MATERNAL ADIPOSITY, INSULIN SENSITIVITY AND INFLAMMATION, AND INFANT ADIPOSITY: ASSOCIATIONS WITH OFFSPRING AUTONOMIC NERVOUS SYSTEM DEVELOPMENT

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ABSTRACT

Objective: The prevalence of maternal overweight and obesity is high and the impact is far reaching. Recent studies suggest that fetal exposure to maternal obesity is related to poor neurodevelopmental outcomes in childhood. However, limited data have been collected on the developing fetus. Studies also suggest that the adverse effect was mediated through insulin resistance (IR) and inflammation. We investigated if maternal pre-pregnancy BMI, gestational weight gain (GWG) and newborn adiposity, were associated with fetal autonomic nervous development as indicated by fetal heart rate variability (HRV) and newborn behavior. We also investigated if maternal IR and inflammation were associated with fetal HRV.

Material and methods: A total of 48 mother-offspring pairs were analyzed. Short-term (SD1) and overall (SD2) fetal HRV were measured at 32 and 36 weeks of pregnancy using fetal magnetocardiogram. Maternal plasma levels of glucose, insulin, and inflammatory cytokines (TNF-α and IL-6) were analyzed from maternal peripheral blood collected at 36 weeks of pregnancy. Newborn body composition was measured using air-displacement plethysmography (PeaPod®). Multiple linear regression models predicted fetal HRV measures (SD1 and SD2 at 36 week, and SD1 and SD2 change between 32 and 36 week) and NBAS clusters from maternal and newborn factors. Infant gender and pre-pregnancy BMI were included as confounders.

Results: In the adjusted regression model, greater GWG was associated with greater SD1 (β=0.442; R^2=0.19; p=0.003) and with SD1 change (β =0.511; R^2=0.27; p=0.007). Lower newborn %fat was associated with greater SD2 change, but the association was diminished in the

adjusted model (β=-0.426; R^2=0.17; p=0.055). No correlation was found between any of NBAS cluster scores and maternal or infant predictors, and between fetal HRV and maternal IR and blood inflammatory markers.

Conclusions: Greater GWG was associated with better fetal autonomic nervous development, while greater newborn %fat was associated with poorer fetal autonomic nervous development. Further studies are needed to elucidate the impact of maternal and fetal/newborn adiposity on fetal neurodevelopment.

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CHAPTER 1

INTRODUCTION AND SPECIFIC AIMS

1.1. Introduction

Maternal obesity and excessive gestational weight gain (GWG) are now considered major public health concerns due to obesity associated health outcomes and a high prevalence of occurrence. A 2011 Pregnancy Nutrition Surveillance System report stated the prevalence of maternal overweight or obesity (pre-pregnancy body mass index (BMI) > 25 kg/m^2) in the US is 53.6%, and 48% of women gain greater than ideal weight during pregnancy [1]. Both maternal pre-pregnancy BMI and GWG are positively correlated with the offspring's adiposity and adverse cardiovascular risk factors at birth and later life [2-10]. Despite convincing evidence that the intrauterine environment influences growth and development of the offspring, it is not fully understood how obesity and excessive GWG influence offspring health.

Independent of pregnancy, obesity is associated with poor autonomic nervous system (ANS) function [11-14]. The ANS plays an important role in the pathophysiology of various diseases, including hypertension [15], atherosclerosis [16], and type-2 diabetes mellitus [17, 18]. Studies suggest that ANS dysfunction is associated with body fat mass (FM) and contributes to the development of insulin resistance (IR) and diabetes [13, 14, 17, 19-21]. Heart rate variability (HRV) is an index of ANS development and function. In fetuses, higher HRV is considered an indicator of a more mature ANS [22]. In healthy, non-pregnant adults and in those with cardiovascular disease, a decrease in HRV is associated with higher levels of inflammatory markers [16, 23-26].

A previous study links fetal HRV to postnatal developmental outcomes [27]. A higher fetal HRV is related to a higher HRV at one month of age, suggesting that the ANS is programmed during gestation. Because ANS dysfunction is associated with various diseases, exposure to events during intrauterine development of the ANS may program offspring

susceptibility to disease development later in life. Therefore, fetal HRV could predict later disease risk.

ANS function in neonates and young infants is reflected by self-regulation of behavior and assessed in part by the Neonatal Behavioral Assessment Scale (NBAS) [28]. While many studies confirm the role of the ANS in the pathophysiology of diseases, less is known about the interaction between the intrauterine environment and fetal ANS development. Human observational studies have shown that pre-pregnancy obesity is associated with IR, impaired metabolism, and inflammation in both the mother and fetus which results in greater infant adiposity. This association is stronger in women who gain excessive gestational weight [29, 30]. However, the effects of these factors on fetal ANS development have yet to be investigated.

Evidence from animal studies show that maternal adiposity and higher placental glucose transport to the fetus is associated with impaired offspring ANS development [31, 32]. Additionally, a recent study indicated that increased GWG is associated with decreased fetal HRV [33]. Although the study was not designed to investigate the association between GWG and fetal HRV, the finding supports previous animal studies that suggest an association between maternal adiposity and offspring ANS development [31]. We speculate that greater neonatal adiposity and maternal pre-pregnancy obesity and/or excessive GWG may induce alteration in ANS function. The relationship could occur through a direct mechanism or indirectly by activation of placental inflammatory pathway or fetal metabolic changes induced by maternal adiposity may alter the fetal ANS function independent from fetal adiposity.

Understanding how maternal conditions relate to fetal ANS development will help to identify the pathways that can be targeted for nutrition or lifestyle intervention. Therefore, the

study aims are to investigate the relationships between maternal adiposity, maternal IR and inflammation, and infant adiposity on ANS development of the fetus and neonates.

1.2. Specific aims

The general aim of this study is to analyze the relationships of intra-uterine factors during pregnancy and ANS and newborn behavior of the offspring. Intra-uterine factors include maternal adiposity (pre-pregnancy BMI and GWG), newborn adiposity (newborn FM), maternal inflammation and maternal IR. ANS and behavior of the offspring include non-linear fetal HRV measures (SD1 and SD2) at 36 weeks of gestational age, the change in the fetal HRV measures (SD1 at 36 weeks – SD1 at 32 weeks, and SD2 at 36 weeks – SD2 at 32 weeks) and NBAS score. In specific, the study aims are as follow:

Aim 1. To determine the relationships between maternal adiposity and fetal HRV and newborn behavior, and between neonatal adiposity and fetal autonomic development and newborn behavior.

Hypothesis 1.1: Pre-pregnancy BMI is negatively associated with the fetal HRV.

Hypothesis 1.2: GWG is negatively associated with the fetal HRV.

Hypothesis 1.3: Pre-pregnancy BMI is negatively associated with fetal HRV change.

Hypothesis 1.4: GWG is negatively associated with the change of the fetal HRV.

Hypothesis 1.5: Pre-pregnancy BMI is negatively associated with NBAS score.

Hypothesis 1.6: GWG is negatively associated with NBAS score.

Hypothesis 1.7: Newborn FM is negatively associated with the fetal HRV.

Hypothesis 1.8: Newborn FM is negatively associated with fetal HRV change.

Hypothesis 1.9: Newborn FM is negatively associated with the NBAS score.

Aim 2. To determine the relationships between maternal IR and inflammation during pregnancy and fetal HRV.

Hypothesis 2.1: Maternal IR is negatively associated with the fetal HRV.

Hypothesis 2.2: Maternal IR is negatively associated with the fetal HRV change.

Hypothesis 2.3: Maternal blood TNF-α is negatively associated with the fetal HRV.

Hypothesis 2.4: Maternal blood TNF-α is negatively associated with fetal HRV change.

Hypothesis 2.5: Maternal blood IL-6 is negatively associated with the fetal HRV.

Hypothesis 2.6: Maternal blood IL-6 is negatively associated with the fetal HRV change.

CHAPTER 2 LITERATURE REVIEW

2.1. Obesity in pregnancy

Obesity is a major public health problem in both developed and developing countries. Worldwide, it is estimated that more than 1.4 billion people are obese [34]. The risk of obesity for cardiovascular disease, type 2 diabetes mellitus, cancer and inflammatory diseases has been well documented [35-38]. Because recent studies have suggested that obesity is passed through generations, there is an interest in studying the impact of maternal obesity on the offspring. The impact of maternal obesity on offspring development may interact with or be independent of the effects of excessive GWG. Both conditions are related to increased adiposity in the offspring, as well as to increased risk of comorbidities such as type 2 diabetes and cardiovascular diseases.

2.1.1. The problem of pre-pregnancy obesity

Within the past 30 years, the prevalence of overweight and obesity in women worldwide has increased from 29.8% to 38.0% [38, 39]. World Health Organization 2014 statistics indicate that more than half of women in 38 out of 136 countries have a BMI greater than recommended (> 25 kg/m^2) [35]. In the US, about 35.8% of women of reproductive age are obese [40] and about one out of five women were obese when they became pregnant [41]. PRAMS data from 20 states showed that the prevalence of pre-pregnancy obesity has increased by 0.5 percentage points per year up to 20.7% in 2009 [42]. The Pregnancy Nutrition Surveillance System 2011 report stated that the prevalence was 27.6% [1]. Collectively, these statistics highlight the developing problem of maternal obesity in the United States and worldwide. In fact, a growing body of data links maternal obesity to offspring adiposity at birth and beyond.

2.1.2. Impact of maternal obesity on offspring adiposity at birth

The trans-generational effect of obesity is demonstrated by studies that show a relationship between pre-pregnancy obesity and offspring adiposity at birth. For example, Hull et al. [9] measured newborn (2-4 weeks old) body composition using air displacement plethysmography (ADP) and compared the body composition of newborns born to obese and normal weight mothers. While there was not a difference in birth weight between infants of obese and normal weight mothers, FM and percentage body fat (%FM) were higher in newborns born to obese mothers compared to newborns born to normal weight mothers.

Studies suggest the association between maternal pre-pregnancy BMI and offspring adiposity differs by gender. In a cross-sectional study involving 599 mother-infant pairs, newborn body composition was measured using ADP within 48 hours of birth. Au and colleagues [43] found a positive association between maternal pre-pregnancy BMI and neonatal %FM. Similar to other studies, males were found to have a lower %FM when compared to females [10, 44]. Pereira et al. [45] measured newborn body composition within 72 hours after birth using ADP in 100 infants. The study tested the independent effects of maternal diet (total energy and macronutrient distribution), maternal pre-pregnancy BMI (dichotomized as <25 kg/m^2 vs. > 25 kg/m^2) and GWG on offspring body composition. The multiple regression models were run separately for males and females therefore a model including infant gender as a dichotomous variable was not completed. They found a positive association between pre-pregnancy BMI and neonatal FM in males but not females.

Another study used an advanced imaging technique (MRI) to assess total, regional and liver adipose tissue content in 105 infants with a wide variability in age (1 to 28 days old) [46]. A positive association was found between maternal pre-pregnancy BMI and infant adiposity. For

every one unit increase in maternal pre-pregnancy BMI, they found an increase in neonatal total, abdominal and non-abdominal adipose tissue by 8 mL, 2 mL and 5 mL, respectively. Another study used less sophisticated methodology to quantify infant adiposity in a larger sample size. Tikellis et al. [47] found that independent of birth weight, higher maternal pre-pregnancy BMI was associated with greater neonatal sub-scapular skinfold (n=7,945). These data support that a relationship exists between maternal pre-pregnancy BMI and offspring adiposity at birth. Data has also been published reflecting that this relationship persists into childhood and beyond [48-51].

2.1.3. Impact of maternal obesity on offspring adiposity beyond infancy

The influence of maternal pre-pregnancy BMI on infant adiposity at birth extends beyond infancy into childhood and adulthood. Gaillard et al. 2014 [48] reported data from the Generation R Study in 4,871 6-year-old children whose body composition was measured using DXA. In modeling adjusting for multiple confounders, a positive relationship was found between maternal pre-pregnancy BMI and offspring total FM at 6 years old. Similarly, Gale et al. [49] assessed body composition using DXA in 216 children at 9 years old. Fat mass and fat-free mass indexes (FMI and FFMI, respectively) were calculated (FM (kg)/Height (cm^2) and FFM (kg)/Height (cm^2)). They found a positive relationship between maternal pre-pregnancy BMI and offspring FMI at 9 year of age. Kaar and colleagues used MRI to assess total and regional adiposity in 313 offspring at 10 years old [50]. They dichotomized maternal pre-pregnancy BMI as normal (<25 kg/m^2) or overweight/obese (>25 kg/m^2). Infants born to overweight/ obese women had greater visceral and abdominal subcutaneous adipose tissue compared to offspring born to normal weight women. Perng et al. [52] studied 1,090 mother-child pairs from the

Project Viva cohort. Body composition was measured from 6-10 years old using DXA. For every 5 kg/m^2 increase in maternal pre-pregnancy BMI, total FM and trunk FM of the offspring increased by 0.92 kg and 0.39 kg, respectively. In summary, data confirm that the impact of maternal pre-pregnancy weight tracks from birth into childhood.

Few cohorts have been followed from birth into adulthood with data available from both maternal pregnancy and offspring body composition. Reynold et al. 2010 [53] reported data from 216 participants enrolled in the Motherwell Birth Cohort collected from 1967-68 in the United Kingdom. Body fat mass was estimated using skinfolds in the offspring at 30 years old. A positive relationship was found between maternal BMI and adult offspring FM. However, maternal characteristics from the Motherwell Birth Study are different from today's pregnant population. In the Motherwell Birth Cohort, the average BMI at 17 weeks gestation was 23.3 kg/m^2 and the average GWG was between 9.4-10 kg. The average maternal BMI before pregnancy is now 25.0 kg/m^2 and the average GWG is 15.7 kg [54]. The older cohort data may underestimate the impact of maternal BMI and GWG on FM compared to more recent pregnancies.

2.1.4. The problem of excessive GWG

In response to the growing number of women entering pregnancy overweight or obese and in response to data that suggest the effect of GWG varies by pre-pregnancy BMI group, the Institute of Medicine (IOM) published updated GWG guidelines in 2009. Updates in the guidelines include aligning the BMI categories with WHO BMI categories and a specific range of recommend weight gain for obese women (5-9 kg vs. ≥7 kg from 1990 recommendations) [55]. Based on the new guidelines, data have shown that the problem of excessive GWG is high.

PNSS data from 2011 found that 48% of pregnant women, regardless of pre-pregnancy BMI category, gained more than recommended. Similarly, PRAMS 2009-2010 data from 30 states found 47.8% of pregnant women gained an excessive amount of body weight [56]. Though the overall percentage of women gaining excessive body weight is high, there are disparities for excessive GWG between pre-pregnancy BMI groups. Greater percentages of overweight and obese women (58.8% and 55.6%, respectively) gain more body weight than recommended when compared to normal weight and underweight women (38.6% and 26.2%, respectively) [57]. These data highlight the problem of excessive maternal GWG. More concerning are data that link excessive maternal GWG and adverse offspring health including greater offspring adiposity at birth.

