Relationship between metabolic and anthropometric maternal parameters and the fetal autonomic nervous system

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Dedicated to my beloved mother, Hanishah Yaacob and my beloved husband, Azlan Mohd Nor. Each of whom has been an incomparable inspiration, blessed me with abundant love and wisdom, who helped to learn what is important in life.

To my beloved son, Aqidd and my beloved daughter Humaira, you are the best things that ever happened in my life.

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Summary

Pre-pregnancy obesity, defined as a body mass index (BMI) greater than or equal to 30 kg/m^2, can have adverse effects on the health of newborns and can also lead to metabolic, cardiovascular and neurological diseases in the offspring as they grow older. In the area of fetal origins and disease in adult life, a large number of studies have reported a critical role for maternal weight and metabolism before or during gestation in shaping the health of their offspring. Maternal obesity is recognised as a major modifiable contributor to obesity and metabolic syndrome in offspring, but the underlying factors remain unclear. The fetal autonomic nervous system (ANS) is subject to programming during developmental periods and is considered one of the processes by which early programming of disease can take place.

The main goal of the present work was to use the fetal heart rate (HR) and heart rate variability (HRV) as proxies for the fetal ANS to study the effects of metabolic and anthropometric maternal (MAM) parameters before and during gestation on the fetuses of healthy, normoglycemic mothers. A total of 184 women in their second/third trimesters of uncomplicated pregnancies were included in this study. Pre-pregnancy BMI and maternal weight gain during pregnancy were recorded. In a subsample (n = 104), maternal insulin sensitivity was measured during an oral glucose tolerance test. Fetal HR and HRV were determined by magnetic recording in all subjects. The influence of pre-pregnancy BMI, maternal weight gain and maternal insulin sensitivity on fetal HR and HRV was evaluated. Associations between MAM parameters and maternal HR and HRV were also assessed. ANCOVA, partial correlation and mediation analysis were applied, all of which were adjusted

for gestational age, gender and parity. A regression on fetal HR using a machine learning approach was tested to explore which maternal factor is the driving factor programming the fetal ANS. Four models were tested: Linear regression, Regression Tree, Support Vector Machine and Random Forest.

The fetal HR was higher in fetuses of mothers with high pre-pregnancy BMI (overweight/obese) than in mothers with normal weight. The fetal HRV was lower in mothers with high weight gain than in mothers with normal weight gain. The fetal HR was negatively correlated with maternal weight gain and maternal insulin sensitivity. Pre-pregnancy BMI was positively correlated with fetal high frequency and negatively correlated with low frequency and the low to high frequency ratio. Maternal weight gain was associated indirectly with birth weight through fetal HR, while maternal insulin sensitivity was associated with fetal HR through fetal HRV. Separately, fetal HRV was associated with birth weight through the fetal HR. The Random Forest ensemble tree-based model outperformed linear regression as the fetal HR regression model. Fetal HR can be predicted using the following nine relevant variables (sorted from the most important to the least important): pre-pregnancy BMI, gender, maternal fasting insulin, maternal insulin sensitivity, gravidity, maternal age, maternal fasting glucose, gestational age and maternal weight gain. Pre-pregnancy BMI appeared to be the major factor predicting fetal HR. In conclusion, the fetal ANS is sensitive to maternal metabolic and anthropometric influences, and particularly maternal weight before pregnancy. These findings support the concept of the “Developmental Origin of Health and Disease” and increase our knowledge about the importance of the intrauterine environment in the programming of the ANS and the possible programming of disease in later life.

Contents

Abbreviations

BMI	Body mass index
BPM	Beat per minute
DOHaD	Developmental Origins of Health and Disease
ECG	Electrocardiogram
fMEG	Fetal magnetoencephalography
GDM	Gestational diabetes mellitus
GLU	Maternal fasting blood glucose
HF	High frequency power
HR	Heart rate
HRV	Heart rate variability
Hz	Hertz
INS	Maternal fasting blood insulin
ISI	Insulin sensitivity
IUGR	Intrauterine growth restriction
LF	Low frequency power
LF/HF	Ratio of low frequency to high frequency power
MAM	Metabolic and anthropometric maternal
MCG	Magnetocardiogram
MEG	Magnetoencephalography
MS	Millisecond
MWG	Maternal weight gain
NW	Normal weight
OGTT	Oral glucose tolerance test
OW	Overweight
OB	Obese
PT	Pico Tesla
PREBMI	Pre-pregnancy BMI
RMSSD	Root mean square of successive differences of RR intervals
RR	Succesive R waves between QRS complexes
SDNN	Standard deviation of RR interval
UW	Underweight

Chapter 1

General introduction

Obesity, defined as a body mass index (BMI) greater than or equal to 30 kg/m^2, has become a worldwide epidemic and continues to rise (WHO, 2018) . Some studies have reported that maternal obesity might expose women's offspring to long-term health effects, including obesity (Alfaradhi & Ozanne, 2011; Drake & Reynolds, 2010; Lecoutre & Breton, 2015; Poston, Harthoorn, & Van Der Beek, 2011; Wankhade, Thakali, & Shankar, 2016). Taking a closer look at the societal "overnutrition" trend, infants of women with obesity often have higher birth weights and a greater risk of obesity in later life when compared with infants born to women without obesity (Godfrey et al., 2017; Taylor & Poston, 2007). Women with obesity before pregnancy also appear to have offspring with obesity in childhood and adulthood (Drake & Reynolds, 2010). Moreover, recent meta-analyses suggest that excessive maternal weight gain can predispose the offspring to develop obesity in later life (Leonard, Petito, Rehkopf, Ritchie, & Abrams, 2017; Mamun, Mannan, & Doi, 2014; Nehring, Lehmann, & von Kries, 2013).

For the past thirty years, epidemiological studies have shown that diseases are programmed during early life development and involve interactions between inheritance, both genomic and development, and environment (Gluckman, Hanson, & Buklijas, 2010; Hanson & Gluckman, 2014). A study of the Dutch Hunger Winter victims was the earliest to link early life nutrition to later obesity (Ravelli, Stein, & Susser, 1976). The researchers found that offspring born to women exposed to famine in early pregnancy were more likely to become obese in later life. The emergence of the fetal programming theory emerged from a later series of epidemiological studies by David Barker and colleagues in 1986 and onwards (Barker & Osmond, 1986; Barker, Osmond, Golding, Kuh, & Wadsworth, 1989; Barker, Osmond, Winter, Margetts, & Simmonds, 1989). They proposed that maternal undernutrition during pregnancy might interrupt metabolic signalling in fetuses and may cause dysfunction of the metabolic systems controlling fetal food intake and storage, thereby resulting in cardiovascular diseases in adulthood (Barker, 1995; Godfrey & Barker, 2001). Further studies broadened this concept by including additional stressors like maternal stress and environmental chemical exposures, and provided further evidence for the possibility that a variety of metabolic, cardiovascular and neurological diseases can be triggered during fetal development (Barouki, Gluckman, Grandjean, Hanson, & Heindel, 2012; Reynolds, 2013). This theory is known today as DOHaD, short for “Developmental Origins of Health and Disease” (Gluckman & Hanson, 2006; Suzuki, 2018).

Overall, these previous findings suggest that maternal weight and metabolism could shape offspring development. Despite many studies reporting evidence of unfavourable outcomes in offspring of mothers with obesity and excessive maternal

weight gain, the possible underlying processes that could link changes in maternal weight and metabolism to the health of offspring are not well understood.

Autonomic nervous system (ANS) is the main system responsible for energy balance and regulation of body weight (Hall, 2015; Landsberg, Saville, & Young, 1984; Young, 2002). A comparably low HRV, is a common feature of childhood and adulthood obesity, indicating an imbalance in the ANS (Thayer, Yamamoto, & Brosschot, 2010). Adults with low birth weight tend to have a high resting pulse rate (Phillips & Barker, 1997). These observations suggested that the programming of the ANS is established during fetal development and might be one of the system involve in the disease development in adulthood. Also, Young suggested that programming of ANS in utero is linked to the development of obesity (2002). Although limited data are available relating birth weight and ANS in adults (Phillips & Barker, 1997; Weyer, Pratley, Lindsay, & Tataranni, 2000), more studies have emerged showing an association of undernourishment during fetal development and fetal HRV, an indirect measure of fetal ANS. Several studies have reported a lower fetal HRV in growth-restricted fetuses than in normal fetuses (Nijhuis et al., 2000; Schneider, Fiedler, Liehr, Kahler, & Schleussner, 2006). Conversely, fetuses of women with gestational diabetes mellitus (GDM), also had lower postprandial fetal HRV (Fehlert et al., 2016). These researchers proposed an impaired development of the ANS in fetuses exposed to hyperglycaemia. GDM and maternal insulin resistance also have a known relationship with the postprandial response in the fetal central nervous system (Linder et al., 2014; Linder et al., 2015), while pre-pregnancy obesity appears to have long-term impacts on offspring neurodevelopment (Casas et al., 2013; Hinkle et al., 2012). Furthermore, women with higher pre-pregnancy BMI also have higher perinatal insulin resistance

(measured from cord blood), suggesting a relationship within utero development of peripheral insulin resistance (P. M. Catalano, Presley, Minium, & Hauguel-de Mouzon, 2009), which could potentially program the fetal metabolic phenotype.

Given the potential for a negative effect of early life metabolic influences on the development of fetal ANS, and coupled with the fact that maternal weight or weight gain can, at least to a point, be modified, this modifiable risk factor for impairment in fetal ANS is particularly worthy of study. The objective of this thesis was to broaden the current knowledge of the potential role of intrauterine challenges linked with maternal obesity in fetuses of healthy mothers on the development of fetal ANS – a process by which early programming of disease might take place. Therefore, the research aim of this study was to explore the relationship between various metabolic and anthropometric maternal (MAM) parameters before and during gestation and the fetal HR and HRV in healthy pregnancies. Specifically, various analysis methods were used to investigate the association between maternal factors (pre-pregnancy BMI, maternal weight gain and glucose metabolism during the fasting state), and the fetal HR and HRV. The existence of the inter-relationships between MAM parameters, fetal HR, HRV and neonatal birth weight were evaluated by mediation analysis. Gestational age and parity were added as covariates in the analysis of fetal HRV, and a gender effect was tested as a potential control factor. The MAM parameters and maternal HRV were then used as features in the prediction of fetal HR using machine learning regression models. Fetal HR and HRV based on fetal magnetocardiograms (MCGs) were examined in a large sample of subjects. High pre-pregnancy BMI, excessive maternal weight gain and maternal insulin resistance in healthy pregnancies were expected to alter fetal HR and HRV.

1.1. The autonomic nervous system

The ANS regulates the internal body functions that maintain body homeostasis (Squire et al., 2008; Wehrwein, Orer, & Barman, 2016). Its nerve cells leave the spinal cord and brainstem and connect to all the major organs and glands to either inhibit or stimulate their activity (Hall, 2015). The ANS is composed of two subsystems: the sympathetic nervous system (SNS) and parasympathetic nervous system (PNS). Functionally, each system is dominant under certain conditions. The SNS is stimulated during stressful situations and elicits what are known as “fight-or-flight” responses. The PNS is responsible for quiet “rest-and-digest” activities, and opposes the effects of the SNS. Overall, the PNS conserves and stores energy to regulate basic body functions.

The main transmitter produced and released by sympathetic post-ganglionic neurones is norepinephrine, whereas the parasympathetic post-ganglionic neurones produce and release acetylcholine. This difference in neurotransmitters causes the different functions between SNS and PNS (Squire et al., 2008). Figure 1.1 shows the connection of SNS and PNS to organs in human body. The ANS consists of a series of two neurones that conduct impulses away from the central nervous system (CNS). The axon of the first neuron (called a pre-ganglionic neuron) synapses with a second neuron within an autonomic ganglion located outside the CNS. The axon of the second neuron (called a post-ganglionic neuron) extends from autonomic ganglion synapses to an organ tissue. The ANS conducts impulses from pre-ganglionic to the post-ganglionic neuron to stimulate the organ. Most organs are innervated by both systems, but some are innervated by only the SNS. The organs, such as the heart, that receive dual innervation show opposing effects

of the SNS and PNS, where an increase in the activity of one system simultaneously decreases the activity of the other. This antagonistic effect allows for the precise control of a tissue's function, resulting in the state of activity of the organ. Dynamic interplay between both systems is important for body homeostasis as well as serving as a survival mechanism (Squire et al., 2008; Wehrwein et al., 2016).

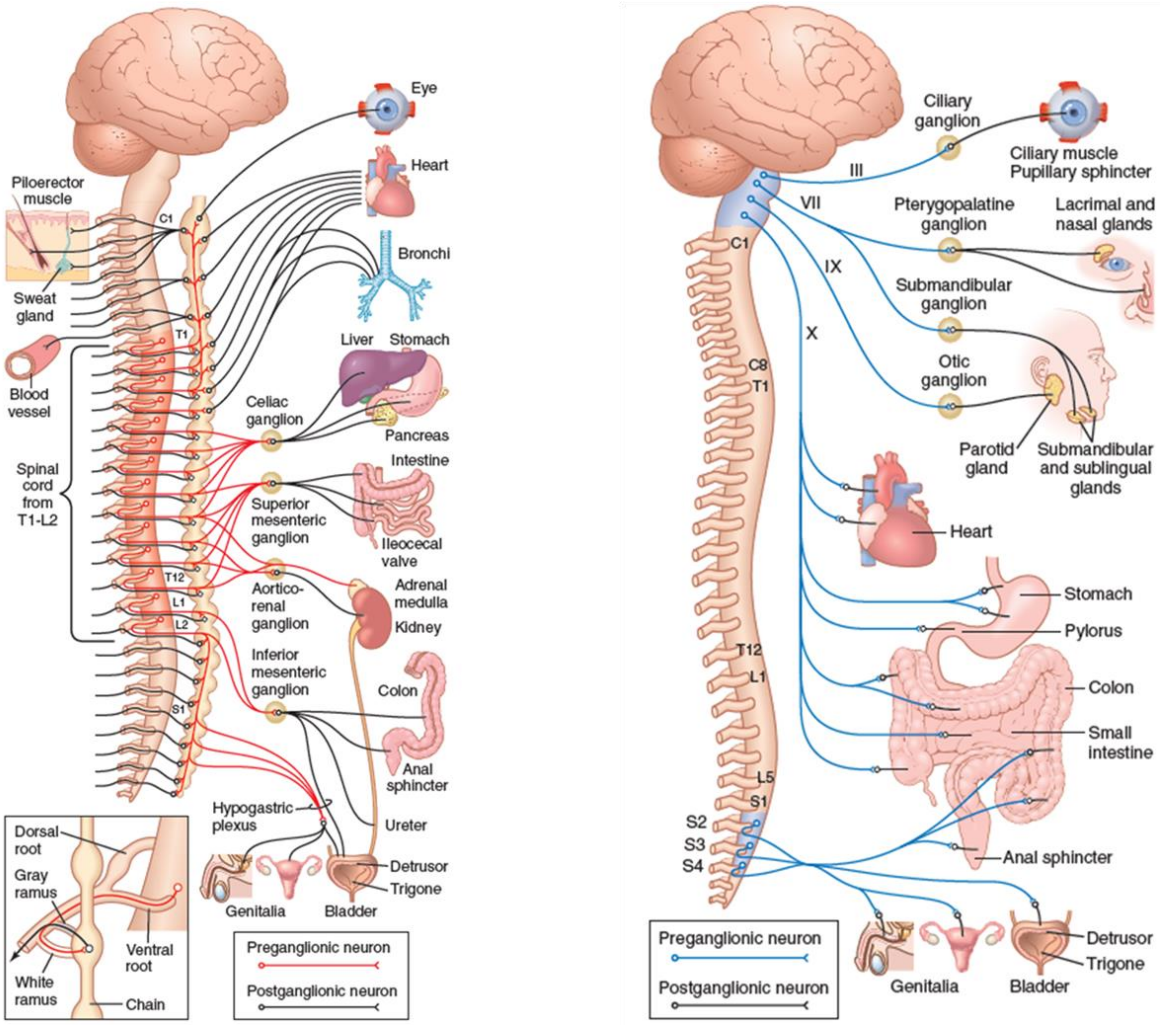


Figure 1.1 Autonomic nervous system and its two branches. Left: Sympathetic nervous system. Right: Parasympathetic nervous system. Image from Hall (2015) used by permission of Elsevier.

1.2. Autonomic control of the heart rate

Baseline HR is controlled at an internal pace that is determined by the sinoatrial (SA) node, while the ANS controls the heart rhythm through the SNS and the PNS (Figure 1.2).

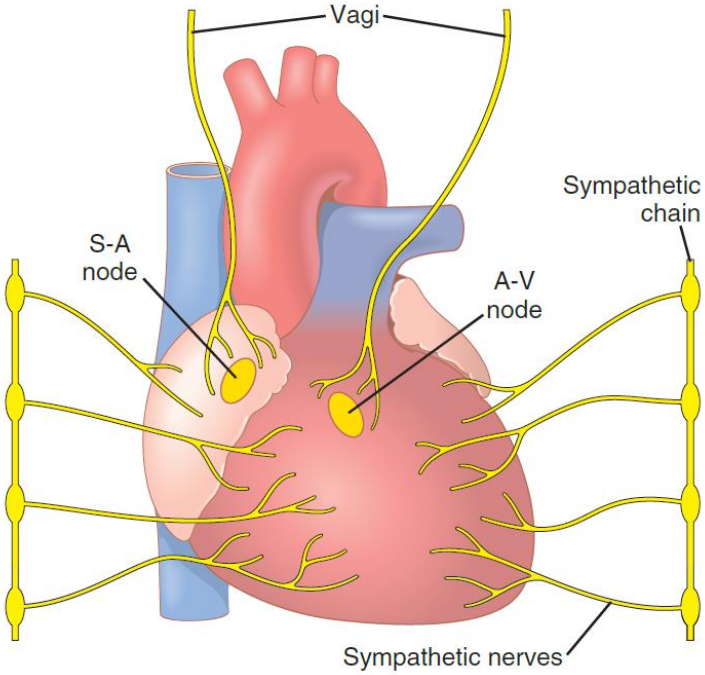


Figure 1.2 Control of the heart by sympathetic and parasympathetic nerves. Image from Hall (2015) used by permission of Elsevier.

