MATERNAL BODY COMPOSITION LATE IN PREGNANCY AND INFANT BODY COMPOSITION AT BIRTH

By

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ABSTRACT

Background: Fat mass (FM) is significantly higher in neonates born to overweight and obese women, while no difference is found in fat free mass (FFM). Higher gestational weight gain (GWG) is also related to a greater neonatal birth weight and FM gain. However, no study has reported the relationship between maternal body composition during gestation and neonatal body composition at birth.

Objectives: The primary aim of this study was to evaluate the relationship between maternal body composition late in pregnancy and neonatal body composition at birth. The secondary aim of this study was to investigate the association between maternal trimester-specific GWG and neonatal body composition at birth.

Methods: Healthy pregnant women with a pre-pregnancy body mass index (BMI) between 18.5 to 39.99 kg/m^2 were recruited. Maternal body composition (percentage body fat (% fat), FM, FFM, and total body water (TBW)) was measured using the four-compartment model during 34 to 39 weeks gestation and infant body composition (% fat, FM, and FFM) was measured using air-displacement plethysmography (ADP) within 72h after birth. Maternal GWG during the 1^{st} (0 to 13 weeks), 2^{nd} (14 to 28 weeks) and 3^{rd} (29 weeks to delivery) trimesters were calculated using extracted body weight from medical records minus their self-reported pre-pregnancy weight. Multiple linear regression models were used to determine the relationship between maternal factors and neonatal body composition. Neonatal % fat, FM, and FFM were used as dependent variables. Maternal % fat, FM, FFM and TBW were used as independent variables for the primary aim and the 1^{st}, 2^{nd}, and 3^{rd} trimester gestational weight gains were used as independent variables for the secondary aim. Maternal age, neonatal age at test, gender and gestational age were controlled in the models for the primary aim and maternal pre-pregnancy BMI, neonatal age at test, gender and gestational age were controlled in the models for the secondary aim.

Results: Forty women completed visits for the primary aim and forty-five women completed visits for the secondary aim. Maternal body FFM and TBW were related to neonatal birth weight (r^2 = 0.280, p = 0.011; r^2 = 0.330, p = 0.007, respectively) and FFM (r^2 = 0.521, p = 0.011; r^2 = 0.519, p = 0.011, respectively). A trend of significance was found between maternal FM and neonatal birth weight (r^2 = 0.224; p = 0.053) and FM (r^2 = 0.052; p = 0.085). The relationship between trimester-specific GWG and neonatal body composition varied by maternal pre-pregnancy BMI category.

Conclusions: Maternal body composition was related to neonatal birth weight, while maternal FFM and TBW were related to neonatal FFM but not FM at birth. The relationship between maternal GWG and neonatal body fat at birth was dependent on maternal pre-pregnancy BMI.

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LIST OF ABBREVIATIONS

% fat: percentage body fat

ADP: air-displacement plethysmography

AGA: appropriate for gestational age

BIA: bioelectrical impedance analysis

BMI: body mass index

DXA: dual-energy x-ray absorptiometry

FFM: fat free mass

FM: fat mass

GDM: gestational diabetes mellitus

GWG: gestational weight gain

IOM: Institute of Medicine

LGA: lager for gestational age

MRI: magnetic resonance imaging

NO: nitric oxide

OR: odds ratio

RR: relative risk

SDS: standard deviation score

SGA: small for gestational age

TBK: total body potassium

TBW: total body water

TGV: thoracic gas volume

TOBEC: total body electrical conductivity

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

CHAPTER 1

INTRODUCTION

The intention of this study was to observe the relationship between the maternal environment and infant body composition. The primary purpose of this study was to determine the relationship between maternal body composition late in pregnancy and infant body composition at birth.

1.1. Maternal obesity and obesity development in offspring

Obesity is an epidemic in the industrialized world as economies have matured. The obesity prevalence in the US is 36.3% (1), and about one-third of females enter pregnancy obese (2). Obesity in women of childbearing age is a serious public health problem. From 1991 to 2001, the incidence of women being obese before pregnancy increased from 25% to 35% (3), and in 2008, the prevalence of pre-pregnancy obesity in the US increased to 28.5% based on the data from the Pregnancy Nutrition Surveillance System (4).

The time period surrounding pregnancy presents a critical window related to the immediate and future health of the mother and child. An obese pregnancy is associated with a heightened risk of pregnancy complications such as preeclampsia (5-7), gestational diabetes mellitus (GDM) (8), miscarriage (9), as well as the future development of chronic disease in mother (10). Maternal pre-pregnancy obesity presents a potential modifiable risk factor to avoid adverse outcomes for both mother and baby.

An obese maternal environment also impacts the health of the offspring. Negative offspring health effects stemming from maternal obesity include increased risk of childhood obesity (11) and type 2 diabetes (12). Maternal obesity is strongly related to offspring obesity (13, 14), creating a viscious cycle for the perpetuation of obesity from generation to generation. Epidemiologic data have shown that the prevalence of obesity is highest among children from obese parents (11), and children who have obese mothers are more at risk of being overweight or obese than those who have obese fathers (15). For women with a body mass index (BMI) of over 30, the prevalence of childhood obesity in their offspring at ages 2, 3 and 4 was 15%, 21% and 25%

respectively. This was 2.4 to 2.7 times the prevalence of obesity observed in children of mothers whose BMI was in the normal range (11).

1.2. Metabolic and hormonal occurrences and disturbances during pregnancy

During a healthy pregnancy, several hormonal and metabolic adaptations occur in order to support fetal growth and development. Pregnancy is characterized by increases in blood glucose levels, insulin resistance and circulating lipids, which make energy available to the fetus (16). However these adaptations appear to go away in maternal obesity. In maternal obesity, there is an exaggerated lipid response, which leads to lipotoxicity (17) and an even greater degree of insulin resistance (17). Jarvie *et al*. (18) proposed that lipotoxicity, which influences placental metabolism and function, is the pathological link between maternal obesity and adverse pregnancy outcomes leading to offspring obesity. Lipotoxicity is a metabolic syndrome that results from the accumulation of lipids, particularly fatty acids, in non-adipose tissue, leading to cellular dysfunction and death. The lipid abnormalities arising from excessive free fatty acids may be responsible for the observed endothelial dysfunction and placental complications of obese pregnancy.

Catalano *et al.* (17) found a decrease in maternal insulin sensitivity. Obese women were less insulin sensitive than lean women. Exposure to elevated or frequently fluctuating glucose concentrations may cause embryonic developmental abnormalities. Maternal pre-pregnancy insulin sensitivity has the strongest relationship with infant fat mass (FM) at birth (17). Catalano *et al.* (17) speculated that decreased insulin sensitivity and increased beta cell response affect early placenta development and function, and these changes relate to up-regulated lipid and cytokine gene expression, which in later pregnancy affect both maternal lipid metabolism and placental transport of nutrients. In the hyperlipidemic states of controlled type 1 diabetes and GDM, placental expression of genes coding for the transport and activation of fatty acids are up-

regulated (19). This might be the mechanistic by which offspring from obese women have greater FM compared to offspring from normal weight women.

Placentas from obese pregnancies have macrophage accumulation and inflammation analogous to adipose tissue (20). The placenta is a source of cytokines which can induce maternal gestational insulin resistance and alter nutrient transport to the fetus. Thus the placenta is a likely place of ectopic fat accumulation in obese pregnancy that may induce lipotoxicity in the fetus. **Figure 1.1** provides a description of the possible mechanisms relating maternal obesity to fetal programming of obesity.

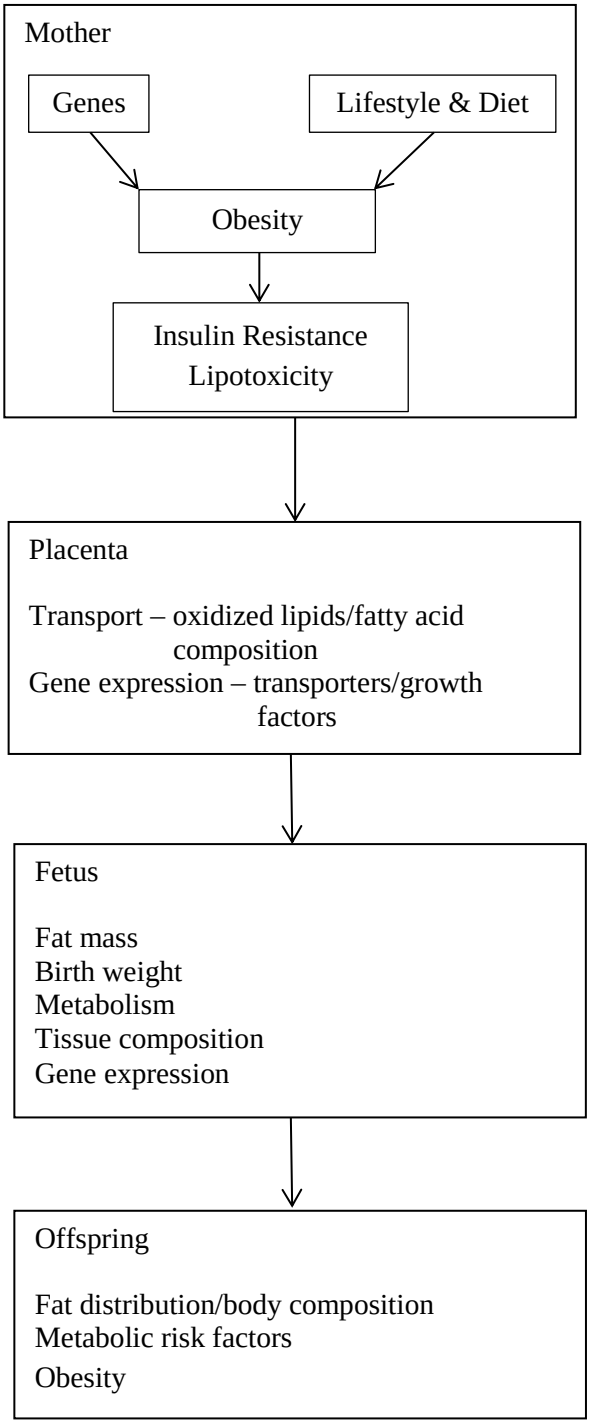

Figure 1.1 Fetal programming of obesity

1.3. The "*fetal origins of adult disease*" hypothesis

In the late 1980's, Barker introduced the "*fetal origins of adult disease*" hypothesis (21). The "*fetal origins of adult disease*" hypothesis linked low birth weight to the development of metabolic syndrome in adulthood. Barker (21) hypothesized that adult disease was a result of fetal growth restraint during gestation due to malnutrition of the mother or impaired nutrient transfer to the fetus.

Undernutrition was thought to cause an insult during a critical period of early life to the fetus that led to impaired fetal organ development with permanent or long term effects on organ structure or function (21). Barker proposed that fetal exposure to undernutrition caused a physiological adaptation to ensure survival, which resulted in preserving the development of critical organs such as brain, over the development of less critical organs such as kidneys, pancreas, liver and muscle. The altered growth changed the metabolism, perfusion and innervation of organs initiating long lasting consequences to organ function that made the fetus more susceptible to developing chronic diseases later in life (21).

Fetal malnutrition is related to impaired development and function of critical organs such as the liver, kidneys and pancreas, which are important for metabolic balance and circulatory system function involved in blood pressure control (22). This phenotype persists beyond the prenatal period and is associated with increased central adiposity in childhood, increasing the risk of hypertension and cardiovascular disease in adulthood (23). For example, suboptimal nutrition during pregnancy may damage the beta cells of the pancreas, giving rise to defects in the structure and function of the pancreas, resulting in insufficient insulin release and hyperglycemia. Impaired pancreatic function predisposes the individual to type 2 diabetes development, and pancreatic damage is exacerbated by age and a natural decline in organ function (24).