2.1.5. Impact of excessive GWG on offspring adiposity at birth

Maternal pre-pregnancy obesity is positively associated to higher offspring adiposity at birth. Additionally, whether a woman gains an appropriate or excessive amount of gestational weight as classified by IOM guidelines has also been shown to impact offspring FM. Crozier et al. [58] classified 948 women as gaining an inadequate, adequate or excessive amount of gestational weight and then used DXA to measure offspring body composition at birth. They found a significant positive association between GWG and neonatal fat mass (β=0.14 (-0.01 – 0.29), p=0.06). An effect of excessive GWG on offspring FM was also shown in a small study including only women with normal pre-pregnancy BMI [59]. Thirty eight women with normal pre-pregnancy BMI and their infants were assessed. Newborn FM and %FM were measured within 72 hours after birth using ADP. The study found that newborns born to women that

gained excessively GWG (n=11) had significantly greater FM than newborns born to women that gained an appropriate amount of weight (n=27) (348 vs. 525 g, p=0.009).

However, other research has found this relationship varies between pre-pregnancy BMI groups. Hull et al. [10] studied 306 newborns and categorized mothers by pre-pregnancy BMI status (normal, overweight and obese) and by 2009 IOM weight gain classifications (appropriate or excessive). Newborn body composition was measured using ADP at 1-2 days after birth. The results showed that in the excessive group, newborns born to overweight and obese mothers had significantly greater %FM than did those who were born to normal BMI mothers. Most interesting were the within group effects. For both offspring born to normal weight or obese women, it did not matter whether the mother gained appropriately or excessively. No within group difference was found (i.e., appropriate vs. excessive). For example, FM was not different between an offspring born to an obese mother that gained appropriately when compared to offspring born to an obese mother that gained excessively. However, there was a striking difference in FM between the offspring born to overweight women that gained appropriately vs. excessively. Offspring born to overweight women that gained excessively had FM similar to offspring born to obese women (highest in the sample) and offspring born to overweight women that gained appropriately had FM similar to offspring born to normal weight women (lowest in the sample). These studies demonstrate a programming effect of maternal GWG on offspring adiposity and suggest this effect may vary by pre-pregnancy BMI group. Furthermore, growing evidence suggest that the adverse impact of excessive maternal GWG at neonatal period extends to childhood and adulthood.

2.1.6. Impact of excessive GWG on offspring adiposity beyond infancy

The association between maternal GWG and offspring adiposity in childhood was shown in 313 mother-child pairs involved in EPOCH study by Kaar et al. [50]. Measures of offspring adiposity (average 10.4 years) included BMI, waist circumference (WC) and visceral adipose tissue (VAT) and subcutaneous adipose tissue (SAT) measured by MRI. The interaction between maternal pre-pregnancy BMI and maternal GWG category (adequate vs excessive) was reported for offspring adiposity measures. The authors reported that higher pre-pregnancy BMI was associated with higher child adiposity measures (BMI, WC, SAT and VAT) in all GWG category, but the effect was stronger in the excessive GWG group and reached significant level for child's BMI, WC and SAT.

The associations between GWG and childhood adiposity by anthropometric indicators were reported by two large studies. Ensenauer et al. [60] reported the results from a retrospective study of 6,837 mother-child dyads from Germany. Maternal GWG was categorized as inadequate, adequate or excessive, and the offspring BMI and waist circumference at 5.8 years were categorized as overweight (BMI $\geq 90^{th}$ percentile) and excessive abdominal adiposity (WC $\geq$ 90 age and sex specific percentile), respectively. In this study, 53.6% women gained excessively. Results showed that when compared to adequate maternal GWG, excessive GWG was related to both childhood overweight (OR 1.57) and abdominal adiposity (OR 1.39). Disparities were also found when examining within pre-pregnancy group differences. When comparing to offspring born to normal weight women that gained appropriately, offspring born to normal weight women that gained excessively had a greater likelihood to be overweight (OR 1.29) and have excessive abdominal adiposity (OR 1.35). Similarly, offspring born to overweight

women that gained excessively were more likely to be overweight (OR 1.64) though no relationship to excessive abdominal adiposity was found.

Dello Russo et al. [61] studied 12,775 mother-child pairs. The mothers were divided by GWG tertiles (interquartile range; tertile 1=8-11 kg, tertile 2=13-15 kg, tertile 3=18-24 kg), and anthropometric indices (waist circumference, subscapular and triceps skinfolds) of the children were measured at age 2-9 years. ANCOVA found that all indices were increased across GWG tertiles. Taken together, these studies clearly demonstrate that a significant association exists between maternal GWG and childhood adiposity.

Studies have shown that the effect of GWG tracts into adulthood. However, only a few studies have measured body fat in adulthood using a more direct indicator than BMI such as skinfolds. The Motherwell Birth Cohort [51] reported on 276 mother-offspring pairs where the mothers had been enrolled during pregnancy from 1967-1968. When offspring were 27-30 years old, body composition was measured by skinfolds (biceps, triceps, subscapular and suprailiac). They found significant correlations between maternal GWG and offspring adult %FM ($r=0.14$, $p=0.03$) and FM index ($r=0.14$, $p=0.03$). Other studies used BMI as the proxy to measure body fat and similar trends were found. In a prospective study established in 1959–1961, 1,540 offspring of mothers included in "The Copenhagen Perinatal Cohort" were followed from birth until 41 years old. The study found that GWG was associated with offspring BMI, and there was an increasing risk of offspring obesity (OR 1.08) per kg increase in GWG ($p=0.003$) [62]. Analysis of data from a study with cohort born in Brisbane between 1981-1983 showed an increase of offspring BMI by 0.3 kg/m^2 for every 0.1 kg/week increase in maternal GWG [3]. The impact of excessive maternal GWG on offspring body composition and risk of obesity development tracks from birth to childhood and adulthood. These studies highlight the

importance for women to gain appropriate body weight during pregnancy for their given pre-pregnancy BMI.

2.2. Maternal adiposity and infant developmental outcomes

While it is demonstrated that pre-pregnancy obesity and/or excessive GWG are associated with greater infant, child and adult adiposity, there is growing evidence to suggest that maternal pre-pregnancy obesity may impact infant cognitive function and development. Recent studies found that infants born to obese women are at increased risk of poor cognitive function and developmental disorders including lower cognitive development scores at 11-22 months [63], autism spectrum disorder and neurodevelopmental delay at 2-5 years of age [64], and attention deficit hyperactivity disorder at 7 years old [65]. In a study involving 30,212 women-children pairs, Huang et al. [66] reported that pre-pregnancy obesity was associated with lower offspring IQ at 7 years of age and this association was strengthened by controlling for maternal excessive GWG. Given the increasing prevalence of maternal obesity, and women gaining excessive GWG, these two maternal factors may contribute significant risk for cognitive problems in the offspring.

Cognitive function is closely related to the autonomic nervous system (ANS). Studies showed that an alteration in the ANS can influence cognitive function and in conditions where the cognitive function is impaired, the ANS function is also decreased [67-71]. Next we will discuss the relationship between ANS and cognitive function.

2.2.1. Overview of the autonomic nervous system

The autonomic nervous system (ANS) regulates the body's internal environment to achieve homeostasis. The system helps the body in the regulation of homeostatic mechanism (heart rate, circadian rhythm) and its malfunction is associated with of various diseases [72, 73]. In newborns, the system is particularly important for adaptation to the *ex utero* environment.

The ANS consists of central and peripheral components. The peripheral ANS consists of sympathetic and parasympathetic nervous systems and is under control of central nervous system through the central autonomic network (CAN). The CAN is an integral component of the regulation system at the brain and the brain stem [74, 75]. The neurons of the ANS release neurotransmitters, with acetylcholine (Ach) and norepinephrine (NE) being the most common. Cholinergic fibers (nerve fibers that release Ach) include sympathetic and parasympathetic preganglionic fibers, all parasympathetic postganglionic fibers, and sympathetic postganglionic fibers of the sweat glands. Adrenergic fibers (nerve fibers that release NE) consist of mostly sympathetic postganglionic fibers [72].

The first area of the ANS that develops in the human fetus is unmyelinated vagal fibers, which are part of parasympathetic nervous system, that arise from the dorsal motor nucleus (DMN) of vagus nerve (VN). Immature DMN can be identified at 9 weeks of gestation in the brainstem and reach maturity at 28 weeks [76]. The myelinated vagal system which arises from the nucleus ambiguus (NA) develops last in the fetus. The mature neurons appear as early as 8-9 weeks gestation but the axons do not reach the cardiac tissue and do not show inhibitory effect until the myelination begins at the third trimester and continues to increase up to 1 year after birth [77]. The development of the sympathetic nervous system begins sometime between the development of the older DMN and the NA vagus. In accordance with the development of the

ANS, the fetal heart rate is primarily under the influence of sympathetic nervous system at early gestation and as the myelinated vagal system develops, the fetal heart rate decreases towards the end of gestation from 175 bpm at the first trimester to 140 bpm at term [78].

The vagus nerve (VN) holds the physiological significance of the parasympathetic nervous system as it makes up 75% of all parasympathetic fibers. Myelinated fibers of the VN function as a vagal brake which inhibits the sinoatrial (SA) node, the heart pacemaker that regulates the heart rate. The high vagal tone inhibits the pacemaker and substantially decreases the heart rate. When the vagal tone is low, there is no or limited inhibition of the pacemaker and the heart rate will increase [79]. In contrast to parasympathetic influence, sympathetic action increases heart rate through its influence on the pacemaker and its innervation to the ventricular myocardium where it increases cardiac contractility. The sympathetic effect on the heart rate is much slower than the parasympathetic effects, and therefore, the changes in the heart beat to beat timing is mostly under parasympathetic influence [73]. Based on the influence of sympathetic and parasympathetic functions on heartbeat, analysis of the variability in the heart rate, therefore, can be used to assess the ANS function.

2.2.2. Heart rate variability as indicator of the ANS

Heart rate variability (HRV) is considered as a reliable indicator of the interplay between the sympathetic and parasympathetic systems [53, 80]. The cardiac cycle consists of contraction (systolic) and relaxation (diastolic) periods. The calculation of HRV indices are based on the time sequences of the beat to beat intervals. HRV analysis can be computed in linear domain, i.e. time- and frequency-domains, and non linear domain.

The time domain is an analysis of the continuous monitoring of cardiovascular parameters while the frequency domain is a spectral analysis that expresses heart rate oscillations [54]. The most commonly used components are: 1) standard deviation of normal sinus beat-to-beat intervals (SDNN), expressed in milliseconds; and 2) Root mean square of successive difference between two consecutive inter beat intervals (RSMMD), also expressed in milliseconds and reflects parasympathetic activity [54]. On the other hand, the frequency domain is derived from the conversion of the time domain through a mathematical transformation called power spectral analysis. The spectrum consists of three components; a very low frequency (VLF) which is modulated predominantly by the sympathetic nervous systems, a low frequency (LF) band which reflects both sympathetic and parasympathetic activity [22], and a high frequency (HF) band which reflects parasympathetic activity [44]. In fetal HRV analysis, the frequency band cut offs are 0.02-0.08 Hz for VLF, 0.08-0.20 for LF, and 0.40-1.70 Hz for HF [81]. Respiratory Sinus Arrhythmia (RSA), the change in heart rate due to respiration, is another measure used in HRV analysis that reflexes parasympathetic activity.

The non-linear domain includes several measures of HRV. One commonly used is the Poincaré plot. Relative to other non linear methods, this method is simple to interpret. It is a plot of the correlation between successive RR, that is, RR_{j+1} against RR_j (Figure 1). The plots make an ellipse shape that is oriented to the line-of-identity ($RR_j = RR_{j+1}$). The standard deviation of the plots that are perpendicular to the line (SD1) is closely related to the standard deviation of successive RR interval differences (RMSSD) and describes short-term HRV which is mainly affected by RSA. On the other hand, the standard deviation of the plots along the line (SD2) is related to SDNN and SDSD and describes overall HRV [82, 83].

Postnatal studies showed that the HRV is controlled by the central nervous system at the highest level (cerebral cortex) that transmits signals to the CAN at the nuclei in the brain stem and the spinal cord [84]. The output signals from the CAN is controlled at the nucleus solitary tract which is the site where the afferent vagus meet with efferent vagus [22]. The regulation of the vagal brake by myelinated vagal fibers is crucial for an individual to survive as it improves the ingestive vagal reflex during feeding, increases the capacity to self-regulate and calm, and the ability to develop social behavior [85].

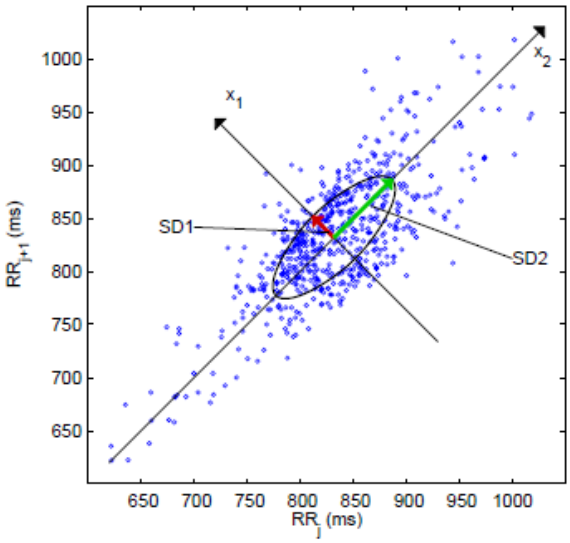

Figure 1. An example of Poincaré plot. The width (SD1) and length (SD2) of the ellipse are the standard deviation of the distance of the plots from each axis [82, 83].

Fetal ANS function is measured by fetal HRV, which essentially reflects fetal cardiac autonomic control. As gestation develops, fetal HRV increases due to increased innervation of parasympathetic fibers into the heart and the influence of the autonomic control from the medulla oblongata to the cerebral cortex [27]. An anatomical study using human fetuses suggested that the critical period of brain development related to the fetal heart rate control is around 28-30 week of gestation [86]. This is also the time period where fetal behavior states emerge.

There is evidence that individual differences in fetal HRV measured at the third trimester of pregnancy is conserved until the end of pregnancy and possibly after birth. Di Pietro et al. [27] measured fetal HRV of 137 fetuses of healthy mothers longitudinally from 20 to 38 week of pregnancy. They found little variation in individual HRV growth trajectory at 20-28 weeks and 28-38 weeks, suggesting that those with lower or higher fetal HRV growth rates at earlier period tended to have lower or higher rates at the end of the pregnancy. Another study reported that higher fetal HRV is associated with higher HRV measured at 1 year of age [87].