SNS stimulation increases the HR, while PNS stimulation decreases the HR. The sympathetic nerves innervate the heart muscle throughout the atria and ventricles to provide higher pumping ability during stress (Hall, 2015; Squire et al., 2008). SNS stimulation releases norepinephrine at the nerve endings and increases the permeability to sodium and calcium ions. This causes a more positive resting potential of the heart cells at the SA node, thereby increasing the rate of self-excitation, which then leads to an increase in HR. The vagus nerve mainly

innervates the heart at the SA node and the atrioventricular (AV) node. Parasympathetic stimulation releases acetylcholine at the vagal ending. This increases the permeability of the fiber membranes to potassium, thereby leading to a decrease in the resting membrane potential of the SA node to a lower value than usual. The time needed to reach the self-excitation rate increases, resulting in slower heart beats. Parasympathetic activation to change the heart rate takes around 1.5 seconds, while sympathetic activation takes longer, around 24 seconds (Berntson et al., 1997). The differences in the speed and frequency of parasympathetic and sympathetic activation serves as the basis of spectral analysis of fetal HRV, which will be discussed in the next section.

1.3. Fetal heart rate and heart rate variability

The activity of the ANS can be estimated noninvasively by HRV analysis (Akselrod et al., 1981; Malik, 1996; Sayers, 1973). This analysis is based on consecutive heart beat variations of sinus origin. The interval between the R wave onsets of electrocardiogram (ECG) or MCG traces are typically used as the reference wave (i.e. the RR interval) to mark the heart beats since this has the largest amplitude compared to the neighbouring P, Q, S and T waves. A standard for HRV measurement, interpretation and clinical use was established in 1996 (Malik, 1996). The analysis involves deriving time and frequency domain parameters using geometrical and nonlinear methods. The time and frequency domain analyses were the methods used in this dissertation.

Fetal HR and HRV can be assessed noninvasively with magnetic sensors (Brändle et al., 2015; Fehlert et al., 2016; Van Leeuwen, Lange, Bettermann, Gronemeyer, &

Hatzmann, 1999; Wakai, 2004) or electrodes (David, Hirsch, Karin, Toledo, & Akselrod, 2007; Karin, 1994). These methods provide the necessary temporal resolution for reliable assessment of fetal HRV measures. In 1960, Hon introduced fetal HR monitoring, and he was the first to describe HRV as a clinical application in relation to maternal contractions (Hon, Reid, & Hehre, 1960). Later, he showed that fetal distress was preceded by alterations in fetal HRV even before the changes in the mean HR could be observed (Hon & Lee, 1963). Since then, fetal HRV has gained interest, particularly in pathologic conditions like intrauterine growth restriction (IUGR) pregnancy and preterm birth (Aziz, Schlindwein, Wailoo, Biala, & Rocha, 2012; Huhn et al., 2011; Schneider et al., 2006; Sriram et al., 2013). Fetal HR has been used as standard clinical procedure for fetal monitoring (Banta & Thacker, 1979; Dawes, Moulden, & Redman, 1996; Jenkins, 1989; Macones, Hankins, Spong, Hauth, & Moore, 2008) and is known as an important indicator of fetal well-being.

The normal fetal HR ranges from 120 to 160 beats per minute (bpm) (Pildner von Steinburg et al., 2013). The regulation of the fetal HR is mainly driven by parasympathetic and sympathetic innervation (David et al., 2007). The synchronization of PNS and SNS neural activity at the SA node is thought to increase or decrease the fetal HR. Decreases in fetal HR and increases in HRV indicate that parasympathetic activity is dominant, while the opposite changes occur when sympathetic activity is dominant. Such changes in both HR and HRV have been used as indicators of fetal ANS maturation (Schneider et al., 2018; Schneider et al., 2009). In healthy developing fetus, the fetal HR decreases and HRV increases in a more complex pattern throughout gestation (Pildner von Steinburg et al., 2013; Serra, Bellver, Moulden, & Redman, 2009; Van Leeuwen et al., 1999;

Wakai, 2004). In the normally developing fetal ANS, the regulation of the HR is mostly under the control of SNS because the maturation of the PNS mainly occurs after birth. Only in the third trimester, starting from 31 weeks of gestation, does the parasympathetic regulation start to increase (Pildner von Steinburg et al., 2013; Van Leeuwen, Cysarz, Edelhauser, & Gronemeyer, 2013; Wakai, 2004).

The response of fetal HR and HRV could be influenced by several other factors. Fetal HR and gestational age have been suggested to have strong influence on the fetal HRV (Lange, Van Leeuwen, Geue, Hatzmann, & Gronemeyer, 2005). In addition, gender also has an influence on the fetal HR, even though some inconsistencies exist in the reported findings. For example, no differences in fetal gender were detected at any gestational age (Bracero et al., 2016; Druzin, Hutson, & Edersheim, 1986; Fleisher, DiPietro, Johnson, & Pincus, 1997; Genuis, Genuis, & Chang, 1996; Lange et al., 2005; Ogueh & Steer, 1998), whereas DiPietro described differences in fetal HR at the second and third trimester, with a faster HR in female than in male fetuses (2015).

The following is a short overview of the current state of research regarding the influence of maternal weight and metabolism on fetal HR and HRV: Earlier studies on fetal HRV in human fetuses were mainly performed in studies on maternal undernutrition (Bekedam, Visser, Mulder, & Poelmann-Weesjes, 1987; Nijhuis et al., 2000; Schneider et al., 2006; Sriram et al., 2013). Later studies reported fetal HRV in pregnancies associated with diabetes and GDM (Costa, Nomura, Reynolds, Miyadahira, & Zugaib, 2009; Fehlert et al., 2016; Sirico, Sarno, Zullo, Martinelli, & Maruotti, 2019; Tincello, White, & Walkinshaw, 2001). Data are limited that relate maternal obesity and fetal HRV. To my current knowledge, only one study has

investigated the effect of maternal obesity on fetal HRV (Voegtline, Costigan, Henderson, & DiPietro, 2016). These researchers found lower fetal HRV and lower fetal HR acceleration in the fetuses of women with pre-pregnancy obesity than in the fetuses of women with normal weight, suggesting an impairment in fetal cardiac and motor development. One study tested the relationship between maternal insulin resistance and maternal inflammation with fetal HRV, but found no association (Dewi et al., 2017). Another study found a positive association between pre-pregnancy BMI and fetal HRV, the low to high frequency ratio (Ojala et al., 2009).

1.4. Fetal magnetoencephalography

Fetal magnetoencephalography (fMEG) is a non-invasive technique for recording magnetic fields generated by electrical currents in human tissue, with special adaptation for fetal and neonatal measurements (Preissl, Lowery, & Eswaran, 2005). The method allows the simultaneous recording of fetal brain (magnetoencephalography, MEG) and fetal heart (MCG) signals through a sensor array fit onto the mother‘s abdomen. MEG for fetal measurement was first reported in 1976 to measure fetal heart signals (Hukkinen et al., 1976) and was later used by Blum to record fetal evoked brain responses (Blum, Saling, & Bauer, 1985). The fetal adapted systems were built later. The high temporal resolution of the fetal heart signal recorded by fMEG device allows the investigation of the fetal ANS through HRV analysis (Brändle et al., 2015; Fehlert et al., 2016; Sriram et al., 2013). Fetal heart signal amplitudes range from 1 to 10 pico Tesla and are stronger than the fetal brain signals, which range from 10 to 80 femto Tesla (Vrba, Robinson, McCubbin, Murphy, et al., 2004).

1.5. Goals and hypotheses

The specific goal of this dissertation was to provide insight into the relationship of variations in MAM parameters—specifically pre-pregnancy BMI, maternal weight gain and maternal insulin sensitivity—and the ANS during fetal development. A second goal was to evaluate whether the associations of MAM and birth weight are mediated through the fetal ANS. A third goal was to predict fetal HR using regression-based machine learning algorithms from MAM parameters and maternal HRV as features.

In this dissertation, the following hypotheses were tested:

1. High pre-pregnancy BMI, excessive maternal weight gain and maternal insulin resistance in healthy pregnancies are associated with an increase in fetal HR and/or a decrease in fetal HRV.
2. Fetal ANS is one of the processes linking MAM and weight at birth.
3. Among the MAM parameters, pre-pregnancy BMI (as weight before pregnancy) has a stronger role in the programming of fetal ANS.

Chapter 2

Material and methods

2.1. Study population

An MCG was recorded for the duration of 15 minutes in 184 pregnant women. In six of the recordings, the MCG could not be extracted due to the low signal to noise ratio, resulting in a total number of 178 included datasets. In a subsample comprising 104 participants, an oral glucose tolerance test (OGTT) was performed. For the anthropometric and metabolic characteristics of the participants, see the Results and Table 3.2.

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

perinatal outcomes. Exclusion criteria included hypertension, cardiovascular disease, diabetes mellitus and GDM.

Pre-pregnancy BMI is a measure of preconception body mass index in units of kg/m^2. Based on the BMI category from the World Health Organization (WHO, 2000), participants were assigned to four pre-pregnancy BMI groups: underweight (UW, BMI < 18.5 kg/m^2; n = 9), normal weight (NW, BMI 18.5–24.9; n = 120), overweight (OW, BMI 25.0–29.9; n = 32) and obese (OB, BMI ≥ 30.0; n = 17).

Maternal weight gain, which is a measure of weight gain during gestation (in kg/week) in the 2nd and 3rd trimester, was calculated according to the following formula:

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

For the weight gain during the 1st trimester (12 weeks), participants were assumed to gain the average recommended weight of 1.25 kg (Rasmussen & Yaktine, 2009) (see Table 2.1). This value was subtracted from the total weight gain and divided by the number of gestational weeks the pregnancy had progressed in the 2nd and 3rd trimesters (gestational age during visit minus 12 weeks for the 1st trimester).

On the basis of the Institute of Medicine recommendations for maternal weight gain in the respective pre-pregnancy BMI groups (Rasmussen & Yaktine, 2009), the participants were assigned to three maternal weight gain groups: below the recommended weight gain (Low; n = 66), within the recommended weight gain

(Normal; $n = 53$) and above the recommended weight gain (High; $n = 59$). Table 2.1 summarises the recommendations and absolute values for maternal weight gain according to the pre-pregnancy BMI and maternal weight gain groups.

Table 2.1 Maternal weight status categories

Pre-pregnancy BMI (kg/m^2)		Recommended weight gain in 2nd and 3rd trimester (kg/week)*	Maternal weight gain (kg/week)		
			Low (Below limit)	Normal (Within limit)	High (Above limit)
Underweight	< 18.5	0.51 (0.44 - 0.58)	0.28 ±0.04	0.51 ±0.05	0.71 ±0.00
Normal weight	18.5 - 24.9	0.42 (0.35 - 0.50)	0.22 ±0.06	0.42 ±0.01	0.64 ±0.02
Overweight	25.0 - 29.9	0.28 (0.23 - 0.33)	0.03 ±0.06	0.26 ±0.01	0.54 ±0.03
Obese	≥ 30.0	0.22 (0.17 - 0.27)	0.05 ±0.03	0.26 ±0.01	0.45 ±0.06

Adapted from 2009 Institute of Medicine recommendations for weight gain during pregnancy. *assumes a first trimester weight gain of 0.5 - 2.0 kg. Data presented are mean ±SEM.

2.2. Laboratory measurements and calculations

In a subsample comprising 104 participants an OGTT (75 g glucose challenge) was performed. Blood samples were obtained at three time points: before glucose ingestion and 60 and 120 minutes after the challenge. Calculations and laboratory procedures of the blood measures are detailed in previous publications (Linder et al., 2014; Linder et al., 2015). Maternal fasting blood glucose and insulin were determined using the ADVIA 1800 autoanalyzer (Siemens Healthcare Diagnostics) and the ADVIA Centaur XP immunoassay system (Siemens AG), respectively. Maternal ISI is defined as the ability of insulin to increase glucose uptake to

maintain glucose homeostasis (Wilcox, 2005). Maternal ISI were calculated in units of µmol kg^{-1} min^{-1} pmol/l, according to the formula Stumvoll (2001):

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

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

2.3. Data acquisition

Figure 2.1 shows a flow chart from fMEG data acquisition to MCG data processing and HRV analysis. All MCG measurements were performed with the SARA (SQUID Array for Reproductive Assessment, VSM MedTech Ltd., Port Coquitlam, Canada) system in the fMEG Center at the University of Tübingen; this system was specifically developed for fetal measurements. The system consists of 156 primary magnetic sensors and 29 reference sensors. The primary magnetic sensors are distributed over a concave array that is shaped to match the form of the gravid abdomen (Figure 2.2). 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 (Preissl, Lowery, & Eswaran, 2004). Spontaneous MCG without any stimulation was recorded continuously for a period of 15 minutes at a sampling frequency of 610.4 Hz.

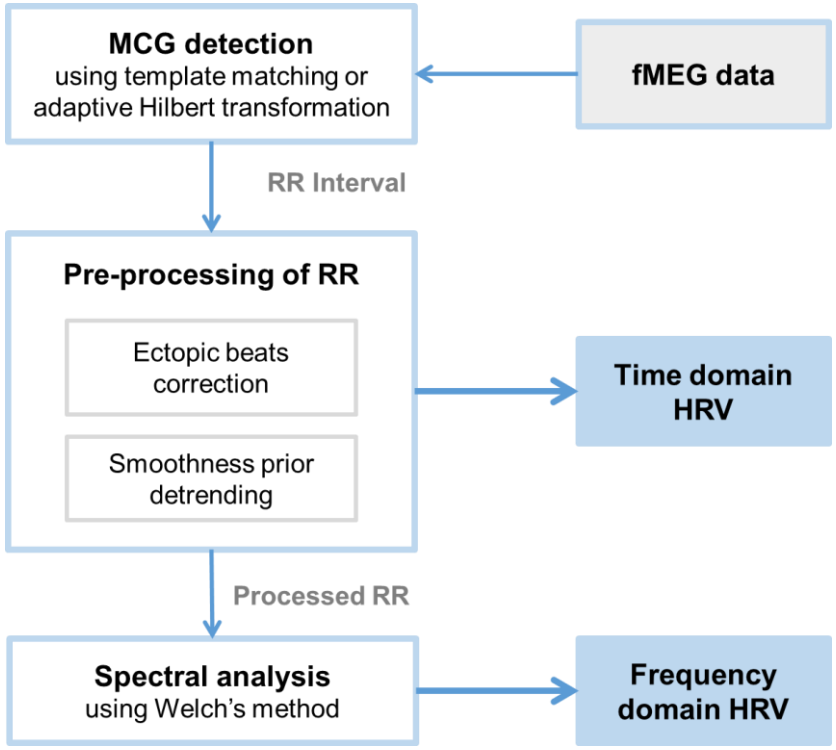


Figure 2.1 Flow chart from fMEG data acquisition to MCG data processing and HRV analysis

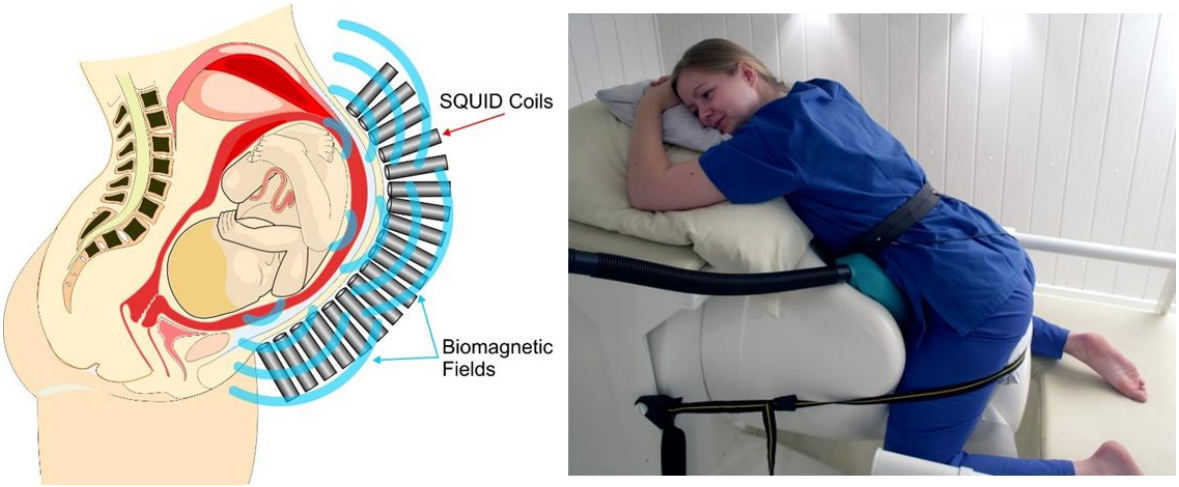


Figure 2.2 Left: Schematic of a pregnant woman on the fetal MEG device with SQUID coils that allow recording of biomagnetic fields; for example, fetal brain and fetal heart activity. Right: a pregnant mother is seated on the fetal MEG device. © University Hospital Tübingen

2.4. MCG detection and RR intervals extraction

Signals recorded from the fetal MEG device contain magnetic fields generated by electrical activity from various biological sources in the maternal body. Two major signals come from the maternal and fetal hearts—the maternal MCG and fetal MCG. For the analysis of maternal and fetal HRV, time differences between consecutive R waves (RR intervals) were acquired as an input signal and extracted from the maternal MCG and fetal MCG.

The RR intervals of the maternal and fetal heart signals were extracted using the following methods: First, the maternal MCG was detected and marked with a template matching technique (Vrba, Robinson, McCubbin, Lowery, et al., 2004) or with adaptive Hilbert transformation (Ulusar et al., 2009). The maternal RR intervals were extracted and the maternal MCG was attenuated by signal space projection (McCubbin et al., 2006; Vrba, Robinson, McCubbin, Lowery, et al., 2004). After removal of the maternal MCG, the fetal MCG was marked in the resulting dataset and the fetal RR intervals were obtained by identical methods. Before extraction of the maternal RR intervals, the data were high-pass filtered at 0.5 Hz and the fetal RR data were extracted after application of a band-pass filter between 1 and 50 Hz. Figure 2.3 shows the overlaid time series of all MEG channels before and after the maternal MCG extraction. Image in Figure 2.3 from Preissl et al. (2004) is used by permission of Elsevier.

