of the blood measures have been detailed in previous publications (18,19). Maternal fasting blood glucose and insulin were determined using the ADVIA 1800 autoanalyzer (Siemens Healthcare

TABLE 2 Description of assessed heart rate variability (HRV) parameters

	Parameters	Description	Associated autonomic function
Time domain	HR (bpm)	Heart rate	Sympathetic and parasympathetic
	Standard deviation of normal-to-normal interval, SDNN (ms)	SD of R-R intervals	Overall HRV, sympathetic and parasympathetic
	RMSSD (ms)	Root mean square of successive differences of R-R intervals	Short-term HRV, parasympathetic
Frequency domain	LFn (normalized)	Low frequency power normalized fetal: 0.08 to 0.20 Hz; maternal: 0.04 to 0.15 Hz	Sympathetic and parasympathetic
	HFn (normalized)	High frequency power normalized fetal: 0.40 to 1.70 Hz; maternal: 0.15 to 0.40 Hz	Primarily parasympathetic
	LF/HF	Ratio of LF to HF power	Sympathovagal balance

[a]HFn, normalized high frequency; HR, heart rate; HRV, heart rate variability; LF/HF, low to high frequency ratio; LFn, normalized low frequency component; RMSSD, root mean square of successive differences of R-R intervals; SDNN, standard deviation of normal-to-normal interval.

Diagnostics, Los Angeles, California) and the ADVIA Centaur XP immunoassay system (Siemens AG, Munich, Germany), respectively. Maternal ISI was calculated in units of μmol · kg^{-1} · min^{-1} · pmol/L, according to the following formula (21):

$$\begin{aligned}&\text{Maternal insulin sensitivity}\\&=0.156-0.0000459\cdot \text{Ins}_{120\text{min}}-0.000321\cdot \text{Ins}_{0\text{min}}\\&\quad-0.00541\cdot \text{Glu}_{120\text{min}}\end{aligned}$$

$\text{Ins}_{120\text{min}}$ and $\text{Glu}_{120\text{min}}$ are insulin and glucose levels 120 minutes after glucose challenge, and $\text{Ins}_{0\,\text{min}}$ is fasting-state insulin level.

Data acquisition

All MCG measurements were performed with the "SARA" (or SQUID Array for Reproductive Assessment, VSM MedTech Ltd., Port Coquitlam, Canada) system in the fMEG Center at the University of Tübingen, a system specifically developed for fetal magnetoencephalography measurements. The system consists of 156 primary magnetic sensors and 29 reference sensors. Primary magnetic sensors are distributed over a concave array that is shaped to match the form of the gravid abdomen. During the measurements, the mother leans forward in a comfortable resting position, with minimal pressure on the abdomen. The system is located in a magnetically shielded room (Vakuumschmelze, Hanau, Germany) to attenuate external magnetic fields, and it allows for simultaneous recording of maternal and fetal MCG signals (22). Spontaneous MCG without any stimulation was recorded continuously for a period of 15 minutes at a sampling frequency of 610.4 Hz.

Extraction of time intervals between consecutive R peaks

Time differences between consecutive R peaks (R-R intervals) of the maternal and fetal heart signals were extracted using the following methods: First, maternal R peaks were detected and marked with the template matching technique (23) or with adaptive Hilbert transformation (24), and maternal R-R intervals were extracted. To extract the fetal R peaks, maternal MCG was attenuated by signal space projection (23,25). In the resulting data set, fetal R peaks were detected, and fetal R-R intervals were extracted by identical methods. Before extraction of maternal R-R intervals, data were high-pass filtered at 0.5 Hz, and fetal RR data were extracted after application of a band-pass filter between 1 and 50 Hz.