1.3.1. Programming of vascular disease

Martyn *et al.* (25) proposed that the programming of vascular disease occurs early in life. He hypothesized fetal undernutrition causes impairment of kidney function through nephron damage and impaired elastin in the blood vessel walls. These pathologies lead to an inability to regulate blood pressure resulting in the development of hypertension. Low birth weight is related to the development of hypertension in adulthood (26-28). Blood pressure is regulated by vasodilation of blood vessels. Vasodilation is mediated by endothelial secreted nitric oxide (NO). NO is a potent vasodilator of smooth muscle. NO mediated endothelial vasodilation is impaired in low birth weight infants (26, 27), suggesting a higher risk of developing hypertension in future

life. The sympathic nervous system is up-regulated in low birth weight infants (29). Overstimulation of the sympathetic nervous system leads to higher plasma levels of catecholamines, dopamine, norepinephrine and epinephrine. Catecholamines are neuromodulators that cause vasoconstriction of smooth muscle therefore causing an increase in blood pressure (30). High levels of catecholamines in the blood cause biochemical abnormalities that lead to hypertension.

1.3.2. Programming of obesity

Abnormal environmental factors during pregnancy may lead to extremes of fetal growth that can induce developmental programming, which leads to metabolic imprinting for energy homeostasis (31). Animal studies indicate that an obese phenotype is influenced by the *in utero* environment (31-33), which indicated the importance of embryonic environment because impaired *in utero* environment may program obesity on the next generation.

There is a U shaped relationship between birth weight and adult BMI (34). Individuals with a birth weight at either the low end (< 5 lbs) or at the high end (> 7 lbs) of the birth weight distribution have a higher risk of later obesity (34, 35). Many studies have established a link between low birth weight and adult disease development (21, 36-38). However, only 5% of neonates in the US are born with a low birth weight (< 2500g) (39). Overnutrition is much more prevalent. Catalano *et al.* (40) found a mean increase of 116 g in term singleton birth weight over the past 30 years. The increase in maternal pre-pregnancy weight was the factor most strongly correlated with the increase in birth weight.

A positive relationship is found between maternal BMI and neonatal birth weight (41-45). A birth weight $\geq 90^{th}$ percentile increases the risk for obesity in adolescents as well as adults (46-52). Obese women tend to have heavier infants with a birth weight ≥ 4000 g (macrosomic) or classified as large for gestational age (LGA). These infants have an increased risk for development of obesity (13, 14), hypertension (34), and diabetes (35) later in life.

Over the next several sections, studies will be discussed and data will be presented to support a relationship for fetal programming of later obesity.

1.4. Birth weight as a marker of disease

When trying to discern the pathways for programming of adult disease, birth weight is often used as a marker of the *in utero* experience (53). A low birth weight is suggestive of poor nutrient transfer to the fetus and therefore poor growth while a high birth weight is suggestive of fetal overnutrition (53). Both low and high birth weight relate to later disease development (53).

1.4.1. Low birth weight and later disease

Low birth weight is defined as a weight at delivery less than 2500 g (54). Term infants with a low birth weight is related to decreased muscle mass, altered adipocyte differentiation and accelerated postnatal growth (55).

Term infants with a low birth weight have decreased muscle mass and a high fat preservation in adulthood (56, 57). Yliharsila *et al.* (57) showed a strong correlation between birth weight and adult lean body mass after adjusting for adult age and BMI (β = 1.89, $p < 0.001$; β = 1.97, $p < 0.001$ for men and women, respectively), and higher percentage body fat (% fat) was predicted by low birth weight in a BMI-adjusted model (β = -1.38, $p < 0.001$; β = -1.36, $p < 0.001$ for men and women, respectively). The authors hypothesized that this was due to a lower amount of muscle mass accretion during fetal development that persisted into childhood. There is little cell replication in muscle after approximated 1 year of age, thus relatively speaking, the number of muscle fibers accumulated during infancy would be reflective of the number of muscle fibers attained in adulthood. *In utero*, muscle fibers are staring formed between 6 to 8 weeks during gestation and completed by 18 weeks (58). Inadequate energy intake by pregnant women in this window has been shown to decrease the number of fetal muscle fibers (59, 60). This may contribute to hyperglycemia and insulin resistance since skeletal muscle is important for glucose disposal (58). Eriksson *et al.* (61) found insulin resistance in adults was associated with low birth weight (p = 0.02). They suggested that altered sensitivity of the muscle to insulin might be the

explanation that underlies the association between low birth weight and insulin resistance during adult life.

Undernutrition during pregnancy also affects adipocyte differentiation. Low energy intake in the first and second trimesters may increase adipocyte differentiation and low energy intake in the third trimester may decrease adipocyte differentiation (62). Permanent alteration in the number of adipocytes would affect adipocyte secreted hormones, such as leptin. Leptin binds to sites both centrally in the brain and peripherally to decrease food consumption and increase energy utilization (58). Therefore, maternal undernutrition during early gestation could result in greater secretion of leptin and maternal undernutrition late in pregnancy could result in lower secretion of leptin in the offspring. High amounts of leptin have been found in adult obesity and may cause an inhibition of insulin secretion and stimulate adipogenesis (63). Research suggests a link between birth size and leptin (64). Cord blood leptin is positively related to birth weight and body fat of infants. The correlation was stronger in infants exposed to hyperinsulinemia and hyperglycemia *in utero* (62). Low birth weight is also associated with higher levels of plasma leptin in adulthood (65). Phillips *et al.* (65) found adults with a lower birth weight had higher leptin concentration than those with a higher birth weight even after adjusting for adult BMI (p = 0.02). These results suggested that low birth weight is associated with higher leptin that may correlate to some leptin resistance, which is related to overweight or obese phenotype.

Undernutrition during early fetal development is typically followed by improved or adequate postnatal nutrition. Postnatal accelerated or compensatory growth often occurs in infants born with a low birth weight or born prematurely (66). Undernutrition during gestation and small size at birth followed by rapid childhood weight gain is linked to cardiovascular disease and type 2 diabetes in adulthood (67, 68). Rapid growth and associated hormonal and metabolic changes may disrupt cell function and impose excessive metabolic demand on organs that are underdeveloped or small due to undernutrition during gestation and slow fetal growth (69). For example, low birth weight infants have limited pancreatic beta cell numbers; however, the

glucose induced insulin response is increased in infants with compensatory growth (70). Therefore, these infants could be at increased risk of developing insulin resistance in adulthood.

In conclusion, low birth weight is association with a reduction of muscle fibers, more adipocytes, higher leptin levels, and accelerated postnatal growth. Malnutrition related low birth weight is not a common problem in developed countries. Overnutrition during pregnancy is more common and is related to a high birth weight. In a similar manner as low birth weight relates to later disease, a high birth weight is also related to abnormal development and later adverse health.

1.4.2. High birth weight and development of diseases

The health consequences related to a low birth weight are serious (21, 36-38); however, a high birth weight is also a concern. High birth weight is related to increased risk of obesity development, type 2 diabetes and metabolic syndrome later in life (71). High birth weight is defined as a birth weight > 4000 g and LGA is defined as birth weight > 90^{th} percentile for gestational age. Rates for high birth weight and LGA were 9.2% and 10%, respectively (72-74). Obese females are more likely to deliver an LGA infant compared to normal weight women (75, 76). Epidemiological data suggests a relationship between the occurrence of LGA and adult disease (77-79). Both human and animal studies show increased adipocyte differentiation and fetal metabolism when exposed to maternal overnutrition (62, 80-84).

Hediger *et al.* (77) showed that children who were born LGA were prone to increased fat accumulation and remained heavier through at least 47 months of age. This suggested a relationship between intrauterine growth and risk of obesity in early childhood. Boney *et al.* (78) evaluated the major components of metabolic syndrome in a longitudinal cohort study of children aged 6, 7, 9 and 11 years old who were born LGA or appropriate for gestational age (AGA). They found children who were LGA at birth had a trend toward ($p = 0.08$) higher incidence of insulin resistance and a 2-fold increased risk of developing metabolic syndrome at 11 years old compared to children who were AGA at birth. Wang *et al.* (79) also found the prevalence of metabolic syndrome was significantly higher in obese children born LGA compared to obese children born

AGA. The prevalence of hypertension, hypertriglyceridemia and hypercholesterolemia was significantly different between LGA-obese children and AGA-obese children (adjusted odds ratio (OR) = 2.77, 2.32 and 3.11, respectively).

Maternal overnutrition late in pregnancy may change fetal adipose tissue differentiation and promote offspring obesity development (62). Infant fat accumulation mainly occurs during the third trimester and increased maternal food intake during this time increases fetal adipose tissue deposition (85). Maternal overnutrition late in gestation could increase maternal leptin levels in serum, which plays a key role in the regulation of neonatal body FM and body weight (80). Kiess *et al.* (81) showed leptin levels in cord blood were positively correlated with birth weight ($r = 0.57$; $p = 0.03$). Neonatal weight and skinfold thickness accounted for about 35% to 70% of the variance of leptin levels in cord blood. Increased leptin levels resulting from maternal overnutrition during late gestation may lead to higher fetal fat deposition and higher birth weight.

Animal studies have shown that maternal overnutrition causes detrimental infant health effects that impact offspring health (86-92). Increased adiposity was found in offspring born to dams fed a high fat diet (45% fat) throughout pregnancy (82). Another study in non-human primates showed that a maternal high fat diet (35% fat compared to 15% fat in the control diet) during pregnancy led to significant increases in plasma free fatty acid levels and liver triglycerides content in the fetus (83). Higher plasma free fatty acid levels in the fetus could lead to later insulin resistance (84). These results suggest diet impacts offspring phenotype and maternal overnutrition during pregnancy can program the offspring.

There are many similar effects between offspring exposed to either maternal under- or overnutrition. Growing evidence is suggesting programming during fetal life because of maternal malnutrition (over or undernutrition) at specific stages of gestation may result in permanent adaptive responses that lead to physiological changes and subsequent development of offspring hypertension, insulin resistance and hypertriglyceridemia. However, more research is needed to clarify the relationships between maternal nutrition and offspring health.

1.5. Birth weight and later BMI

1.5.1. Birth weight and BMI in adulthood

Studies have reported a direct relationship between birth weight and adult BMI (14, 34, 35, 46-49, 93, 94). Many are large longitudinal cohort studies or reports using census or registry data to analyze the relationship between birth weight and BMI in adulthood.

Rasmussen and Johansson (46) analyzed the relationship between birth weight and later BMI in 165,109 males born from 1973 to 1976. Birth weight was obtained from the Swedish Medical Birth Registry and weight and height at 18 years old were obtained from the Military Service Conscription Registry. A direct relationship was found between weight for gestational age and BMI at the age of 18 years old. A high birth weight was related to a higher risk of overweight at 18 years old after adjusting for the living area, maternal age, educational level and maternal parity. Those with a birth weight between the 95^{th} and 99^{th} percentile had an OR for overweight in adulthood of 1.50 and those with a birth weight over 99^{th} percentile had an OR for overweight in adulthood of 1.67 when using birth weight between 25^{th} and 50^{th} percentile as the reference.

Sorensen *et al.* (47) identified 4300 births using the Danish Medical Birth Registry for men born after 1972. Their birth weight, height and weight at between 18 to 26 years old was recorded by the Danish draft board. They found a relationship between birth weight and later BMI and a continuous increase in young adulthood BMI with increasing birth weight after controlling for maternal age, marital status and occupation. The prevalence of obesity in this population from 18 to 26 years old was 3.5% for the group with a birth weight $\leq$ 2500g and 11.4% for the group with a birth weight $\geq$ 4500g.

Similar studies were conducted in Norway and Sweden. Eide *et al.* (48) collected the birth weight of 348,706 males using the Medical Birth Registry of Norway and the height and weight in adulthood from information collected during the military draft. A positive association was found between birth weight and adult BMI for birth weight > 2500g. In Sweden, Tuvemo *et*

al. (49) collected the birth weight of 39,901 males using the Swedish birth registry and the weight and height in adulthood were collected using draft from the Swedish conscript registry. The group with a birth weight < 2500g had a mean adult BMI of 21.93 kg/m^2 while the group with a birth weight ≥ 4500g had a mean adult BMI of 23.02 kg/m^2. Men with a high ponderal index (birth weight (g)/length $(cm)^3$) had an OR for obesity as 1.8 when using men with a low ponderal index as reference.

Seidman *et al.* (93) used data from the Israeli draft medical exam to identify 33,413 infants of both genders born between 1964 and 1971 and followed until 17 years old. A positive association was found between birth weight and BMI during adolescence. The OR for being overweight at 17 years old was 2.16 for males and 2.95 for females with a birth weight > 4500g compared to a normal birth weight (3000 to 3500g). This is the first study that included females when analyzing the relationship between birth weight and BMI in adulthood.