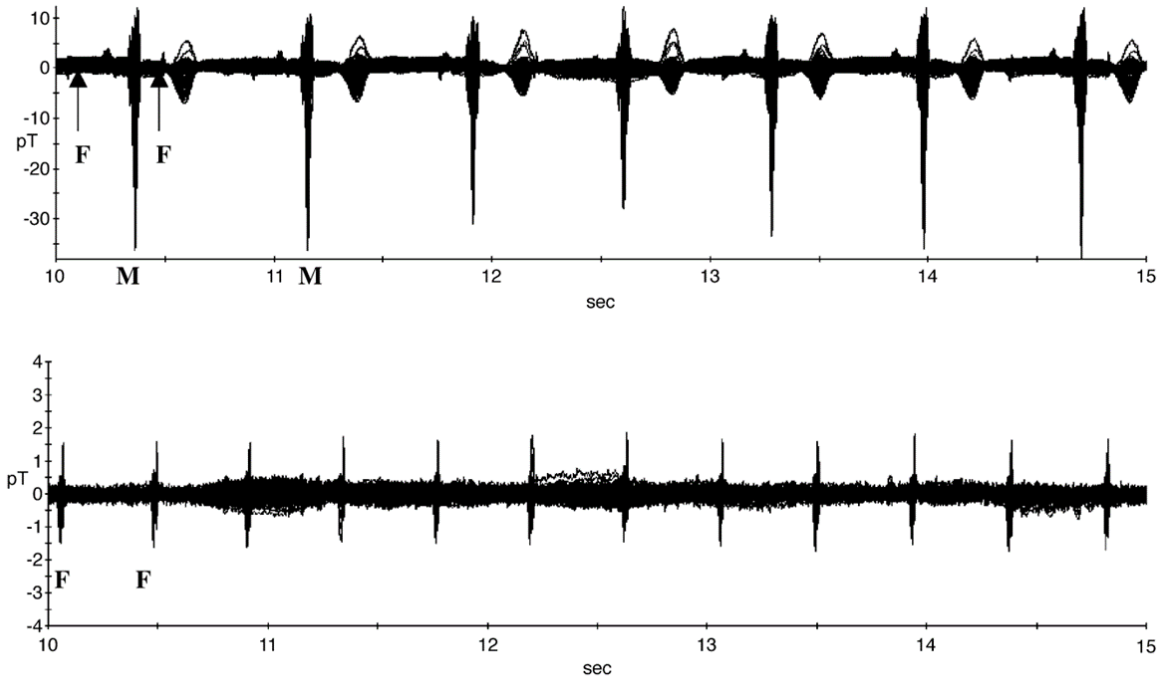


Figure 2.3 Upper: a 15 second time series of all overlaid MEG channels with visible maternal MCG (marked by "M") and fetal MCG signals (marked by "F"). Lower: the resulting time series with fetal MCG as the main component, after the extraction of maternal MCG. Image from Preissl et al. (2004) used by permission of Elsevier.

2.5. HRV analysis

The analyses, including pre-processing of RR time series and short-term HRV analysis in both the time and frequency domains, were developed and performed by in-house routines in MATLAB (Mathworks, Inc., Natic, MA, USA). The in-house routines were based on existing standard approaches and tailored for use with fetal MEG data on our system. The details of the techniques are described in the following sections.

2.5.1. Pre-processing of the RR time series

The RR interval, determined from consecutive R waves, is shown in Figure 2.4.

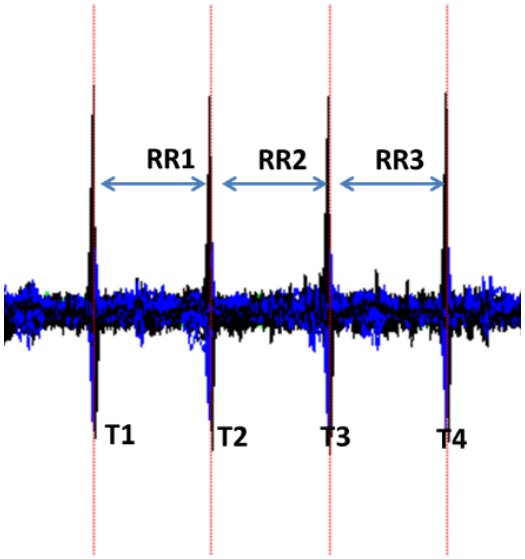


Figure 2.4 An MCG segment of overlaid all MEG channels containing 4 fetal heart beats showing the extraction of beat-to-beat interval from consecutive R waves. T is the time corresponding to the RR.

The RR time series of an MCG segment containing i beats is given by:

$$RR_i = T_{i+1} - T_i$$

where RR_i is the i_{th} RR interval in the segment, T_i is the time of the i_{th} beat occurs. The HRV is defined as the variation in the beat-to-beat interval during sinus rhythm. Therefore, any cardiac fluctuations that are not caused by the sinus node (known as ectopic beats) need to be excluded from the HRV analysis. The pre-processing steps were therefore intended to filter out noise effects and minimize the number of incorrectly detected R-peaks due to the occurrence of ectopic beats. Ectopic beats were detected and replaced by an adaptive filtering method (Wessel et al., 2000). The process is illustrated in Figure 2.5.

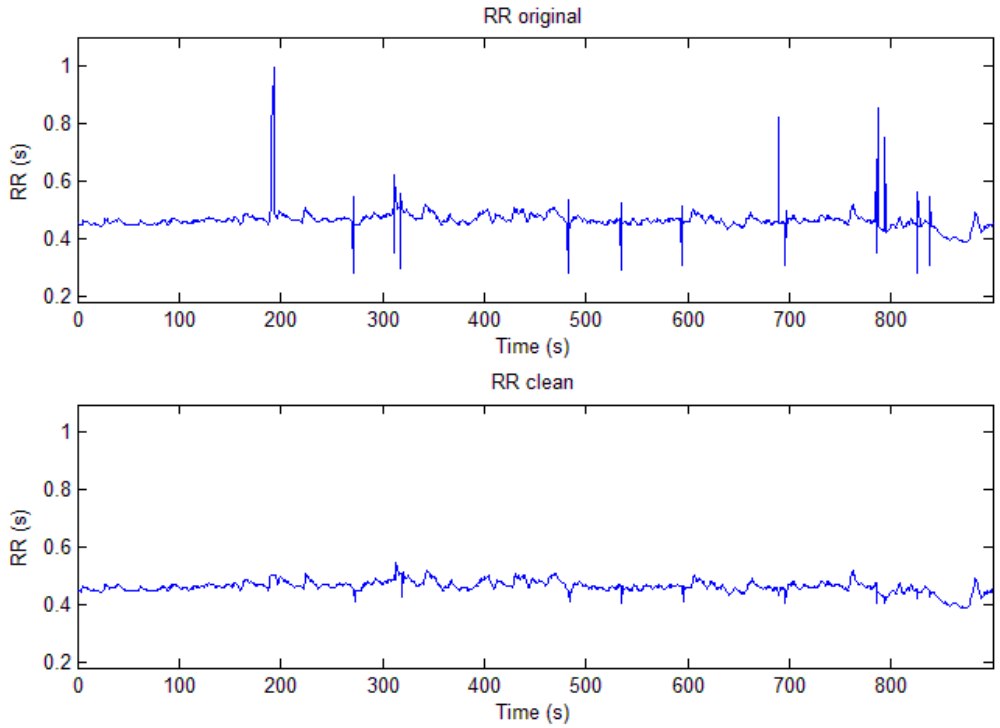


Figure 2.5 Correction of ectopic beats in a segment of RR time series. Upper: original RR time series. Lower: resulting time series after ectopic beat replacement using adaptive filtering.

A detrending method called “smoothness prior detrending”, as proposed by Tarvainen et al. (2002), was applied to eliminate the unwanted low frequency trends in the RR time series. Detrending is a common pre-processing step applied in HRV analysis. It was performed through high-pass filtering the non-stationary trends to minimize the non-stationarities within the RR time series. The stationarity of the RR time series is required for power spectral density estimation. Trends in physiological signals, like HR signals, contain complex components and strongly affect the low frequency components (Colak, 2009; Yoo & Yi, 2019). A lambda value of 500 was used to remove the unwanted low-frequency trend components. This value was chosen to have a high pass filter with cut-off frequency of less than 0.04 Hz (the lower limit of the low frequency band) to avoid

distorting the frequency band of interest. Figure 2.6 shows the detrending of an RR time series.

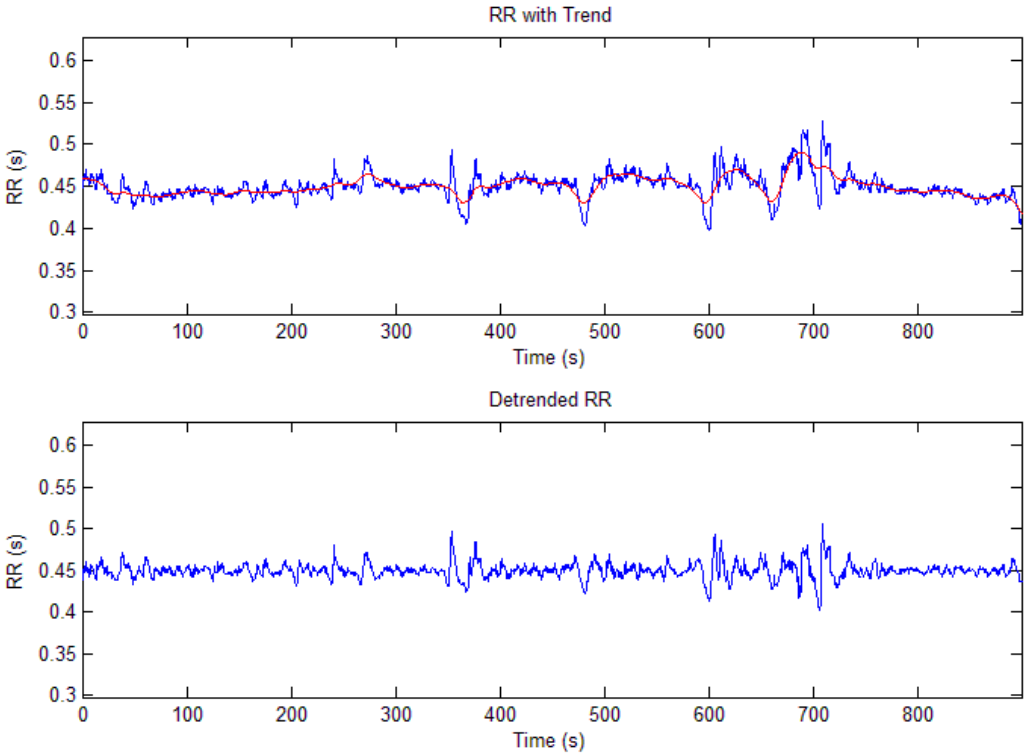


Figure 2.6 Detrending a segment of an RR time series. Upper: original RR time series. The red line shows the low-frequency trend component of the time series. Lower: resulting time series after removal of the trends by “smoothness prior detrending”.

The short-term HRV analysis was performed according to a standard approach (Malik, 1996) in both time and frequency domains. For maternal HRV, the 15-minute data were segmented into 5-minute segments to reduce the non-stationarity, and the HRV was computed for each segment. The average of the HRVs of the three periods was then used in the analysis. Since the fetal HR is usually much higher than the maternal HR, shorter segments were used for fetal data to reduce the issue of non-stationarity. Based on the procedure by Schneider

et al. (2009), the fetal HRV was analyzed from segments of 256 fetal heart beats. Assuming an average fetal HR of 140 bpm, a segment was roughly 2 minutes long.

2.5.2. Time domain HRV measures

Time domain analysis estimates the changes in the HR time series. For time domain measures, we calculated the mean HR in beats per minute (bpm). The variability within the RR time series was investigated by including the standard deviation of RR intervals (SDNN) and the root mean square difference of successive RR intervals (RMSSD) in the analysis.

2.5.3. Frequency domain HRV measures

Frequency analysis estimates the fluctuation of HR at different frequencies. In the frequency domain, the power spectral density was estimated using fast Fourier transform (FFT), based on Welch's method. To calculate the FFT, the RR interval time series has to be transformed into an equidistant set of data points. Prior to the analysis, the unevenly sampled RR intervals were resampled with cubic spline interpolation at the resampling frequency of 4 Hz. This 4 Hz sampling frequency is sufficient to satisfy the Nyquist criterion to avoid the aliasing effect. The steps for performing FFT using Welch's method consist of partitioning the time series data into overlapping windows, computing the periodogram for each window separately, and then averaging the periodogram segments to estimate the power spectrum. The segments overlapping by 50% and the Hamming window were applied to each data segment of 512 points in size (length of the Discrete Fourier Transform) before the computation of the periodogram. Welch's method was implemented using the "pwelch" function in MATLAB.

For maternal HRV, the spectral components of the different frequency bands typically used for adults (Malik, 1996) were evaluated: low frequency (LF: 0.04 to 0.15 Hz), and high frequency (HF: 0.15 to 0.40 Hz). For fetal HRV, the respiratory motion of the fetus is reported to occur at a different frequency range than that of adults. Therefore, the frequency bands proposed by David et al. (2007) were used for HRV analysis in the fetus (LF: 0.08 to 0.2 Hz and HF: 0.4 to 1.7 Hz). The frequency components were expressed in absolute (LF and HF) as well as normalized (LFn and HFn) measures. SDNN, LF and LFn reflect both sympathetic and parasympathetic activity, while RMSSD, HF and HFn are assumed to be influenced by parasympathetic activity only. The LF/HF ratio reflects the balance between sympathetic and parasympathetic activity (David et al., 2007; Groome, Mooney, Bentz, & Wilson, 1994; Malik, 1996). The time and frequency domain parameters, with their formulas and their assumed influence of the ANS branches, are summarized in Table 2.2.

Table 2.2 Heart rate variability parameters and their definitions

	Parameters	Formula	Description	Associated autonomic function
Time domain	HR (bpm)	$HR = \frac{60000}{\frac{1}{n}\sum_{i=1}^{n} RR_i}$	Mean of heart rate	Sympathetic and parasympathetic
	SDNN (ms)	$SDNN = \sqrt{\frac{1}{n-1}\sum_{i=1}^{n}(RR_i - \widehat{RR})^2}$	Standard deviation of RR intervals	Overall HRV, sympathetic and parasympathetic
	RMSSD (ms)	$RMSSD = \sqrt{\frac{1}{n}\sum_{i=1}^{n-1}(RR_{i+1} - RR_i)^2}$	Root mean square of successive differences of RR intervals	Short-term HRV, parasympathetic
Frequency domain	LF (ms^2) LFn (normalized)	$LF = Power\ spectrum\ in\ the\ LF\ range$ $LFn = \frac{LF}{LF + HF} * 100$	Low frequency power in absolute and normalized value Fetal: 0.08 to 0.20 Hz Maternal: 0.04 to 0.15 Hz	Sympathetic and parasympathetic
	HF (ms^2) HFn (normalized)	$HF = Power\ spectrum\ in\ the\ HF\ range$ $HFn = \frac{HF}{LF + HF} * 100$	High frequency power in absolute and normalized value Fetal: 0.40 to 1.70 Hz Maternal: 0.15 to 0.40 Hz	Primarily parasympathetic
	LF/HF	$\frac{LF}{HF}$	Ratio of LF to HF power	Sympathovagal balance

2.6. Regression analysis using machine learning

Many studies have been performed to understand the behaviour of the fetal ANS and gain insight into neurodevelopment and fetal maturation (DiPietro, Costigan, Shupe, Pressman, & Johnson, 1998; DiPietro et al., 2015; Schneider et al., 2018). Multiple factors are assumed to contribute to the development of the fetal ANS, but the actual factors involved and how they interact remain to be established (DiPietro et al., 2015; Samuelsson et al., 2010; Young, 2002, 2006). Studies using fetal HRV indices themselves are now increasingly adopting machine learning. For example, Random Forest, an ensemble tree-based method, was used in the assessment of fetal maturation (Tetschke, Schneider, Schleussner, Witte, & Hoyer, 2016). In another study, Support Vector Machine was used for detecting fetal distress (Warmerdam et al., 2018).

Regression analysis is an approach for modelling the relationship between a continuous response and another set of regressor variables (also called independent variables). A regressor can be one continuous variable (known as single regression) or more variables (known as multiple regression). In a complex system like fetal ANS, the outcome most likely depends on multiple input variables. We conducted multiple regressions to examine whether fetal HR (as proxy of fetal ANS) is a function of multiple factors.

Specifically, we used a machine learning approach to relate various maternal factors with the fetal HR to obtain insight into the possible factors driving fetal ANS behaviour. To avoid overlooking factors that might contribute to the ANS development, we included several maternal anthropometric parameters (for example, height and age) at the beginning of the analysis and then excluded the

factors that did not contribute to the model. The hypothesis was that the fetal ANS, operationalized as fetal HR, can be represented with a non-linear model and is driven by multiple maternal factors (for example, MAM and maternal ANS). The following techniques with non-linear functions were deemed as potentially beneficial and were applied: Random Forest and Support Vector Machine. Thus, this regression analysis sought to extract patterns from multiple maternal factors that are potentially useful for characterizing fetal ANS. The findings can provide knowledge about potential factors involved in the complex ANS process to better understand the developmental programming of the fetal ANS and its relationship with the weight at birth.

In designing a model of fetal ANS, several supervised learning algorithms were used to perform the regression analysis. The workflow for building regression model of fetal HR is shown in Figure 2.7. The modelling, tuning and prediction for each algorithm was applied using the R "caret" package (M. Kuhn, 2008; Max Kuhn & Johnson, 2013). Caret, short for "Classification and Regression Training" is a streamline of multiple tools for modelling processes, including data splitting, feature selection and prediction.

2.6.1. Data splitting and feature selection

The subsamples containing 104 cases were included in the regression analysis. Testing the model on the unseen data will ensure that the model makes an unbiased prediction (James, Witten, Hastie, & Tibshirani, 2013; M. Kuhn, 2008). As an initial step, we randomly partitioned the dataset into two parts; "Training dataset" and "Testing dataset". The training dataset is 70% of the data that were used for model training, evaluation and tuning (n = 73). The remaining 30% of the

data is the testing dataset (n = 31). The testing dataset was used for testing the prediction performance of the models. We used the function called "createDataPartition" in the R caret package (M. Kuhn, 2008) to perform the random splitting of the testing and training dataset.