HRV analysis

The analyses, including preprocessing of RR time series and HR and HRV analysis, were performed by in-house routines in MATLAB (Mathworks, Inc., Natic, Massachusetts). The in-house routines were based on the following standard methods: Ectopic beats were detected and replaced by an adaptive filtering method (26). Low frequency trends in the RR time series were eliminated by detrending (27). The short-term HRV analysis was performed using a standard approach (28) in both the time and frequency domains. For maternal HRV, we segmented the 15 minutes of data into 5-minute segments and computed the HRV for each segment. We then took the average of the HRV from the three periods for our analysis. On the basis of the procedure by Schneider et al. (29), fetal HRV was analyzed from segments of 256 fetal heart beats. Assuming an average fetal HR of 140 beats per minute (bpm), the segments were roughly 2 minutes long. For our analysis, we calculated the HR and time- and frequency-related parameters for HRV (see Table 2, which also displays the assumed influence of sympathetic and parasympathetic influence of ANS on each parameter). Frequency bands as proposed by David et al. (30) were used for the HRV analysis in the fetus.

Statistical analysis

All statistical analyses were performed with SPSS Statistics software version 23 (IBM Corp., Armonk, New York), and results at a significance level of $P<0.05$ were regarded as statistically significant. A one-way ANCOVA was performed separately for the main factors prepregnancy BMI (four levels) and maternal weight gain (three levels). In addition, a partial correlation analysis was performed to investigate the associations between anthropometric and metabolic maternal parameters and maternal and fetal HR and HRV. Data are presented in mean (SEM). Bonferroni-Holm correction was applied, and corrected significance levels were used for multiple comparisons. To determine possible causal effects, we performed mediation analysis using the PROCESS macro in SPSS (31). Bootstrapping with 95% confidence intervals was applied to determine the significance of mediation effects (32).

Results

Participants

In the main sample of 184 participants, gestational age ranged between 26 and 38 weeks (mean [SEM], 30.37 [2.91]), maternal age was 31.7 (5.3) years, prepregnancy BMI was 23.59 (3.96), and weight gain

TABLE 3 Fetal autonomic nervous system (ANS) parameters in prepregnancy BMI and maternal weight gain groups

	Prepregnancy BMI					Maternal weight gain			
Variable	UW ($n=9$)	NW ($n=120$)	OW ($n=32$)	OB ($n=17$)	*P* value	Low ($n=66$)	Normal ($n=53$)	High ($n=59$)	*P* value
HR (bpm)	143.41 ± 2.27	140.25 ± 0.62	140.37 ± 1.20	144.99 ± 1.66	**0.040**	141.73 ± 0.86	139.75 ± 0.95	140.95 ± 0.91	0.305
SDNN (ms)	8.19 ± 0.98	9.52 ± 0.27	9.16 ± 0.52	8.78 ± 0.72	0.471	9.11 ± 0.36	10.34 ± 0.39	8.64 ± 0.38	**0.007**
RMSSD (ms)	4.13 ± 0.75	5.30 ± 0.21	5.60 ± 0.40	5.54 ± 0.55	0.375	5.22 ± 0.28	5.92 ± 0.31	4.88 ± 0.29	0.048
LFn	0.84 ± 0.04	0.81 ± 0.01	0.78 ± 0.02	0.76 ± 0.03	0.174	0.80 ± 0.020	0.81 ± 0.02	0.79 ± 0.02	0.859
HFn	0.16 ± 0.04	0.19 ± 0.01	0.22 ± 0.02	0.24 ± 0.03	0.171	0.20 ± 0.02	0.20 ± 0.02	0.21 ± 0.02	0.857
LF/HF	6.20 ± 1.14	5.95 ± 0.31	4.99 ± 0.61	4.69 ± 0.83	0.304	5.72 ± 0.43	5.89 ± 0.47	5.41 ± 0.45	0.763

Data presented are mean ± SEM. *P* values represent ANCOVA main effects of prepregnancy BMI and maternal weight gain (covariates: gestational age, gender, and parity) on fetal ANS parameters. *P* values < 0.05 are considered statistically significant and are marked in bold.
ANS, autonomic nervous system; bpm, beats per minute; HFn, normalized high frequency; High, above recommended weight gain; HR, heart rate; LF/HF, low to high frequency ratio; LFn, normalized low frequency component; Low, below recommended weight gain; Normal, within recommended weight gain; NW, normal weight; OB, obesity; OW, overweight; RMSSD, root mean square of successive differences of R-R intervals; SDNN, standard deviation of normal-to-normal interval; UW, underweight.