In the US, Curhan *et al.* (34, 35) did two large scale studies in males and females that provided information related to birth weight and adult BMI. They obtained information for 51,289 men using data from the Health Professional Follow-up Study and for 164,040 women using data from the Nurses' Health Study I and II. In the Health Professional Study, those with a birth weight over 4.5 kg had a higher risk of having a BMI over 28.2 kg/m^2 in adulthood (OR = 2.08) as well as those with a birth weight between 3.86 and 4.5 kg (OR = 1.50) compared to those with a birth weight between 3.2 to 3.8 kg (35). In the Nurses' Health Study I and II, the OR of being in the highest BMI quintile (BMI > 29.2 kg/m^2) were 1.19 and 1.62 for women born with a birth weight 3.86 to 4.5 kg and over 4.5 kg, respectively (34).

Parson *et al.* (14) reported that infant birth weight was independently related to adult BMI based on a study of 10,683 infant males and females born in 1958 in Scotland, England and Wales. They found a J shape relationship between birth weight and later BMI. Infants who had a birth weight in the heaviest quintile had a high BMI in adulthood regardless of childhood growth. This relationship was largely predicted by maternal pre-pregnancy BMI and independent of

paternal height, socioeconomic status or maternal smoking habits. Fall *et al.* (94) also found a J relationship between birth weight and adult BMI in a group of 297 women born between 1923 and 1930. They found BMI in these women rose with increasing birth weight ($p = 0.05$).

In summary, birth weight is positively related to BMI in adulthood. This suggests that the maternal environment has the potential to substantially increase the risk of offspring obesity later in life.

1.5.2. Birth weight and later BMI in childhood

Studies have assessed birth weight as a predictor of being an overweight and obese child (95-103). Epidemiological studies show that childhood obesity is related to adulthood obesity. A child who is obese as an adolescent has an 80% chance of being obese as an adult (103, 104).

Fisch *et al.* (95) collected data prospectively in 1,786 Minnesota children and related birth weight to obesity at ages 4 and 7. An infant was classified as obese if his/her birth weight was at or above the 95th percentile. Obesity at birth was positively related to weight/ height index (weight (kg) /height (cm)) at both 4 and 7 years old. Another prospective longitudinal study was done in Australia. Mothers were interviewed pre-delivery and children were followed for visits immediately after delivery, at 6 months and 5 years. Complete data were collected on 4,602 mother-child pairs. Moderate obesity was defined as a BMI between the 85th and 95th percentile while severe obesity was defined as BMI > 95th percentile. Birth weight was an independent predictor of both moderate and severe obesity. The OR of severe obesity was 1.8 for a birth weight ≥ 95th percentile (96). These results were consistent with another more recent study done in Denmark. Lausten-Thomsen *et al.* (97) found birth weight was a predictor of severe childhood obesity in 1,171 obese children aged 3 to 18 years old in Denmark. They defined childhood obesity as BMI above 95th percentile for age and gender according to Danish BMI charts. Weight and height were collected at clinic visit in the Children's Obesity Clinic at Copenhagen University Hospital Holbaek in Denmark from 3 to 18 years old. Childhood BMI-standard derivation scores (SDS) were calculated according to Danish BMI charts. Birth weight was

collected from the hospital record and ponderal index was calculated. In a generalized linear model adjusted for socioeconomic status, a significant correlation between BMI-SDS at the time of enrollment and birth weight ($p < 0.0001$) was found. They also found BMI-SDS at the time of enrollment correlated with birth weight for gestational age ($p < 0.0001$) and infant ponderal index ($p = 0.020$) after adjusting for socioeconomic status and breastfeeding duration. Instead of a U-shape relationship, their data supported a linear association between birth weight and childhood BMI. They also found that birth weight was a better predictor for childhood obesity than ponderal index and suggested that the trend of increasing birth weight might underlie the increasing childhood obesity incidence.

Some studies used large data registries to analyze the association between birth weight and child's BMI. Binkin *et al.* (98) used birth certificates of participant's from the Women, Infant and Children special supplement food program for low income families in Tennessee to obtain infant birth weight. Birth weight was stratified in 500 g increased from 1000 to 4999 g. Higher birth weight was directly related to a greater risk of obesity development at 3 years old. Only one percent of children were obese at 36 to 41 months old if they had a birth weight between 1000 and 1499 g while 8.7% children were obese at the same age if they had a birth weight between 4500 and 4999 g. Zive *et al.* (99) used data from the Study of Children's Activity and Nutrition Project to examine the relationship between birth weight and child's BMI at the age of 4 in 331 Anglo- and Mexican-Americans. Birth weight correlated with child's BMI ($r = 0.28$. $p < 0.001$) and the sum of skinfold thickness ($r = 0.16$, $p < 0.01$). Hui *et al.* (100) collected birth weight data of 6496 full term infants from a Hong Kong birth cohort in 1997. Weight and height of these children were measured by the Department of Student's Health Service when children were 7 years old. Overweight and obesity were defined as BMI $\geq$ 25 and 30 kg/m^2, respectively. Children with a higher birth weight and a faster growth rate had a greater risk for being overweight at 7 years old for both genders, especially for the first 3 months. The OR for infants in

the highest birth weight tertile (mean birth weight = 3.6 kg) to be overweight at 7 years of age was 2.00 for girls and 2.31 for boys compared to low birth weight girls.

Some population-based studies showed an association between macrosomia and childhood overweight. Rugholm *et al.* (102) studied 124,615 girls and 128,346 boys born between 1936 and 1983. Overweight was defined by BMI in relation to internationally accepted criteria. Compared to children with a birth weight of 3.0 to 3.5 kg, the risk of overweight increased consistently with each increase in birth weight category among girls and boys and at all ages between 6 and 13 years old. Furthermore, the association between birth weight and increased risk of overweight in childhood remained stable across a 48-year period. Kromeyer-Hauschild *et al.* (103) studied 1,901 German boys and girls aged 7 to 14 years old using cross sectional surveys completed in 1975, 1985 and 1995. They found a significant relationship between childhood overweight (BMI > 90^{th} percentile) and birth weight in boys ($p = 0.04$) and girls ($p = 0.035$), when controlling for socioeconomic status. Another population-based study done by He *et al.* (101) examined 748 preschool boys and 574 preschool girls in China aged from 0 to 7 years old. Birth weight ≥ 4000 g was identified as a major risk factor for obesity development ($p < 0.05$).

Birth weight has been shown to be related to both adult and childhood BMI and it is also one of the most common characteristics used to evaluate fetal growth, which links maternal environment to offspring's long-term health. However, standard weight and length measurements provide only estimates of infant adiposity but fail to quantify what comprises infant body mass. Moulton *et al.* (105) demonstrated that the variability in birth weight within mammalian species was explained by the amount of adipose tissue whereas the amount of lean body mass was relatively constant and changed in a consistent manner over time. Catalano *et al.* (106) found FM in term neonates accounted for only 14% of birth weight but explained 46% of the variance in birth weight. For those reasons, neonatal body composition may be a better way to assess fetal growth instead of birth weight. Recent advancement has allowed for the assessment of neonatal

body composition (107-109). Analyses of neonatal body composition will be beneficial to explore the relationships between the maternal and the fetal environment.

1.6. Assessment of neonatal body composition

The available methods to assess body fat indirectly are based on theoretical models (110). The accuracy of these techniques depends on the model and the associated assumptions. Neonates violate the underlying assumption of a constant hydration of fat free mass (FFM) because they have a higher body water content and a lower density in body FFM. During the initial days of life, the hydration level of the body fluctuates as the newborn adapts to life outside the intrauterine environment (111). This must be taken into account when the assessment is conducted. The Pea Pod® software takes into account the fluctuations of neonatal hydration when determining FFM during the first few days of life. The Pea Pod® uses age and gender-specific equations to calculate neonatal body density based on results obtained from multi-compartment models (112, 113).

Various methods are used to assess neonatal body composition including anthropometry (114-119), magnetic resonance imaging (MRI) (120), total body water (TBW) (112, 120-122), total body potassium (TBK) (112, 121), total body electrical conductivity (TOBEC) (116, 121-125), dual energy x-ray absorptiometry (DXA) (114, 117, 118, 121, 126-128) and densitometry using air-displacement plethysmography (ADP) (107-109, 129-132). Densitometry is a safe, quick and easy method to assess neonatal body composition (129). The Pea Pod® is the only technology currently available to assess neonatal body composition and it assesses densitometry using ADP (129). Measurement of neonatal body composition involves assessment of body mass and body volume.

Several studies of neonates have been done using ADP since the Pea Pod® was introduced in 2003(107-109, 129-132). Sainz and Urlando (107) used 24 phantoms made from pig muscle and fat to assess the precision and accuracy of the Pea Pod® compared with chemical

analysis and hydrostatic weighing. No differences were found between the Pea Pod® and chemical analysis. Yao and colleagues (108, 109) assessed within and between day reliability of the Pea Pod® in 17 neonates on two consecutive days. The study showed no between or within day difference for percent fat. In addition, the investigator compared the body fat measures from Pea Pod® and from TBW using deuterium and no differences for % fat were found between these two techniques.

Most studies that assessed neonatal body composition examined maternal anthropometric variables as predictors. Typically these include pre-pregnancy weight, pre-pregnancy BMI, and gestational weight gain (GWG). Anthropometrics do not take into account the composition (FM or FFM) of body weight. Quantification of maternal body fat may be a better predictor because it provides more detailed information that describes the underlying relationships between the maternal and intrauterine environment.

1.7. Assessment of maternal body composition

Assessment of body composition during pregnancy is complicated because the composition of lean tissue changes during pregnancy. The hydration of FFM changes during pregnancy due to the increase of plasma volume (133) and the increase in amniotic fluid volume (134) to support fetal growth. Plasma volume increases by 1.2 to 1.5 L by 34 weeks of gestation (133) and the mean amniotic fluid volume from 22 to 39 weeks is about 0.8 L (134). Water gain is the largest component (average around 70% at 38 weeks (135, 136)) of maternal weight gain. The amount of water in FFM in pregnant women varies between 72.5% and 76.2% (137) compared with 73.8% in non-pregnant women (138), because of the increase in maternal body water.

The two-compartment model is the most common model used to measure body composition in non-pregnant population. It divides the body into FM and FFM. The two-compartment model estimates the body fat based on the assumption that the densities of FM and

FFM are 0.900 g/cm^3 and 1.10 g/cm^3, respectively. Increases in FFM (mainly TBW) during pregnancy lower the density of FFM compared with non-pregnancy status and invalidate the standard two-compartment model conversion factors complicating body composition assessment during pregnancy. Kopp-Hoolihan *et al.* (138) and Hopkins *et al.* (139) found that the two-compartment model of assessing body composition was not valid during pregnancy, even when correcting for altered hydration status that occurs in normal pregnancy.

Multi-compartment models give reliable and valid estimates of body fat in studies of subjects with varied body composition during pregnancy (140, 141). The four-compartment model measures water, fat, mineral lean and protein independently. This direct approach does not rely on assumptions about the fractional contributions of body water and bone mineral mass to FFM, nor about the density of the FFM. Such models provide more accurate estimates of FM for pregnant women. Lederman *et al.* (142) reported the first study using the four-compartment model to determine longitudinal changes in body composition occurring during pregnancy in the early 1990s. They measured body weight, total body water and body density at 14 weeks and again at 37 weeks during gestation. Bone mineral was measured at 3 weeks postpartum to avoid radiation exposure during pregnancy.

1.7.1. Total body water

TBW is measured using stable radiolabeled isotope, either deuterated water (D_2O) or oxygen-18, and measuring its dilution. The hydration of FFM is estimated as 73% in non-pregnant women however there is wide variation during pregnancy (137, 143, 144). Two early studies (143, 144) estimated the amount and composition of tissues gained to calculate changes in FFM hydration in pregnant women using Hytten's equation (145). Both of the studies found the TBW did not change before week 10 and rose gradually to 76% at 40 weeks (143, 144). Hopkinson (139) and Paxton and colleagues (146) measured TBW as part of four-compartment model during pregnancy and both reported a hydration of 76% at 36 weeks. Forsum *et al.* (147) showed FFM hydration was 73.2% at 16 to18 weeks and rose to 74.8% at 30 and 36 weeks

gestation, which is within the range reported by van Raaij *et al.* (144) The hydration of FFM dropped back to 73.2% at 2 or 6 months postpartum (147).The results of these studies show similar values and agreement among the different theoretical hydration estimates. Women with different pre-pregnancy FFM would have different hydration of the FFM even if they gain the same FFM during pregnancy (144). Thus, large and small pregnant women classified by pre-pregnancy BMI may not have the same change in hydration with a given FFM increment. Depending on the women's pre-pregnancy FFM and whether she develops edema, FFM at term could range from 74.6% to 77.1%. (144).