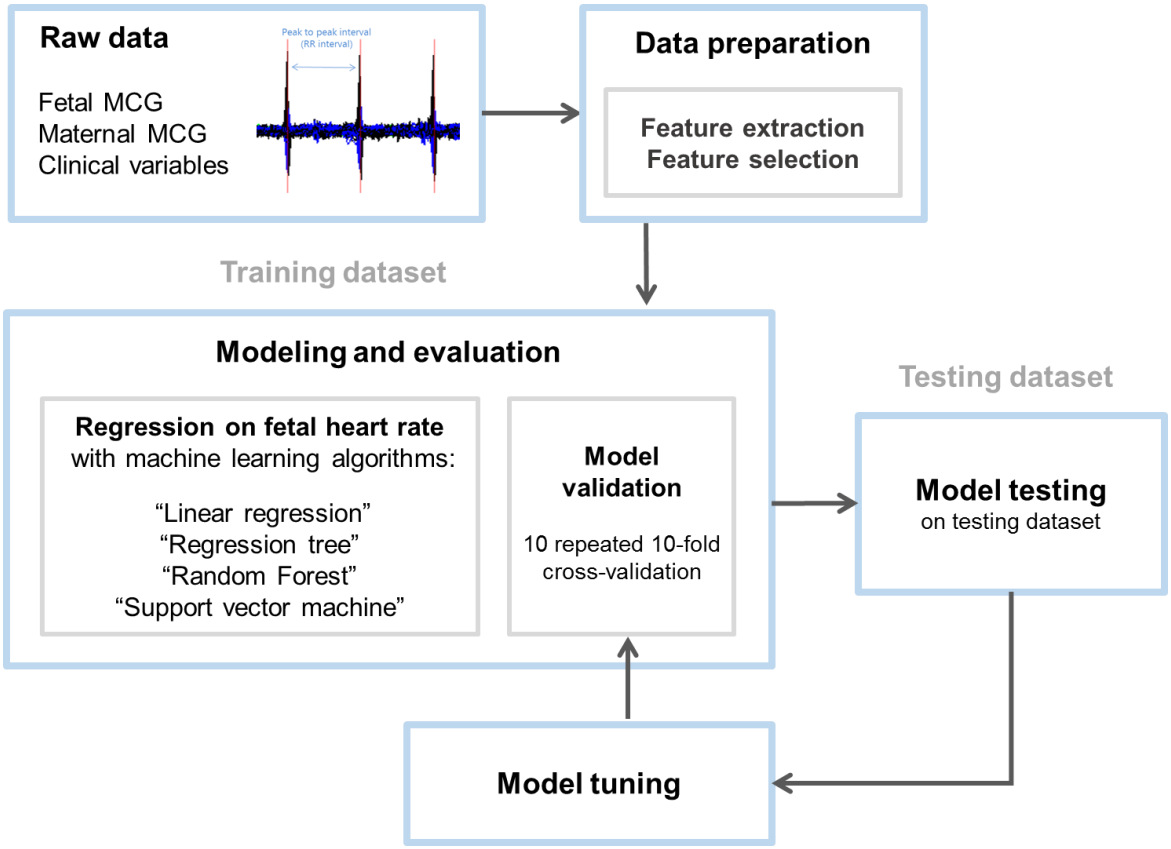


Figure 2.7 Workflow of the machine learning approach for building a regression model of fetal heart rate

For the analyses, 11 clinical variables (pre-pregnancy BMI, maternal weight gain, maternal insulin sensitivity, maternal fasting glucose, maternal fasting insulin, gravidity, parity, maternal age, maternal height, gestational age during the measurement and fetal gender) and 8 maternal HRV variables (mean HR, SDNN, RMSSD, LF, HF, LFn, HFn and LF/HF) were included as regressors on fetal HR.

Abbreviations for descriptions of each variable are provided in Table 2.3. The model was trained according to three sets of features:

1. *Clinical variables* consist of 11 features (upper part of Table 2.3).
2. *Maternal HRV* consists of 8 HRV features (lower part of Table 2.3).
3. *Combined clinical/maternal HRV* consists of 19 features.

Table 2.3 Features and descriptions

Features	Descriptions
Clinical variable consists of 11 features	
PreBMI	Body mass index of mother before pregnancy (kg/m^2)
MWG	Weight gain of mother during pregnancy (kg/week)
ISI	Maternal insulin sensitivity index
Glu	Fasting plasma glucose (mg/dl)
Ins	Fasting plasma insulin (pmol/l)
Grav	Gravidity
Para	Parity
Age	Age of mother (years)
Height	Height of mother (m)
GA	Gestational age during measurement (week)
Gender	Fetal sex
Maternal HRV consists of 8 features	
HR	Mean of heart rate (bpm)
SDNN	Standard deviation of RR intervals (ms)
RMSSD	Root mean square of successive differences of RR intervals (ms)
LF	Absolute low frequency power (ms^2)
HF	Absolute high frequency power (ms^2)
LFn	Normalized low frequency power
HFn	Normalized high frequency power
LF/HF	Ratio of low to high frequency power

2.6.2. Machine learning algorithms

The Decision Tree, Random Forest (RF) and Support Vector Machine (SVM) algorithms were utilized to find one that best fit the data. The main concept of each algorithm is described in this section.

Decision tree. Decision tree, introduced by Breiman, builds classification or regression models in the form of a tree structure (1984). The algorithm partitions a dataset into subsets through recursive partitioning. The partitioning process starts with a binary split and continues until no further splits can be made. The final result is a tree with various branches of variable lengths, called decision nodes and leaf nodes (also known as terminal nodes). The topmost node in a tree is a decision node (also known as a root node). It corresponds to the best predictor and has two or more branches. A leaf node contains a small subset of observations that represents a classification. Each leaf node can be presented as an "if-then" rule, and this makes the tree interpretable. For regression, the algorithm select the partition to minimize sum of squared error (James et al., 2013). As models with small trees are easier to interpret and produce decisions faster than large trees, the depth of the tree should be controlled (James et al., 2013). The regression tree was implemented using the "rpart" package provided within the R caret package (M. Kuhn, 2008; Therneau, Atkinson, & Ripley, 2019).

Random forest. Random forest (RF) is a tree-based method that combines many decision trees into a single model. Individual predictions made by decision trees may not be accurate, but the predictions can be improved using combined decision trees; thus, the name "Random Forest" (James et al., 2013). The technique was created by Breiman (2001) and has a powerful performance in comparison to other

supervised learning methods (Lavecchia, 2015; Strobl, Malley, & Tutz, 2009). In addition, RF also provides a measure of the variable importance of each feature (Breiman, 2001). Especially in clinical research, this feature importance has been widely used as a selection tool to remove features that contribute less in the prediction process (Hapfelmeier & Ulm, 2013; Kursa, 2014; Touw et al., 2019). This is important because, in machine learning, a higher number of features in a model often leads to overfitting (Zhang & Ma, 2012). Therefore, RF is suitable due to its increased accuracy and its ability to overcome overfitting problems (James et al., 2013). Each decision tree in the forest considers a random subset of input features in making predictions (Breiman, 2001). This increases diversity in the forest, leading to more robust overall predictions. For regression, the RF is formed by growing trees depending on a random vector of numerical values, and it takes an average of all the individual decision tree estimates (Zhang & Ma, 2012). To implement the RF algorithm, the package "randomForest" within the R caret package (M. Kuhn, 2008; Liaw & Wiener, 2018) was used with *ntree* = 1000 (*ntree* is the total number of trees in each forest) and *mtry* = default value (*mtry* is total number of randomly selected variables for each tree). The default value of *mtry* for regression is the total number of features divided by 3.

Support vector machine. The goal of the support vector machine (SVM) algorithm is to find an optimum boundary that separates data points of one class from the other class (James et al., 2013). It is mainly used in classification problems, but for the case of regression, similar to the classification, it optimizes the generalization bounds given for regression (James et al., 2013). The boundary is known as a "hyperplane". If the data are linearly separable with 2 dimensions, the best hyperplane is a straight line with the largest distance between two nearest data

points from either class. The distance between the hyperplane and the nearest data point from either set is known as a "margin". These two data points, also known as "support vectors", are the most critical elements, since the optimum hyperplane depends on their positions for dividing the two classes. For nonlinearly separable data, SVM transforms the data into a higher dimensional space where a hyperplane can be formed to separate the two classes. This transformation of data into a higher dimension is known as kernelling (James et al., 2013). Regression analysis using SVM was performed using Radial Basis Function kernel from the "kernlab" package provided in the R caret package (Karatzoglou, Smola, Hornik, & Karatzoglou, 2018; M. Kuhn, 2008; Schölkopf & Smola, 2001).

2.6.3. Model training and validation

Two metrics were used to evaluate the model performance: the mean absolute error (MAE) and the root mean squared error (RMSE). The MAE is the average of the absolute differences between predicted values and actual values. The formula for this metric is:

$$\text{MAE} = \frac{1}{n}\sum_{i=1}^{n} |y_i - \hat{y}_i|$$

where y_i is the i_{th} actual outcome in the training dataset, $\hat{y}_i$ is the model prediction.

The RMSE is the average of the squared differences between predicted values and actual values. It can be obtained using:

$$\text{RMSE} = \sqrt{\frac{1}{n}\sum_{i=1}^{n}(y_i - \hat{y}_i)^2}$$

Using the linear regression model as a baseline and based on the value of RMSE and MSE, we compared each model performance with the linear model.

Due to the relatively small sample size for this approach, a robust resampling method called “k-fold cross-validation” was applied to maximize the usage of each data point (Max Kuhn & Johnson, 2013). The data were randomly partitioned into k subsets (k-folds) of approximately equal size. One subset (one fold) is reserved for model testing and all other subsets (k-1 folds) are used for evaluating the model performance. As suggested by Kuhn (2013), k = 10 was used to minimize bias and variance in the error estimation due to the small dataset. The partitioning of data was repeated 10 times, which resulted in n = 100 resamples to fit each model. The cross-validation error metrics were obtained using the following formula:

$$MAE_k = \frac{1}{k}\sum_{i=1}^{k} MAE_i$$

$$RMSE_k = \frac{1}{k}\sum_{i=1}^{k} RMSE_i$$

where MAE_i and $RMSE_i$ are each the error of the i_{th} fold in the training dataset. The function “trainControl” in R caret package was used for setting the parameter of cross-validation. The random number generator was reset to a common seed using

the “set.seed” function to create the same folds of resampling datasets prior to each model fitting.

2.6.4. Model testing, tuning and feature reduction

The prediction performance of the model was tested using the “predict” function in the R caret package (M. Kuhn, 2008). The best model was further improved by tuning the model parameters, and the new tuned model was called the “tuned RF model”. In order to build a model with a small number of features, the features that effectively contributed to the prediction performance were additionally evaluated using the “varImp” function to obtain a variable importance score (Breiman, 2001; Genuer, Poggi, & & Tuleau-Malot, 2019; M. Kuhn, 2008). The variable importance score represents the increment in the sum of the squared error from the permutation of each feature (M. Kuhn, 2008). Features with a higher variable importance score are more important and make larger contributions to the model performance. We referred to the model with a lower number of features as the “tuned RF model with reduced features”.

2.7. Statistical analysis

All statistical analyses were performed with SPSS version 23 (IBM SPSS Statistics, NY, USA) and results with a significance level of $P < 0.05$ were regarded as statistically significant. Pearson’s correlations and partial correlations were performed to investigate the associations between MAM parameters and maternal and fetal HR and HRV. Pearson’s correlations were also performed to test the correlation between the predicted and the actual values of fetal HR in the machine learning models. A one-way analysis of covariance (ANCOVA) was performed

separately for the main factors pre-pregnancy BMI (4 levels), pregnancy weight gain (3 levels) and gender (2 levels). A one-way analysis of variance (ANOVA) was performed for the machine learning regression models (5 levels). A paired t-test was used in the case-control subsample between the matched groups of pre-pregnancy BMI. Fetal HR and HRV were compared between pre-pregnancy BMI groups matched in gestational age, gender and parity. An independent t-test was used to compare the effect of fetal gender on fetal HR and HRV. For all analyses, except for Pearson's correlations, the adjusted values with gestational age, gender and parity as covariates were reported. Data are presented as mean ± standard error of the mean (SEM). Bonferroni-Holm correction was applied for multiple comparisons and corrected significance levels were used. To explore the possible mediation relationship between MAM parameters and fetal HR, HRV and birth weight, a mediation analysis was performed with a simple mediation model using the PROCESS macro in SPSS (Hayes, 2017). The significance of mediation effects was determined using bootstrapping with 95% confidence intervals (Preacher & Hayes, 2008). Figure 2.8 illustrates a general model for testing mediation effects.

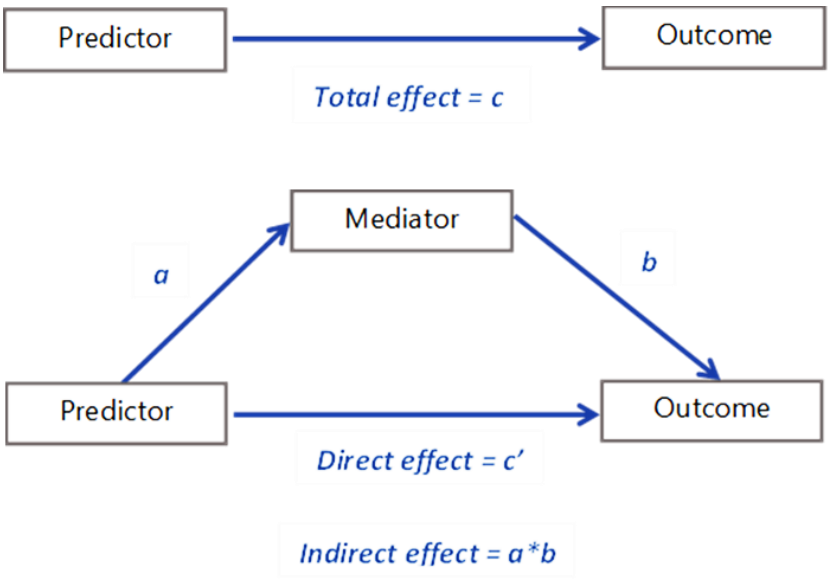


Figure 2.8 General model for testing mediation effects. Total effect = path c, the effect between the predictor and the outcome. Direct effect = path c', the effect between the predictor and the outcome after including the mediator in the model. Indirect effect = path ab, is the multiplication between the effect of the predictor and the mediator (path a) multiplied by the effect of the mediator and the outcome (path b).

Chapter 3

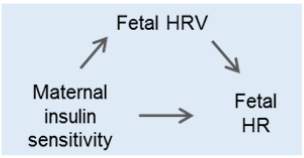
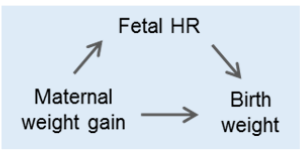
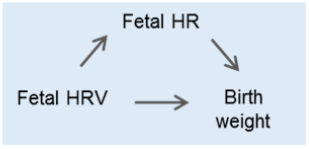
Results

Section 3.1 presents the descriptive characteristics of study participants, followed by the results for the gender effect on fetal HR and HRV in section 3.2. Sections 3.3 to 3.5 present the main results of the association between fetal HR, HRV and maternal weight factors, maternal glucose metabolism and birth weight. An overview of the analysis performed for the main results is provided in Table 3.1.

Results in section 3.3, 3.4 and 3.5 presented in this chapter are published as:

Mat Husin, H., Schleger, F., Bauer, I., Fehlert, E., Kiefer-Schmidt, I., Weiss, M., Kagan, K. O., Brucker, S., Pauluschke-Fröhlich, J., Eswaran, H., Häring, H. U., Fritsche, A. and Preissl, H. (2020). Maternal weight, weight gain and metabolism are associated with changes in fetal heart rate and variability. *Obesity*.

Table 3.1 Overview of analysis and results

Section/ analysis	**3.3 Maternal weight factors**	**3.4 Maternal metabolism**	**3.5 Birth weight**
Correlation and/or ANOVA	Maternal HRV and maternal weight variables Fetal HR and pre-pregnancy BMI Fetal HR and maternal weight gain Fetal HRV and pre-pregnancy BMI Fetal HRV and maternal weight gain	Maternal weight variables and maternal fasting metabolism Maternal HR, HRV and maternal fasting metabolism Fetal HR and maternal fasting metabolism Fetal HRV and maternal fasting metabolism	Fetal HR, HRV and birth weight
Mediation analysis			 

3.1 Study participants

In the main sample of 184 subjects, the gestational age ranged between 26 and 38 weeks (mean ± standard deviation, 30.37 ± 2.91), maternal age was 31.7 ± 5.3 years, pre-pregnancy BMI was 23.59 ± 3.96 kg/m^2 and weight gain was 0.38 ± 0.21 kg/week. Neonatal birth weight was 3387.9 ± 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 3.2.

Table 3.2 Maternal anthropometric, metabolic characteristics and birth outcomes for pregnant women with normal pregnancies

Variables	Range	Mean	Standard deviation
Study population, n = 178			
Gravidity	1 - 5	1.72	0.96
Parity	0 - 5	0.56	0.80
Age (years)	21 - 45	31.72	5.16
Height (m)	1.52 - 1.85	1.67	0.06
Gestational age (week)	26 - 38	30.42	2.92
Gestational age at birth (week)	37 - 42	40.21	1.27
Birth weight (gram)	2500 - 4670	3390.98	445.60
Birth length (cm)	44 - 56	51.28	2.32
Weight before pregnancy (kg)	44 - 115	66.09	12.36
Weight during pregnancy (kg)	53 - 117	74.29	12.02
BMI before pregnancy (kg/m^2)	16.8 - 42.4	23.53	3.98
BMI during pregnancy (kg/m^2)	18.2 - 42.9	26.470	3.88
Maternal weight gain (kg/week)	-0.22 - 0.93	0.38	0.21
Subsample from OGTT participants, n = 104			
Fasting plasma glucose (mg/dl)	63 - 90	78.78	5.41
Fasting plasma insulin (pmol/l)	22 - 311	68.70	38.23
Insulin sensitivity index	-0.52 - 0.12	0.07	0.03

BMI: Body mass index, OGTT: oral glucose tolerance test

3.2 Fetal HR, HRV and fetal gender

When testing the effect of fetal gender as a potential covariate, an ANCOVA using gestational age as a covariate revealed a significantly higher fetal HR in female than in male fetuses (142.34 ± 0.74 vs. 139.46 ± 0.73 bpm, $F(1) = 7.54$, $P = 0.007$). The significant difference remained even after birth weight was additionally added as a covariate (142.11 ± 0.74 vs. 139.68 ± 0.73 bpm, $F(1) = 5.33$, $P = 0.022$). However,

fetal gender showed no significant difference with respect to any fetal HRV parameter ($P > 0.2$). When analysing fetal HR and HRV, we therefore included fetal gender as a covariate, together with gestational age and parity.

3.3 Maternal weight factors

3.3.1 Maternal HR, HRV and maternal weight factors

No association was found between maternal heart parameters and any maternal weight factors. Some concerns have been raised that HRV is mainly driven by HR (Antelmi et al., 2004; Billman, 2013; Sacha, 2014; Tsuji et al., 1996); therefore, we tested the correlation between maternal HR and maternal HRV. This test confirmed a significant correlation between the maternal HR and all maternal HRV measures ($P < 0.001$). Further analyses were conducted for maternal HRV by adjusting for maternal HR. Even after adjusting for maternal HR, no significant correlation was noted between maternal weight factors and maternal HRV as a continuous variable (Table 3.4). Consideration of pre-pregnancy BMI and maternal weight gain as a group factor revealed no significant differences in maternal HR and HRV. Moreover, tests of the relationship between continuous variables revealed a negative correlation between pregnancy BMI and maternal weight gain ($r = -0.254$, $P = 0.001$).