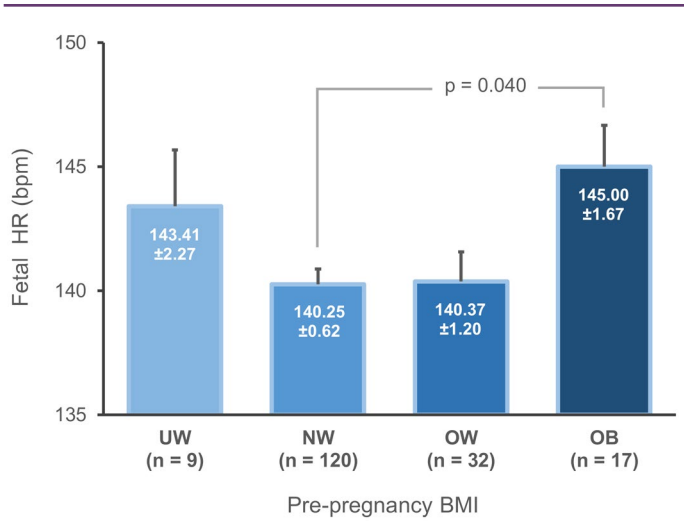

Figure 1 Relationship between fetal HR and prepregnancy BMI. Heart rate differed significantly between the different prepregnancy BMI groups ($P=0.040$). This effect was mainly due to a difference between mothers with obesity and normal weight ($P=0.040$; not significant after Bonferroni-Holm correction). Data presented are mean ± SEM. bpm, beats per minute; HR, heart rate; NW, normal weight; OB, obesity; OW, overweight; UW, underweight. [Color figure can be viewed at wileyonlinelibrary.com]

was 0.38 (0.21) kg/wk. Neonatal birth weight was 3,387.9 (440.72) g. Detailed anthropometric and metabolic characteristics of the study population ($n=178$) and the subsample from OGTT participants ($n=104$) are depicted in Table 1.

Maternal weight and weight gain

Fetal HR and prepregnancy BMI. The ANCOVA containing the four weight groups (covariates: gestational age, gender, and parity) revealed a significant main effect of prepregnancy BMI on fetal HR ($F(3)=2.84$; $P=0.040$; Table 3). Post hoc analysis showed that fetuses of mothers with obesity had a higher HR than those of normal-weight mothers (145.00 [1.66] vs. 140.25 [0.62]; $P=0.040$). This is, however, not significant after Bonferroni-Holm correction. Figure 1 shows a U-shaped pattern of fetal HR with lower values in the normal-weight group than in the groups outside the normal weight range in either direction. Partial correlations showed no significant relationships between prepregnancy BMI and fetal HR. Maternal HR and HRV (covariate: maternal HR) showed no significant correlations with any maternal weight variables ($P>0.3$; Table 4).

Fetal HR and maternal weight gain. The ANCOVA for the three weight gain groups (covariates: gestational age, gender, and parity) revealed no significant main effect on fetal HR ($F(2)=1.20$; $P=0.305$; Table 3). Maternal weight gain was significantly negatively correlated with fetal HR ($P=0.041$; covariates: gestational age, gender, and parity; Table 4). However, after prepregnancy BMI was added as an additional covariate, no significant correlation was found ($r=-0.132$; $P=0.083$).