1.7.2. Body density

Body density is measured by hydrodensitometry (commonly referred to as underwater weighing) and ADP. In a two-compartment model, body fat is estimated by measuring body volume and using the densities of fat (0.900 g/cm^3) and FFM (1.10 g/cm^3) to derive body composition. The change in hydration during pregnancy invalidates the body density measure because if hydration of FFM changes, then a FFM density of 1.10 g/cm^3 is not correct. Lederman *et al.* (142) showed the mean density of the FFM for pregnant women was 1.099 g/cm^3 at 14 weeks and 1.089 g/cm^3 at 37 weeks. As a result, a single method using an assumed density of FFM would not reflect the changes in the density of FFM across pregnancy. Therefore FFM would be underestimated during pregnancy resulting in an overestimation of FM using two-compartment model.

Adjusted equations correcting for altered values in pregnancy could provide more accurate values for body composition changes during pregnancy. For example, average values for fetal, placental and amniotic fluid weight and composition have been used to correct the mean density of FFM during pregnancy (143, 144); however, many other variables that may influence body composition during pregnancy are still not clear. The pre-pregnancy FFM and degree of edema during pregnancy could be other factors that affect the density of FFM during pregnancy. Van Raaij *et al.* (144) showed the density of the total FFM at term would range from 1.0895 to

1.0850 g/cm^3 in women who did not developed edema during pregnancy; however, the range would be 1.0830 to 1.0785 g/cm^3 for women who did develop generalized edema. The FFM gain during pregnancy represented 16.6% to 25% of the pre-pregnancy FFM.

Multi-compartment models are particularly useful in measuring body composition during pregnancy because they can improve the accuracy of each subject's measurements and decrease the number of subjects needed. It is a challenge to use the four-compartment model in pregnant women because hydrodensitometry is difficult for many women to successfully complete near term. ADP rather than water displacement to measure body volume is a useful alternative and has a wider application than hydrodensitometry. Notably, there have been no data published using ADP in a multi-component model to assess maternal body composition to date. This is the first study that will use ADP to measure maternal body volume in a four-compartment model.

1.8. Maternal BMI and infant birth weight

Maternal pre-pregnancy BMI is directly related to the risk of developing a series of pregnancy related complications and macrosomia (17). Maternal pre-pregnancy BMI is frequently used as an indicator of the conditions experienced *in utero* and it has an impact on infant birth weight (148-153).

Koepp *et al.* (148) examined the relationship between maternal pre-pregnancy BMI and offspring birth weight in 58,383 Norwegian women. Maternal pre-pregnancy weight and height were self-reported and infant birth weight was obtained from the medical records. They found the birth weight of offspring increased with increasing maternal pre-pregnancy BMI. For every unit increase in maternal pre-pregnancy BMI (1 kg/m^2) infant birth weight increased by 25.9 g (95% CI: 25.0, 26.9). Kalk *et al.* (149) conducted a similar study in 2,049 German mother-infant pairs. Maternal pre-pregnancy BMI was calculated based on self-reported body weight before pregnancy and maternal height was extracted from medical records. The results showed an increased risk of having a macrosomic (OR: 1.5 to 2) or LGA infant (OR: 1.6 to 2.5) in

overweight and obese women. This association was independent of child gender and gestational age.

These results are consistent with another large epidemiological study of 325,395 pregnant women in London conducted by Sebire *et al.* (150). Maternal pre-pregnancy BMI was calculated using weight and height measured at the first antenatal visit and infant birth weight was expressed as the number of standard deviations by which the measured birth weight differed from the expected mean for gender and gestational age. Infant birth weight was positively associated with increasing maternal pre-pregnancy BMI and the mean birth weight was significantly increased in offspring from overweight ($t = 39.2$, $p < 0.0001$) and obese women ($t = 53.1$, $p < 0.0001$) compared to offspring born to normal weight women. The prevalence of LGA infants was almost twice as high in offspring born to obese women compared to offspring born to normal weight women (OR: 2.36; 99% CI: 2.23, 2.50).

Frederick *et al.* (151) suggested an independent role of pre-pregnancy BMI as a determinant of infant birth weight, as well as complex relationships between pre-pregnancy BMI, GWG, and other maternal factors with fetal growth, as measured by size at birth. They analyzed the impact of pre-pregnancy BMI on infant birth weight in 2,670 women in the US. Pre-pregnancy weight and height were self-reported and confirmed with medical records and infant birth weight was obtained from the infants' medical records. In this study, the quadratic term of pre-pregnancy BMI (pre-pregnancy BMI^2) accounted for 27.3% of the variation in infant birth weight (adjusted $R^2 = 0.273$). Both pre-pregnancy BMI and pre-pregnancy BMI^2 were significantly associated with birth weight (pre-pregnancy BMI, $\beta = 44.67$, $p = 0.001$; pre-pregnancy BMI^2, $\beta = -0.51$, $p = 0.029$). When using a women with a normal pre-pregnancy BMI as a reference, underweight women had a 50% reduced risk of delivering a macrosomic infant (adjusted relative risk (RR): 0.50; 95% CI: 0.35, 0.71) but obese women had a 1.65-fold increased risk of delivering a macrosomic infant (adjusted RR = 1.65; 95% CI 1.29, 2.11).

Neggers *et al.* (152) did a prospective study to determine whether maternal anthropometric measurements during pregnancy as well as pre-pregnancy weight and pre-pregnancy BMI predicted newborn measures at birth. They studied 1,205 low income African-American women and their babies in Alabama. Maternal pre-pregnancy weight was self-reported and height was measured at the first prenatal visit. Body weight and skinfold thickness of the infant were measured within 24 hours after birth. Maternal pre-pregnancy weight had the greatest relationship to infant birth weight. A difference in maternal pre-pregnancy weight from the 10^{th} to 90^{th} percentile resulted in a 295 g increase in adjusted infant birth weight. However, in a regression model of infant body fat estimated by skinfold thickness, maternal pre-pregnancy BMI was the strongest predictor. A difference in maternal pre-pregnancy BMI from the 10^{th} to 90^{th} percentile resulted in a 12 to 15% increase in infant skinfold thickness. Multiple linear regression showed a 1 kg increase in maternal pre-pregnancy weight was associated with a 7.3 g increase in birth weight after adjusting for maternal GWG.

Yu *et al.* (153) did a meta-analysis systematic review to examine the relationship between maternal pre-pregnancy BMI and infant birth weight. Thirty-four of the 45 analyzed articles investigated the association between maternal pre-pregnancy BMI and infant birth weight. Sixteen studies assessed the association between pre-pregnancy BMI and a prevalence of small-for-gestational-age (SGA) and low birth weight. They found women who were underweight before pregnancy classified by BMI had a higher risk of having an SGA infant (OR: 1.81; 95% CI: 1.76–1.87; $p < 0.001$) compared to women who were normal weight before pregnancy. In contrast, an overweight or obese pre-pregnancy BMI decreased the risk of low birth weight (OR: 0.83; 95% CI: 0.81–0.84; and OR: 0.81; 95% CI: 0.80–0.83; respectively $p < 0.001$). Twenty-two studies assessed the association between pre-pregnancy BMI and high birth weight and macrosomia. When compared to women with a normal pre-pregnancy BMI, women who were overweight or obese before pregnancy had an increased risk of having either a high birth weight

(OR: 1.53; 95% CI: 1.44–1.63; and OR: 2.00; 95% CI: 1.84–2.18; $p < 0.001$) or macrosomic infant (OR: 1.67; 95% CI: 1.42–1.97; and OR: 3.23; 95% CI: 2.39–4.37; $p < 0.001$).

In summary, maternal pre-pregnancy BMI is directly related to infant birth weight. Women who were overweight or obese before pregnancy have a higher risk of delivering an infant with a high birth weight or delivering an infant classified as macrosomic.

1.9. Maternal BMI and infant body composition

Birth weight is not a precise indicator of fetal nutritional status (58), though birth weight provides a crude estimate of the intrauterine environment. Infant body composition may be a better biomarker reflecting or mediating the development of disease later in life. Neonatal body composition correlates with childhood body composition (128). Crozier *et al.* (128) show moderate correlations between neonatal FM and childhood FM at ages 4 and 6 years old. Stronger correlations were found between neonatal FFM and childhood FFM at ages 4 and 6 years old. Neonatal body composition may reflect or mediate the development of disease later in life.

A few studies have assessed neonatal body composition in relation to maternal factors (125, 131, 132, 154, 155). FM is significantly higher in neonates born to overweight and obese women, while no difference is found in FFM (125, 131). Sewell *et al.* (125) compared body composition of neonates using TOBEC from pre-pregnant normal weight and overweight/obese women. Neonates born to pre-pregnant overweight or obese women had greater birth weight (3436g vs. 3284g; $p = 0.051$), body fat (11.6% fat vs. 9.7% fat; $p = 0.03$), and FM (420g vs. 380g; $p = 0.01$) compared with neonates from normal weight women. Sewell *et al.* (125) showed the differences of birth weight were attributed to increased FM but not FFM. This was the first study to investigate the relationship between maternal pre-pregnancy BMI and neonatal body composition. Hull *et al.* (131) compared pre-pregnancy BMI and neonatal body composition using ADP in a total of 72 neonates (33 from normal weight women and 39 from overweight/obese women). Significant differences in body fat (12.5% vs. 13.6%; $p \leq 0.0001$) and

FM (414.1g vs. 448.3g; $p \leq 0.05$) were found between neonates from normal weight women and overweight/obese women. However, no significant differences were found in birth weight between groups in this study. More recently, Andres *et al.* (155) measured body composition using ADP in 65 infants at 2 weeks of age (46 born to normal weight women and 19 born to overweight women). They found infant % fat and absolute FM were significantly higher in infants born to normal weight women compared to infants born to overweight women at 2 weeks of age (11.9% vs. 15.3%, $p = 0.01$; 0.44 vs. 0.61 kg; $p = 0.005$). The effects on FFM were not described in this study. All of these studies showed a positive relationship between maternal pre-pregnancy BMI and neonatal % fat and FM (125, 131, 155).

One study found an interaction of maternal pre-pregnancy BMI and GWG on neonatal body composition. Hull *et al.* (132) compared GWG in normal, overweight and obese women and neonatal body fat using ADP in 306 neonates. More than 70% of overweight and obese women gained an excessive amount of weight whereas just 40% normal weight women gained excessive weight. Neonatal body fat differed depending on whether overweight women gained an appropriate or excessive amount of weight during pregnancy. Neonates born to overweight women who gained excessively had similar body fat when compared to neonates born to obese women regardless of weight gain. However, a neonate born to an overweight woman who gained an appropriate amount of weight had similar body fat to a neonate born to a normal weight woman. Regardless of appropriate or excessive gains, neonates born to normal weight women had the lowest body fat while neonates born to obese women had the highest.

Maternal pre-pregnancy BMI is associated with neonatal body composition. If a woman has an overweight or obese BMI before pregnancy, her neonate is more likely to have higher %fat and FM compared to neonates born to normal weight women. However, none of these studies analyzed maternal body composition during pregnancy and how that related to neonatal body fat. Currently, we do not know if either maternal FM or FFM influences neonatal body fat at birth.

1.10. Maternal GWG and infant birth weight

Previous studies associate maternal genetic, socio-culture, demographic and behavior factors with infant birth weight (156-158). For example, maternal pre-pregnancy BMI and GWG influence infant birth weight and play significant roles in pregnancy outcomes. Low GWG is associated with SGA (159) and preterm birth (160) whereas high GWG is associated with risk of macrosomia (159) and caesarean section births (161). To optimize birth weight, the Institute of Medicine (IOM) guidelines (**Table 1.1**) for GWG recommend a higher GWG for underweight women and a lower GWG for obese women (see **Table 1.1**) (85). Overweight and obese women are more likely to gain excessive weight during pregnancy compared to women with a normal weight pre-pregnancy BMI (132, 162). Excessive GWG is associated with higher maternal weight retention (135) and birth weight (163, 164).