3.3.2 Fetal HR and pre-pregnancy BMI

The ANCOVA for the four pre-pregnancy BMI groups (covariates: gestational age, gender and parity) revealed a significant main effect of pre-pregnancy BMI on HR ($F(3) = 2.84$, $P = 0.040$, Table 3.3). Post hoc analysis showed that the HR was higher

in fetuses of mothers with obesity than in fetuses of mothers with normal weight (145.00 ± 1.67 vs. 140.25 ± 0.62, P = 0.040). However, after Bonferroni-Holm correction, the differences did not reach significance (P = 0.024). Figure 3.1b shows a U-shaped pattern of fetal HR in the different pre-pregnancy BMI groups, with lower values in the normal weight group compared to the groups outside the normal weight range in both directions. Pre-pregnancy BMI as a continuous variable showed no significant correlation with fetal HR (Table 3.4). Image from Figure 3.1b has been previously published in Mat Husin et al. (2020).

Given the imbalanced sample sizes for pre-pregnancy BMI groups, with a very small sample of underweight (UW) subjects, further analysis was performed on matched subsamples in two larger groups: a normal weight (NW) group and a combined overweight and obese (OW+OB) group. Forty-nine subjects matched in gestational age, gender and parity were included. A paired t-test showed a significantly higher fetal HR in the combined overweight and obese group than in the normal weight group (142.00 ± 0.83 vs. 139.25 ± 1.04, $t(96) = 1.96$, P = 0.050). The results are shown in Figure 3.1c. We additionally performed an ANCOVA with three groups, by excluding the UW group (NW, OW, and OB). The main effect remained significant ($F(2) = 3.51$, P = 0.032). Post hoc analysis showed a significantly higher fetal HR in the fetuses of the OB group than in those of the NW group (145.00 ± 1.67 vs. 140.29 ± 0.62, P = 0.028), but the difference was not significant after Bonferroni-Holm correction (P = 0.084).

3.3.3 Fetal HR and maternal weight gain

The ANCOVA containing three maternal weight gain groups (covariates: gestational age, gender and parity) revealed no significant difference in fetal HR

($F(2) = 1.20$, $P = 0.305$, Table 3.3). Maternal weight gain as a continuous variable (covariates: gestational age, gender and parity) had a significant negative correlation with fetal HR ($r = -0.138$, $P = 0.041$, Figure 3.2a, Table 3.4). However, after pre-pregnancy BMI was added as an additional covariate, no significant correlation was found between maternal weight gain and fetal HR ($r = -0.132$, $P = 0.083$).

3.3.4 Fetal HRV and pre-pregnancy BMI

The ANCOVA revealed no significant main effect of pre-pregnancy BMI group on any HRV parameter ($P > 0.2$, covariates: gestational age, gender and parity, Table 3.4). For continuous variables, pre-pregnancy BMI had a positive correlation with the HFn component ($r = 0.175$, $P = 0.021$), and an inverse correlation with the LFn ($r = -0.175$, $P = 0.021$) and the LF/HF ratio ($r = -0.168$, $P = 0.026$, Figure 3.1, Table 3.4).

3.3.5 Fetal HRV and maternal weight gain

The ANCOVA (covariates: gestational age, gender and parity) showed a significant main effect of maternal weight gain group on fetal SDNN and absolute LF component (SDNN: $F(2) = 5.17$, $P = 0.007$; LF, $F(2) = 5.28$, $P = 0.006$). Post hoc analysis revealed that SDNN and LF were significantly lower in fetuses of mothers with high weight gain than in fetuses of mothers with normal weight gain, even after Bonferroni-Holm correction (SDNN: 8.64 ± 0.38 vs. 10.34 ± 0.39, $P = 0.006$, after Bonferroni-Holm correction, $P = 0.018$; LF: 24.85 ± 2.65 vs. 36.54 ± 2.77, $P = 0.008$, after Bonferroni-Holm correction, $P = 0.024$). Remarkably, the overall effect of the maternal weight gain group on both SDNN and LF were depicted as an

inverted U-shaped relationship, with lower values in weight gain lower and higher than the recommended range (Table 3.3, Figure 3.2b and 3.2c). Maternal weight gain as a continuous variable showed no significant correlation with any fetal HRV variable (Table 3.4). Image in Figure 3.2b has been previously published in Mat Husin et al. (2020).

Table 3.3 Fetal HRV in pre-pregnancy BMI and maternal weight gain group

Variables	Pre-pregnancy BMI				*P* value	Maternal weight gain			*P* value
	UW (n = 9)	NW (n = 120)	OW (n = 32)	OB (n = 17)		Low (n = 66)	Normal (n = 53)	High (n = 59)	
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
LF (ms^2)	23.62 ±6.91	30.59 ±1.89	27.32 ±3.66	24.91 ± 5.05	0.541	26.94 ±2.50	36.54 ±2.77	28.85 ±2.65	**0.006**
HF (ms^2)	4.22 ±2.61	7.37 ±0.71	7.75 ±1.38	8.83 ±1.91	0.547	7.70 ±0.97	8.47 ±1.07	6.15 ±1.02	0.278
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 pre-pregnancy BMI and maternal weight gain (covariates: gestational age, gender, and parity) on the fetal ANS parameters. *P* values < 0.05 are considered statistically significant, and are marked in bold. HRV, heart rate variability; High, above recommended weight gain; Low, below recommended weight gain; Normal, within recommended weight gain; NW, normal weight; OB, obesity; OW, overweight; UW, underweight.

Table 3.4 Partial correlation coefficients between maternal HRV, fetal HRV and maternal nutrition

	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 (bpm)[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
HF (ms^2)	-0.026	0.088	-0.012	-0.106	-0.173	-0.111	0.020	0.101	**-0.215***	-0.004
LF (ms^2)	-0.085	-0.083	-0.013	0.014	0.069	0.078	-0.083	-0.062	-0.103	0.079
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

r_{fet} is partial correlation coefficient for fetal variables, adjusted for gestational age, parity and gender. r_{mat} is partial correlation coefficient for maternal HRV adjusted for maternal HR. [a] r_{mat} is Pearson's correlation coefficient for maternal HR. Correlation coefficients with *P* values < 0.05 are considered statistically significant, and are marked in bold. * $P < 0.05$, ** $P < 0.01$. HRV: heart rate variability, PreBMI: pre-pregnancy BMI, MWG: maternal weight gain, ISI: insulin sensitivity, Ins: maternal fasting insulin, Glu: maternal fasting glucose.

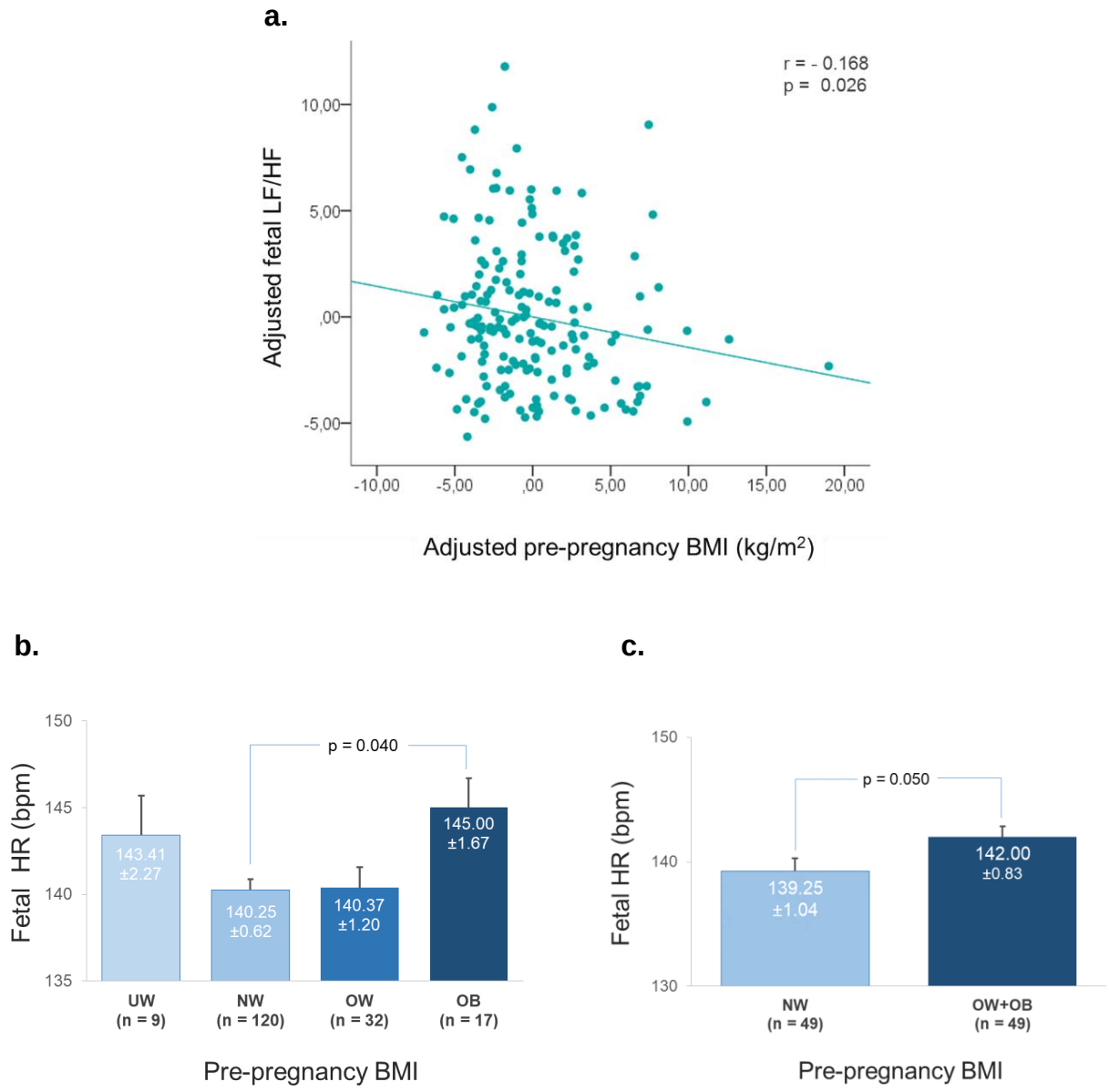


Figure 3.1 Relationship between fetal HR, HRV and pre-pregnancy BMI. a. Associations between pre-pregnancy BMI as a continuous variable and fetal LF/HF, with r as a partial correlation coefficient adjusted for gestational age, parity and gender. b. HR differed significantly between the different pre-pregnancy BMI groups. This effect was mainly due to a difference between the obese and normal weight group and was not significant after Bonferroni-Holm correction. A U-shaped pattern appears between pre-pregnancy BMI and fetal HRV in overall populations. c. When case-control matched for gestational age, parity and gender, HR differed significantly between normal weight and overweight/obese group. Data presented are mean ± SEM. HR: heart rate, bpm: beat per minute, BMI: body mass index, UW: underweight, NW: normal weight, OW: overweight, OB: obese. Figure 3.1b has been previously published in Mat Husin et al. (2020).

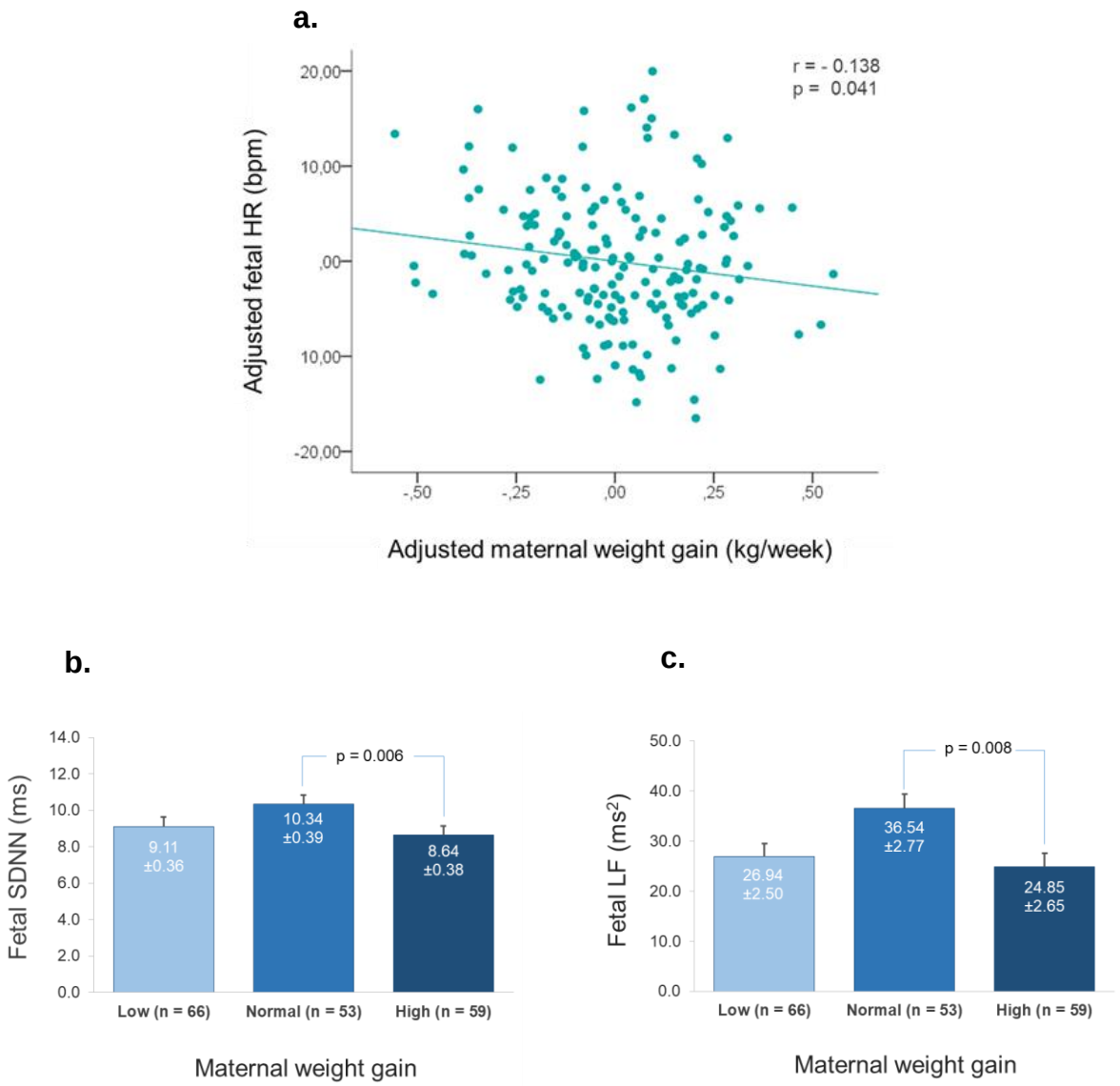


Figure 3.2 Relationship between fetal HR, HRV and maternal weight gain. a. Associations between fetal HR and maternal weight gain, with r as a partial correlation coefficient adjusted for gestational age, parity and gender. Significant differences between the b. SDNN and c. LF in fetuses of high weight gain mothers and that of normal weight gain mothers (significance remained after Bonferroni-Holm correction). An inverted U-shaped pattern appears between maternal weight gain and fetal HRVs in overall populations. Data presented are mean ± SEM. HRV: heart rate variability, LF/HF: ratio of low frequency to high frequency, BMI: body mass index, SDNN: standard deviation of normal-to-normal interval, LF: low frequency, ms: milliseconds. Figure 3.2b has been previously published in Mat Husin et al. (2020)

3.4 Maternal glucose metabolism in the fasting state

3.4.1 Maternal weight factors and maternal metabolism

The pre-pregnancy BMI had a positive correlation with maternal fasting glucose and insulin (Glucose: $r = 0.198$, $P = 0.042$, Insulin: $r = 0.536$, $P = 0.000$). As expected, pre-pregnancy BMI was negatively correlated with maternal insulin sensitivity ($r = -0.350$, $P = 0.000$). No significant correlation was observed between any measure of maternal fasting metabolism and maternal weight gain (Glucose: $r = 0.105$, $P = 0.286$; Insulin: $r = -0.121$, $P = 0.220$; Insulin sensitivity: $r = 0.060$, $P = 0.544$).

3.4.2 Maternal HR, HRV and maternal metabolism

Maternal HR was significantly correlated with maternal fasting insulin ($r = 0.252$, $P = 0.010$) and insulin sensitivity ($r = -0.218$, $P = 0.026$). For maternal HRV parameters, HF was negatively correlated with maternal fasting glucose ($r = -0.199$, $P = 0.040$) and LF/HF had a positive correlation with maternal fasting insulin ($r = 0.193$, $P = 0.050$). As in the previous section for the whole population, further analyses were conducted by including HR as covariate to exclude the influence of HR on HRV. After adjusting for HR, HF remained significantly correlated with maternal fasting glucose ($r = -0.215$, $P = 0.029$); however, no significant correlation remained between LF/HF and maternal fasting insulin ($r = 0.084$, $P = 0.399$). In addition, RMSSD and HFn now showed a negative correlation with insulin sensitivity (RMSSD: $r = -0.203$, $P = 0.040$; HFn: $r = -0.228$, $P = 0.020$). LFn was positively correlated with insulin sensitivity ($r = -0.228$, $P = 0.020$). Table 3.4 summarizes the correlation results.

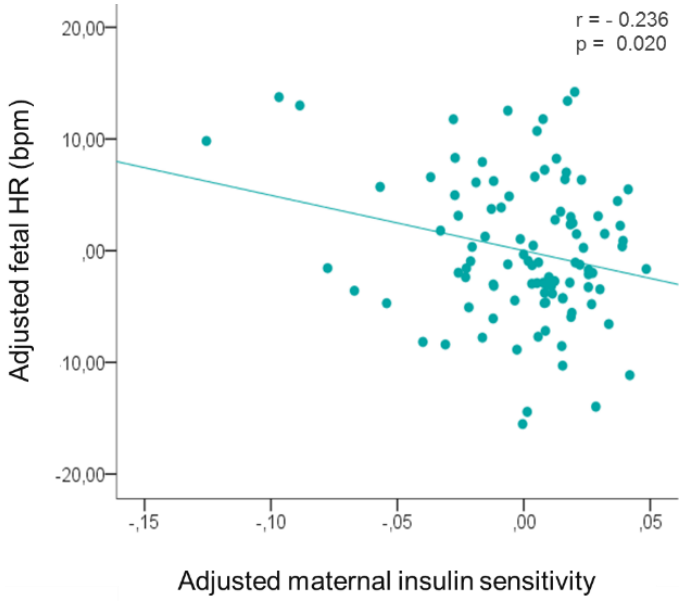
3.4.3 Fetal HR and maternal metabolism

As observed for the maternal HR, the fetal HR was significantly correlated with maternal fasting insulin (r = 0.241, P = 0.017) and insulin sensitivity (r = -0.236, P = 0.020, Figure 3.3a). No significant correlation was observed between fetal HR and maternal fasting glucose (r = 0.169, P = 0.097). The correlation results are shown in Table 3.4.