A large body of evidence shows that higher HRV is associated with better cognitive function and development [70, 80, 88-90]. In developmental studies, HRV has been used as a physiological indicator of the child's performance including information processing, development and cognitive assessment [68, 91-93].

2.2.3. The association between HRV and cognitive and developmental outcomes

Child developmental studies have shown that the fetal and neonatal HRV can predict the neurodevelopmental outcomes of a child. Fox et al. [91] used ECG to record the heart rate pattern in 80 full term neonates. From the recording, they quantified RSA, one of the HRV components, using spectral analysis. At 8 and 12 month of age, Bayley scales were applied to assess developmental outcomes. They reported that all infants with high vagal tone, as indicated by high RSA, had positive developmental outcomes at both 8 and 12 month, while those with low vagal tone had varied results. In a study including very low birth weight pre-term infants, Doussard-Roosevelt et al. [94] measured various HRV components including RSA at birth and developmental outcomes at 3 years of age. They reported significant associations between higher RSA and better social and motor skills, and better mental processing. A similar trend was also

demonstrated in a study with normal healthy infants by DiPietro et al. [27]. In the study, fetal HRV measured at 28 weeks of gestation or later was shown to predict mental and psychomotor development as well as language ability of the infants at 2 years - 2.5 years of age.

Collectively, these studies demonstrate that an association exists between the fetal HRV and cognitive function in infancy. The exact mechanism that underlies the association remains unclear; however, the fact that the ANS is closely related to cognitive function has been confirmed.

2.2.4. The ANS and cognitive aspects interplay

Two theories were proposed to explain the interaction between the ANS and the cognitive responses, i.e., polyvagal theory and neurovisceral integration theory [95, 96]. The polyvagal theory, introduced by Porges in 1995 [95], proposed that there are two distinct origins of vagal fibers, the NA and the DMN. Both pathways end at the SA node, the heart pacemaker, and contribute to the RSA; however, the NA, which is the primitive one, is thought to be dominant. On the other hand, the fibers from DMN are more evolved and linked to social interaction and self-behavior. The theory follows the phylogenetic hierarchy and suggests that the ANS develops according to the environmental challenge and responds accordingly. Thus, the theory emphasizes the role of physiological state in regulation of behavior and psychological responses. The neurovisceral integration theory, proposed by Thayer and Lane in 2000 [96], suggests an integration of the cognitive, behavioral and physiological factors regulations. The theory emphasizes the importance of the inhibitory mechanism by the CAN system to sympathetic responses through vagal activity.

The association between the ANS and cognitive function is further confirmed by anatomical and physiological studies. Studies in animal models have identified neural pathways

that transmit visceral information to the cerebral cortex and identified sites in the cerebral cortex that regulate the autonomic response [97, 98]. In human studies using functional MRI, these specific sites in cerebral cortex have been identified and in addition to autonomic regulation, the sites were also shown to have significant roles in cognitive function [80, 84, 96]. These findings further highlight the close interaction between the ANS and cognitive function.

The proper function of the ANS in young infants is reflected by their capacity to regulate their mental state, i.e. their behavior, in accordance with the environment challenges. The neonatal behavior assessment scale (NBAS), developed by T Berry Brazelton and his colleagues in 1973, is a method to assess self-regulation behavior in newborns and has been proven to predict developmental disabilities at later ages in low birth weight and premature infants [99]. In healthy infants, the method is also suggested to be useful to predict later development [27]. The scale includes behavioral items for optimal performance, reflex items for neurological function, and supplementary items that assess the general performance of the newborns [100].

Taken together, the data suggest that the ANS is associated with cognitive function, and in young infants, it is an important predictor for cognitive function and development. The development of the ANS starts as early as 8 weeks *in utero* and maternal factors, particularly nutrition, have been shown to shape this process.

2.2.5. Maternal nutritional factors and infant autonomic development

In animal studies, maternal nutritional factors during pregnancy influence offspring nervous system development. The effect of refined carbohydrate intake on the fetal ANS was shown in a study by Young et al. [31]. Using a rodent model, the investigators observed an increase in NE content of the pancreas and retroperitoneal fat in the fetus of rats whose mothers

were fed diets high in refined carbohydrate, indicating activation of sympathetic system in those organs. A similar effect was shown with a high fat diet intervention. Prior et al. [101] reported an increase in renal sympathetic activity in 4 month old rabbit offspring whose mothers were fed a high fat diet. In primates, offspring exposed to high fat diet during pregnancy showed alteration in their central serotonergic system as well as increased anxiety [102].

Human studies showing the effect of maternal nutrition on fetal ANS development are limited. DHA is one nutrient that has been studied and shown to impact fetal HRV. In a randomized controlled clinical trial by Gustafson et al. [81], pregnant women were supplemented with either 600 mg/day DHA or placebo starting at 14.4 ($\pm$4) week of pregnancy until delivery. The study found that offspring of the supplemented group had significantly higher fetal HRV than the control group as well as better scores on the NBAS in autonomic and motor behavior domains which reflect better cognitive function. Thus, the study demonstrated a direct effect of the intrauterine environment, that is, DHA intake, on fetal autonomic and cognitive development.

While nutrition appears to be important for proper development of fetal ANS, maternal obesity, a state of over nutrition, is thought to negatively impact fetal ANS development.

2.2.6. Maternal adiposity and infant autonomic development

Maternal adiposity during pregnancy has been shown to influence cognitive function of the offspring in many studies [64-66, 103]. However, the effect of maternal adiposity on the fetal ANS, to our knowledge, has not been clearly demonstrated. A study by Ojala et al. [104] found a positive correlation between fetal cardiac sympathetic activity recorded by fetal electrocardiogram (ECG) during delivery to maternal pre-pregnancy BMI. This suggests that higher maternal adiposity is related to poor fetal autonomic function. However, the observed

autonomic response using this method was limited and therefore the complete feature of the fetal HRV could not be obtained.

Although the evidence of an association between maternal obesity and fetal ANS alteration is limited, there have been biochemical and cellular studies that suggest such an association.

2.3. Potential mechanisms by which maternal adiposity influences the fetal ANS

The mechanism behind how maternal adiposity influences fetal ANS development remains largely unknown. Since excessive adipose tissue induces many hormonal and biochemical alterations, it is suggested that these changes, particularly inflammation and insulin resistance, mediate the observed fetal ANS and cognitive disturbances.

2.3.1. Obesity induces insulin resistance and inflammation

Excess adipose tissue found in obesity not only functions as a fat depot for energy storage but also acts as an active endocrine organ which releases various active substances that are collectively called adipocytokines [105]. As obesity develops, the adipose tissue is infiltrated by macrophages that releases inflammatory mediators and brings about a state of systemic low grade inflammation [106]. In contrast with adipose tissue in lean individuals which preferentially releases adipocytokines that are anti-inflammatory, adipose tissue in obesity secretes mainly pro-inflammatory adipocytokines which can induce IR. Some common pro-inflammatory adipocytokines include Retinol Binding Protein (RBP4), Tumor Necrosis Factor-α (TNF-α), Interleukin-6 (IL-6), Monocyte Chemoattractant Protein-1 (MCP-1), and leptin [107].

In addition to the pro-inflammatory cytokines, free fatty acids (FFAs) released from adipose tissue also contribute to adipose induced inflammation and insulin resistance. The

increased release of FFA stimulates the adipose tissue macrophages to produce larger amounts of inflammatory mediators [108] while the elevated level of circulating FFA induces insulin resistance in various organs [109]. The great flux of plasma FFAs is followed by their uptake in myocytes, adipocytes, and hepatocytes where the FFAs and the fatty acids intermediates induce IR [110]. This obesity-induced inflammation and IR can further contribute to ANS dysfunction.

2.3.2. Inflammation and insulin resistance induce ANS dysfunction

In non-pregnant obese individuals, inflammation and IR induced by excess adipose tissue was shown to underlie autonomic dysfunction [11-14, 19, 20, 111-116]. Inflammatory adipocytokines released from adipose tissue directly stimulates the central sympathetic nervous system in the hypothalamus, or indirectly induces a low-grade inflammation state that stimulates sympathetic activation [117]. Obesity-induced IR results in elevation of plasma insulin, glucose, and glycation end products that can cause autonomic dysfunction. Moreover, insulin stimulates sympathetic excitation in the hypothalamus [118] and chronically elevated glucose levels were shown to induce parasympathetic loss [21], resulting in an autonomic imbalance. The effect of obesity-induced inflammation and insulin resistance on fetal ANS development in pregnancy, to our knowledge, has not been reported. However, there is evidence that maternal adiposity causes inflammation and IR in the fetus.

2.3.3. Maternal adiposity induces fetal inflammation and insulin resistance

Data from animal studies show that changes in metabolism leading to inflammation and IR that are induced by excess adipose tissue observed in non-pregnant subjects were also observed in late pregnancy and considered physiologic [119-121]. These changes are more

prominent in an obese pregnancy and shown to induce inflammation and IR in the offspring. In a sheep model, fetuses of obese mothers have a lower number of pancreatic beta cells and lower insulin levels when compared to offspring born to lean mothers [122]. Also, the placental fatty acid transporter expression of the fetuses of obese mothers were increased as well as the cytokine expression [123]. Maternal adiposity, both prior to conception and gained during the gestational period, determined the level of maternal insulin sensitivity, and fetal size and adiposity [124].

Similarly, in a rat model a maternal high fat diet (HFD)-induced adiposity during pregnancy was shown to induce hyperleptinemia and IR of the fetus. In addition, fetal subcutaneous adipose tissue was hypertrophic with an increase in the levels of inflammatory cytokines but a decrease in the level of glucose transporter-4 mRNA [121, 125].

The influence of maternal pre-pregnancy adiposity on IR and inflammation of the offspring in human was first reported by Catalano et al. [126]. In a study involving 68 obese and 53 lean pregnant women who went through an elective cesarean section, they found a positive association between maternal pre-pregnancy BMI and fetal adiposity and IR, and between fetal adiposity and fetal IR. Therefore, offspring born to obese mothers were fatter and more insulin resistant. Obese mothers had higher levels of leptin, IL-6, and CRP than did the lean mothers, while fetuses of obese mothers had higher leptin and CRP levels. The study demonstrated that obese pregnant women and their fetuses had higher levels of inflammation, and that the fetuses of obese mothers developed insulin resistance *in utero*.

To test if maternal adiposity influenced inflammation in the fetus, Aye et al. [127] collected cord plasma following cesarean delivery and analyzed maternal and fetal pro-inflammatory cytokines, and placental activation of inflammatory pathways. They found that higher maternal pre-pregnancy BMI was associated with higher maternal cytokines. Moreover,

they found that TNF-α appeared to induce activation of certain inflammatory pathways. The study suggested that maternal adiposity-induced inflammation alters placental function by activation of specific inflammatory pathways which further influences the fetal growth.

2.3.4. Obesity induced-inflammation in pregnancy and offspring cognitive outcomes

The role of obesity-induced inflammation during pregnancy on cognitive disruption of the offspring was demonstrated in animal studies. In HFD induced-obese pups, Bilbo et al. [128] observed inflammation in the brain as indicated by increase in microglial activation markers in the hippocampus at birth, and pro-inflammatory cytokine expression at weaning and adulthood, as well as anxiety and spatial learning changes. Similarly, Kang et al. [129] found that maternal diet-induced obesity resulted in brain inflammation of the offspring as marked by increased brain IL-1, TNF-α and microglial activation, as well as behavior deficits including anxiety, hyperactivity and decreased sociability. While studies have shown that the intrauterine environment, i.e. maternal adiposity, influences infant autonomic and cognitive outcomes possibly through inflammation and insulin resistance, the association has not been confirmed in human studies.

2.4. Conclusion

The problem of maternal pre-pregnancy obesity and excessive GWG are high and still increasing. This brings great concern as both conditions are known risk factors for various adverse health effects on both mother and infant. Maternal obesity and excessive GWG are associated with higher infant adiposity at birth, and a greater risk of obesity in childhood and adulthood. Recent findings suggested that maternal obesity is associated with poor infant

cognitive function and development. However, whether this is a direct effect of maternal adiposity or mediated through increased infant adiposity is yet to be elucidated. Cognitive function is closely related to the ANS and fetal HRV, an indicator of fetal ANS, has been used to predict the cognitive and developmental outcomes of the child. It is suggested that maternal obesity serves as an *in utero* factor that influences the fetal ANS, and that the alterations in cognitive function and development of the child seen after birth are shaped by the *in utero* environment. How maternal obesity can influence the fetal ANS is largely unknown, but it is suggested through maternal inflammation and insulin resistance induced by obesity during pregnancy.

CHAPTER 3 METHODS

3.1. Subjects

This study included a total of 48 mother-fetus/newborn pairs from two observational studies, i.e. "Factors affecting growth pattern and body composition of infants (HSC#: 13126)" and "Developmental origin of fetal autonomic control (HSC#: 0312)". Recruitment was conducted by approaching patients from the KUMC Obstetric and Gynecology clinic that were screened from the hospital O2 software system prior to their visit, and by sending study advertisement email blast to KUMC academic community.

The inclusion criteria were 18-35 years of age and singleton pregnancy. Candidates were excluded if they have one or more of these conditions: 1). BMI<18.5 kg/m^2, 2). twins, triplet or more, 3). gestational diabetes mellitus and 4). pre-existing chronic diseases that can influence fetal growth and or development. Eligible candidates were enrolled at 20-30 weeks of pregnancy.

3.2. Data collection

3.2.1. Maternal pre-pregnancy BMI and GWG

Pre-pregnancy weight was determined by pre-pregnancy self-reported body weight. Body height was measured using a wall stadiometer to the nearest cm. Maternal pre-pregnancy BMI was calculated by dividing pre-pregnancy body weight in kilogram by the square of body height in meters. GWG was calculated by subtracting self-reported highest body weight during the pregnancy from self-reported pre-pregnancy body weight.

3.2.2. Blood assay

Blood was drawn from the subject's vein and collected in a vacuum tube containing heparin after an overnight fast at 36 weeks of pregnancy. The heparin containing blood was centrifuged and the plasma was separated and stored at -80 C° in a liquid nitrogen tube until analysis. Plasma glucose level was analyzed by enzymatic colorimetric method (Cayman®). Analysis of plasma insulin (Alpco®), TNF-α (Quantikine, R&D®) and IL-6 (Ebioscience®) were conducted using ELISA method. Insulin resistance was determined by homeostasis model assessment (HOMA) calculated using the mathematical formula for approximate estimation [130]:

HOMA index = (fasting blood insulin (μIU/mL)*fasting blood glucose) /22.5

3.2.3. Infant body composition assessment

Newborn FM was assessed using air displacement plethysmography (Pea Pod®) within 2 – 5 days after delivery. Prior to the assessment, the baby's clothing is removed including the diaper. A tight cap is put over the baby's head to reduce the hair volume that might cause inaccuracy in the body volume measurement.