Table 1.1 Institute of Medicine gestational weight gain recommendations, 2009

Pre-pregnancy body mass index, kg/m^2	Gestational weight gain, kg (lbs)
Underweight (<18.5)	12.5 – 18 (28 – 40)
Normal weight (18.5-24.99)	11.5 – 15.9 (25 – 35)
Overweight (25-29.99)	7 – 11.5 (15 – 25)
Obese (>30)	5 – 9 (11 – 20)

Frederick *et al.* (151) investigated the effect of GWG on infant birth weight in a prospective study. They collected GWG and birth weight in 2,670 mother-infant pairs in the US. GWG within 1990 IOM guidelines was associated with reduced risk of both low birth weight and macrosomia. About half the women in their population gained weight in excess of the 1990 IOM guideline, and 75.7% of overweight women and 61.8% of obese women gained weight above the 1990 IOM guideline. Women who gained above the 1990 IOM guideline experienced a 76% increased risk of delivering macrosomic infants compared to women who gained below the guideline (adjusted RR = 1.76; 95% CI: 1.40 to 2.22). After adjusting for maternal pre-pregnancy

BMI, race, and complications during pregnancy, GWG below 15.9 kg was associated with a 51% lower risk of delivering macrosomic infants (adjusted RR = 0.49; 95% CI: 0.40 to 0.60).

Liu *et al.* (165) investigated the combined association of pre-pregnancy BMI and GWG on infant birth weight in 292,568 Chinese women. All information was obtained from a population-based Perinatal Health Care Surveillance Survey. GWG was defined as the weight difference between the last prenatal visit and the first prenatal visit. GWG was categorized based on 2009 IOM GWG guidelines. With increasing GWG, the risk of delivering a low birth weight or SGA infant decreased and the risk of delivering a macrosomic or LGA infant increased. Weight gain above 2009 IOM recommendations was associated with an increased risk of LGA (OR: 1.9; 95% CI: 1.8 to 1.9) and macrosomia (OR: 2.0; 95% CI: 1.9 to 2.1). A statistically significant interaction was found between pre-pregnancy BMI and GWG for the outcomes of low birth weight, SGA, macrosomia and LGA ($p < 0.01$), but not for other outcomes. Mamun *et al.* (166) analyzed GWG based on 2009 IOM guidelines and birth weight in 6,632 women participating in the Mater-University Study of Pregnancy in Australia. Compared to women who gained adequate weight, women who gained inadequate weight delivered a 190.63 g (95% CI: -221.05 to -161.20) lighter baby, while women who gained excessive weight delivered a 206.45 g (95% CI: 178.82 to 234.08) heavier baby. For 0.1 kg/week increase of GWG, each woman delivered an 81.51 g heavier baby.

Several studies analyzed the effects of GWG on birth weight in different pre-pregnancy BMI categories (167-169). Thorsdottir *et al.* (167) identified the effects of different GWG among women of normal pre-pregnancy BMI on infant birth weight in 200 women. They found high weight gain during pregnancy resulted in greater birth weight. Infants born to women who gained 18 to 24 kg during pregnancy weighed 286 ± 66 g more than infants born to women who gained 9 to 15 kg during pregnancy ($p < 0.001$). Sixty-two percent of women who gained 9 to 15 kg during pregnancy had an infant greater than 3500 g while 80% of women who gained 18 to 24 kg during pregnancy had an infant greater than 3500 g. They also found GWG to be related to birth weight

when a woman's weight gain was between 9 and 15 kg ($r = 0.19$, $p < 0.001$) but not if a woman's weight gain was over 18 kg ($r = 0.01$, $p > 0.05$). Net GWG and birth weight were also correlated ($r = 0.18$, $p < 0.001$).

Langford *et al.* (168) analyzed the association between GWG and infant outcomes in 35,576 overweight women (pre-pregnancy BMI: 26 to 29 kg/m^2) using Missouri birth certificate data from 1990 to 2004. They found only 21% women met 1990 IOM recommendations for GWG and 74% exceeded the recommendations. The cumulative incidences of preeclampsia (RR: 1.71; 95% CI: 1.54 to 1.89), cesarean section (RR: 1.30; 95% CI: 1.24 to 1.36) and macrosomia (RR: 2.13; 95% CI: 1.94 to 2.33) were higher among women who gained more than recommended compared to women who gained less or within the recommendations. Jensen *et al.* (169) analyzed GWG and pregnancy outcomes in 481 glucose-tolerant obese women in Denmark. They divided women into 4 groups according to their GWG (< 5.0 kg, 5.0 to 9.9 kg, 10.0 to 14.9 kg, and > 15kg). They found infant birth weight increased significantly across the groups ($p < 0.001$). The risk of LGA and macrosomia also increased with GWG ($p = 0.001$) while the risk of SGA was similar across the groups ($p = 0.63$).

Several studies have assessed the association between trimester-specific weight gain and birth weight (170-172). Abrams *et al.* (170) investigated the maternal weight gain pattern and birth weight in 2,994 non-obese Caucasian women. They found each kg of maternal gain in the first, second, and third trimesters to be associated with increases in birth weight of 18.0, 32.8, and 17.0 g, respectively. When compared to weight gain that was adequate in all trimesters, inadequate weight gain in the first and second trimesters was associated with a significant decrease in birth weight of 133.0 g and inadequate weight gain in the second and third trimesters was associated with a significant decrease in birth weight of 88.5 g. Brown *et al.* (171) examined 389 women and their infants and found each kg of weight gain was related to 20 g increase in birth weight ($p < 0.0001$). In the first and second trimester, each kg of weight gained by the woman predicted a 31 g and 26 g increase in newborn weight, respectively. Maternal weight gain

of 0 to 4 kg in the first trimester and 4 to 10 kg in the second trimester was strongly and positively related to newborn weight. Weight gain in the third trimester was not predictive of infant birth weight.

A recent study (Child Health and the Development Study) in the US (172) found women with a lower pre-pregnancy BMI had a higher weight gain during the second and third trimesters but lower weight gain during first trimester. However women with a higher pre-pregnancy BMI had a lower weight gain throughout the pregnancy. They found that each kg of GWG was associated with an increase in birth weight percentile of 1.60 (95% CI: 1.39 to 1.82), a significant decrease in OR of SGA of 0.89 (95% CI: 0.86 to 0.91), and a significant increase in OR of LGA of 1.13 (95% CI: 1.10 to 1.17). GWG across all trimesters was significantly and independently associated with a lower OR of SGA and a higher OR of LGA. Each kg of first trimester weight gain was associated with an increase in birth weight percentile by 1.94 (95% CI: 0.70 to 3.19) for women with underweight pre-pregnancy BMI, 1.59 (95% CI: 1.28 to 1.90) for women with normal pre-pregnancy BMI, and 0.92 (95% CI: 0.43 to 1.40) for women with overweight/obese pre-pregnancy BMI. No interactions were found between maternal pre-pregnancy BMI and GWG in the second and third trimesters. Each kg of second trimester GWG was associated with an increase in birth weight percentile by 1.99 (95% CI: 1.61 to 2.37), and each kg of third trimester GWG was associated with an increase in birth weight percentile by 1.55 (95% CI: 1.18 to 1.93). Second trimester GWG was associated with a larger difference in OR for LGA and SGA than the first and third trimester GWG.

In summary, an independent effect of GWG and a combined effect of pre-pregnancy BMI and GWG on infant birth weight are found. Increased GWG is associated with an increased infant birth weight. The effects of the timing of GWG on infant birth weight were not consistent. This may be due partly to differences between study designs and study populations.

1.11. Maternal GWG and infant body composition

Maternal GWG is positively related to neonatal birth weight (151, 163-172). However, few studies have investigated the changes in the compartments of body weight (amount of FM and FFM) and no previous study has assessed the relationship between the changes in the compartments of maternal body weight and neonatal body composition at birth. Two studies analyzed the association between GWG and maternal body composition (135, 173) and two studies assessed the relationship between GWG and neonatal body fat (128, 132).

Butte *et al.* (135) assessed body composition in 63 women using a multi-compartment model. The compartments measured included TBW by deuterium dilution, body volume by hydrodensitometry at 9, 22 and 36 weeks and bone mineral content by DXA before or after pregnancy. GWG was positively correlated with gains in TBW (r = 0.39; p = 0.003), FFM (r = 0.50; p = 0.001) and FM (r = 0.76; p = 0.001). FM gain was the highest in the high BMI group (BMI 28.8 $\pm$2.6 kg/m^2) compared to the normal BMI group (22.1 $\pm$1.5 kg/m^2). Birth weight was positively correlated with changes in TBW (r = 0.37; p = 0.006) and FFM (r = 0.39; p = 0.003) but not FM (r = 0.05; p = 0.74). Lederman *et al.* (173) also measured body composition in 200 pregnant women using a four-compartment model. Body density was measured by hydrodensitometry and TBW was measured by deuterium dilution during pregnancy at weeks 14 and 37. Total bone mineral was measured at 2-4 weeks postpartum by DXA. Women were divided into 4 groups: underweight (BMI < 19.8 kg/m^2; n=21), normal weight (BMI 19.8-26 kg/m^2; n=118), overweight (BMI 26-29 kg/m^2; n=29) and obese (BMI > 29 kg/m^2; n=28) by their pre-pregnancy BMI. Lederman *et al.* (173) found fat gain between gestational week 14 to 37 was correlated negatively with pre-pregnancy body weight (r = -0.25; p < 0.0005) and positively correlated with pregnancy weight gain (r = 0.81; p < 0.0001). A smaller change in FM was found in the obese group compared to all other groups. Both of these studies reported the composition of weight gain during pregnancy along with maternal pre-pregnancy BMI. Butte *et al.* (135)

investigated the relationship between GWG and neonatal body composition measured by DXA at 2 weeks after birth, but did not find any relationship.

Two recent studies have shown direct relationship between excessive GWG and neonatal body fat (128, 132). Crozier *et al.* (128) reported an association between neonatal and childhood FM and GWG in a sample of 566 children from the Southampton Women's Survey. In this study, about one-half of the women gained excessive weight during pregnancy based on IOM recommendations (see **Table 1.1**). Offspring born to women who gained excessive weight during pregnancy had a greater FM at birth (SD: 0.17; $p = 0.03$), at 4 years old (SD: 0.17; $p = 0.05$), and at 6 years old (SD: 0.30; $p = 0.002$) compared with offspring born to women with appropriate GWG. Hull *et al.* (132) examined the interactive effects of GWG and maternal pre-pregnancy BMI on neonatal body composition in 306 neonates. About 2/3 of overweight and obese women gained excessive weight while only 1/3 of normal weight women gained excessively. Regardless of the GWG, neonates born to obese women had the greatest body fat when compared to offspring born to normal weight and overweight women. An interaction between GWG and pre-pregnancy BMI for neonatal body fat was detected in offspring from overweight women. If an overweight woman gained excessively, her neonate had body fat similar to a neonate born to an obese woman. This study suggests a relationship between neonatal body fat and GWG with the relationships varying by maternal pre-pregnancy BMI. Both of the studies showed a positive relationship between GWG and neonatal body fat, but none of the studies related the maternal weight gain to maternal body composition (FM, FFM, and TBW) during late pregnancy.

Maternal GWG influences maternal fat retention, neonatal birth weight and neonatal body composition. The TBW gain is positively related to neonatal birth weight (135). Higher GWG is related to a greater neonatal birth weight (135) and FM gain (132), which may relate to childhood body composition (128). However, the relationship between trimester-specific GWG and infant body composition has not been reported and more studies are needed to elucidate the association between the timing of GWG and infant body composition.

1.12. Maternal body composition and infant birth weight

Studies have related maternal body composition to neonatal birth weight (135, 174-181) though the results are inconsistent. Lederman *et al.* (174) measured maternal body weight and body composition in early (14 weeks) and late (37 weeks) pregnancy in 200 women to examine the relationship between maternal body fat and TBW in relation to neonatal birth weight. The results indicate maternal weight and body water at term are significantly associated with neonatal birth weight, but maternal body fat at term is not. Butte *et al.* (135) show maternal FFM gains in the first, second and third trimesters contributed independently to birth weight. Changes in maternal TBW were independent predictors of birth weight during the second and third trimesters. However, the gains in maternal FM were not correlated with birth weight.