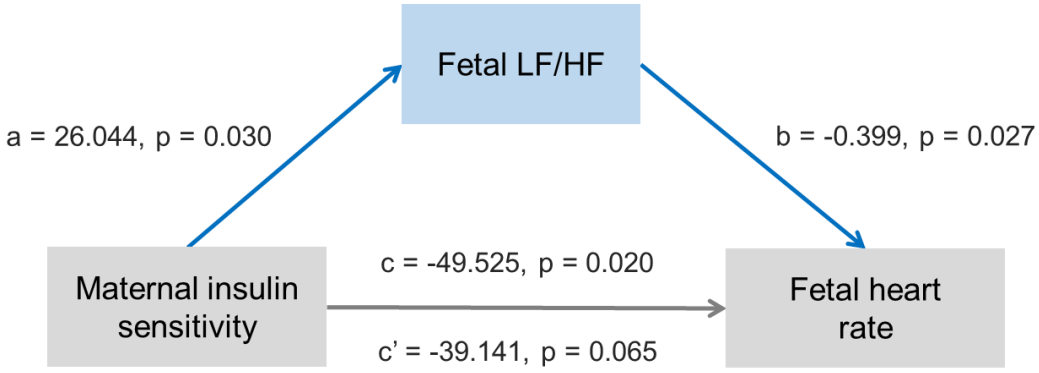
3.4.4 Fetal HRV and maternal metabolism

Maternal fasting insulin had a positive correlation with HFn (r = 0.253, P = 0.012) and an inverse correlation with LFn (r = -0.253, P = 0.012). A positive correlation was noted between maternal insulin sensitivity and LFn (r = 0.269, P = 0.008), as well as LF/HF (r = 0.220, P = 0.030), whereas a negative correlation was observed with HFn (r = -0.269, P = 0.008). As pre-pregnancy BMI was shown to be significantly associated with maternal insulin sensitivity, the test was repeated to control for the influence of pre-pregnancy BMI. The correlation remained significant for HFn (r = -0.222, P = 0.031) and LFn (r = 0.222, P = 0.031), but not for LF/HF (r = -0.199, P = 0.054). No associations were detected between fetal HRV and maternal fasting glucose. The results are presented in Table 3.4.

a.



b.



Indirect effect, ab = -10.383, 95% CI [-26.613, -2.369]

Figure 3.3 Relationship between maternal insulin sensitivity, fetal HR and HRV. a. Associations between fetal HR and maternal insulin sensitivity with r as a partial correlation coefficient adjusted for gestational age, parity and gender. b. Fetal HRV mediating the association between maternal insulin sensitivity and fetal HR. LF/HF: the ratio of low to high frequency, CI: confidence intervals. Figure 3.3b has been previously published in Mat Husin et al. (2020)

Fetal HRV mediating the association between maternal insulin sensitivity and fetal HR

We proposed fetal LF/HF as another possible mediator in the association between maternal insulin sensitivity and fetal HR. As illustrated in Figure 3.3b, fetal LF/HF significantly mediated the maternal insulin sensitivity in predicting fetal HR (*ab* = -10.383, 95% CI [-26.613, -2.369]). The direct relationship between maternal insulin sensitivity and fetal HR was significant (*c* = -49.525, *P* = 0.020), but was reduced and no longer significant after adding fetal LF/HF as the mediator (*c'* = -39.141, *P* = 0.065). Image in Figure 3.3b has been previously published in Mat Husin et al. (2020).

3.5 Birth weight

3.5.1 Fetal HR, HRV and birth weight

Fetal HR was significantly negatively correlated with birth weight (*r* = -0.187, *P* = 0.013). No correlation was observed between any fetal HRV measure and birth weight.

Fetal HR mediating the association between fetal HRV and birth weight

In partial correlation, only fetal LF/HF was significantly correlated with fetal HR (*r* = -0.157, *P* = 0.039). Here, we tested the possibility of a mediation relationship between fetal HR and birth weight via fetal HR. As depicted in Figure 3.4a, a significant indirect association was apparent between fetal LF/HF and birth weight mediated by fetal HR (*ab* = 3.668, 95% CI [0.124, 10.470]). The association between fetal LF/HF and birth weight was not significant (*c* = 7.058, *P* = 0.470), and

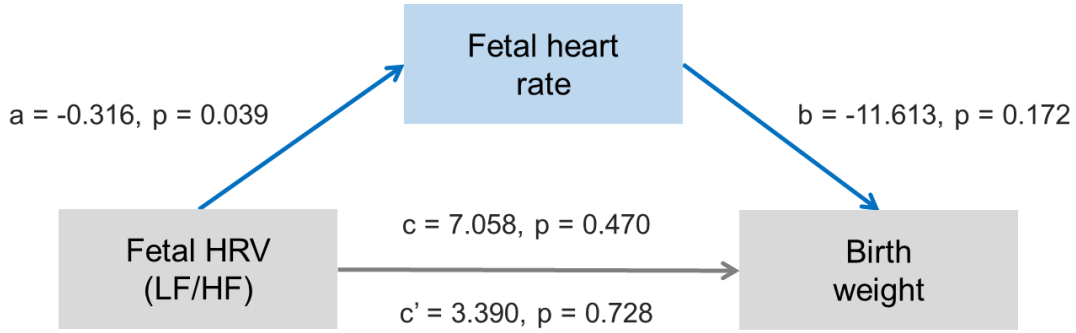
remained not significant with further reduced effect size, after adding fetal LF/HF as the mediator (c' = 3.390, P = 0.728).

3.5.2 Fetal HR, maternal weight gain and birth weight

Fetal HR mediating the association between maternal weight gain and birth weight

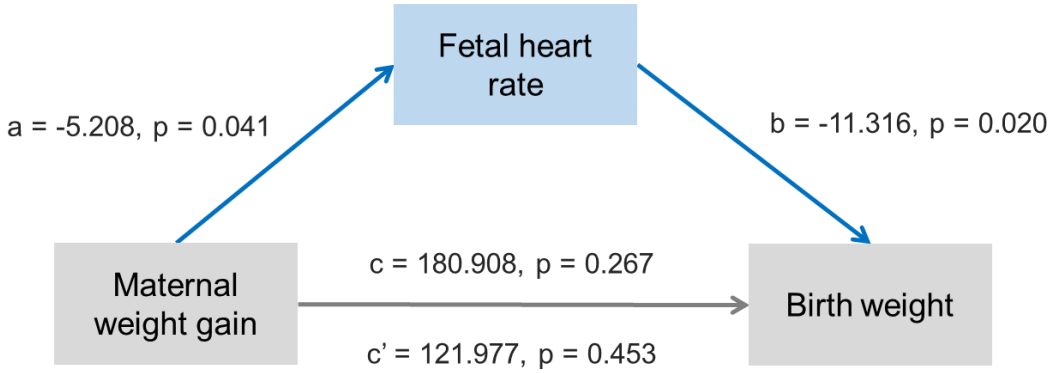
Based on the inter-correlation between parameters from the results of partial correlation, we proposed that maternal weight gain predicts birth weight through fetal HR as the mediator. This model, as shown in Figure 3.4b, revealed a significant indirect association between maternal weight gain and birth weight, through fetal HR (ab = 58.931, 95% Cl [4.809, 159.590]), with a significant relationship shown for maternal weight gain predicting fetal HR (a = -5.208, P = 0.041) as well as for fetal HR predicting birth weight (b = -11.316, P = 0.020). No significant relationship was indicated for the total effect between maternal weight gain and birth weight (c = 180.908, P = 0.267). The association was reduced after adding fetal HR as the mediator (c' = 121.977, P = 0.453), suggesting that fetal HR mediates the relationship between maternal weight gain and birth weight.

a.



Indirect effect, ab = 3.668, 95% CI [0.124, 10.470]

b.



Indirect effect, ab = 58.931, 95% CI [4.809, 159.590]

Figure 3.4 Relationship between maternal weight gain, fetal HR, HRV and birth weight. a. Fetal HR mediating the association between fetal HRV and birth weight. b. Fetal HR mediating the association between maternal weight and birth weight. CI: confidence intervals.

3.6 Fetal HR regression models

In this section, the prediction performance of fetal HR regression models is compared by three different set of features: Clinical variables, maternal HRV and combined clinical/maternal HRV. Model predictions on the testing dataset are examined based on the correlation between the predicted and actual values. The prediction performance of the tuned model and the tuned model with reduced number of features are then evaluated. The regression performance from the results of the cross-validation are examined.

Decision tree: In each set of features, when we trained the regression model with the decision tree, the tree did not find a good split and took the average of the fetal HR outcome as the predictor. Therefore, we excluded the results from the decision tree. The results for the performance of Linear regression (Linear), Support Vector Machine (SVM), Random Forest (RF), tuned RF and tuned RF with reduced features are reported.

3.6.1 Feature set of clinical variables

Prediction performance on testing dataset

The predicted values of the RF model were significantly correlated with the actual fetal HR values (r = 0.378, P = 0.036). Other models showed no significant correlation between the predicted and the actual fetal HR. Given that the RF model showed the prediction of fetal HR closest to the actual fetal HR values, we examined the two RF model parameters; *mtry* and *ntree*, to find their best values to improve the model prediction performance. Using “grid search”, we obtained

the best value of *mtry* = 2, which is the same value used in the original RF model. Tuned RF model with *ntree* = 200 had a lower MAE (Figure 3.5a) and RMSE compares with the model using *ntree* = 1000 (*ntree* used in the original RF model). Therefore, the smaller value of *ntree* was chosen and the prediction performance of the tuned RF model was tested on the testing dataset. The model prediction was significantly correlated with the actual fetal HR ($r = 0.360$, $P = 0.049$); however, the prediction of the original RF was better than the tuned RF model. The reduction in the significance level was expected due to the lower number of trees used in the tuned RF model in comparison with the original RF model. This can be seen from the cross-validation results as shown in Figure 3.5. The original RF model with *ntree* = 200 had a higher MAE and lower r^2 compares with the tuned RF model using the same number of trees. For *ntree* = 1000, the MAE was lower in the tuned RF, however the r^2 are similar.

To test the model with a smaller number of features, we excluded 2 of the 11 features with a cut-off of importance score lower than zero: parity and maternal height (the importance score is shown in Table 3.7). The model fitting using only 9 features was repeated on the previously tuned RF model. The prediction performance remained significant ($r = 0.382$, $P = 0.034$). The prediction results are detailed in Table 3.5. The predictions of the three RF based models (RF, tuned RF and tuned RF with reduced features) and the linear model are presented in Figure 3.6.

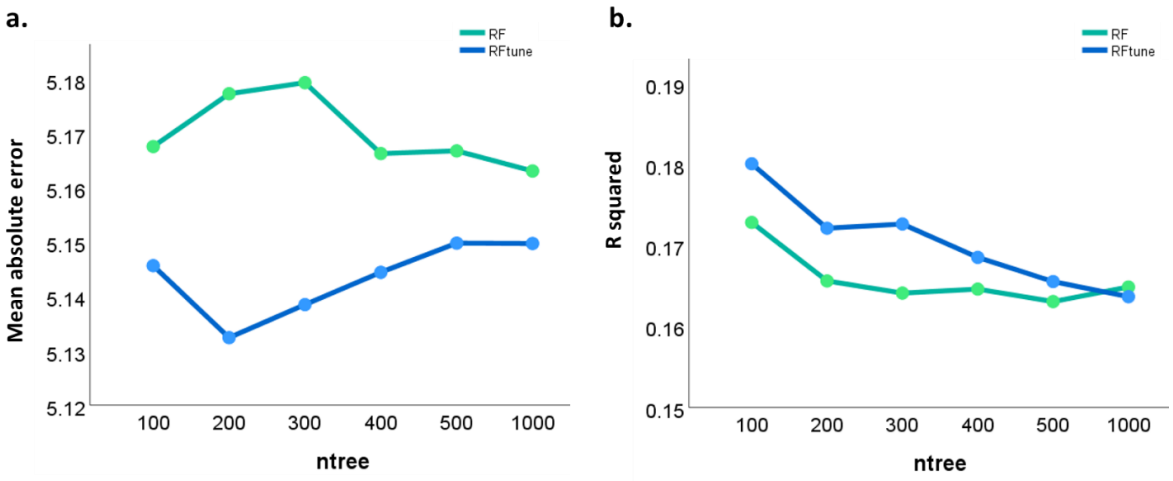


Figure 3.5. Evaluation of clinical variables based model between original RF model and tuned RF model from cross-validation. a. mean values of MAE and b. mean values of r^2 versus number of trees.

Model prediction performance from cross-validation

The ANCOVA for the five models using clinical variables showed a significant main effect on MAE ($F(4) = 6.75$, $P < 0.001$. Post hoc analysis revealed a significantly lower MAE for all four models; RF (5.16 ± 1.10, $P = 0.002$), SVM (5.00 ± 1.01, $P < 0.001$), tuned RF (5.16 ± 1.10, $P = 0.002$) and tuned RF with reduced features (5.26 ± 1.13, $P = 0.011$) than for the linear model (5.75 ± 1.15). All results remain significant even after Bonferroni-Holm correction. The cross-validation results are shown in Table 3.6.

Table 3.5 Correlation between predicted and actual values of fetal heart rate on testing dataset across feature sets and five separate models

Model	CV		mHRV		CV/mHRV	
	r	*P* value	*r*	*P* value	*r*	*P* value
Linear	0.317	0.082	0.035	0.853	0.219	0.238
Support vector machine	0.278	0.130	-0.156	0.403	0.222	0.223
Random forest	**0.378**	**0.036**	0.178	0.338	**0.376**	**0.037**
Tuned random forest	**0.360**	**0.047**	0.158	0.397	**0.397**	**0.027**
Tuned Random Forest with reduced features	**0.382**	**0.034**	0.250	0.174	0.312	0.088

Correlation coefficients, *r* with *P* values < 0.05 are considered statistically significant, and are marked in bold. Random forest model for all set of features uses ntree = 1000, mtry = 2. Tuned and tuned with reduced features random forest model for CV based model: ntree = 200, mtry = 2; mHRV based model: ntree = 100 ,mtry = 2; CV/mHRV based model: ntree = 100 ,mtry = 2. CV: clinical variables, mHRV: maternal heart rate variability, CV/mHRV: combined clinical and maternal heart rate variability.

Table 3.6 Performance of regression models from 10 repeated 10-fold cross-validation

Model	CV		mHRV		CV/mHRV	
	MAE	RMSE	MAE	RMSE	MAE	RMSE
Linear	5.75 ±1.15	7.13 ±1.41	5.87 ±1.31	7.42 ±1.58	6.35 ±1.41	7.66 ±1.63
Support vector machine	4.50 ±1.01	6.53 ±1.44	5.71 ±1.02	7.19 ±1.31	5.39 ±1.02	6.80 ±1.39
Random forest	5.16 ±1.10	6.71 ±1.43	6.04 ±1.25	7.65 ±1.47	5.41 ±1.14	6.89 ±1.44
Tuned random forest	5.21 ±1.11	6.77 ±1.46	6.11 ±1.29	7.70 ±1.50	5.46 ±1.18	6.91 ±1.56
Tuned random forest with reduced features	5.17 ±1.14	6.77 ±1.49	6.04 ±1.36	7.60 ±1.64	5.42 ±1.12	6.92 ±1.39
P value	**< 0.001**	0.054	0.140	0.107	**< 0.001**	**< 0.001**

Data presented are mean ±SD. *P* values represent ANOVA main effects for five models on error metrics. *P* values < 0.05 are considered statistically significant, and are marked in bold. Random forest model for all set of features uses ntree = 1000, mtry = 2. Tuned and tuned with reduced features random forest model for CV based model: ntree = 200, mtry = 2; CV/mHRV based model: ntree = 100 ,mtry = 2. CV: clinical variables, mHRV: maternal heart rate variability, CV/mHRV: combined clinical and maternal heart rate variability.

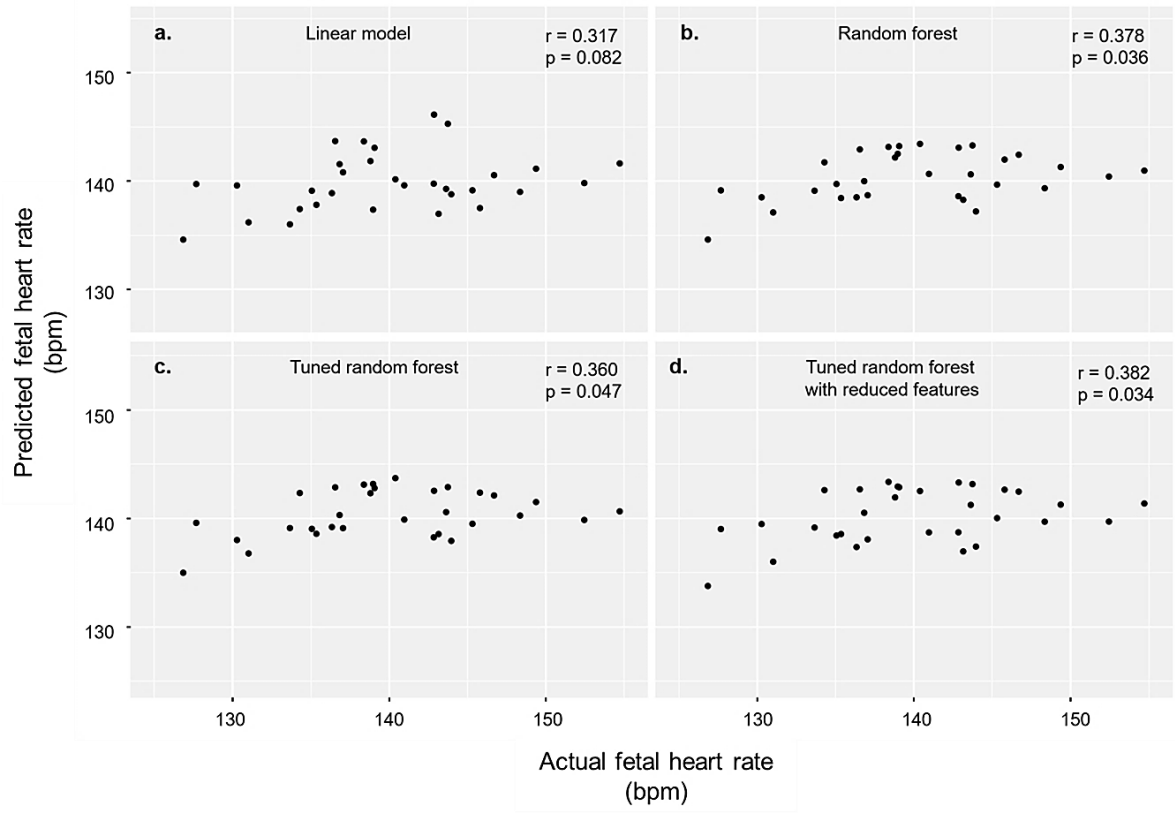


Figure 3.6 Regression using a feature set of clinical variables. Predicted versus actual fetal heart rate values using testing dataset for the a. linear model, b. random forest model, c. tuned random forest model and d. tuned random forest model with a reduced number of features.