Newborns were placed naked on a scale to measure body weight for 6-20 seconds and then placed in the Pea Pod chamber for 2 minutes to measure body volume. Body density was calculated from body weight and volume, and then converted to newborn FM using equations developed by Fomon et al [131].

3.2.4. Fetal HRV assessment

The fetal HRV tests were conducted at 32 and 36 weeks of gestation. Prior to the test, the subject was instructed to remove any metal such as underwire bra and jewelries. The test was conducted in a magnetically shielded room. The subject sat in the exam chair with her abdomen resting next to the fetal magnetocardiogram (MCG) surface with minimum pressure. Magnetic signals from the abdomen were recorded by 83 axial gradiometer sensors that were spatially spread on the MCG surface. The data were recorded in 20 minutes using 500 Hz sampling rate and 0-75 Hz recording filter. The MCG recordings were presented to Kubios HRV Analysis 2.1 software (University of Kuopio, Kuopio, Finland). Fetal MCG was separated from maternal MCG and artifacts, and the non-linear domain was analyzed using Poincaré plot method to calculate SD1 and SD2. The change in fetal HRV was defined as the difference in SD1 and SD2 between those measured at 36 weeks and 32 weeks of gestation.

3.2.5. NBAS

The assessment of neonatal behavior was conducted by a certified psychologist using the NBAS within 1-5 days after delivery. The scale consists of 7 clusters developed by Lester et al. 1984 [132], including habituation, orientation, motor, range of state, regulation of state, autonomic stability and reflexes. The total score for each cluster is the sum of the item scores under the corresponding cluster. The score of the items range from 0-9 with higher scores reflect better performance except for those under reflexes cluster. The reflex clusters scored 0-4 with higher scores reflect non optimal response. The whole test takes about 30 minutes.

3.3. Statistical analysis

Descriptive statistics for maternal and offspring variables were calculated as means and standard deviations. The associations between outcome and predictor were analyzed using bivariate correlations. For aim 1, bivariate correlations were analyzed between 3 predictor variables of interest (pre-pregnancy BMI, GWG, and newborn FM) x 5 outcomes (SD1, SD2, SD1 change, SD2 change, and NBAS scores). For aim 2, bivariate correlations were analyzed between 3 predictor variables of interest (maternal HOMA index, TNF-α and IL-6) x 4 outcomes (SD1, SD2, SD1 change, and SD2 change).

Predictor variables with significant associations and the highest coefficient of determination (R^2) were included in multiple regression analysis for each outcome variable. The following potential confounding variables were explored in the models: maternal pre-pregnancy BMI, infant gender and neonatal age (for models with NBAS score outcome only).

Pair-wise correlation coefficients among independent variables were assessed to identify collinearity. Level of significance is set at $\alpha = 0.05$. Logarithmic transformation of data was planned to normalize the distribution if necessary. Statistical analyses was performed using IBM statistics, SPSS version 20.0.

CHAPTER 4 RESULTS

A total of 80 women participated in the study. However, a complete dataset was not available for all participants. The following list includes the number of samples collected for each outcome: fetal HRV at 32 weeks was collected in 30 women, fetal HRV at 36 weeks was collected in 48 women, data on blood analysis were available from 44 subjects, and data on infant body composition was available from 42 newborns (Figure 2). The levels of maternal TNF-α in 3 subjects were not detectable and therefore were excluded from the analysis leaving n=41 viable samples. The NBAS assessments were conducted in 20 newborns. However, due to an unsuitable newborn alert state when the test was performed, only data from 6 and 7 newborns were valid for habituation and orientation clusters, respectively. For other NBAS clusters, data from 13 newborns were available.

4.1. Subject characteristics

Descriptive statistics for maternal and offspring variables are listed in Table 1. Most women were Caucasian (83.33%), and the rest were African American (8.33%), Hispanic and Asian (4.17% each). The average pre-pregnancy BMI was 27.16 kg/m^2. About half of the women (47.9%) had a normal pre-pregnancy BMI, while 18.7% and 33.3% were overweight and obese, respectively. The average GWG was 15.61 kg. Based on the 2009 IOM GWG guidelines, 54.2% and 39.6% of women gained excessively and adequately, respectively, and 6.2% gained inadequately. Forty eight percent of the newborns were male, and the average birthweight was 3.45 kg. The mean infant age at body composition assessment was 3.7 days.

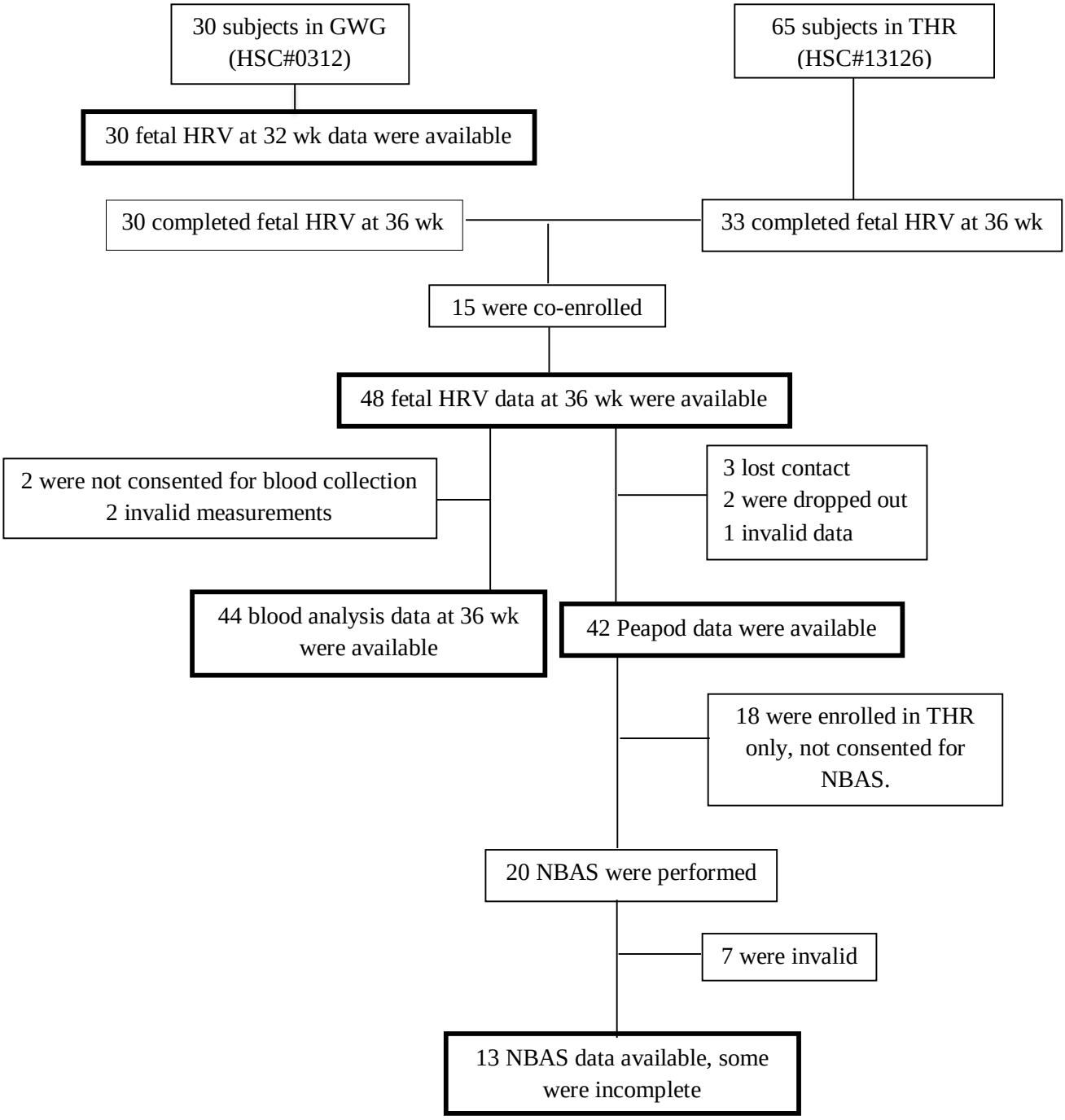

Figure 2. Consort diagram

Table 1. Characteristics of subjects

Characteristics	Values*
Mother (n=48)	
Race	
Caucasian	40 (83.33)
African	4 (8.33)
Hispanic	2 (4.17)
Asian	2 (4.17)
Age (year)	28.96 ± 3.20
Family income ($/year)	60,430 (41,021-80,547.25)
GA of delivery (week)	39.4 ± 0.9
Pre-pregnancy BMI	27.16 ± 6.35
Pre-pregnancy BMI category	
Normal (18.5-24.9)	23 (47.9)
Overweight (25-29.9)	9 (18.8)
Obese (≥30)	16 (32.7)
GWG (kg)	15.61 ± 6.04
GWG category (IOM 2009)	
Inadequate	3 (6.25)
Adequate	19 (39.6)
Excessive	26 (54.2)
Blood biomarkers (n=44)	
Glucose level (mg/dL)	93.6 ± 22.55
Insulin level (μIU/mL)	6.00 (3.43-8.10)
HOMA-IR index	1.84 (0.74-1.93)
IL-6 level	1.18 (0.95-1.49)
TNF-α level (pg/mL), n=41	1.31 (0.56-2.75)
Newborns (n=42)	
Male	23 (48)
Birthweight (kg)	3.454 ± 0.40
Body composition	
Age at assessment (day)	3.7 ± 3.9
Body Mass (kg)	3.302 ± 0.377
%Fat Mass (%)	11.7 ± 3.4
Fat Free Mass (kg)	2.910 ± 0.299
F-HRV	
36 wks, n=48	
SD1 (msec)	4.39 ± 1.91
SD2 (msec)	32.66 ± 10.97
32 wks, n=30	
SD1 (msec)	3.21 ± 0.83
SD2 (msec)	27.62 ± 10.91
NBAS scores	
Habituation (n=6)	21.7 ± 2.4
Orientation (n=6)	34.1 ± 9.5
Motoric (n=13)	25.7 ± 4.4
State organization (n=13)	13.5 ± 2.9
State regulation (n=13)	16.7 ± 4.2
Autonomic (n=13)	17.2 ± 2.7
Reflexes (n=13)	2.3 ± 1.6

*values are n (%), mean ± SD, or median (Q1-Q3)

4.2. Maternal and newborn factors to predict fetal HRV

Bivariate correlations between fetal HRV measures and maternal and newborn factors are listed in Table 2. Greater GWG was associated with a greater SD1 and a greater SD1 change ($r=0.42$; $p=0.003$ and $r=0.48$; $p=0.008$, respectively). These associations were further tested by multiple linear regression models to determine if correlations between GWG and fetal HRV remained significant while controlling for pre-pregnancy BMI and infant gender. We found that pre-pregnancy BMI and infant gender were not correlated to either SD1 or SD1 change. When controlling for pre-pregnancy BMI and infant gender, the positive associations between GWG and SD1 as well as between GWG and SD1 change remained significant (Table 3). Correlations between GWG and SD1 and SD1 change were also assessed within pre-pregnancy BMI category (normal and overweight/obese). GWG was significantly associated SD1 (Figure 3B) and SD1 change (Figure 4B) within overweight/obese group, but not within normal group.

The negative relationship between maternal TNF-α and fetal SD2 approached significance ($r=-0.29$, $p=0.07$). Similarly, the negative relationship between newborn %FM and fetal SD2 change approached significance ($r=-0.29$, $p=0.061$). A negative correlation was found between newborn %FM and SD2 change ($r=-0.41$, $p=0.040$). However, when performing multiple linear regression and adjusting for maternal pre-pregnancy BMI and infant gender, the correlation weakened ($\beta=-1.2$, $p=0.055$). Correlations between newborn %FM and fetal SD2 change was also assessed within pre-pregnancy BMI category (normal and overweight/obese). The significant inverse association between newborn %FM and fetal SD2 change was observed within overweight/obese group, but not within normal group (Figure 5).

We found no significant association between any of the fetal HRV measures and pre-pregnancy BMI, maternal HOMA-IR, maternal IL-6 level, newborn FFM and newborn BM (Table 2).

4.3. Maternal and newborn factors to predict newborn neurobehavior

Using Spearman's correlation analysis, we found no significant correlations between the NBAS cluster scores and maternal factors as well as newborn factors (Table 4). However, two correlations approached significance: GWG and newborn state of organization (r=-0.53; p=0.065) and newborn BM and newborn orientation (r=0.75; p=0.052). The characteristics of subjects with NBAS data are listed in Table 5.

Table 2. Pearson correlations between fetal HRV components their predictors

Predictors	Fetal HRV[a]			
	SD1	SD2	SD1 change	SD2 change
Pre-pregnancy BMI	-0.003 (-0.300-0.294)	-0.055 (-0.351-0.241)	0.141 (-0.247-0.530)	-0.022 (-0.415-0.369)
n	48	48	30	30
GWG	0.420** (0.151-0.689)	0.220 (-0.070-0.510)	0.481**(0.137-0.826)	0.040 (-0.350-0.432)
n	48	48	30	30
HOMA-IR	-0.257 (-0.573-0.059)	-0.186 (-0.502-0.125)	-0.069 (-0.429-0.290)	-0.047 (-0.403-0.309)
n	43	43	27	27
IL-6	0.174 (-0.140-0.489)	0.089 (-0.222-0.400)	0.266 (-0.103-0.551)	0.114 (-0.220-0.447)
n	44	44	28	28
TNF-α	-0.034 (-0.378-0.309)	-0.285 (-0.617-0.026)	-0.232 (-0.693-0.229)	-0.016 (-0.463-0.432)
n	41	41	27	27
%FM	-0.208 (-0.531-0.114)	-0.294 (-0.601-0.014)	-0.086 (-0.535-0.364)	-0.343*(-0.669- -0.018)
n	42	42	25	25
FFM	0.156 (-0.170-0.481)	0.241 (-0.072-0.553)	0.151 (-0.293-0.595)	-0.013 (-0.368-0.343)
n	42	42	25	25
BM	0.072 (-0.256-0.401)	0.117 (-0.202-0.437)	0.097 (-0.324-0.518)	-0.119 (-0.450-0.212)
n	42	42	25	25

[a] Values are correlation coefficient (confidence interval)

** Correlation is significant at the 0.01 level (2-tailed).

* Correlation is significant at the 0.05 level (2-tailed).