Other studies have used less sophisticated techniques or techniques that have questionable validity during pregnancy to explore the relationship between maternal and infant outcomes. Mardones-Santander *et al.* (175) measured maternal body composition at 36 weeks in 224 women using deuterium dilution technique and found TBW was the major maternal body component associated with birth weight. Larciprete *et al.* (176) examined the link between maternal body composition and newborn birth weight using bioimpedance analysis (BIA) in 29 women at 36 weeks of gestation. Farah *et al.* (177) conducted a similar study in 184 non-diabetic pregnant Caucasian women. These 2 studies were consistent with the results shown by Lederman *et al.* (174) and Mardones-Santander *et al.* (175), i.e., TBW but not FM is the main maternal body component associated with the newborn weight at term.

Forsum *et al.* (178) measured maternal body composition in 19 women using the two-compartment model based on TBW and related maternal body composition to neonatal birth weight. Neonatal birth weight was positively correlated with maternal total body fat content before pregnancy and at gestational week 32. In addition, Villar *et al.* (179) found a significant association between maternal fat gain early in pregnancy and neonatal birth weight. In this study, maternal body composition was evaluated 8 times during gestation using anthropometric methods

and BIA. Body fat and FFM were calculated with equations specific for pregnant women. Gale *et al.* (180) and Ogbonna *et al.* (181) found a trend of association between maternal fat gain estimated by the mid-upper arm circumference and neonatal birth weight.

In summary, the results of studies comparing maternal body composition to neonatal birth weight are inconsistent. Some report a positive relationship between maternal fat free mass and neonatal birth weight (135, 174-177) while others report a positive relationship between maternal fat mass and neonatal birth weight (178-181). However, except for the studies by Lederman *et al.* (174) and Butte *et al.* (135), who used a multi-compartment model, all other studies were based on a two-compartment model, which has been shown to not be valid during pregnancy. Based on the two studies that used the multi-compartment model to assess maternal body composition, TBW gain is related to neonatal birth weight (135, 174).

1.13. Maternal body composition and infant body composition

Neonatal body fat is related to childhood body fat (128), which in turn relates to the development of a variety of health outcomes (182). The majority of research relating maternal and neonatal body composition has relied on maternal BMI as a marker of adiposity. Body mass index does not take into account the composition of an individual's body weight. Forsum *et al.* (178) assessed maternal body composition in 23 women before pregnancy, at 32 weeks and 2 weeks postpartum using a two-compartment model based on TBW to assess maternal body composition. FFM was calculated from TBW using the hydration factors 0.718, 0.747, 0.734 before pregnancy, at 32 weeks and 2 weeks postpartum, respectively. FM was body weight minus FFM. The average skinfold thickness from 10 sites was used as a surrogate marker of FM in the neonates. The study found neonatal birth weight was explained by maternal body fat at 32 weeks and the average of the 10 neonatal skinfold thickness. Neonatal body composition was not measured.

One published study investigated the relationship between maternal body composition and neonatal body composition. Butte *et al.* (135) assessed the body composition of 63 women (n = 17; low weight, n = 34; normal weight and n = 12 as high) using the four-compartment model. Neonatal body composition was assessed at 2 and 27 weeks of age by DXA. Neonatal body composition at 2 and 27 weeks of age did not differ among BMI groups. Neonatal body composition (FM, FFM, %fat) at 2 weeks of age was not correlated with maternal body composition before, during and after pregnancy. A potential reason for no relationship found may be due to the BMI groups. The BMI groups and mean ranges Butte *et al.* (135) used were: low (18.9 ± 0.8 kg/m^2), normal (22.1 ± 1.5 kg/m^2) and high (28.8 ± 2.6 kg/m^2). Therefore the low group was more closely represented by an underweight BMI, the normal was very lean and the high was mainly comprised of overweight subjects and a few in the obese BMI range. Likely Butte's study was not powered to show a difference or possible relationships that are present in an obese population. A second potential problem is that Butte *et al.* (135) measured neonatal body composition 2 weeks after birth. Neonatal body weight fluctuates after the neonate is born with gains of about 6 g/per day of fat during the first month of life (113). Many factors influence neonatal body composition after birth, such as the feeding method. These factors will confound the evaluation of the relationship between maternal and neonatal body composition. As a result, measurement of neonatal body composition at birth is most accurate to elucidate the relationship between maternal and neonatal body composition than measurement at 2 weeks or later after birth. No study has reported the relationship between maternal body composition and neonatal body composition at birth. The influence of maternal adipose tissue on neonatal body composition has yet to be elucidated.

In summary, the maternal environment is very important for fetal growth. Because both under- and overnutrition during gestation are linked to "metabolic programming" of the fetus, which may relate to the offspring's long-term health. Birth weight is the most common outcome

measure reflecting fetal experience and maternal anthropometrics such as pre-pregnancy BMI and GWG reflect maternal energy status. Assessments of maternal and infant body composition are more sensitive indicators of the maternal and *in utero* environment. These assessments can provide more detailed information to clarify how the maternal environment influences fetal growth and contribute to elucidating the pathways for programming of adult disease.

CHAPTER 2

METHODS

2.1. Study population

The study population included pregnant women (18-45 years old) without known infectious disease or illness (e.g. autoimmune disease) regardless of their ethnic and racial background. The subjects included women who were normal weight (BMI 18.5-24.99 kg/m^2) overweight (BMI 25-29.99 kg/m^2) and obese (BMI 30-40 kg/m^2) before pregnancy. The pre-pregnancy BMI was calculated using self-reported maternal pre-pregnancy body weight and measured height at the study visit. We recruited 80 women in the study. Forty women (n=22 normal weight, n=10 overweight and n=8 obese) completed all assessments for the primary aim and 45 women (n=27 normal weight, n=8 overweight and n=10 obese) completed all assessments for the secondary aim.

2.1.1. Inclusion/exclusion criteria

Women with a pre-pregnancy BMI from 18.5 to 40 kg/m^2, carrying a singleton pregnancy and planned delivery at KU Hospital were recruited. Women with an underweight pre-pregnancy BMI (< 18.5 kg/m^2) or a pre-pregnancy BMI over 40 kg/m^2 were excluded from the study, as well as women whom were diagnosed with preeclampsia or GDM. Women who reported smoking, illicit drug use during their pregnancy or who could not communicate in English were excluded.

2.1.2. Subject recruitment

Women were identified at 28-39 weeks gestation using the IDXterm software by trained study coordinators. Charts were screened for medically related inclusion and exclusion criteria under a waiver approved by the Human Subjects Committee at the University of Kansas Medical Center in Kansas City, KS (KUMC) (#12793). The study coordinators approached eligible women in the clinic of the Department of Obstetrics and Gynecology at KUMC. If they were interested, the study was explained and the informed consent obtained. Women completed a general questionnaire about their health throughout the pregnancy.

2.2. Research design

This was an observational study. The primary aim was to identify the relationship between maternal body composition (FM, FFM and TBW) in late pregnancy and neonatal body fat at birth. The secondary aim of this study was to determine the relationship between GWG during the different trimesters and neonatal body composition at birth.

2.2.1. Study visits

There were three planned contacts for this study. The first contact occurred between 34 and 39 weeks gestation when maternal body composition was measured. All of the subjects fasted at least 1 hour before the visit. TBW and body volume were measured during that visit. The second contact occurred after delivery when the subject was discharged from the hospital. Neonatal body composition was measured at this visit (within 24-72 hours after delivery). The last study visit occurred 2-6 weeks postpartum. Bone mineral content was measured by DXA at the last study visit.

2.2.2. Measurement of maternal body composition

Maternal body composition was estimated using the four-compartment model of Selinger (183). The four-compartment model uses the deuterated water technique, a DXA scan, and the Bod Pod® to quantify water, mineral lean, and body volume, respectively. All subjects had their FM measured using the four-compartment model late in pregnancy (34 to 39 weeks). The equation is: % fat = (2.747/BD – 0.714*W/BW + 1.129*B/BW – 2.037)*100 (173); where BD is body density in g/ml measured by the Bod Pod® using ADP, W is the volume of total body water in liters measured by deuterium dilution, BW is body weight in kilograms and B is bone mineral in kilograms measured by DXA. This method is accurate for measuring body composition in women during pregnancy (173).

2.2.2.1. Body volume

Body volume was measured by the Bod Pod® (CosMed, Concord, CA). Subjects wore minimal tight fitting clothing (e.g. one-piece swimsuit) and a fitted hat (Allentown Scientific

Associates, Inc., Allentown, PA). Body weight was obtained to the nearest 0.01 kg using the Bod Pod® system's electronic scale (Tanita, Tokyo, Japan). Body volume was corrected for thoracic gas volume (TGV), surface area artifact (183) and body density calculated as BD = body mass/ body volume (184). The coefficient of variation for the Bod Pod® is about 0.07-0.22% and has been validated in adults (185). Measurement of body volume using the Bod Pod® occurred in the Department of Dietetics and Nutrition Clinical Laboratory at KUMC.

2.2.2.2. Total body water

Deuterium oxide (D_2O) was used to assess TBW. Blood samples were collected at baseline and three hours after oral administration of the D_2O between 34 and 39 weeks. TBW was calculated from D_2O by correcting for proton exchange (186). Each subject was given an oral dose of D_2O (ICON, Summit, NJ) at 0.1 g per kg of body weight, weighed in a dose cup (accurate to ± 0.002 g). At this dose, the D_2O concentration is at equilibrium at <0.03 % of body water (187). The concentration of D_2O was measured after lyophilization using a single frequency infrared-spectrophotometer (Nicolet 380; Thermo Electron, Madison, WI). Total body water volume was calculated by dividing the dose by net D_2O concentration in the specimen. The typical precision for the TBW measurement is ± 1.0 % (188). The deuterated water method is not associated with increased risk to the pregnant woman. There is a no observed adverse effect level of 14 mg/m^3, and the deuterium given in this study was much lower than that value. The blood was processed immediately after collection and plasma was stored at -80 ℃ for analysis. The plasma was sent as a batch of all samples by overnight FedEx on dry ice for analysis at St. Luke's Hospital in New York City.

2.2.2.3. Bone mineral mass

Bone mineral mass were measured by iDXA (GE Healthcare, Madison, WI) with encore software (Version 13.50). iDXA simultaneously measures the mass of lean (bone and non-bone lean tissue) and fat tissue (189) and provides information on bone mineral content and soft tissue content of the total body and of its regions, such as arms, legs and trunk. Bone and soft tissue

composition is determined by variations in the attenuation of the x-ray beam. A total body scan was used to assess the bone mineral mass of each subject. Because the DXA uses x-ray technology, subjects were exposed to a radiation during the whole body scan. The dose of the radiation is 0.004 mSv (189), which is comparable to taking a flight from Los Angeles to New York City and considered to be well within safe limits. The DXA scans of women took place in the Division of Endocrinology, Metabolism and Genetics at KUMC in 2 to 6 weeks following delivery.

2.2.3. Measurement of maternal anthropometry

The height of all women was measured in centimeters using a wall stadiometer (Health o meter®, Bradford, MA). The participant stood with their back to the stadiometer shoeless, heels together, back straight, heels, buttocks, shoulders, and head touching the wall and looking straight ahead. Body weight was measured using the Bod Pod® system's electronic scale (Tanita, Tokyo, Japan). The participant was measured with minimal clothing and no shoes. BMI (kg/m^2) was calculated. All of the anthropometry measurements of the pregnant women occurred at late pregnancy (34 to 39 weeks) in the Department of Dietetics and Nutrition Clinical Laboratory at KUMC.

2.2.4. Neonatal adiposity and anthropometry

Neonatal crown to heel length was measured to the nearest 0.1 cm using a neonatal Shorr board (Shorr Productions, Olney, MD) and neonatal body weight was measured during the measurement of body composition using the Pea Pod® neonatal scale to the nearest 0.01 kg. Neonatal body composition was measured within 24-72 hours following birth. The Pea Pod® Body Composition System (CosMed, Concord, CA) was used to assess body volume and to calculate body density. All clothing and the diaper were removed during the body volume measurement. Direct measurement of thoracic gas volume (TGV) is not feasible in neonates so it is estimated (190, 191). Neonatal body composition measurements with the Pea Pod® have been validated (108, 192). Once a volume measure is obtained, body density is calculated and

converted to % fat by the equation %fat = (FM/BM)*100% (193) (BM is body weight). FM is calculated by the equation BM/BD = FM/D_{fm} + FFM/D_{ffm} (194) (BD is body density, D_{fm} is FM density, and D_{ffm} is FFM density). D_{fm} is constant throughout life as 0.9007 g/ml (194). D_{ffm} changes during growth (195) so age and gender specific D_{ffm} values are used in calculating %fat (112). All of these tests were conducted by trained staff when the mother is discharged from the hospital in Department of Dietetics and Nutrition Clinical Laboratory at KUMC.