Fetal HRV and prepregnancy BMI. The ANCOVA revealed no significant main effect of prepregnancy BMI group on any HRV parameter ($P>0.2$; covariates: gestational age, gender, and parity; Table 3). Prepregnancy BMI showed a positive correlation with the normalized high frequency component (HFn) ($P=0.021$) and a negative correlation

TABLE 4 Partial correlation coefficients between maternal HRV, fetal HRV, and maternal outcomes

	preBMI		MWG		ISI		Ins		Glu	
	r_{mat}	r_{fet}	r_{mat}	r_{fet}	r_{mat}	r_{fet}	r_{mat}	r_{fet}	r_{mat}	r_{fet}
HR (beats/min)[a]	0.052	0.110	−0.070	**−0.138***	**−0.218***	**−0.236***	**0.252****	**0.241****	0.027	0.169
SDNN (ms)	−0.023	−0.077	−0.015	−0.011	−0.146	0.117	0.023	−0.107	−0.166	−0.017
RMSSD (ms)	−0.003	0.076	−0.034	−0.091	**−0.203***	−0.050	0.083	0.069	−0.175	−0.014
LFn	−0.027	**−0.175***	−0.072	0.082	**0.228***	**0.269****	−0.031	**−0.253***	−0.044	−0.029
HFn	0.027	**0.175***	0.072	−0.081	**−0.228***	**−0.269****	0.031	**0.253***	0.044	0.029
LF/HF	0.032	**−0.168***	−0.077	0.090	0.147	**0.220***	0.084	−0.178	−0.051	0.002

[a]Values for maternal HR are Pearson correlation coefficients.
*$P<0.05$.
**$P<0.01$.
Correlation coefficients with P values <0.05 are considered statistically significant and are marked in bold.
r_{mat} is partial correlation coefficient for maternal HRV adjusted for maternal heart rate. r_{fet} is partial correlation coefficient for fetal variables, adjusted for gestational age, parity, and gender.
bpm, beats per minute; Glu, maternal fasting glucose; HFn, normalized high frequency; HR, heart rate; HRV, heart rate variability; Ins, maternal fasting insulin; ISI, maternal insulin sensitivity; LF/HF, low to high frequency ratio; LFn, normalized low frequency component; MWG, maternal weight gain; preBMI, prepregnancy body mass index; RMSSD, root mean square of successive differences of R-R intervals; SDNN, standard deviation of normal-to-normal interval.

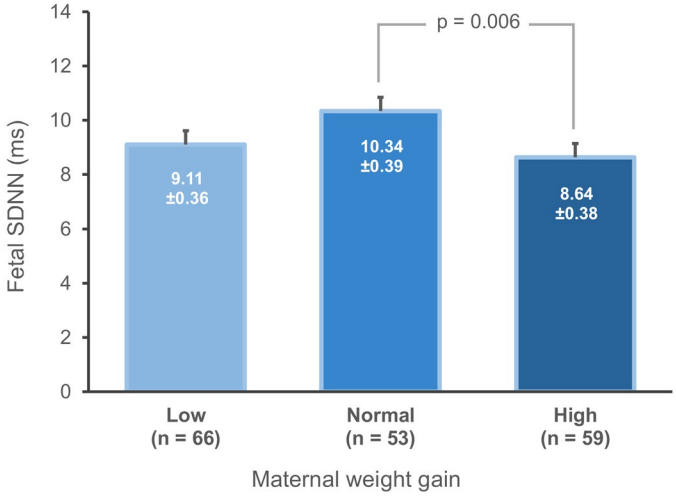

Figure 2 Relationship between fetal HRV and maternal weight gain. Fetal SDNN differed significantly between maternal weight gain groups ($P=0.010$). There were significant differences between SDNN in fetuses of mothers with high weight gain and normal weight gain ($P=0.010$). Data presented are mean ± SEM. HRV, heart rate variability; Low: below recommended weight gain; Normal: within recommended weight gain; High: above recommended weight gain; SDNN, standard deviation of normal-to-normal interval. [Color figure can be viewed at wileyonlinelibrary.com]

with the normalized low frequency component (LFn) ($P=0.021$) and the low to high frequency ratio (LF/HF) ($P=0.026$; Table 4).