Table 3. Multiple linear regressions to predict fetal HRV measures

Independent	N	Dependent	β (CI)	p-value	R^2
GWG	48	SD1	0.442 (0.161-0.724)	0.003	0.19
Pre-pregnancy BMI			0.088 (-0.200-0.377)	0.540	
Infant gender			-0.033 (-0.315-0.249)	0.813	
GWG	30	SD1 change	0.511 (0.155-0.867)	0.007	0.27
Pre-pregnancy BMI			0.198 (-0.157-0.554)	0.262	
Infant gender			0.077 (-0.293-0.447)	0.672	
Newborn %FM	25	SD2 change	-0.426 (-0.715-0.009)	0.055	0.17
Pre-pregnancy BMI			0.023 (-0.354-0.394)	0.914	
Infant gender			0.034 (-0.349-0.410)	0.869	

Table 4. Spearman's correlations NBAS cluster scores and maternal and newborn adiposity

Predictors	Habituation	Orientation	Motoric	St. Organization	St. Regulation	Autonomic	Reflexes
	(n=6)	(n=7)	(n=13)	(n=13)	(n=13)	(n=13)	(n=13)
Maternal							
Pre-pregnancy BMI	-0.03	-0.11	-0.36	0.01	0.17	-0.07	-0.01
GWG	0.20	0.36	0.21	-0.53*	0.12	0.00	-0.18
Newborn							
%FM	0.61	0.46	0.14	-0.37	-0.04	-0.40	-0.21
FFM	0.52	0.61	0.26	0.41	-0.25	-0.08	0.05
BM	0.52	0.75**	0.25	0.29	-0.33	-0.18	0.06

*p = 0.065
**p = 0.052

Table 5. Characteristics of the 13 subjects with NBAS data

Characteristics	Mean ± SD/Q2(Q1-Q3)/n
Mother	
Age (year)	27.23 ± 3.52
Family income ($/year)	45,977.5 (35,212.5-53,454.5)
Pre-pregnancy BMI	24.67 ± 4.23
Pre-pregnancy BMI category	
Normal (18.5-24.9)	8
Overweight (25-29.9)	3
Obese (≥30)	2
GWG (kg)	16.84 ± 6.11
GWG category (IOM 2009)	
Inadequate	0
Adequate	6
Excessive	7
Newborns	
Male	4
Birthweight (kg)	3.290 ± 0.36

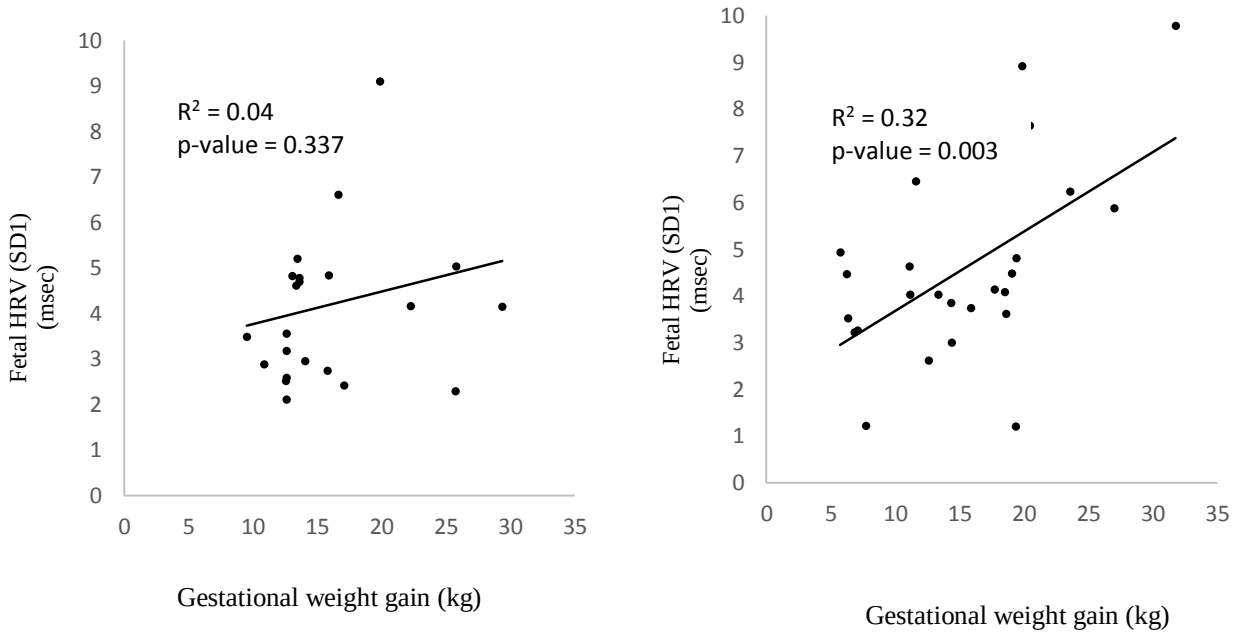

Figure 3. Relationship of fetal HRV (SD1) at 36 week of gestational age and gestational weight gain in women with: A. normal weight (n=23), and B. overweight/obese (n=25).

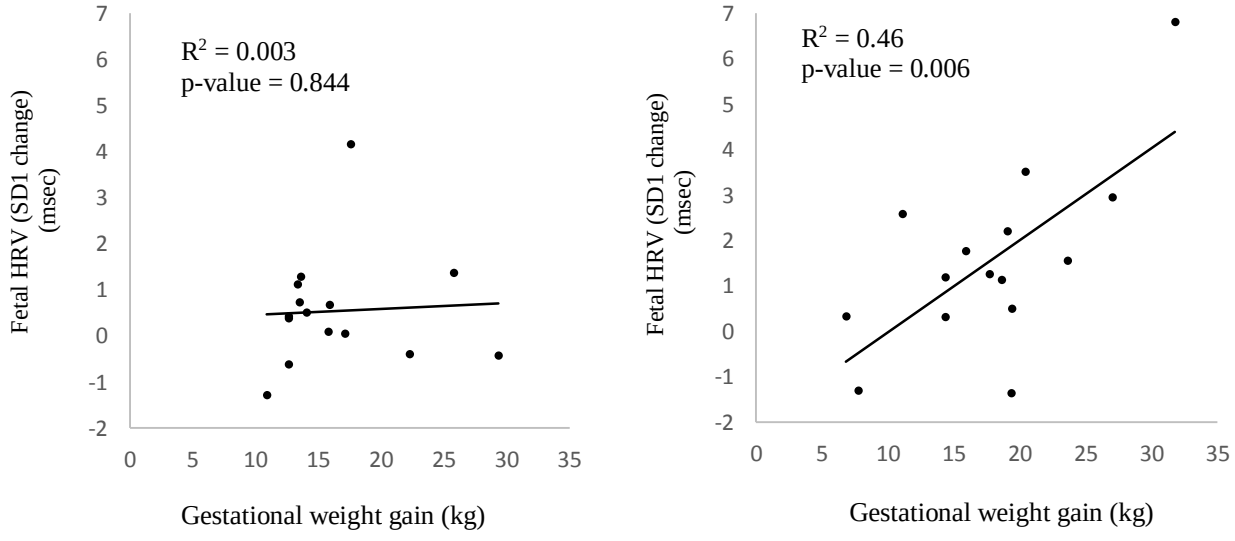

Figure 4. Relationship of fetal HRV difference between 36 and 32 week of gestational age (SD1 change) and gestational weight gain in women with: A. normal weight (n=15), and B. overweight/obese (n=15).

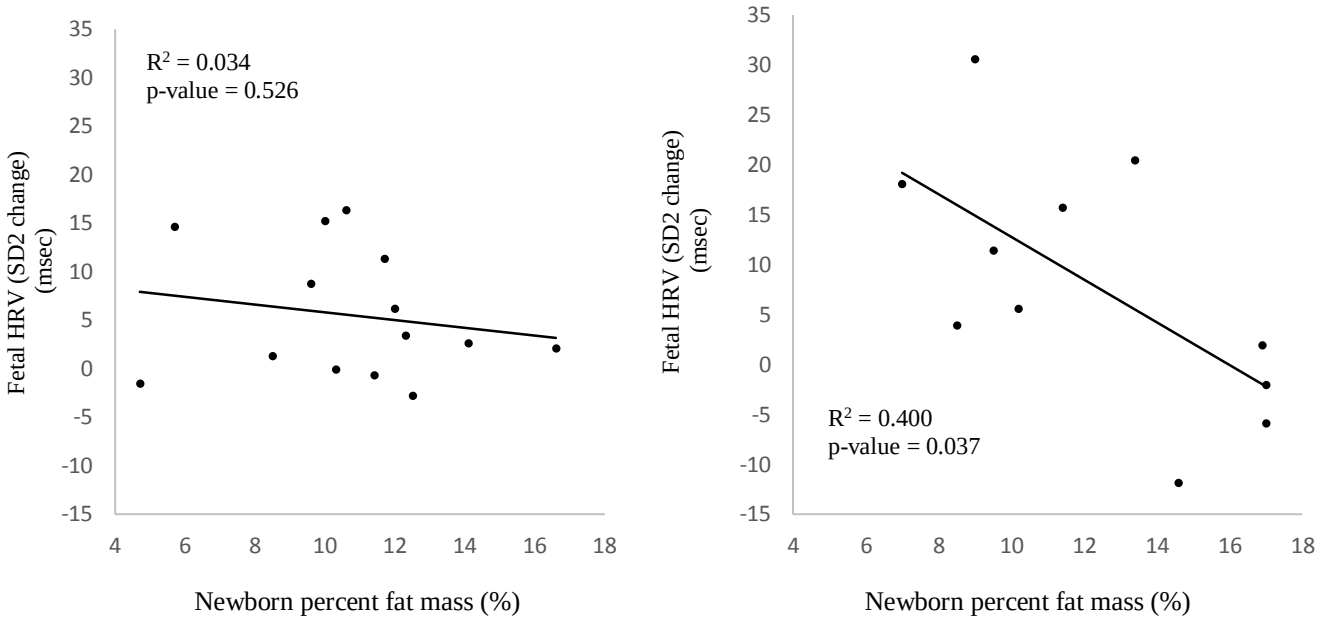

Figure 5. Relationship of fetal HRV difference between 36 and 32 week of gestational age (SD2 change) and newborn percent fat mass born to mothers with: A. normal weight (n=14), and B. overweight/obese (n=11).

CHAPTER 5 DISCUSSION

Neurodevelopment during the gestational period and in early life is critical to program offspring long-term cognitive performance, social skill, language, and motor function. In the present study, we asked whether fetal exposure to maternal obesity impacts *in utero* offspring autonomic nervous system development or infant behavior early in the postnatal period. We also asked if maternal inflammation and IR were associated with fetal autonomic nervous system development. We assessed if fetal HRV, a proxy of fetal autonomic nervous system, is predicted by maternal and infant adiposity, and maternal IR and inflammation. We found that greater GWG was associated with greater short-term variability of fetal HRV (SD1) and the change in fetal HRV from 32-36 weeks of gestation. These relationships remained significant after controlling for the effect of maternal pre-pregnancy BMI and infant gender. Greater newborn %FM was associated with lower change in overall variability of fetal HRV (SD2), but the association was diminished after adjusting for pre-pregnancy BMI and offspring gender. Infant behavior was assessed using NBAS. However, we found no correlation between any of NBAS cluster scores and maternal or infant predictors. Thus, we found that fetal HRV measures were positively associated with maternal adiposity but negatively associated with infant adiposity. We found no evidence of an association between fetal HRV and maternal IR nor maternal inflammation state during pregnancy.

5.1. Maternal pre-pregnancy BMI and offspring neurodevelopment

Maternal pre-pregnancy BMI is inversely associated with child and infant neurodevelopmental outcomes in many large studies [103, 133-136]. For example, Jo et al. [134] measured offspring development at 6 years of age in 1,311 mother-child pairs and reported increased risks for having emotional symptoms, peer problems, psychosocial difficulties, and

other developmental problems for children born to severely obese women. Similar results were reported in a study by Hinkle et al. [133] which analyzed data on mental developmental index (MDI) and psychomotor development index (PDI) of 6,850 children at 2 years of age. Children born to women with a very high maternal pre-pregnancy BMI (obese class II and III) had reduced MDI compared to those of normal pre-pregnancy BMI. Casas et al. [103] assessed cognitive development scores in a total of 2,379 infants aged 11-22 months from two birth cohorts and found an inverse association between maternal pre-pregnancy obesity (BMI >30 kg/m^2) and infant cognitive development.

Although maternal pre-pregnancy BMI is inversely associated to offspring neurodevelopment outcomes, there is inconsistent evidence when studies are replicated in other cohorts or when confounding factors (i.e. family education and socio-economic status) are considered [137, 138]. To determine if the relationships were driven by intrauterine factors, the assessment of the outcomes needs to be performed in perinatal period i.e. in fetuses or newborns. To our knowledge, studies investigating the relationship in newborns have never been reported. We attempted to study such relationships by examining newborn behavior as a proxy for newborn neurodevelopment using NBAS. We did not find any significant associations between maternal adiposity and any of the NBAS cluster scores. However, since our sample was reduced due to technical problems, our small sample size may have reduced our ability to detect relationships. The relationship between maternal adiposity and offspring autonomic nervous development measured in fetuses was investigated in two studies [104, 139] and are discussed below.

5.2. Maternal pre-pregnancy BMI and fetal HRV

Though many studies have investigated the relationship between maternal adiposity and offspring neurodevelopment in childhood, only two studies [104, 139], to our knowledge, have investigated the relationship in fetuses. Fetal HRV, an index of cardiac autonomic control, was used as a proxy for fetal neurodevelopment. In theory, greater HRV reflects more mature autonomic control and potentially better fetal neurodevelopment. In agreement to studies conducted in infants and children, the two studies by Voegtline et al. [139] and Ojala et al. [104] reported an inverse relationship between maternal pre-pregnancy BMI and fetal HRV. In Voegtline et al. [139] study, pregnant women were grouped based on their pre-pregnancy BMI into normal weight (n=382), overweight (n=129), or obese (n=67). Fetal autonomic nervous system development was indicated by overall fetal HRV and the data were recorded using fetal actocardiograph. At 36 weeks of pregnancy, fetal HRV of the obese group was significantly lower than the normal group ($p=0.024$). In the other study, Ojala et al. [104] analyzed fetal HRV in 41 fetuses of normal pregnancies (pre-pregnancy BMI ranged from 18.2 to 34.9 kg/m^2) using fetal ECG during delivery. A positive association was found between maternal pre-pregnancy BMI and the LF/HF ratio, a measure of autonomic balance ($r=0.3$, $p<0.05$). While overall fetal HRV is a reflection of both sympathetic and parasympathetic nervous system, the high LF/HF ratio indicates increased sympathetic activity or decreased parasympathetic drive. Hence, both Voegtline et al. [139] and Ojala et al. [104] studies showed that greater pre-pregnancy BMI is associated with reduced function of fetal autonomic nervous system.