3.6.2 Feature set of maternal HRV

Prediction of the testing dataset and model performance from cross-validation

No model based on maternal HRV variables showed significant correlation between the predicted and actual fetal HR values (Linear: $P = 0.853$, SVM: $P = 0.403$, RF: $P = 0.338$, Table 3.5). We tuned the RF model using *ntree* = 100 and *mtry* = 2 and reduced the number of features from 8 to 6 features based on the scores of variable importance. Correlations between the predicted and actual fetal HR values remained not significant even after the hyperparameter tuning and features

reduction (Table 3.5). The ANOVA showed no significant difference of MAE and RMSE between the five models (Table 3.6).

3.6.3 Feature set of combined clinical/maternal HRV

Prediction performance of the testing dataset

The predicted values of the RF model were significantly correlated with the actual fetal HR values (r = 0.376, P = 0.037). Models using linear regression and SVM showed no significant correlation between the predicted and the actual values of the fetal HR. The prediction results are shown in Table 3.5. We tuned with the RF model, which was the model with the best prediction performance. The value of the *ntree* = 100 and *mtry* = 2 were chosen because they had the lowest RMSE and MAE (the original RF model used *ntree* = 1000 and the same *mtry* value). When the tuned RF model was tested on the testing dataset, we observed that prediction was significantly correlated with the actual fetal HR (r = 0.397, P = 0.027). The prediction performance was better than the original RF model. In an attempt to improve the model prediction performance, we reduced the number of features from 19 to 13 based on the scores of variable importance. A zero cut-off of the importance score was used for feature reduction (Table 3.7). We then performed the model fitting again on the previously tuned RF model. The prediction was no longer significantly correlated with the actual fetal HR (r = 0.312, P = 0.088). Predictions of the three RF based models and the linear model are presented in Figure 3.7.

Model performance from cross-validation

An ANCOVA revealed a significant main effect of MAE and RMSE between the models of the combined clinical/maternal HRV feature set (MAE: $F(4) = 12.51$, $P < 0.001$, RMSE: $F(4) = 5.64$, $P < 0.001$). Post hoc analysis showed that the MAE was significantly lower for all models (all $P < 0.001$) compared to the linear model. The RMSE values were significantly lower for all models (all $P < 0.005$) than for the linear model. All results remained significant even after Bonferroni-Holm correction. The results of cross-validation are listed in Table 3.6.

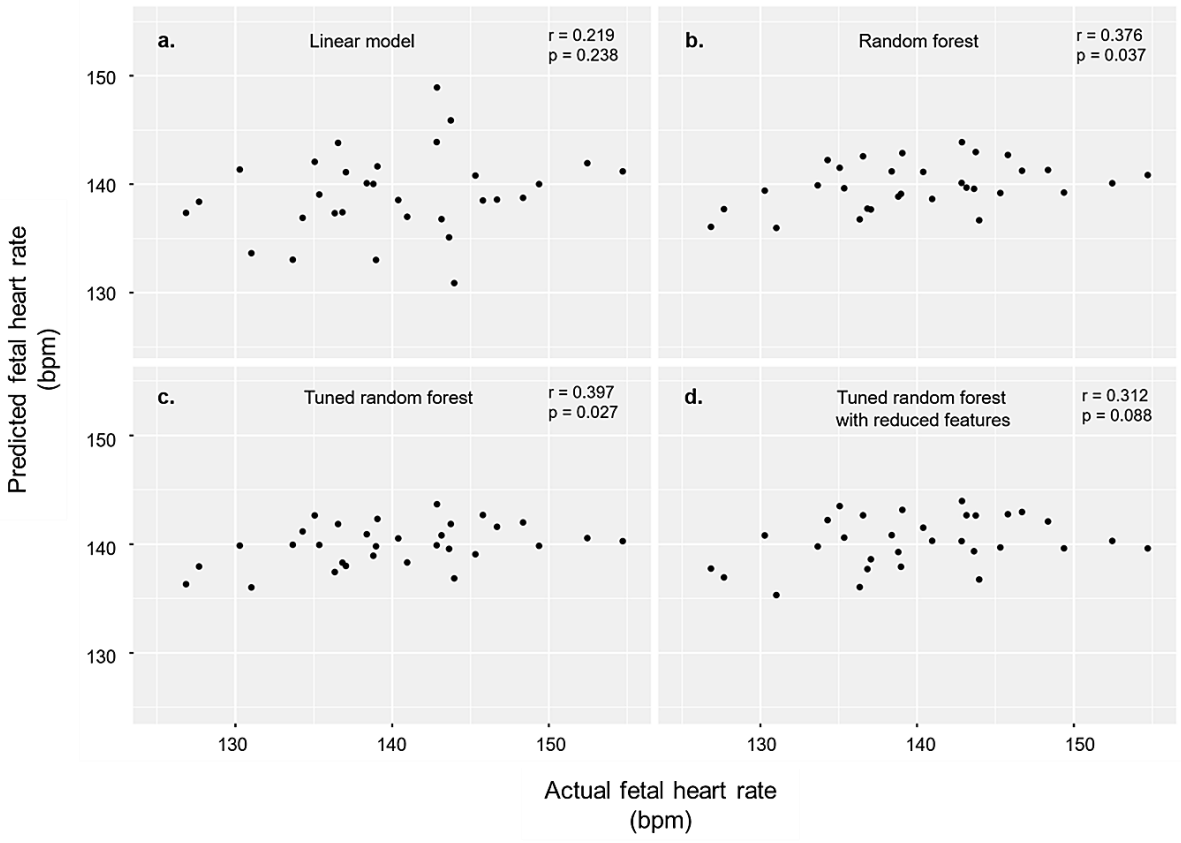


Figure 3.7 Regression using a combined clinical/maternal HRV feature set. Predicted versus actual fetal heart rate values using testing dataset for the a. linear model, b. random forest model, c. tuned random forest model and d. tuned random forest model with a reduced number of features.

Table 3.7 Variable importance score by feature set

CV				CV/mHRV			
Tuned RF		Tuned RF reduced features		Tuned RF		Tuned RF reduced features	
Feature	Imp	Feature	Imp	Feature	Imp	Feature	Imp
PreBMI	8.49	PreBMI	8.01	PreBMI	6.73	RMSSD	6.67
Gender	2.93	Gender	3.35	RMSSD	4.33	PreBMI	6.61
Ins	1.96	Ins	2.74	HF	4.21	LFn	5.39
Age	1.62	ISI	1.12	LF/HF	4.02	HF	5.22
ISI	1.41	Grav	0.91	HFn	3.55	LFHF	4.39
GA	1.41	Age	0.87	Gender	2.20	HFn	3.61
Grav	0.79	Glu	0.68	SDNN	2.01	GA	3.03
Glu	0.58	GA	0.63	LFn	1.88	Gender	2.29
MWG	0.04	MWG	0.01	ISI	1.80	HR	1.33
Para	-0.64			GA	1.67	Para	-0.05
Height	-0.73			Para	1.23	SDNN	-0.60
				HR	0.91	ISI	-0.10
				Height	0.33	Height	-1.23
				Age	-0.15		
				LF	-0.28		
				Glu	-0.43		
				Grav	-0.98		
				Ins	-1.07		
				MWG	-1.58		

CV: Clinical variables, CV/mHRV: combined clinical variables and maternal heart rate variability, Imp: variable importance score, RF: Random Forest

Chapter 4

General discussion

4.1. Discussion of the main findings

Table 4.1 Main findings

Pre-pregnancy BMI, maternal weight gain and maternal insulin sensitivity related to fetal autonomic nervous system

Fetal heart rate	Fetal heart rate variability
Higher fetal HR in fetuses of pre-pregnancy obese mothers	Fetal HRV (SDNN and LF) is lower in fetuses of high maternal weight gain mothers
Fetal HR has a U- shaped relationship with pre-pregnancy BMI (pre-pregnancy BMI in the range from 16.8 to 42.4 kg/m^2)	Fetal HRV has an inverted U- shaped relationship with pre-pregnancy BMI (maternal weight gain in the range from -0.24 to 0.93 kg/week)
Fetal HR mediates the effect of maternal weight gain on birth weight	Fetal HRV mediates the effect of maternal insulin sensitivity on fetal HR
Fetal HR mediates the effect of fetal HRV on birth weight	
Pre-pregnancy BMI is the driving factor of fetal HR	

Changes in pre-pregnancy BMI, maternal weight gain and maternal insulin sensitivity alter fetal HR and HRV

Our results provide evidence supporting our first hypothesis that variations in pre-pregnancy BMI, weight gain during pregnancy and maternal insulin sensitivity can alter the development of fetal ANS. Table 4.1 summarizes the findings. We found a significant effect for the four pre-pregnancy BMI groups with regard to fetal HR. Normal weighted mothers had the lowest fetal HR. However, the point should be borne in mind that the normal fetal HR is in the range of 120 to 160 bpm (Pildner von Steinburg et al., 2013). Although the mean HR in the fetuses of mothers with obesity was still in the normal range (the mean fetal HR is 142 bpm), the value was higher than in the fetuses of their lean counterparts. The fetuses in the underweight group showed the same pattern, albeit not significant, which is possibly due to the small sample size in the underweight group. This finding indicates a U-shaped relation, suggesting that pre-pregnancy BMI should be in the normal range to prevent alterations in fetal HR. Furthermore, with regard to 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 the SDNN was reduced by 16.5 percent. Although the maternal weight gain group was computed according to pre-pregnancy 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. (2016), in which maternal weight gain was reported to have no predictive power on fetal HR and fetal HRV beyond pre-pregnancy BMI. This reduction in fetal HRV in mothers with high weight gain is an indicator of an adverse effect on fetal ANS, since reduced fetal HRV is also observed in IUGR (Nijhuis et al., 2000) and GDM fetuses (Fehlert et al., 2016).

In general, the lower the pre-pregnancy BMI, the more weight gain during pregnancy is expected, and the ranges of a recommended weight gain for women with normal pre-pregnancy BMI are higher compared with women with obesity. Therefore, the observed relationship between pre-pregnancy 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 pre-pregnancy BMI is associated not only with fetal ANS changes but also with increased maternal weight gain, even in women with normal pre-pregnancy BMI. Different, potentially adverse intrauterine environments—whether pre-pregnancy underweight or obesity—as well as inadequate or excessive maternal weight gain may therefore result in altered trajectories of normal ANS development. HR and HRV alterations are driven by sympathetic and parasympathetic activity. The U-shaped and the inversed U-shaped curves in the effect of pre-pregnancy BMI and maternal weight gain could indicate immaturity of fetal ANS, implicating an anomalous ANS development in less favourable intrauterine environments.

In healthy fetal development, fetal HR decreases gradually with advancing gestational age (Pildner von Steinburg et al., 2013; Serra et al., 2009). Maturation of the parasympathetic vagal tone commences at approximately 31 weeks of gestation and continues after birth (Nederend, Jongbloed, de Geus, Blom, & ten Harkel, 2016). This stronger parasympathetic influence causes a reduction in HR and an increase in HRV during gestation (Pildner von Steinburg et al., 2013; Schneider et al., 2009; Van Leeuwen et al., 2013; Wakai, 2004). Higher fetal HR and lower fetal HRV can be due either to an increase in sympathetic regulation or to a

decrease in parasympathetic regulation. Since the majority of our study population was 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 obese/overweight pre-pregnancy BMI is most probably influenced by activity in the sympathetic rather than in the parasympathetic nervous system. In addition, the observed effects on fetal HRV are seen in measures associated with both sympathetic and parasympathetic activity, but not in those associated primarily with parasympathetic activity. This indicates that the observed alterations are very probably the result of enhanced sympathetic nervous system activity.

In addition, reduced maternal insulin sensitivity was indirectly associated with an increased fetal HR through fetal HRV, suggesting that the fetuses of healthy mothers with decreased insulin sensitivity are already exposed to a less favourable metabolic environment. As of now, only one study has studied the relationship between fetal heart parameters and maternal insulin sensitivity, but no association was observed (Dewi et al., 2017). These researchers suggested that their sample size (44 samples) was too small to show an effect. The correlation analysis in the present work revealed that decreased maternal insulin sensitivity and increased maternal fasting insulin are associated with an increased maternal HR and fetal HR. Furthermore, HR is higher in mothers with GDM than in normoglycemic mothers (Fehlert et al., 2016), and HR was higher in the fetuses of mothers with GDM both during the fasting state and after an oral glucose load. At 120 minutes after a glucose load, the SDNN, as the measure of overall variability, was lower in fetuses of mothers with GDM. The researchers also suggested that the decrease in the fetal HR in fetuses of mothers with GDM might be a reflection of fetal insulin

resistance (Fehlert et al., 2016). Based on these observations, the increased maternal and fetal HR associated with lower maternal insulin sensitivity and higher maternal fasting insulin already seen in normoglycemic mothers could be related to higher sympathetic activity in both mother and fetus.

Fetal HR mediates the relationship between maternal weight gain and birth weight

The role of MAM influences and fetal ANS on neonatal outcome, specifically whether fetal HR is mediating the relationship between maternal weight gain and birth weight, would be interesting to know. The absence of a linear association between maternal weight gain as a continuous variable and birth weight indicated that maternal weight gain has no association with the birth weight. However, mediation analysis revealed a significant effect of maternal weight gain on birth weight after adding fetal HR as the mediator. In other words, an increase in maternal weight gain associated with an increase in birth weight, but indirectly through the decrease in fetal HR. Based on the birth weight guidelines from Erich-Saling Institute of Perinatal Medicine (2013), the majority of fetuses in this study have birth weights within the normal recommended range (97.8%, mean birth weight 3388 gram, recommended range is from 2500 to 4499 gram). The mean fetal HR (96.7 %, mean fetal HR 140.9 bpm) was also within the normal range (Pildner von Steinburg et al., 2013). Nonetheless, the fetal HR mediated the association between maternal weight gain and birth weight. In an impaired environment, the fetal ANS control may adapt to compensate for the changes in metabolism, perhaps through alterations in the fetal HR. This would appear to indicate that high sympathetic nervous system activity in fetuses may be involved in the fetal growth that is reflected in birth weight. Additionally, this finding

provides further supporting evidence that an immature ANS in the fetus may be an important key linking the increased birth weight and large-for-gestational-age offspring seen in women with excessive maternal weight gain. In the second mediation model on birth weight, besides maternal weight gain, the fetal HR also mediates the relationship between fetal HRV and birth weight. Birth weight is assumed to also have an optimal range and has a U-shaped relationship with fetal HR.

Pre-pregnancy BMI is the driving factor of fetal HR

The regression analysis using the advanced machine learning method showed that pre-pregnancy BMI has a major impact on the fetal HR and the impact was stronger than the maternal weight gain during pregnancy. Focusing first on the technical aspects of model performance, the results from both the clinical variables and the combined clinical/maternal HRV features show that the RF model outperforms multiple linear regression. The models successfully predicted the fetal HR as representing the complexity and dynamics of fetal ANS. Even clinical features alone—including multiple clinical variables such as gestational age, maternal weight and metabolic measures—were able to predict fetal HR.

Given the best prediction performance of RF in the two feature sets (clinical variables and combined clinical/maternal HRV), model tuning was performed by choosing the best tree depths for making the prediction. The tuned model in the combined clinical/maternal HRV based model with a smaller number of trees produced an even better prediction than the clinical variable based model. By limiting the number of trees to 100 (which is the best total number of branches grows after each split), the forest is less deep, thereby increasing the model

generalization. We further reduced the complexity of the model by choosing a cut-off of zero for the importance score to filter out the less important features. With only nine features, the clinical variables-based model achieved a higher accuracy than the model with thirteen features in the combined clinical/maternal HRV.

During training, the model learned the patterns in the training dataset to make predictions, including the data that do not contribute to the predictive value. Even though in the cross-validation the SVM showed a similar performance to that of the RF model, the correlation results between prediction and the actual fetal HR values indicated that the RF model could describe the fetal ANS better than the SVM model. The RF technique probably produced a prediction closest to actual values compared to other assessed techniques because of the generalization created from the random sampling of subset and the selection of the features to make the split at each tree node. In several cross-validation folds using the decision tree algorithm, fetal HR values in the test subset were not within the range of the training set. In these cases, the decision tree could not find the best split and thus, took the average of fetal HR as the prediction outcome. The error metrics could not be calculated for these particular folds because the variance of the prediction becomes zero.

From the ranking of the variable importance score, pre-pregnancy BMI has the largest impact on the prediction performance of the fetal HR models (Table 3.5). Maternal weight gain appeared to be among the least important features, with a negative importance score. After testing for collinearity, only maternal age, pre-pregnancy BMI and gestational age had significant correlations with maternal weight gain, but the correlations were low (all parameters had $r < 0.25$). Moreover,

the RF algorithm produces diverse sets of trees and automatically removes the interaction and association between the features through the random sampling of features in the subunit of trees (Strobl et al., 2009). Therefore, the low importance of maternal weight gain in the model may not be due to collinearity between the included variab21les in the models. A possible reason could be that, among the MAM factors, a factor before pregnancy has a stronger influence than that factor during pregnancy in predicting the fetal HR. The importance of weight before pregnancy compared to weight during pregnancy has been reported in many studies (Kuzawa, 2005; Van Lieshout, Taylor, & Boyle, 2011). Among the maternal HRV features, RMSSD appeared to have the greatest on the fetal HR and was among the top features in the combined clinical/maternal HRV model. However, no maternal HRV-based model was able to predict the fetal HR even after the model tuning. When we combined maternal HRV with clinical features (clinical/maternal HRV), the model prediction performance became similar to the clinical variables based model. By integrating clinical variables like pre-pregnancy BMI, gender and maternal fasting insulin in the fetal HR model, we were able to predict fetal HR with high accuracy, even without maternal HRV. Furthermore, this finding also showed that maternal HRV features alone play a less important role than the MAM factors in the development of fetal ANS.