2.2.5. Measurement of GWG

GWG was calculated from self-reported pre-pregnancy weight and the delivery weight from the medical record. Good agreement has been reported between maternal recall of pre-pregnancy weight and medical records for pre-pregnancy weight (196, 197). Self-reported pre-pregnancy weight is considered a satisfactory substitute when medical chart extraction is unavailable (197). Maternal GWG during the 1st (0 to 13 weeks), 2nd (14 to 28 weeks) and 3rd (29 weeks to delivery) trimesters was calculated using data from the medical records minus their pre-pregnancy weight. Since the time between body weight measures varied, the absolute weight gain within each trimester was divided by the weeks within that period to derive a weight gain/week for each trimester.

2.3. Statistical analyses

Linear regression modeling was used to explore the relationships between the maternal and infant variables. The specific analysis for each aim is described below. To determine which maternal and infant confounding variables to include in the models, bivariate correlations were performed between infant body composition (%fat, FM, FFM) and the following maternal and infant variables known to be related to infant body composition: maternal age, pre-pregnancy BMI, maternal ethnicity, parity, smoking history, neonatal age at test, gender and gestational age. Once the significant variables were identified, correlations between the confounding variables were performed to identify any inter-relationships between confounding variables (collinearity).

Once the final model was identified, stepwise hierarchical regression was performed to find and remove the least significant variable. Only statistically significant variables were retained in the final model. Significance level was $p \leq 0.05$ and analyses were performed using SPSS v20.0 (SPSS Inc., Chicago, IL).

Aim 1: Determine the relationship between maternal body composition (FM, FFM and TBW) and neonatal body fat at birth.

Multiple linear regression analysis was used to determine which maternal factors were related to neonatal outcome variables. The dependent variable in the model was neonatal body composition (% fat, FM, FFM). The independent variables were maternal FM, FFM and TBW. The following variables were included as covariables in the final models: maternal age, neonatal age at test, gender, and gestational age.

Aim 2: Examine the relationship between maternal GWG during each trimester and neonatal body composition at birth.

Multiple linear regression modeling was used to examine the relationship between GWG during the 1^{st}, 2^{nd} and 3^{rd} trimesters and neonatal body composition (FM, FFM, and %fat). Neonatal FM, FFM and %fat were the dependent variables. The independent variables were maternal GWG in 1^{st}, 2^{nd}, and 3^{rd} trimester. The following variables were included as covariables in the final models: maternal pre-pregnancy BMI, neonatal age at test, gender and gestational age. Maternal pre-BMI was explored as a continuous variable and categorized as normal weight, overweight and obese. Interaction between trimester-specific GWG and each maternal pre-pregnancy BMI category were also explored. Simple linear regression models were used when assessing the interaction since our sample is small, no covariables was included in the models.

2.3.1. Statistical power

The primary aim of this study was to determine the relationship between maternal body composition and neonatal body composition. Power calculations were based on prior research examining the impact of maternal pre-pregnancy BMI on the offspring's adiposity (131). The

mean difference in neonatal % fat between infants born to overweight/obese women and normal weight women was 2.0 ± 2.5%. For a single test of correlation between neonatal FM and a given maternal factor of interest, a sample size of 40 provides sufficient (> 90%) power to detect a correlation of at least $r = 0.5$ at the 0.05 level of significance (**Figure 2.1**); that is, this study has sufficient power to detect a linear relationship where the maternal factor of interest explains at least 25% of the total variation in neonatal FM.

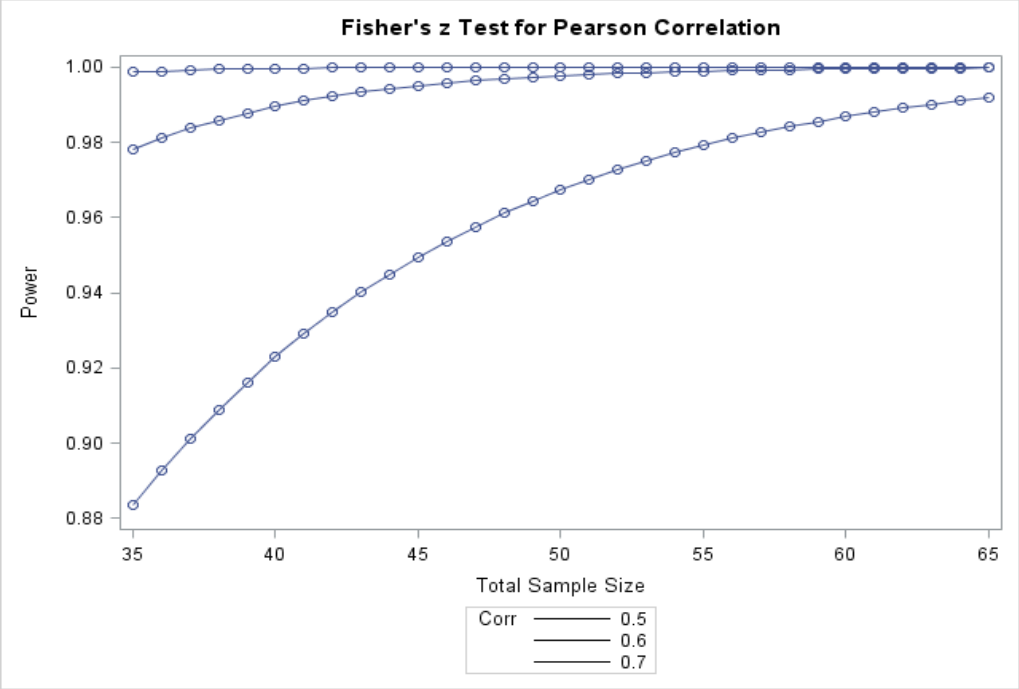

Figure 2.1 Power calculations for the primary aim

CHAPTER 3

MATERNAL BODY COMPOSITION LATE GESTATION AND NEONATAL BODY COMPOSITION AT BIRTH

3.1. Abstract

Background: Infant birth weight and body composition is directly related to maternal pre-pregnancy BMI but it is unknown how infant body composition measured at birth relates to maternal body composition.

Objective: To evaluate how maternal body composition late in pregnancy relates to neonatal body composition at birth.

Methods: Healthy pregnant women with a pre-pregnancy BMI between 18.5 and 39.99 kg/m^2 were enrolled in the study at 28 to 39 weeks gestation. Maternal body composition was measured using the four-compartment model between 34 to 39 weeks gestation. Neonatal body composition was measured using air displacement plethysmography (Pea Pod®) within 1-3 days after birth. Multiple linear regression models were used to assess the relationship between maternal body composition and neonatal body composition. Neonatal body composition measures (fat mass (FM), fat free mass (FFM), and percentage body fat (% fat)) were the dependent variables and maternal body composition measures (FM, FFM, total body water (TBW) and % fat) were the independent variables. Maternal age, neonatal age at test, gender and gestational age were used as cofounding variables in this model. The level of statistical significance was set at $p \leq 0.05$.

Results: We measured the body composition of 40 mother-infant pairs. Maternal FFM predicted neonatal birth weight ($r^2 = 0.280$, $p = 0.011$) and neonatal FFM ($r^2 = 0.521$, $p = 0.011$). Maternal TBW also predicted neonatal birth weight ($r^2 = 0.330$, $p = 0.007$) and neonatal FFM ($r^2 = 0.519$, $p = 0.011$). A nonsignificant trend was found between maternal FM and neonatal birth weight ($r^2 = 0.224$; $p = 0.053$) and neonatal FM ($r^2 = 0.052$; $p = 0.085$).

Conclusion: Maternal FFM and TBW are related to neonatal FFM but not FM.

3.2. Introduction

Suboptimal intrauterine environment affects the fetal development. Baker *et al.* (21) have proposed that fetal growth altered by maternal factors may be in part responsible for long-term ramifications such as obesity (198), cardiovascular diseases (23) and type 2 diabetes (199). The intrauterine environment is crudely assessed by neonatal birth weight, which could also be used as a marker for future diseases development. It is known that Individuals with a birth weight at either the low end (< 5 lbs) or at the high end (> 7 lbs) of the birth weight distribution have a higher risk of later obesity (34, 35).

Studies have related maternal body composition to neonatal birth weight (135, 174, 177). Maternal total body water (TBW) late in gestation (at 37 weeks) but not fat mass (FM) has been shown positively related to infant birth weight (174). However, birth weight does not quantify what comprises neonatal body mass: FM and fat free mass (FFM). Catalano *et al.* (106) found FM in term neonates accounted for only 14% of birth weight but explained 46% of the variance in birth weight. For those reasons, infant body composition may be a better marker to assess fetal growth compared to birth weight.

Recent advancements have allowed for the assessment of neonatal body composition (107-109). Understanding maternal factors that influence the amount of adiposity at birth is critical as neonatal FM is related to childhood levels of FM (128). Evidence suggests a direct relationship between maternal pre-pregnancy body mass index (BMI) and neonatal FM at birth (125, 131). Infants born to overweight and obese women before pregnancy have higher body fat compared to infants born to normal weight women (125, 131). However, standard weight and height measurements provide only estimates of maternal adiposity but do not quantify maternal body composition (FM and FFM). Variance in the composition of pregnant women's body may be crucial in determining the relationship between maternal environment and fetal growth. It is not clear whether maternal fat or water contributes to infant body fat at birth. Only one study evaluated the association between maternal and infant body composition (135) and no

relationship was found between maternal and infant body composition; however, infant body composition was measured at 2 weeks of age, which could be influence by *ex utero* environment. No study has related maternal body composition during pregnancy to neonatal body composition when measured at birth.

The objective of this study was to relate maternal body composition measured late in pregnancy and body composition of their neonates at 1-3 days following birth and to evaluate the relationship between maternal environment and neonate body fat at birth.

3.3. Materials and Methods

3.3.1. Study population

Pregnant women (18-45 years old) without known infectious disease or illness (e.g. autoimmune disease) were recruited from 28 to 39 weeks gestation regardless their ethnic and racial background. Exclusion criteria included: Women with a pre-pregnancy BMI < 18.5 kg/m^2 or $\geq$ 40 kg/m^2 , women who had multiple gestation (twins or triplets), women who were diagnosed with preeclampsia or gestational diabetes mellitus (GDM), self-reported smoking and illicit drug use during their pregnancy and women who did not speak English. Women were recruited between January 2012 and March 2013 at their prenatal care visits to clinics of University of Kansas Hospital in Kansas City. The study was approved by the University of Kansas Medical Center (KUMC) Institutional Review Board (IRB) (#12793), and all subjects provided written informed consent. A questionnaire was distributed at enrollment to collect women's demographic and baseline information. Pre-pregnancy BMI was calculated using self-reported maternal pre-pregnancy body weight and measured height at the study visit.

3.3.2. Measurement of maternal anthropometry

Height was measured in centimeters using a wall stadiometer (Health o meter®, Bradford, MA). The participants stood with their backs to the stadiometer shoeless, heels together, back straight, heels, buttocks, shoulders, and head touching the wall and looking straight ahead. Body

weight was measured during the measurement of body composition using the Bod Pod® system's electronic scale (Tanita, Tokyo, Japan). The participant was measured with minimal tight fitting clothing (e.g. one-piece swimming suit) and no shoes.

3.3.3. Measurement of maternal body composition

Maternal body composition was measured between 34 and 39 weeks gestation using the four-compartment model of Selinger (183). The four-compartment model measures TBW, bone mineral content (BMC), and body volume using the deuterated water technique, dual x-ray absorptiometry (DXA), and the Bod Pod®, respectively. All subjects had their FM measured using the four-compartment model late in pregnancy (34 to 39 weeks). To determine the % fat we used the equation (2.747/BD – 0.714*W/BW + 1.129*B/BW – 2.037)*100 (173); where BD is body density in kg/L determined by the equation BW (kg)/body volume (L), W is TBW in liters, BW is body weight in kilograms measured by an electronic scale (Tanita, Tokyo, Japan) and B is BMC in kilograms. This method is a valid measure of body composition during pregnancy (173).