Our results were different from the studies of Voegtline et al. [139], Ojala et al. [104] and others conducted in infancy or beyond [103, 133-135]. We did not find a relationship between pre-pregnancy BMI and fetal HRV. The different results were unexpected since women in our

study and the studies by Voegtline et al. [139] and Ojala et al. [104] were similar in some respects; they were not underweight and were healthy. Some women in Voegtline et al. [139] developed gestational diabetes, but the trend of the results did not change when these women were excluded from the analysis. Compared to our study, the Voegtline et al. [139] study had a considerably larger sample size (578 vs 48) and therefore was more sensitive to detect differences. Ojala et al. [104] had a similar sample size as our study (n=41), but the study was focused on fetal sympathetic activity which is predictive for future cardiovascular diseases risk. Therefore, the fetal HRV components measured in their study (LF/HF ratio) did not sufficiently indicate the overall function of the fetal autonomic nervous system. Compared to both studies, we used a more sensitive and accurate method to assess fetal HRV. We also provided a more complete description of fetal HRV by measuring short-term and overall HRV.

Our unexpected results might be due to maternal factors that were not controlled in the study. Outside of maternal adiposity, other maternal factors have been related to fetal HRV. Maternal intake of certain nutrients including Zinc [140-142] and DHA [81] and maternal physical activity level [143] during pregnancy were reported to influence fetal HRV. Although maternal excessive adiposity before pregnancy has been proposed to influence offspring neurodevelopment, the mechanism remains unknown. Beside the studies of Voegtline et al. [139], Ojala et al. [104], and ours, we are not aware of other studies investigating the relationship between maternal adiposity and fetal neurodevelopment. Thus, the programing effect of maternal adiposity on offspring neurodevelopment needs further examination.

5.3. Maternal GWG and offspring neurodevelopmental outcomes

We found that greater GWG was associated with better fetal HRV. Our finding was in accordance with studies that investigated the association in children [136, 138]. Gage et al. [136] analyzed data from Avon Longitudinal study cohort. The outcomes measured were school entry assessment (SEA) score at age of 4 years (n=5,832), intelligent quotient (IQ) at 8 years old (n=5,191) and school final-exam score at age 16 years (n=7,339). Analysis was controlled for confounders including maternal age, pre-pregnancy BMI, smoking status during pregnancy, parity, mode of delivery, maternal education gestational age, offspring's age at outcome assessment, and offspring gender. Gestational weight gain in early (0-18 weeks), mid (18-28 weeks), and late pregnancy (28 weeks – delivery) were positively associated with IQ, while GWG in early and mid-pregnancy was positively associated with SEA scores. Since a significant interaction was found between pre-pregnancy overweight/obesity and GWG regarding final-exam score, the associations between GWG and the final-exam score were tested within each pre-pregnancy BMI category. In normal pre-pregnancy BMI, greater GWG at early pregnancy was associated with decreased odds of adequate final-exam score. However, in overweight and obese pre-pregnancy BMI, greater GWG in late pregnancy was associated with increased odds of adequate final-exam score. Thus, the study found that greater GWG was associated with better offspring developmental outcomes. In addition, the study also suggested that the relationship between GWG and offspring neurodevelopment differs based on pre-pregnancy BMI category, gestational period, and the type of neurodevelopmental aspect being measured.

Another study investigating GWG and child neurodevelopment was conducted by Keim et al. [138] which included over 30,000 individuals who participated in the U.S. Collaborative Perinatal Project. Similar to the Gage et al. [136] study, Keim et al. [138] study suggested a

positive association. Children were assessed for cognitive development at 4 years of age by Stanford-Binet Intelligent Scale and Block sort test, and at 7 years of age by the wide range achievement test (WRAT) and the Weschler Intelligence Scales for Children (WISC). Multiple regression with general estimating equation (GEE) and fixed-effects (FE) models were used. In FE models, in addition to potential confounders that are controlled in GEE model (pre-pregnancy BMI, race, maternal age, smoking, parity, and socio-economic status), confounding factors shared in siblings, i.e. proportion of genetic factors and parenting practices, were also controlled. In GEE models, they found U-shaped associations between GWG and most of the cognitive outcomes. However, the associations were diminished when FE was applied. Using FE model, higher GWG was linearly associated with better scores in WRAT spelling (β=0.15, 95% CI: 0.01-0.29) and WRAT arithmetic (β=0.15, CI: 0.02-0.28).

In accordance with our findings, both Gage et al. [136] and Keim et al. [138] studies suggested a positive association between GWG and offspring neurodevelopment. However, it is important to note that apparently, the association shown in each of the studies applied to a range of GWG that was less than that in our study. Women in our study gained more than those in Keim et al. [138] study (mean GWG 15.6 kg vs 10.1 kg). Also, the proportion of women who gained inadequately in Keim et al. [138] study was much greater than in our study (58% vs 6.25%). Gestational weight gain in Gage et al. [136] study was presented by gestational period and therefore we do not know the amount of their total GWG. However, from the amount of GWG by gestational period, we estimated that women in their study also gained less than those in our study. Similarly, the proportion of women who gained inadequately in Gage et al. [136] study was greater than that in our study (34% vs 6.25%).

Another study has found GWG both too low and too high are related to poor offspring neurodevelopment outcomes measured in childhood [144]. In the Tavris et al. [144] study, a total of 2,590 mother-child pairs participated in the Child Health and Development Studies at University of California, Berkeley [144]. Women were grouped based on their GWG into <5 lbs, 5 to 29 lbs, and >29 lbs, and the child development outcome measured was Raven colored progressive matrices test at age 5 years. Children born to mothers who gained between 5 to 29 lbs during pregnancy had a better score on the test compared to children of mothers from the other two groups. However, the analysis did not control for pre-pregnancy BMI. Our study findings are in contrary to these findings. While Tavris et al. [144] found that children born to women who gained more than 29 lbs had reduced developmental test scores, we found that offspring exposed to excessive GWG had better fetal neurodevelopment. This is interesting considering women in our study gained a greater amount of weight than those in the Tavris et al. study [144]; our women gained between 12.5 to 70 lbs. The inconsistent findings among our study and others might be due to variations in subject characteristics especially the range of pre-pregnancy BMI and GWG, offspring age, and methods used to assess the neurodevelopmental outcome.

Data suggest that inadequate or excessive GWG is related to offspring neurodevelopment outcomes. To further investigate this finding, studies have used the 2009 IOM guidelines [145] to examine the relationship between appropriate versus excessive GWG and offspring developmental outcomes. However, study results are inconclusive. Gage et al. [136] reported that compared to children exposed to women with adequate GWG, children exposed to inadequate and excessive GWG had lower scores in school final-exam. Different results were reported in Keim et al. [138] study. In women who gained excessively, Keim et al. [138] found a positive

linear association between GWG and offspring WRAT scores at 7 years of age (β=1.41, CI: 0.13-2.70). Our study was not designed to analyze the difference in offspring neurodevelopment by GWG recommendation classification, therefore we cannot compare our results with these findings.

In overweight and obese women, greater GWG is associated with greater gains in maternal fat mass [146-148]. Therefore, we expected that both pre-pregnancy BMI and GWG would be inversely correlated with fetal HRV. Excessive fat mass accumulation, both before and gained during pregnancy, lead to maternal and fetal metabolic disturbances and consequently, results in various adverse pregnancy and offspring health outcomes [8]. We excluded women who were underweight before the pregnancy and almost all women in our study gained either adequately or excessively. Instead of an inverse association, we found a significant positive association between GWG and fetal HRV. As in the case of pre-pregnancy BMI, our unexpected result might be due to maternal factors that were not controlled in the study such as maternal diet [81, 140-142] and physical activity level [143].

Our data and others suggest that maternal adiposity is an important predictor for offspring neurodevelopmental outcomes. Even so, the exact mechanism connecting how maternal adiposity may shape offspring neurodevelopment is unknown. Studies that investigate the association between maternal adiposity and fetal HRV are still limited and therefore more studies are needed to decipher the mechanism. In addition, although fetal HRV is a considered as an indicator of fetal neurodevelopment [149], the evidence that it is predictive for post-natal neurodevelopment is limited [27, 92]. Our results therefore must be interpreted with caution, and we are not able yet to conclude that greater GWG is related to better offspring autonomic and neurocognitive function.

5.4. Newborn Fat Mass and Fetal HRV

Our study found a negative association between newborn %FM and overall fetal HRV. The finding is in agreement with other studies that investigated the association in children and the elderly [13, 14, 150-152]. For instance, Vanderlei et al. [151] analyzed HRV of 121 children aged 8 to 12 years. Children in the obese group had significantly lower HRV in metrics that capture both sympathetic and parasympathetic function, suggesting a generalized decrease in autonomic control, when compared to those in normal-weight group.

Although the inverse association between offspring adiposity measures and HRV have been demonstrated in a number of studies [14, 150, 153, 154], our study is the first to show the association in newborns and therefore suggests an *in utero* programming effect on infant autonomic nervous system development. Increased maternal adiposity is associated with increased infant adiposity [2, 5, 9, 48], and therefore our study also implies that increased infant adiposity might be mediating the relationship between maternal obesity and impaired offspring neurodevelopment demonstrated in numerous clinical studies [103, 133-135, 155, 156].

5.5. Maternal insulin resistance and fetal HRV

Maternal obesity, particularly pre-pregnancy overweight/obesity, is correlated with insulin resistance and inflammation in both the mother and fetus [119, 126, 127, 157]. In addition to their role in predisposing the fetus to develop obesity and metabolic disorders [2, 158, 159], recent studies suggest that maternal insulin resistance and inflammation also play important roles in the mechanism by which maternal adiposity impacts fetal nervous system development [128, 160-163]. Insulin resistance, as in the case of maternal diabetes, was

associated with increased risk of cognitive and developmental problems in the children [160, 163].

Our results, however, did not support that maternal insulin resistance influences fetal autonomic nervous system control. Insulin is known as one of the key regulators for neurodevelopment, and the disruption of insulin regulation in the hippocampus by maternal diabetes was suggested to underlie the cognitive and memory deficit of children born to diabetic mothers [161]. The lack of association in our study might be due to the relatively lower degree of insulin resistance in our subjects as we excluded women with diabetes mellitus. Yet the association of diabetes during pregnancy and fetal HRV, to our knowledge, has never been demonstrated.

5.6. Maternal inflammation and fetal HRV

Maternal obesity induces both maternal and fetal inflammation which in turn results in neurodevelopment disturbances in early childhood and later in life [128, 162]. Increased levels of inflammatory cytokines in fetal circulation and amniotic fluid induced fetal brain inflammation and consequently, impaired neurocognitive and development function [164]. The increase in maternal inflammation, however, is not always followed by increased fetal inflammation as reported by Aye et al. [127]. In a study involving 60 women who had a cesarean section, maternal and fetal cytokines (IL-1β, IL-6, IL-8, MCP 1 and TNF-α) as well as inflammatory pathway activation in the placenta were investigated. They found that maternal pre-pregnancy BMI was associated with increased maternal cytokines and activation of certain placental inflammatory pathways, but the fetal inflammatory profile was not affected.

To our knowledge, our group is the first to report the associations between maternal inflammation and fetal HRV. We did not find evidence for a significant relationship between maternal levels of inflammatory cytokines (IL-6 and TNF-α) and fetal HRV. As in the case of IR, our subjects were relatively healthy and we did not find increased inflammatory cytokines levels with increased pre-pregnancy BMI or GWG (data not shown).

5.7. Study limitations

Our study used sophisticated methods to measure fetal autonomic nervous system development and infant body composition. However, our study also had some limitations. First, our sample size was small and might not be large enough to show significant associations, particularly for those involving the blood biomarkers. Second, we did not measure maternal body composition to measure body fat, and used self-reported pre-pregnancy body weight to calculate BMI. Third, we used only IL-6 and TNF-α among many cytokines to indicate the inflammation status while the true mechanism might involve other types of cytokine. Last, HRV metrics for fetus have not yet been established and so we applied adult HRV metrics which might not be suitable for the developing fetus.

5.8. Conclusion

In summary, based on our data we found that maternal and infant adiposity was associated with fetal autonomic nervous system control. Further, the impact of GWG and maternal pre-pregnancy BMI might have different pathways to exert their impact on the fetal HRV. Even though our study suggested that greater GWG was associated with better fetal HRV, the result has to be taken carefully due to the study limitations. The development of nervous system *in utero* is a complex process that is influenced by many factors not measured in this

study such as maternal diet, physical activity, oxidative stress, and body composition. Further studies with a larger sample size, better measures of maternal body composition, and studies that control for possible confounding factors are needed to provide comprehensive knowledge on how maternal factors during pregnancy impacts fetal neurodevelopment.

REFERENCES

1. *Pregnancy Nutrition Surveillance*. Summary of Health Indicators 2011 [cited 2014 9/15/2014]; Available from: http://www.cdc.gov/pednss/pnss_tables/pdf/national_table2.pdf.

2. Fraser, A., et al., *Association of maternal weight gain in pregnancy with offspring obesity and metabolic and vascular traits in childhood.* Circulation, 2010. **121**(23): p. 2557-64.

3. Mamun, A.A., et al., *Associations of gestational weight gain with offspring body mass index and blood pressure at 21 years of age: evidence from a birth cohort study.* Circulation, 2009. **119**(13): p. 1720-7.

4. Blackmore, H.L. and S.E. Ozanne, *Maternal diet-induced obesity and offspring cardiovascular health.* J Dev Orig Health Dis, 2013. **4**(5): p. 338-47.

5. Howie, G.J., et al., *Maternal nutritional history predicts obesity in adult offspring independent of postnatal diet.* J Physiol, 2009. **587**(Pt 4): p. 905-15.

6. Li, N., et al., *Maternal prepregnancy body mass index and gestational weight gain on offspring overweight in early infancy.* PLoS One, 2013. **8**(10): p. e77809.

7. Oken, E., et al., *Maternal gestational weight gain and offspring weight in adolescence.* Obstet Gynecol, 2008. **112**(5): p. 999-1006.

8. Poston, L., *Gestational weight gain: influences on the long-term health of the child.* Curr Opin Clin Nutr Metab Care, 2012. **15**(3): p. 252-7.

9. Hull, H.R., et al., *Impact of maternal body mass index on neonate birthweight and body composition.* Am J Obstet Gynecol, 2008. **198**(4): p. 416.e1-6.

10. Hull, H.R., et al., *Higher infant body fat with excessive gestational weight gain in overweight women.* Am J Obstet Gynecol, 2011. **205**(3): p. 211.e1-7.

11. Hillebrand, S., et al., *Body fat, especially visceral fat, is associated with electrocardiographic measures of sympathetic activation.* Obesity (Silver Spring), 2014. **22**(6): p. 1553-9.