Fetal HRV and maternal weight gain. The ANCOVA showed a significant main effect of the maternal weight gain group on fetal standard deviation of normal-to-normal interval (SDNN) ($F(2)=5.17$; $P=0.007$; covariates: gestational age, gender, and parity; Table 3). Post hoc analysis revealed that SDNN in fetuses of mothers with high weight gain was significantly lower than those of mothers with normal weight gain, even after Bonferroni-Holm correction (SDNN: 8.64 [0.38] vs. 10.34 [0.39]; $P=0.006$). The overall effect of the maternal weight gain group on SDNN resulted in an inverted U-shaped relationship with lower values for mothers with pregnancy weight gain lower and higher than the recommended range (Table 3; Figure 2). No significant correlation was found between any of the fetal HRV variables and maternal weight gain ($P>0.2$; covariates: gestational age, gender, and parity; Table 4). The correlation remained nonsignificant after adding prepregnancy BMI as an additional covariate ($P>0.3$).

Maternal glucose metabolism

Maternal HR, maternal HRV, and maternal metabolism in fasting state. Maternal HR showed a significant positive correlation with maternal fasting insulin ($P=0.010$) and a negative correlation with ISI ($P=0.026$). Using maternal HR as a covariate for maternal HRV, maternal ISI showed a negative correlation with the root mean square of successive differences of R-R intervals (RMSSD) ($P=0.040$) and HFn ($P=0.020$) and a positive correlation with LFn ($P=0.020$; Table 4).

Fetal HR and maternal metabolism in fasting state. Fetal HR was positively correlated with maternal fasting insulin ($P=0.010$) and negatively correlated with maternal ISI ($P=0.026$; covariates: gestational age, gender, and parity; Table 4). No significant correlation was observed between fetal HR and maternal fasting glucose ($P=0.100$; covariates: gestational age, gender, and parity; Table 4).

Fetal HRV and maternal metabolism in fasting state. Maternal fasting insulin showed a positive correlation with HFn ($P=0.012$) and a negative correlation with LFn ($P=0.012$; covariates: gestational age, gender, and parity; Table 4). There was a positive correlation between maternal ISI and LFn ($P=0.008$) and LF/HF ($P=0.030$) and a negative correlation with HFn ($P=0.008$; covariates: gestational age, gender, and parity; Table 4). Because prepregnancy BMI was shown to be significantly associated with maternal ISI, the test was repeated to control for the influence of prepregnancy BMI. The correlation remained significant in HFn ($r=-0.222$; $P=0.031$) and LFn ($r=0.222$; $P=0.031$) but not in LF/HF ($r=-0.199$; $P=0.054$). No associations

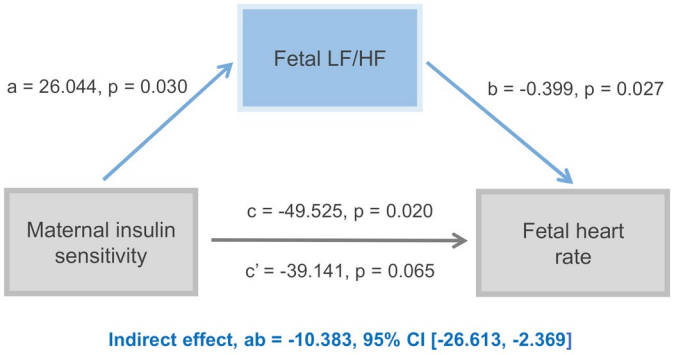

Figure 3 Predicting fetal heart rate (HR) from maternal insulin sensitivity (ISI) mediated by fetal HRV. There is a significant indirect effect of maternal ISI on fetal HR through fetal LF/HF, represented by path ab. Path a, the effect of maternal ISI on fetal low to high frequency ratio (LF/HF) is significant; path b, the effect of fetal LF/HF on fetal HR is significant; path c', direct effect of maternal ISI on fetal HR (after adding fetal LF/HF as the mediator) is no longer significant (covariates: gestational age, gender, and parity) compared with the significant total effect at path c, suggesting that the relationship is completely mediated by fetal LF/HF. $P<0.05$ is considered significant). a, b, c, and c' refer to path coefficient or slope. HR, heart rate; ISI, insulin sensitivity; LF/HF, low to high frequency ratio. [Color figure can be viewed at wileyonlinelibrary.com]

between fetal HRV and maternal fasting glucose were observed (Table 4).