Therefore, the RF model using the nine most relevant features can be concluded to best describe the fetal HR. Fetal HR is thus predicted by the following variables: pre-pregnancy BMI, maternal fasting insulin, insulin sensitivity, gravidity, maternal age, maternal fasting glucose, gestational age and maternal weight gain, with pre-pregnancy BMI being the strongest influence on the fetal HR. From the ranking of variable importance, maternal glucose metabolism (fasting insulin and insulin

sensitivity) also has an important influence on the fetal HR as a maternal metabolic factor during pregnancy.

Fetal HR differs between genders

Female fetuses had a higher HR than the male fetuses, but no gender difference was detected for fetal HRV. The effect of gender on fetal HR appeared to be in line with some data shown previously (DiPietro et al. (2015), but other studies have found no gender differences in fetal HR (Bracero et al., 2016; Druzin et al., 1986; Fleisher et al., 1997; Genuis et al., 1996; Lange et al., 2005; Ogueh & Steer, 1998). Small sample sizes or different inclusion criteria might be the reasons for the inconsistencies. The significant gender difference in fetal HR might reflect variations in behavioural and neurological development by gender during gestation (Buss et al., 2009). Thus, in all fetal HR and HRV analyses of this work, gender has been included as a covariate, together with gestational age and parity. This additional observation of the gender related to fetal HR is important, as it would influence findings in future studies. Hence, taking gender into account as a covariate might be relevant in future fetal HR and HRV analyses.

4.2. Interconnections between maternal weight, weight changes and metabolism, fetal ANS and birth weight

To summarize and connect the main findings, Figure 4.1 presents the relationships connecting MAM variables, fetal ANS and birth weight.

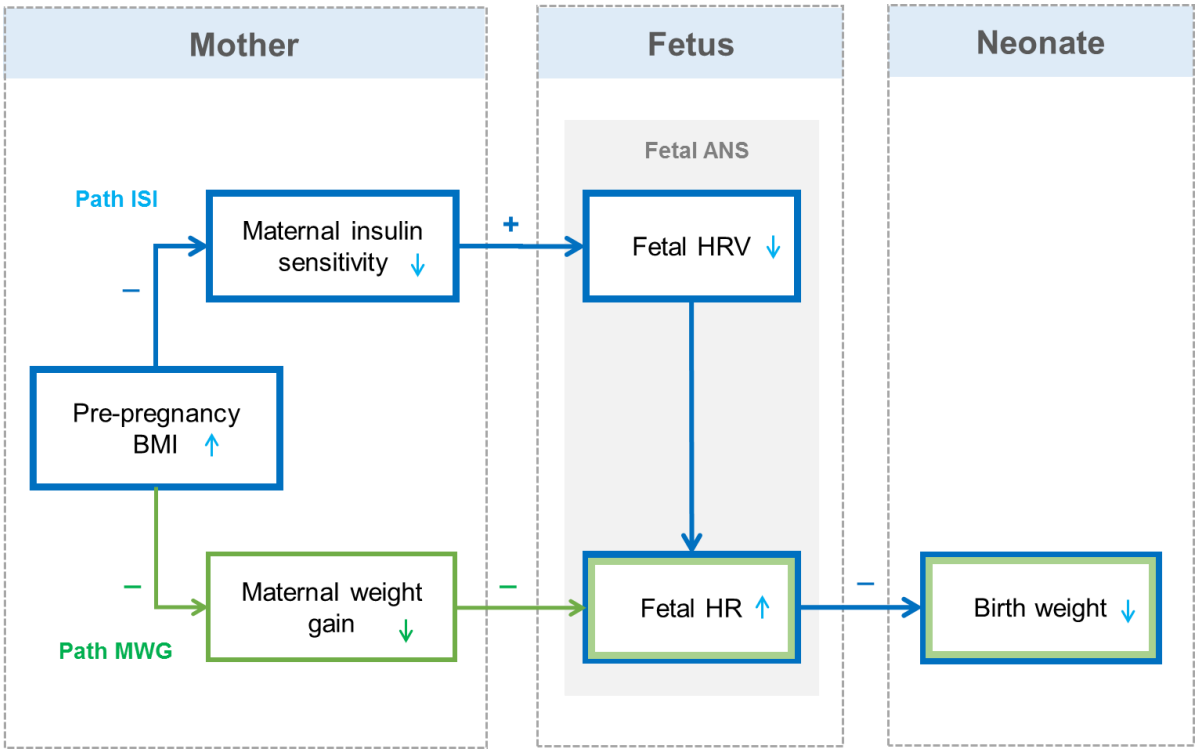


Figure 4.1 Fetal ANS and its association with pre-pregnancy BMI, maternal weight gain, maternal insulin sensitivity and birth weight. Only associations from correlation and mediation analyses are included in the overall relationship.

The relationships shown in the figure indicate two paths linking the factor before pregnancy (pre-pregnancy BMI) and the factors during pregnancy (maternal insulin sensitivity and maternal weight gain), which both connect to the birth weight through the fetal ANS. The first path, “Path ISI”, begins with the association of pre-pregnancy BMI and the maternal insulin sensitivity, through fetal HRV as well as fetal HR and finally birth weight. Through path ISI, for example, a mother with obesity and lower insulin sensitivity (i.e. insulin resistance) would probably have an associated lower fetal HRV and therefore might also show an association with higher fetal HR. This overall relationship might play a role in the decreased birth weight. The second path, “Path MWG”, begins with the relationship between pre-pregnancy BMI and maternal weight gain, as well as fetal HR and finally birth

weight. For the path MWG (i.e. a mother with pre-pregnancy obesity), the lower weight gain might be associated with an increase in the fetal HR and, at the end, might probably be associated with a decrease in the birth weight.

Interestingly, an increase in the pre-pregnancy BMI through both paths was associated with signs of adverse fetal ANS, as indicated by an increase in the fetal HR and/or a decrease in the fetal HRV. However, both paths show a low birth weight as the outcome from a mother with pre-pregnancy obesity. This contrasts with the evidence seen among mothers with pre-pregnancy obesity associated with offspring obesity (Leonard et al., 2017; Mamun et al., 2014; Nehring et al., 2013). A point that should be taken into account is that the majority of fetuses in this sample had birth weights within the normal recommended range (97.8%, mean birth weight 3388 g, the recommended range is from 2500 to 4499 g). We suspected that the relationship between fetal HR and birth weight is not linear.

Combining the interconnections and the findings using machine learning approach, the path ISI appears to play a more important role in the programming of the fetal ANS by pre-pregnancy obesity compares to the path MWG. Pre-pregnancy BMI, followed by maternal insulin sensitivity, are the key factors associated with fetal ANS, while maternal weight gain is the least important factor. Maternal weight gain appeared to be the least important factor not only in the winning model (clinical variables) but also in the other models combining all features (clinical/maternal HRV) as well. A longitudinal study across pregnancy showed that the factor before pregnancy is more important than the factor during pregnancy in representing the intrauterine environment (Hellmuth et al., 2017). Furthermore, Gluckman pointed out that nutrient-dependent signalling (such as

insulin-like growth factor) responds to slow changes in maternal metabolism rather than to acute changes (2010). Maternal long-term environmental signalling also appears to be an important indicator for the developing fetus (Kuzawa, 2005).

In the overall relationship, exposure of fetal ANS to over-nourished condition like high pre-pregnancy obesity, low maternal weight gain and low maternal insulin sensitivity, during fetal development associated with the development at birth - a reduced birth weight. The observed relationship in our findings (as shown in Figure 4.1) appeared to match with the model proposed by Kuh, Ben-Shlomo, Lynch, Hallqvist, and Power (2003), "critical period model". The researchers proposed the model to explain the development of adult disease originating from exposure of the structure/function of organs and tissues/body systems during a sensitive period of early development. This model served as a basic concept of the DOHaD hypothesis (Gluckman & Hanson, 2006; Gluckman et al., 2010).

Programming of fetal ANS related to pre-pregnancy obesity and maternal insulin resistance

To our knowledge, no previous study has analysed the relationship between fetal HR and pre-pregnancy BMI and maternal insulin sensitivity in healthy pregnant mothers. Studies in offspring of mothers with obesity showed that pre-pregnancy BMI is associated with higher insulin levels (Gaillard et al., 2014), but no effect was found on the child's resting HRV (Gademan et al., 2013). The mechanism that could explain how pre-pregnancy BMI and maternal glucose metabolism may lead to changes in fetal HR is not known. The significant association observed between maternal HR and maternal insulin sensitivity and maternal fasting insulin (as shown in Chapter 3), and the maternal RMSSD appeared as a second important

factor after pre-pregnancy BMI in the fetal HR regression model with combined clinical/maternal HRV features. This suggests that maternal ANS probably plays some role in the overall relationship connecting pre-pregnancy BMI, maternal insulin sensitivity and the fetal ANS. Exposure to pre-pregnancy obesity together with insulin resistance may program fetal ANS during early development, thereby possibly interrupting the normal fetal metabolic environment. According to the Pedersen hypothesis, fetal growth is related to a higher transfer of glucose through the placenta, thus stimulating the release of insulin in the fetus and resulting in fetal macrosomia (P. M. Catalano & Hauguel-De Mouzon, 2011; Pedersen, 1971). A higher glucose level in pre-pregnancy obese and insulin resistant mothers causes the transportation of glucose through placental barrier to the fetus (Vogt et al., 2014). The ANS is responsible for regulation of energy and glucose homeostasis (Marino, Xu, & Hill, 2011); therefore, an increase in the sympathetic activity (i.e. an increase in the fetal HR) might be a mechanism for adaptation to the higher glucose environment. In turn, it possibly leads to an increase in insulin secretion in the fetus and may interrupt the normal fetal metabolic development.

4.3. Potential mechanism and implication

Hyperglycemia-hyperinsulinemia

In healthy pregnancies, the basal fetal HR increases and the fetal HRV decreases in a high glucose environment after glucose ingestion (Gillis, Connors, Potts, Hunse, & Richardson, 1992; Weissman, Goldstick, Geva, & Zimmer, 2003; Zimmer, Paz, Goldstick, Beloosesky, & Weiner, 2000). The assumption made in the present study is that the observed changes in fetal HR and HRV are due to an excess of glucose in the maternal circulation, which then elevates the glucose transport

across the placenta, even in normoglycemic mothers who were obese pre-pregnancy. The increase in sympathetic activity could also be a fetal response to stress imposed by high glucose. According to the Pedersen hypothesis, hyperglycemia in mothers can cause an increase in fetal insulin secretion, thus resulting in fetal hyperinsulinemia (Pedersen, 1971). Cohen showed hyperinsulinemia from insulin infusion leads to a high fetal HR in fetal lambs; this may indicate a direct effect of insulin on the peripheral sympathetic nerves (Cohen, Piasecki, Cohn, Susa, & Jackson, 1991). Human studies in healthy adults also support this assumption, as acute insulin stress exerted by a hyperinsulinemic euglycemic clamp has been shown to reduce parasympathetic influence and increase sympathetic activity (Bellavere et al., 1996; Van De Borne, Hausberg, Hoffman, Mark, & Anderson, 1999). Fetal hyperinsulinemia may therefore play some role in the increased fetal HR in the fetuses of mothers with pre-pregnancy obesity. A recent suggestion has been made that insulin resistance and BMI, rather than the insulin level, are the main influencers of autonomic cardiac control in adults (Valensi, Chiheb, & Fysekidis, 2013). Whether this also is similar in the offspring of mothers with obesity remains to be investigated.

Delayed ANS maturation

The progress of the normal fetal ANS maturation is reflected by a decrease in fetal HR and an increase in fetal HRV with advancing gestation, indicating lower sympathetic activity and higher parasympathetic activity (Pildner von Steinburg et al., 2013; Serra et al., 2009; Van Leeuwen et al., 1999; Wakai, 2004). The current findings show that changes in nutrition in utero in relation to MAM factors alter the autonomic fetal cardiac activity. In particular, increases in pre-pregnancy BMI and decreases in maternal insulin sensitivity are associated with an increased fetal

HR and decreased fetal HRV. The alteration of fetal HR and HRV in mothers with pre-pregnancy obesity and insulin resistance might indicate a disturbance in the normal trajectory of fetal ANS maturation. Considerable data support this hypothesis. For example, a recent fMEG study showed that GDM resulted in a high fetal HR during the fasting state and a low fetal HRV postprandially during OGTT (Fehlert et al., 2016). Other studies have reported that GDM was associated with a high baseline fetal HR (Costa et al., 2009; Sirico et al., 2019). Conversely, inadequate intrauterine nutrition in IUGR fetuses elicited a similar response of the fetal HR and HRV (Bekedam et al., 1987; Nijhuis et al., 2000; Schneider et al., 2006). The present findings are in agreement with previous observations of lower fetal HRV in mothers with pre-pregnancy obesity (Voegtline et al., 2016). Therefore, we suspect that excessive intrauterine nutrition due to changes in pre-pregnancy BMI and insulin sensitivity may have important implications in the maturation of the fetal ANS. As the fetal ANS can be programmed during fetal life, fetal adaptation to these nutritional changes during development may disturb the normal maturation of the ANS.

Insulin resistance in fetuses of mothers with pre-pregnancy obesity – a possible missing mediator in the overall relationship

Women with obesity enter pregnancy with higher insulin resistance than normal weight women and, due to pregnancy, the insulin resistance further increases with gestational age (P. Catalano & deMouzon, 2015; P. M. Catalano, Huston, Amini, & Kalhan, 1999). The combination of pregnancy and obesity generates a dysregulation in metabolism that leads to a pathological state of insulin resistance in mothers. In the proposed inter-relationship (as shown in Figure 4.1), mothers with pre-pregnancy obesity and in the state of increased insulin resistance have

fetuses with an altered fetal ANS. An alteration in the fetal ANS could also be associated with the insulin resistance in the fetus that is missing in the overall relationship. This suggestion is supported by the Landsberg hypothesis, which proposes that insulin resistance in obese individuals with sympathetic stimulation is a mechanism for limiting weight gain, but it leads to an increase in blood pressure (Landsberg, 1990, 2001). Specifically, the hypothesis states that "insulin resistance serves to stabilize body weight by limiting fat stored in adipose tissue as well as via the sympathetic nervous system, driving thermogenic mechanisms to restore energy balance". The fetuses of mothers with pre-pregnancy obesity in the proposed relationship who appeared to have lower birth weights agree with this hypothesis. In another observation related to this work, Catalano reported that infants of mothers with obesity have higher peripheral insulin resistance (P. M. Catalano et al., 2009) and the increased fetal insulin resistance was closely associated with increased neonatal body fat mass; however, they did not test the association with the birth weight. Whether the alteration in fetal ANS is a mechanism to prevent weight gain (as proposed by Landsberg) and/or leads to higher fat mass (as observed by Catalano) remains to be established.

Furthermore, recent findings have suggested that insulin resistance in the brain controls not only the body weight, but also glucose metabolism (Hallschmid & Schultes, 2009). Insulin resistance in the fetal brain has been proposed as the cause of the alteration seen in fetal CNS in insulin-resistant mothers (Linder et al., 2014; Linder et al., 2015). A study of adults with obesity showed that insulin apparently influenced parasympathetic heart activity and hypothalamus activation, suggesting an action of insulin in the brain (Heni et al., 2014). Alterations in autonomic cardiac function that precede insulin resistance have been found in

adults, and this might also be true in fetuses (Chang et al., 2010; Masuo, Mikami, Ogihara, & Tuck, 1997; Valensi et al., 2013). Based on this evidence and many theories, a possible explanation is that fetal insulin resistance, as well as fetal fat mass, could be the missing factors in the proposed relationship that links the changes in the fetal ANS towards sympathetic predominance. The state of insulin resistance in the fetus as either a cause or a consequence of the fetal ANS alteration is a subject for future investigation.

4.4. Strengths, limitations and suggestions for future research

One of the strengths of this work is the use of a unique fMEG device that can non-invasively measure the heart signals of the fetus (Preissl et al., 2005), thereby providing a window into fetal ANS development. The fetal HRV recorded with fMEG can be used to monitor fetal ANS, as has been demonstrated in previous studies (Brändle et al., 2015; Fehlert et al., 2016). The present work confirmed that the fMEG device is an excellent tool for detecting changes in fetal ANS associated with maternal metabolism in normoglycemic pregnancies, concerning the potential effect of intrauterine metabolic environment on fetal programming.

The analysis included the use of mediation analysis, which is more complex than linear regression and allows testing of mediation factors in the relationship of interest. Moreover, the implementation of advanced machine learning techniques has provided additional useful information, especially in the study of noisy and complex biological signals. The observed effect of gender on fetal HR has been replicated (DiPietro et al., 2015), suggesting that gender effect should be taken into

account in future fetal HR and HRV studies. Even though the work in this dissertation includes a large population, it consists of cross-sectional measurements. Additionally, the reported significant correlations show 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 non-linear relationships, which should be taken into account in further studies. To advance the understanding of the features responsible for the development of ANS and to draw valid conclusions, long-term studies with HRV measures in offspring throughout childhood are necessary. This is important for tracking changes during development and for observing the relevance of pre-pregnancy BMI, maternal weight gain and maternal insulin sensitivity on the ANS and its contribution to the development of obesity in the offspring during infancy and childhood.

Chapter 5

Conclusion

In conclusion, these findings suggest that an alteration occurs in the development of fetal ANS in response to changes in pre-pregnancy BMI, maternal weight gain and maternal insulin sensitivity. These alterations might indicate a sign of less maturity in the ANS in fetuses of mothers with metabolism disturbances. The state of insulin resistance in the fetuses of mothers with GDM might also be true for fetuses of mothers with pre-pregnancy obesity and insulin resistance. The fact that fetal ANS mediates fetal growth suggests that ANS might potentially be one of the key features involved in the risk the development of disease in offspring. Hence, an alteration in fetal ANS during early development in relation to maternal weight, weight changes and maternal metabolism could have important clinical consequences in this era of rising obesity. Furthermore, this finding highlights the importance of pre-pregnancy weight which, together with maternal insulin sensitivity, could play a crucial role in the developmental programming of the fetal ANS. This may be an early life process driving maternal-fetal transmission of obesity and insulin resistance in offspring. High fetal HR may serve as a marker for an adverse fetal ANS status under the influence of pre-pregnancy weight and maternal insulin sensitivity. Maternal weight and metabolic factors are interconnected and connected to fetal ANS activity, so they might form a part of the complex relationship for fetal adaptation to the intrauterine environment.

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