12. Laitinen, T., et al., *Cardiovascular autonomic dysfunction is associated with central obesity in persons with impaired glucose tolerance.* Diabet Med, 2011. **28**(6): p. 699-704.

13. Tascilar, M.E., et al., *Cardiac autonomic functions in obese children.* J Clin Res Pediatr Endocrinol, 2011. **3**(2): p. 60-4.

14. Yi, S.H., et al., *Differential association of adiposity measures with heart rate variability measures in Koreans.* Yonsei Med J, 2013. **54**(1): p. 55-61.

15. Pal, G.K., et al., *Sympathovagal imbalance contributes to prehypertension status and cardiovascular risks attributed by insulin resistance, inflammation, dyslipidemia and oxidative stress in first degree relatives of type 2 diabetics.* PLoS One, 2013. **8**(11): p. e78072.

16. Pizzi, C., et al., *Autonomic nervous system, inflammation and preclinical carotid atherosclerosis in depressed subjects with coronary risk factors.* Atherosclerosis, 2010. **212**(1): p. 292-8.

17. Liao, D., et al., *Association of vagal tone with serum insulin, glucose, and diabetes mellitus--The ARIC Study.* Diabetes Res Clin Pract, 1995. **30**(3): p. 211-21.

18. Tarvainen, M.P., et al., *Complexity of heart rate variability in type 2 diabetes - effect of hyperglycemia.* Conf Proc IEEE Eng Med Biol Soc, 2013. **2013**: p. 5558-61.

19. Hillebrand, S., et al., *The role of insulin resistance in the association between body fat and autonomic function.* Nutr Metab Cardiovasc Dis, 2014.

20. Sztajzel, J., et al., *Impact of body fat mass extent on cardiac autonomic alterations in women.* Eur J Clin Invest, 2009. **39**(8): p. 649-56.

21. Jaiswal, M., et al., *Impact of glycemic control on heart rate variability in youth with type 1 diabetes: the SEARCH CVD study.* Diabetes Technol Ther, 2013. **15**(12): p. 977-83.

22. Thayer, J.F., et al., *A meta-analysis of heart rate variability and neuroimaging studies: implications for heart rate variability as a marker of stress and health.* Neurosci Biobehav Rev, 2012. **36**(2): p. 747-56.

23. Haensel, A., et al., *The relationship between heart rate variability and inflammatory markers in cardiovascular diseases.* Psychoneuroendocrinology, 2008. **33**(10): p. 1305-12.

24. Kon, H., et al., *Association of decreased variation of R-R interval and elevated serum C-reactive protein level in a general population in Japan.* Int Heart J, 2006. **47**(6): p. 867-76.

25. Lampert, R., et al., *Decreased heart rate variability is associated with higher levels of inflammation in middle-aged men.* Am Heart J, 2008. **156**(4): p. 759.e1-7.

26. Madsen, T., et al., *C-reactive protein is associated with heart rate variability.* Ann Noninvasive Electrocardiol, 2007. **12**(3): p. 216-22.

27. DiPietro, J.A., et al., *Fetal heart rate and variability: stability and prediction to developmental outcomes in early childhood.* Child Dev, 2007. **78**(6): p. 1788-98.

28. Canals, J., et al., *Neonatal Behavioral Assessment Scale as a predictor of cognitive development and IQ in full-term infants: a 6-year longitudinal study.* Acta Paediatr, 2011. **100**(10): p. 1331-7.

29. Nelson, S.M., P. Matthews, and L. Poston, *Maternal metabolism and obesity: modifiable determinants of pregnancy outcome.* Hum Reprod Update, 2010. **16**(3): p. 255-75.

30. Aye, I., et al., *Increasing maternal BMI is associated with systemic inflammation in the mother and the activation of distinct placental inflammatory pathways.* . 2014.

31. Young, J.B., *Developmental origins of obesity: a sympathoadrenal perspective.* Int J Obes (Lond), 2006. **30 Suppl 4**: p. S41-9.

32. Young, J.B., J. Weiss, and N. Boufath, *Effects of dietary monosaccharides on sympathetic nervous system activity in adipose tissues of male rats.* Diabetes, 2004. **53**(5): p. 1271-8.

33. Carlson, S. and K.M. Gustafson, *DHA study.* 2013.

34. *Obesity and overweight: Media centre*. 2014 11/23/2014]; Available from: http://www.who.int/mediacentre/factsheets/.

35. *Global Database on Body Mass Index*. 2014.

36. Aekplakorn, W. and L. Mo-Suwan, *Prevalence of obesity in Thailand.* Obes Rev, 2009. **10**(6): p. 589-92.

37. Mendez, M.A., C.A. Monteiro, and B.M. Popkin, *Overweight exceeds underweight among women in most developing countries.* Am J Clin Nutr, 2005. **81**(3): p. 714-21.

38. Ng, M., et al., *Global, regional, and national prevalence of overweight and obesity in children and adults during 1980-2013: a systematic analysis for the Global Burden of Disease Study 2013.* Lancet, 2014. **384**(9945): p. 766-81.

39. Heslehurst, N., et al., *A nationally representative study of maternal obesity in England, UK: trends in incidence and demographic inequalities in 619 323 births, 1989-2007.* Int J Obes (Lond), 2010. **34**(3): p. 420-8.

40. Flegal, K.M., et al., *Prevalence of obesity and trends in the distribution of body mass index among US adults, 1999-2010.* Jama, 2012. **307**(5): p. 491-7.

41. Chu, S.Y., S.Y. Kim, and C.L. Bish, *Prepregnancy obesity prevalence in the United States, 2004-2005.* Matern Child Health J, 2009. **13**(5): p. 614-20.

42. Fisher, S.C., et al., *Is obesity still increasing among pregnant women? Prepregnancy obesity trends in 20 states, 2003-2009.* Prev Med, 2013. **56**(6): p. 372-8.

43. Au, C.P., et al., *Fetal and maternal factors associated with neonatal adiposity as measured by air displacement plethysmography: a large cross-sectional study.* Early Hum Dev, 2013. **89**(10): p. 839-43.

44. Kuusela, T.A., T.J. Kaila, and M. Kahonen, *Fine structure of the low-frequency spectra of heart rate and blood pressure.* BMC Physiol, 2003. **3**: p. 11.

45. Pereira-da-Silva, L., et al., *The adjusted effect of maternal body mass index, energy and macronutrient intakes during pregnancy, and gestational weight gain on body composition of full-term neonates.* Am J Perinatol, 2014. **31**(10): p. 875-82.

46. Modi, N., et al., *The influence of maternal body mass index on infant adiposity and hepatic lipid content.* Pediatr Res, 2011. **70**(3): p. 287-91.

47. Tikellis, G., et al., *Maternal and infant factors associated with neonatal adiposity: results from the Tasmanian Infant Health Survey (TIHS).* Int J Obes (Lond), 2012. **36**(4): p. 496-504.

48. Gaillard, R., et al., *Risk factors and outcomes of maternal obesity and excessive weight gain during pregnancy.* Obesity (Silver Spring), 2013. **21**(5): p. 1046-55.

49. Gale, C.R., et al., *Maternal size in pregnancy and body composition in children.* J Clin Endocrinol Metab, 2007. **92**(10): p. 3904-11.

50. Kaar, J.L., et al., *Maternal obesity, gestational weight gain, and offspring adiposity: the exploring perinatal outcomes among children study.* J Pediatr, 2014. **165**(3): p. 509-15.

51. Reynolds, R.M., et al., *Maternal BMI, parity, and pregnancy weight gain: influences on offspring adiposity in young adulthood.* J Clin Endocrinol Metab, 2010. **95**(12): p. 5365-9.

52. Aphamis, G., et al., *The relationship between physical fitness and obesity among a sample of adolescents in Cyprus.* Int J Adolesc Med Health, 2014.

53. Rajendra Acharya, U., et al., *Heart rate variability: a review.* Med Biol Eng Comput, 2006. **44**(12): p. 1031-51.

54. Campos, L.A., et al., *Mathematical biomarkers for the autonomic regulation of cardiovascular system.* Front Physiol, 2013. **4**: p. 279.

55. Berggren, E.K., et al., *Are the metabolic changes of pregnancy reversible in the first year postpartum?* Diabetologia, 2015.

56. Tompuri, T., et al., *Measures of cardiorespiratory fitness in relation to measures of body size and composition among children.* Clin Physiol Funct Imaging, 2014.

57. prevention, C.f.d.c.a. *Pregnancy Nutrition Surveillance*. Summary of Health Indicators 2011 [cited 2014 9/15/2014]; Available from: http://www.cdc.gov/pednss/pnss_tables/pdf/national_table2.pdf.

58. Crozier, S.R., et al., *Weight gain in pregnancy and childhood body composition: findings from the Southampton Women's Survey.* Am J Clin Nutr, 2010. **91**(6): p. 1745-51.

59. Li, Y., et al., *Overweight Is Associated With Decreased Cognitive Functioning Among School-age Children and Adolescents.* Obesity, 2008. **16**(8): p. 1809-1815.

60. Ensenauer, R., et al., *Effects of suboptimal or excessive gestational weight gain on childhood overweight and abdominal adiposity: results from a retrospective cohort study.* Int J Obes (Lond), 2013. **37**(4): p. 505-12.

61. Dello Russo, M., et al., *Gestational weight gain and adiposity, fat distribution, metabolic profile, and blood pressure in offspring: the IDEFICS project.* Int J Obes (Lond), 2013. **37**(7): p. 914-9.

62. Schack-Nielsen, L., et al., *Gestational weight gain in relation to offspring body mass index and obesity from infancy through adulthood.* Int J Obes (Lond), 2010. **34**(1): p. 67-74.

63. Hamlin, M.J., et al., *Measurement of cardiorespiratory fitness in children from two commonly used field tests after accounting for body fatness and maturity.* J Hum Kinet, 2014. **40**: p. 83-92.

64. Krakowiak, P., et al., *Maternal metabolic conditions and risk for autism and other neurodevelopmental disorders.* Pediatrics, 2012. **129**(5): p. e1121-8.

65. Buss, C., et al., *Impaired executive function mediates the association between maternal pre-pregnancy body mass index and child ADHD symptoms.* PLoS One, 2012. **7**(6): p. e37758.

66. Huang, L., et al., *Maternal prepregnancy obesity and child neurodevelopment in the Collaborative Perinatal Project.* Int J Epidemiol, 2014. **43**(3): p. 783-92.

67. Al Hazzouri, A.Z., et al., *Reduced Heart Rate Variability Is Associated With Worse Cognitive Performance in Elderly Mexican Americans.* Hypertension, 2013.

68. Benevides, T.W. and S.J. Lane, *A Review of Cardiac Autonomic Measures: Considerations for Examination of Physiological Response in Children with Autism Spectrum Disorder.* J Autism Dev Disord, 2013.

69. Cristea, I.A., et al., *Autonomic effects of cognitive reappraisal and acceptance in social anxiety: Evidence for common and distinct pathways for parasympathetic reactivity.* J Anxiety Disord, 2014. **28**(8): p. 795-803.

70. Frewen, J., et al., *Cognitive function is associated with impaired heart rate variability in ageing adults: the Irish longitudinal study on ageing wave one results.* Clin Auton Res, 2013. **23**(6): p. 313-23.

71. Guaraldi, P., et al., *Cognitive function in peripheral autonomic disorders.* PLoS One, 2014. **9**(1): p. e85020.

72. McCorry, L.K., *Physiology of the autonomic nervous system.* Am J Pharm Educ, 2007. **71**(4): p. 78.

73. Thayer, J.F. and E. Sternberg, *Beyond heart rate variability: vagal regulation of allostatic systems.* Ann N Y Acad Sci, 2006. **1088**: p. 361-72.

74. Benarroch, E.E., *The central autonomic network: functional organization, dysfunction, and perspective.* Mayo Clin Proc, 1993. **68**(10): p. 988-1001.

75. Cersosimo, M.G. and E.E. Benarroch, *Central control of autonomic function and involvement in neurodegenerative disorders.* Handb Clin Neurol, 2013. **117**: p. 45-57.

76. Cheng, G., et al., *Central vagal sensory and motor connections: human embryonic and fetal development.* Auton Neurosci, 2004. **114**(1-2): p. 83-96.

77. Pereyra, P.M., et al., *Development of myelinated and unmyelinated fibers of human vagus nerve during the first year of life.* J Neurol Sci, 1992. **110**(1-2): p. 107-13.

78. Gustafson, K.M., J. Colombo, and S.E. Carlson, *Docosahexaenoic acid and cognitive function: Is the link mediated by the autonomic nervous system?* Prostaglandins Leukot Essent Fatty Acids, 2008. **79**(3-5): p. 135-40.

79. Porges, S.W., *Vagal Tone: A Physiologic Marker of Stress Vulnerability.* Pediatrics, 1992. **90**(3): p. 498-504.

80. Cechetto, D.F., *Cortical control of the autonomic nervous system.* Exp Physiol, 2014. **99**(2): p. 326-31.

81. Gustafson, K.M., et al., *Effects of docosahexaenoic acid supplementation during pregnancy on fetal heart rate and variability: a randomized clinical trial.* Prostaglandins Leukot Essent Fatty Acids, 2013. **88**(5): p. 331-8.

82. Brennan, M., M. Palaniswami, and P. Kamen, *Do existing measures of Poincare plot geometry reflect nonlinear features of heart rate variability?* IEEE Trans Biomed Eng, 2001. **48**(11): p. 1342-7.

83. Tarvainen, M.P.N., Juha-Pekka *Kubios HRV version 2.1 User's Guide*. 2012, The MathWorks, Inc.: Kuopio, FINLAND.

84. Critchley, H.D., J. Eccles, and S.N. Garfinkel, *Interaction between cognition, emotion, and the autonomic nervous system.* Handb Clin Neurol, 2013. **117**: p. 59-77.

85. Porges, S.W. and S.A. Furman, *The Early Development of the Autonomic Nervous System Provides a Neural Platform for Social Behavior: A Polyvagal Perspective.* Infant Child Dev, 2011. **20**(1): p. 106-118.

86. Yoshizato, T., et al., *The relationship between age-related heart rate changes and developing brain function: a model of anencephalic human fetuses in utero.* Early Hum Dev, 1994. **36**(2): p. 101-12.

87. DiPietro, J.A., et al., *Antenatal origins of individual differences in heart rate.* Dev Psychobiol, 2000. **37**(4): p. 221-8.

88. Kim, D.H., et al., *Association between reduced heart rate variability and cognitive impairment in older disabled women in the community: Women's Health and Aging Study I.* J Am Geriatr Soc, 2006. **54**(11): p. 1751-7.

89. Nicolini, P., et al., *Autonomic dysfunction in mild cognitive impairment: evidence from power spectral analysis of heart rate variability in a cross-sectional case-control study.* PLoS One, 2014. **9**(5): p. e96656.