We also investigated whether fetal HRV, quantified by fetal LF/HF ratio, is a mediator in the relationship between maternal ISI and fetal HR (as indicated by the negative correlation between maternal ISI and fetal HR). Fetal LF/HF ratio significantly mediated maternal ISI in predicting fetal HR (standardized indirect effect $ab=-10.383$; 95% bootstrap confidence interval −26.613 to −2.369; covariates: gestational age, gender, and parity). Once fetal LF/HF was added as the mediator, the direct relationship between maternal ISI and fetal HR was no longer significant ($P=0.065$; Figure 3).

Discussion

In a cross-sectional fetal MCG sample, we investigated associations of prepregnancy BMI, maternal weight gain, and maternal ISI with fetal HR and HRV. Our results show that, in uncomplicated pregnancies, prepregnancy BMI and maternal weight gain are both associated with the development of fetal autonomic function. Specifically, we found increased fetal HR in mothers with prepregnancy obesity or overweight and reduced fetal HRV in mothers with high maternal weight gain.

The four prepregnancy BMI groups showed significant differences in fetal HR. Mothers with normal weight had the lowest fetal HR. However, it should be borne in mind that the normal fetal HR is in the range of 120 to 160 bpm (33). Although the mean HR in fetuses of mothers with obesity was still in the normal range (mean fetal HR was 142 bpm), the value was higher than in the fetuses of mothers with normal weight. The fetuses in the group with underweight showed the same pattern, albeit not significant, which is possibly due to the small sample size in this group. This finding indicates a U-shaped relation, suggesting that prepregnancy BMI differences are related to alterations in fetal HR. Furthermore, regarding maternal weight gain, fetal HRV was lower in fetuses of mothers with high maternal weight gain than in those who gained weight within the recommended range, such that SDNN was reduced by 16.5%. Although the maternal weight gain group was computed according to prepregnancy BMI, we still observed a reduction in HRV in fetuses of mothers with high maternal weight gain. This is in contrast to a recent finding by Voegtline et al. (13), in which maternal weight gain was reported to have no predictive power on fetal HR and fetal HRV beyond prepregnancy BMI. The reduction in fetal HRV in mothers with high weight gain could be interpreted as an adverse effect on the fetal ANS, since reduced fetal HRV has also been observed in intrauterine growth-restricted (34) and GDM fetuses (17). In general, the lower the prepregnancy BMI, the more weight gain during pregnancy is expected, and the ranges of recommended weight gain for women with normal prepregnancy BMI are higher compared with women with obesity. Therefore, the observed relationship between prepregnancy BMI and fetal HR versus the association between maternal weight gain and fetal HRV is consistent with the previously stated increased HR in women with obesity (as they probably had a lower weight gain during pregnancy). Therefore, an increase in prepregnancy BMI is associated not only with fetal ANS changes but also with increased maternal weight gain, even in women with normal prepregnancy BMI. Different, potentially adverse intrauterine environments (whether prepregnancy underweight or obesity) as well as inadequate or excessive maternal weight gain might result in altered trajectories of ANS development.

HR and HRV alterations are driven by sympathetic and parasympathetic activity. In healthy fetal development, fetal HR decreases gradually with advancing gestational age (14,35). Maturation of parasympathetic vagal tone commences at approximately 31 weeks of gestation and continues after birth (36). This stronger parasympathetic influence causes a reduction in HR and an increase in HRV during gestation (14,15,29,33). Higher fetal HR and lower fetal HRV can be due either to an increase in sympathetic regulation or to a decrease in parasympathetic regulation. Because the majority of our study population were investigated before 31 weeks of gestation (62%; mean gestational age 30.4 weeks, ranging between 26 and 38 weeks), the observed increase in fetal HR in mothers with prepregnancy obesity or overweight was probably influenced by activity in the sympathetic rather than in the parasympathetic nervous system. In addition, the observed changes in fetal HRV were seen in measures associated with both sympathetic and parasympathetic activity, but not in those associated primarily with parasympathetic activity. This indicates that the alterations observed are probably the result of changes in sympathetic nervous system activity.

In addition, we observed by mediation analysis that reduced maternal ISI was indirectly associated with an increased fetal HR through fetal HRV (Figure 3), suggesting that the fetuses of healthy mothers with decreased ISI were probably already exposed to a less favorable metabolic environment. Our correlation analysis revealed that decreased maternal ISI and increased maternal fasting insulin were associated with an increased maternal HR and fetal HR. We also recently showed that mothers with GDM had higher HR than normoglycemic mothers (17). In addition, the autonomic regulation of fetuses of mothers with GDM also differed from that of fetuses of normoglycemic mothers. Specifically, HR in fetuses of mothers with GDM was higher both during the fasting state and after an oral glucose load. One hundred and twenty minutes after glucose load, SDNN, the measure of overall variability, was lower in fetuses of GDM mothers. On the basis of these observations, we propose that the increased maternal and fetal HR

associated with lower maternal ISI and higher maternal fasting insulin already in normoglycemic mothers could be related to higher sympathetic activity in both mother and fetus.

A meta-analysis reported a lower maternal HR in women with obesity between 31 and 40 weeks of gestation, but not at earlier gestational ages (37); it was suggested that the effect was due to a decrease in parasympathetic activity during pregnancy. We found no association between maternal weight factors and maternal HR and HRV in our sample; this may be because the majority of our participants (62%) were investigated before 31 weeks of gestation. Therefore, changes in the maternal ANS related to maternal weight most probably depend on gestational age.

An excess of glucose in the maternal circulation, even in normoglycemic mothers with prepregnancy obesity, elevates the glucose transport across the placenta, probably resulting in increased fetal insulin secretion that could lead to fetal hyperinsulinemia (38,39). Catalano et al. reported higher insulin resistance and increased adiposity in newborns of mothers with obesity (40). Altered glucose and insulin balance in fetuses may predispose offspring of mothers with obesity for obesity and metabolic disease later in life. In a study of people with obesity, insulin appeared to influence parasympathetic heart activity and hypothalamus activation (41), suggesting that insulin has an influence on the brain. Higher maternal plasma concentrations of glucose, free fatty acids, and amino acids are proposed to contribute to the permanent changes in appetite control, neuroendocrine function, and energy metabolism of the developing fetus, thereby increasing the lifelong risk of obesity (42,43).

A few limitations should be taken into account when interpreting the results: data are cross-sectional, maternal anthropometrics were self-reported, and newborn data were collected from birth records. Additionally, the reported significant correlations showed only weak linear relationships between the maternal and fetal factors, despite our large sample size covering a wide range of maternal values. The grouped analyses indicated nonlinear relationships, which should be taken into account in further studies.

The main strength of the current study is the use of MCG, which allows reliable assessment of fetal HR and HRV because of the high temporal resolution. This is specifically relevant in individuals with obesity, in whom recording with electrocardiography may be difficult because of the low signal to noise ratio. Longitudinal studies of maternal and fetal parameters with long-term follow-ups during further offspring development are necessary to further investigate the relevance of maternal prepregnancy BMI, maternal weight gain, and maternal ISI to the fetal ANS and its contribution to the development of offspring obesity.

Conclusion

In summary, these findings suggest that the development of the fetal ANS may be influenced by maternal weight and metabolic factors in the context of prepregnancy obesity. In addition, excessive maternal weight gain and insulin resistance might contribute to an increase in the offspring's risk of developing obesity. Fetal ANS has been shown to be an important mediating factor by which maternal factors might contribute to the programming of obesity. Maternal metabolic factors are interconnected and are linked to fetal ANS activity, forming a part of the complex mechanism affecting fetal adaptation to the intrauterine environment. To increase our understanding of the mechanisms linking the intrauterine environment and the programming of obesity, long-term follow-ups of mothers and their offspring in a larger cohort would be beneficial to determine which associations persist into childhood. O

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