90. Capuana, L.J., et al., *Factors influencing the role of cardiac autonomic regulation in the service of cognitive control.* Biol Psychol, 2014. **102**: p. 88-97.

91. Fox, N.A. and S.W. Porges, *The relation between neonatal heart period patterns and developmental outcome.* Child Dev, 1985. **56**(1): p. 28-37.

92. Bornstein MH, D.J., Hahn CS, Painter K, Haynes OM, Costigan KA, *Prenatal cardiac function and postnatal cognitive development: An exploratory study.* Infancy 2002. **3**(4): p. 475–494.

93. Spangler, G. and R. Scheubeck, *Behavioral organization in newborns and its relation to adrenocortical and cardiac activity.* Child Dev, 1993. **64**(2): p. 622-33.

94. Doussard-Roosevelt, J.A., et al., *Vagal regulation of heart rate in the prediction of developmental outcome for very low birth weight preterm infants.* Child Dev, 1997. **68**(2): p. 173-86.

95. Porges, S.W., *Orienting in a defensive world: mammalian modifications of our evolutionary heritage. A Polyvagal Theory.* Psychophysiology, 1995. **32**(4): p. 301-18.

96. Thayer, J.F. and R.D. Lane, *A model of neurovisceral integration in emotion regulation and dysregulation.* J Affect Disord, 2000. **61**(3): p. 201-16.

97. Maffei, A., M. Haley, and A. Fontanini, *Neural processing of gustatory information in insular circuits.* Curr Opin Neurobiol, 2012. **22**(4): p. 709-16.

98. Cechetto, D.F. and C.B. Saper, *Evidence for a viscerotopic sensory representation in the cortex and thalamus in the rat.* J Comp Neurol, 1987. **262**(1): p. 27-45.

99. Ohgi, S., et al., *Neonatal behavioral assessment scale as a predictor of later developmental disabilities of low birth-weight and/or premature infants.* Brain Dev, 2003. **25**(5): p. 313-21.

100. BM, L. and B. TB, *Cultural perspectives on child development*. Cross-cultural assessment of neonatal behavior., ed. W. DA and S. HW. 1982, San Francisco: WH Freeman.

101. Prior, L.J., et al., *Exposure to a high-fat diet during development alters leptin and ghrelin sensitivity and elevates renal sympathetic nerve activity and arterial pressure in rabbits.* Hypertension, 2014. **63**(2): p. 338-45.

102. Sullivan, E.L., et al., *Chronic consumption of a high-fat diet during pregnancy causes perturbations in the serotonergic system and increased anxiety-like behavior in nonhuman primate offspring.* J Neurosci, 2010. **30**(10): p. 3826-30.

103. Casas, M., et al., *Maternal pre-pregnancy overweight and obesity, and child neuropsychological development: two Southern European birth cohort studies.* Int J Epidemiol, 2013. **42**(2): p. 506-17.

104. Ojala, T., et al., *Fetal cardiac sympathetic activation is linked with maternal body mass index.* Early Hum Dev, 2009. **85**(9): p. 557-60.

105. Waki, H. and P. Tontonoz, *Endocrine functions of adipose tissue.* Annu Rev Pathol, 2007. **2**: p. 31-56.

106. Hotamisligil, G.S., *Inflammatory pathways and insulin action.* Int J Obes Relat Metab Disord, 2003. **27 Suppl 3**: p. S53-5.

107. Makki, K., P. Froguel, and I. Wolowczuk, *Adipose tissue in obesity-related inflammation and insulin resistance: cells, cytokines, and chemokines.* ISRN Inflamm, 2013. **2013**: p. 139239.

108. Suganami, T., J. Nishida, and Y. Ogawa, *A paracrine loop between adipocytes and macrophages aggravates inflammatory changes: role of free fatty acids and tumor necrosis factor alpha.* Arterioscler Thromb Vasc Biol, 2005. **25**(10): p. 2062-8.

109. Capurso, C. and A. Capurso, *From excess adiposity to insulin resistance: the role of free fatty acids.* Vascul Pharmacol, 2012. **57**(2-4): p. 91-7.

110. Schenk, S., M. Saberi, and J.M. Olefsky, *Insulin sensitivity: modulation by nutrients and inflammation.* J Clin Invest, 2008. **118**(9): p. 2992-3002.

111. Grassi, G., et al., *Effect of central and peripheral body fat distribution on sympathetic and baroreflex function in obese normotensives.* J Hypertens, 2004. **22**(12): p. 2363-9.

112. Lips, M.A., et al., *Autonomic nervous system activity in diabetic and healthy obese female subjects and the effect of distinct weight loss strategies.* Eur J Endocrinol, 2013. **169**(4): p. 383-90.

113. Quilliot, D., et al., *Sympathetic-leptin relationship in obesity: effect of weight loss.* Metabolism, 2008. **57**(4): p. 555-62.

114. Rodriguez-Flores, M., et al., *Relationship of obesity and insulin resistance with the cerebrovascular reactivity: a case control study.* Cardiovasc Diabetol, 2014. **13**: p. 2.

115. Scherrer, U., et al., *Body fat and sympathetic nerve activity in healthy subjects.* Circulation, 1994. **89**(6): p. 2634-40.

116. Troisi, R.J., et al., *Relation of obesity and diet to sympathetic nervous system activity.* Hypertension, 1991. **17**(5): p. 669-77.

117. Janig, W., *Sympathetic nervous system and inflammation: a conceptual view.* Auton Neurosci, 2014. **182**: p. 4-14.

118. Muntzel, M.S., et al., *Mechanisms of insulin action on sympathetic nerve activity.* Clin Exp Hypertens, 1995. **17**(1-2): p. 39-50.

119. Zhang, L., et al., *The inflammatory changes of adipose tissue in late pregnant mice.* J Mol Endocrinol, 2011. **47**(2): p. 157-65.

120. Kondo, E., et al., *Adiponectin mRNA levels in parametrial adipose tissue and serum adiponectin levels are reduced in mice during late pregnancy.* Horm Metab Res, 2004. **36**(7): p. 465-9.

121. Murabayashi, N., et al., *Maternal high-fat diets cause insulin resistance through inflammatory changes in fetal adipose tissue.* Eur J Obstet Gynecol Reprod Biol, 2013. **169**(1): p. 39-44.

122. Zhang, L., et al., *Maternal obesity in ewes results in reduced fetal pancreatic beta-cell numbers in late gestation and decreased circulating insulin concentration at term.* Domest Anim Endocrinol, 2011. **40**(1): p. 30-9.

123. Zhu, M.J., et al., *Maternal obesity markedly increases placental fatty acid transporter expression and fetal blood triglycerides at midgestation in the ewe.* Am J Physiol Regul Integr Comp Physiol, 2010. **299**(5): p. R1224-31.

124. George, L.A., et al., *Different levels of overnutrition and weight gain during pregnancy have differential effects on fetal growth and organ development.* Reprod Biol Endocrinol, 2010. **8**: p. 75.

125. White, C.L., M.N. Purpera, and C.D. Morrison, *Maternal obesity is necessary for programming effect of high-fat diet on offspring.* Am J Physiol Regul Integr Comp Physiol, 2009. **296**(5): p. R1464-72.

126. Catalano, P.M., et al., *Fetuses of obese mothers develop insulin resistance in utero.* Diabetes Care, 2009. **32**(6): p. 1076-80.

127. Aye, I.L., et al., *Increasing maternal body mass index is associated with systemic inflammation in the mother and the activation of distinct placental inflammatory pathways.* Biol Reprod, 2014. **90**(6): p. 129.

128. Bilbo, S.D. and V. Tsang, *Enduring consequences of maternal obesity for brain inflammation and behavior of offspring.* Faseb j, 2010. **24**(6): p. 2104-15.

129. Kang, S.S., et al., *Dietary intervention rescues maternal obesity induced behavior deficits and neuroinflammation in offspring.* J Neuroinflammation, 2014. **11**(1): p. 156.

130. Matthews, D.R., et al., *Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man.* Diabetologia, 1985. **28**(7): p. 412-9.

131. Fomon, S.J., et al., *Body composition of reference children from birth to age 10 years.* Am J Clin Nutr, 1982. **35**(5 Suppl): p. 1169-75.

132. Lester, B.M., H. Als, and T.B. Brazelton, *Regional obstetric anesthesia and newborn behavior: a reanalysis toward synergistic effects.* Child Dev, 1982. **53**(3): p. 687-92.

133. Hinkle, S.N., et al., *Associations between maternal prepregnancy body mass index and child neurodevelopment at 2 years of age.* Int J Obes (Lond), 2012. **36**(10): p. 1312-9.

134. Jo, H., et al., *Maternal prepregnancy body mass index and child psychosocial development at 6 years of age.* Pediatrics, 2015. **135**(5): p. e1198-209.

135. Wylie, A., et al., *Maternal prepregnancy obesity and achievement of infant motor developmental milestones in the upstate KIDS study.* Obesity (Silver Spring), 2015. **23**(4): p. 907-13.

136. Gage, S.H., et al., *Associations of maternal weight gain in pregnancy with offspring cognition in childhood and adolescence: findings from the Avon Longitudinal Study of Parents and Children.* Am J Epidemiol, 2013. **177**(5): p. 402-10.

137. Brion, M.J., et al., *Intrauterine effects of maternal prepregnancy overweight on child cognition and behavior in 2 cohorts.* Pediatrics, 2011. **127**(1): p. e202-11.

138. Keim, S.A. and N.T. Pruitt, *Gestational weight gain and child cognitive development.* Int J Epidemiol, 2012. **41**(2): p. 414-22.

139. Voegtline, K.M., et al., *Fetal heart rate and motor development in overweight and obese pregnant women.* Int J Gynaecol Obstet, 2015.

140. Caulfield, L.E., et al., *Maternal zinc supplementation during pregnancy affects autonomic function of Peruvian children assessed at 54 months of age.* J Nutr, 2011. **141**(2): p. 327-32.

141. Merialdi, M., et al., *Randomized controlled trial of prenatal zinc supplementation and the development of fetal heart rate.* Am J Obstet Gynecol, 2004. **190**(4): p. 1106-12.

142. Spann, M.N., et al., *Deficient maternal zinc intake-but not folate-is associated with lower fetal heart rate variability.* Early Hum Dev, 2015. **91**(3): p. 169-72.

143. May, L.E., et al., *Aerobic exercise during pregnancy influences infant heart rate variability at one month of age.* Early Hum Dev, 2014. **90**(1): p. 33-8.

144. Tavris, D.R. and J.A. Read, *Effect of maternal weight gain on fetal, infant, and childhood death and on cognitive development.* Obstet Gynecol, 1982. **60**(6): p. 689-94.

145. *Institute of Medicine (US) and National Research Council (US) Committee to Reexamine IOM Pregnancy Weight Guidelines; Rasmussen KM, Yaktine AL, editors. Weight Gain During Pregnancy: Reexamining the Guidelines. Washington (DC): National Academies Press (US); 2009. Available from: http://www.ncbi.nlm.nih.gov/books/NBK32813/.*

146. Widen, E.M., et al., *The Pattern of Gestational Weight Gain is Associated with Changes in Maternal Body Composition and Neonatal Size.* Matern Child Health J, 2015. **19**(10): p. 2286-94.

147. Berggren, E.K., et al., *Maternal fat, but not lean, mass is increased among overweight/obese women with excess gestational weight gain.* Am J Obstet Gynecol, 2015.

148. Butte, N.F., et al., *Composition of gestational weight gain impacts maternal fat retention and infant birth weight.* Am J Obstet Gynecol, 2003. **189**(5): p. 1423-32.

149. Wakai, R.T., *Assessment of fetal neurodevelopment via fetal magnetocardiography.* Exp Neurol, 2004. **190 Suppl 1**: p. S65-71.

150. Birch, S.L., M.J. Duncan, and C. Franklin, *Overweight and reduced heart rate variability in British children: an exploratory study.* Prev Med, 2012. **55**(5): p. 430-2.

151. Vanderlei, L.C., et al., *Analysis of cardiac autonomic modulation in obese and eutrophic children.* Clinics (Sao Paulo), 2010. **65**(8): p. 789-92.

152. Wu, J.S., et al., *Epidemiological evidence of altered cardiac autonomic function in overweight but not underweight subjects.* Int J Obes (Lond), 2008. **32**(5): p. 788-94.

153. Eyre, E.L., et al., *The influence of age and weight status on cardiac autonomic control in healthy children: a review.* Auton Neurosci, 2014. **186**: p. 8-21.

154. Koenig, J., et al., *Body mass index is related to autonomic nervous system activity as measured by heart rate variability--a replication using short term measurements.* J Nutr Health Aging, 2014. **18**(3): p. 300-2.

155. Rivera, H.M., K.J. Christiansen, and E.L. Sullivan, *The role of maternal obesity in the risk of neuropsychiatric disorders.* Front Neurosci, 2015. **9**: p. 194.

156. Van Lieshout, R.J., *Role of maternal adiposity prior to and during pregnancy in cognitive and psychiatric problems in offspring.* Nutr Rev, 2013. **71 Suppl 1**: p. S95-101.

157. Pantham, P., I.L. Aye, and T.L. Powell, *Inflammation in maternal obesity and gestational diabetes mellitus.* Placenta, 2015. **36**(7): p. 709-15.

158. Zhou, D. and Y.X. Pan, *Pathophysiological basis for compromised health beyond generations: role of maternal high-fat diet and low-grade chronic inflammation.* J Nutr Biochem, 2015. **26**(1): p. 1-8.

159. Torres-Espinola, F.J., et al., *Maternal Obesity, Overweight and Gestational Diabetes Affect the Offspring Neurodevelopment at 6 and 18 Months of Age--A Follow Up from the PREOBE Cohort.* PLoS One, 2015. **10**(7): p. e0133010.

160. Camprubi Robles, M., et al., *Maternal Diabetes and Cognitive Performance in the Offspring: A Systematic Review and Meta-Analysis.* PLoS One, 2015. **10**(11): p. e0142583.

161. Hami, J., et al., *Some of the experimental and clinical aspects of the effects of the maternal diabetes on developing hippocampus.* World J Diabetes, 2015. **6**(3): p. 412-22.

162. Kang, S.S., et al., *Dietary intervention rescues maternal obesity induced behavior deficits and neuroinflammation in offspring.* J Neuroinflammation, 2014. **11**: p. 156.

163. Xu, G., et al., *Maternal diabetes and the risk of autism spectrum disorders in the offspring: a systematic review and meta-analysis.* J Autism Dev Disord, 2014. **44**(4): p. 766-75.

164. Cai, Z., et al., *Cytokine induction in fetal rat brains and brain injury in neonatal rats after maternal lipopolysaccharide administration.* Pediatr Res, 2000. **47**(1): p. 64-72.