Body volume was measured by the Bod Pod® (CosMed, Concord, CA). Subjects wore minimal tight fitting clothing (e.g. one-piece swimsuit) and a fitted hat (Allentown Scientific Associates, Inc., Allentown, PA). Body weight was obtained to the nearest 0.01 kg using an electronic scale (Tanita, Tokyo, Japan). Body volume was corrected for thoracic gas volume and surface area artifact (183). Body density was calculated as BD = body mass/body volume (184). The coefficient of variation for the Bod Pod® is about 0.07-0.22% and has been validated in adults (185).

To determine TBW, blood samples were collected at baseline and three hours after oral administration of 10g D_2O (ICON, Summit, NJ) that weighed in a dose cup (accurate to ± 0.002 g). At this dose, the D_2O concentration is at equilibrium at <0.03% of body water (187).The blood was processed immediately after collection and plasma was preserved at -80 ℃ for analysis. TBW was calculated from D_2O by correcting for proton exchange (186). The concentration of D_2O was measured after lyophilization using a single frequency infrared-spectrophotometer (Nicolet 380;

Thermo Electron, Madison, WI). TBW volume was calculated by dividing the dose by net D_2O concentration in the specimen. Typical precision for the TBW measurement is $\pm 1.0\%$ (188). The deuterated water method is not associated with increased risk to the pregnant woman. The no observed adverse effect level is 14 mg/m^3, and the deuterium given in this study was much lower.

BMC was measured by iDXA (GE Healthcare, Madison, WI) with encore software (Version 13.50). iDXA simultaneously measures the mass of lean (bone and non-bone lean tissue) and fat (189) and provides information on both BMC and soft tissue content of the total body and of its regions, such as arms, legs and trunk. Bone and soft tissue composition are determined by variations in the attenuation of the x-ray beam. A total body scan was used to assess the bone mineral mass of each subject at 2 weeks following delivery.

3.3.4. Measurement of neonatal body composition and anthropometry

Neonatal crown to heel length was measured to the nearest 0.1 cm using a calibrated length board (Shorr Productions, Olney, MD) and neonatal body weight was measured using the Pea Pod® neonatal scale to the nearest 0.01 kg. Neonatal body composition was measured within 1-3 days following birth. The Pea Pod® Body Composition System (CosMed, Concord, CA) (ADP) was used to measure body volume and to calculate body density. All clothing and the diaper were removed during body volume measurement. Direct measurement of thoracic gas volume is not feasible in neonates so it was estimated (190, 191). Neonatal body composition measurements with the Pea Pod® have been validated (108, 192). Once a volume measure was obtained, body density was calculated and converted to % fat = (FM/BM)*100% (193) (BM is body weight). FM was calculated by the equation $BM/BD = FM/D_{fm} + FFM/D_{ffm}$ (194) (BD is body density, D_{fm} is FM density, and D_{ffm} is FFM density). D_{fm} = 0.9007 g/mL and is constant (194). D_{ffm} changes during growth and differs in male and female (195) therefore age and gender specific D_{ffm} values were used to calculate % fat (112). The neonatal body composition and anthropometrics were obtained at the time of hospital discharge.

3.3.5. Statistical analyses

Multiple linear regression analyses were used to determine which neonatal body composition variables were related to maternal factors. The independent variables were maternal % fat, FM, FFM and TBW. The dependent variables in the model were neonatal % fat, FM, and FFM. Bivariate correlations were performed between infant body composition (%fat, FM, FFM) and the following maternal and infant variables previously related to infant body composition: maternal age, pre-pregnancy BMI, maternal ethnicity, parity, smoking history, neonatal age at test, gender and gestational age. The following variables were included as covariables in the final models: maternal age, neonatal age at test, gender, and gestational age. Correlations between the confounding variables were performed to identify any inter-relationships between confounding variables (collinearity). Pearson correlations between all variables were shown in **Table 3.1**. Stepwise hierarchical regression was performed in the final model and the least significant variable was removed. Only significant variables were retained in the final model. Descriptive statistics and analysis of variance were used to determine if differences between pre-pregnancy BMI groups existed for the maternal and neonatal baseline characteristics. Tukey's post hoc pairwise comparisons between BMI groups were performed. Significance level was $p \leq 0.05$ and analyses were performed using SPSS v20.0 (SPSS Inc., Chicago, IL).

3.4. Results

Eighty pregnant women were enrolled in the study and forty mother-infant pairs completed the study (**Figure 3.1**). Maternal characteristics are presented in **Table 3.2**. Maternal age, smoking history, parity, gestational age at test, height and gestational weight gain (GWG) were not different between the normal weight and overweight/obese groups classified by maternal pre-pregnancy BMI. Maternal ethnicity was not balanced between groups. Most of the African-American women in this study were classified as overweight/obese before pregnancy and most of the Caucasian women in this study were classified as normal weight before pregnancy.

Overweight/obese group had higher maternal % fat ($p < 0.001$), FM ($p < 0.001$), FFM ($p = 0.001$) and TBW ($p = 0.003$) when compared to the normal weight group. Maternal BMC was not different between the groups.

Neonatal characteristics are presented in **Table 3.3**. Infants born to overweight/obese women were older at test compared to infants born to normal weight women ($p = 0.041$). Between the normal weight and overweight/obese groups, unadjusted neonatal gestational age, birth weight, birth length, FM, FFM and % fat did not differ.

3.4.1. Predicting neonatal birth weight

Maternal FFM ($r^2 = 0.280$; $p = 0.011$) and TBW ($r^2 = 0.330$; $p = 0.007$) were correlated with neonatal birth weight after adjusting for neonatal gestational age. When neonatal gestational age was held constant, a unit increase of maternal FFM and TBW led to an increase in birth weight by 21g and 29g, respectively. The relationship between maternal FM and infant birth weight approached significance ($r^2 = 0.224$; $p = 0.053$). Maternal % fat and BMC did not correlate with neonatal birth weight (**Table 3.4**).

3.4.2. Predicting neonatal body composition (%fat, FM and FFM)

Relationships between maternal body composition and neonatal % fat, FM, and FFM are presented in **Tables 3.5**, **3.6** and **3.7**, respectively. Maternal FFM and TBW were related to neonatal FFM ($r^2 = 0.521$, $p = 0.011$; $r^2 = 0.519$, $p = 0.011$, respectively) after adjusting for neonatal gestational age and gender. When neonatal gestational age and gender held constant, a unit increase of maternal FFM and TBW led to an increase in neonatal FFM by 12g and 16g, respectively. Several relationships between maternal body composition and neonatal body fat showed a nonsignificant trend: maternal TBW and neonatal % fat ($r^2 = 0.146$; $p = 0.097$), maternal FM and neonatal FM ($r^2 = 0.052$; $p = 0.085$) and maternal FM and neonatal FFM ($r^2 = 0.466$; $p = 0.098$). Maternal BMC did not relate to neonatal body composition.

3.5. Discussion

This is the first study to show a relationship between maternal body composition late in pregnancy and neonatal body composition at birth. Maternal FFM and TBW were directly related to neonatal FFM. However, maternal %fat and FM were not related to neonatal body composition. The relationship between maternal FM and neonatal FM approached significance. We found that maternal FFM and TBW but not FM was associated with higher neonatal birth weight.

Reports regarding the relationship between maternal body composition and infant birth weight are conflicting. Lederman *et al.* (174) investigated maternal body composition changes between 14 to 37 weeks gestation using the four-compartment model and found maternal weight and TBW, but not maternal FM at 37 weeks was directly related to birth weight. Likewise, Butte *et al.* (135) measured maternal body composition changes from pre-pregnancy to 36 weeks of gestation using the four-compartment model and found birth weight was positively correlated to the total gain in TBW, and FFM, but not FM. Mardones-Santander *et al.* (175) measured maternal body composition at 36 weeks of gestation using deuterium dilution and found that both maternal FFM and FM were related to infant birth weight. The correlation was greater for FFM compared to FM. In our study, we showed that FFM and TBW were the most important maternal body composition compartments that influenced birth weight followed by FM, which was partly consistent with Lederman and Butte's reports. However, both Lederman and Butte captured the changes of maternal body composition and our study was a cross-sectional study that measured maternal body composition late in pregnancy which combined the pre-pregnancy body fat and fat gain during gestation together. It could be the pre-pregnancy body fat but not fat gain during pregnancy that is the important factor predicting neonatal birth weight since we found overweight/obese women had higher body fat and FFM compared to normal weight women.

Neonatal body composition compared to birth weight reflects gestational fetal growth. We found no difference in neonatal body composition among maternal normal weight and overweight/obese pre-pregnancy BMI groups. However, Sewell *et al.* (125) showed maternal

pre-pregnancy BMI was positively associated to neonatal FM but not FFM while Hull *et al.* (131) showed neonates born to overweight/obese women had significantly higher FM but lower FFM compared to neonate born to normal weight women. Small numbers in overweight/obese group might be the reason that we had different results compared to earlier reports. Our study was not powered to find group differences by pre-pregnancy BMI but instead powered to detect a relationship between variables.

Only one previous study assessed the relationship between maternal body composition and neonatal body composition (135). Butte *et al.* (135) reported that maternal body composition before pregnancy and at 2 weeks after delivery did not correlate with neonatal body composition (FFM, FM, % fat, BMC and bone mineral density) at 2 weeks of age in 63 mother-infant pairs. Neither did maternal GWG or TBW, FFM and FM gains during pregnancy related to neonatal body composition at 2 weeks of age. However, during the first 2 weeks of life, infant body weight fluctuated and this fluctuation could be influenced by the postnatal environment, such as feeding patterns. Assessing neonatal body composition at 2 weeks introduces error that may have masked the effect of maternal environment on fetal growth which assessed by neonatal body composition. Our study is the first study linked maternal body composition late in pregnancy to neonatal body composition at birth.

The mechanism underlying the relationship between maternal TBW and neonatal FFM is not clear. One hypothesis we propose is that higher maternal TBW is related to a higher level of inflammation and lower level of maternal adiponectin, which increases the placental transportation of amino acids to fetus. Jansson *et al.* (200) found protein expression of the sodium dependent neutral amino acid transporter 2, which is a system A amino acid transport isoform, was positively related to maternal early pregnancy BMI and birth weight. Jones *et al.* (201) showed maternal adiponectin attenuates insulin signaling in primary human trophoblast cells and inhibits insulin-stimulated placental amino acid transport. It is known that overweight/obese women have lower adiponectin levels when compared to lean women. In our study,

overweight/obese women had higher TBW during the third trimester compared to normal weight women. We also found a negative trend ($p = 0.079$) between maternal TBW and total serum adiponectin level after controlling for the pre-pregnancy BMI (data not shown). As a result, the relationship between maternal adiponectin level and placental amino acid transportation could be an explanation for the association between maternal TBW and neonatal FFM. The association among maternal TBW, adiponectin level and placental transportation of amino acids may have important implications for placental nutrient transport and fetal growth in pregnancy complications.

Maternal hormone changes during pregnancy might be another reason underlying the relationship between maternal and infant body composition. It has been shown that maternal insulin resistance and glucose production rate are associated with fetal growth (202, 203). Research has shown that maternal glucose production explains 31% of the variance in estimating fetal weight (204). Ahlsson et al. (204) found maternal insulin resistance and glucose production were positively related to maternal pre-pregnancy BMI in nondiabetic pregnant women. Since glucose is considered to be the most important fuel for the fetus, the positive association found between maternal glucose production and the fact that insulin is increased with maternal pre-pregnancy BMI may explain the relationship between maternal body composition and infant body composition. However, in our study, we did not measure maternal glucose levels to prove this potential mechanism. Further studies are needed to demonstrate this mechanism.

A strength of our study was that we used four-compartment model to measure maternal body composition during pregnancy. Assessment of maternal body composition during pregnancy is complicated because the composition of lean tissue changes during pregnancy. The hydration of FFM changes during pregnancy due to the increase of plasma volume (133) and the amniotic fluid volume (134). Lederman *et al.* (174) showed the major increase in maternal nonfat tissue during pregnancy is the increase of 6 to 7 liters of water, little or no increase in bone or carbohydrate, and less than 1 kg increase in protein. Thus the common two-compartment model

of assessing body composition is not valid during pregnancy. A second strength of our study was that we assessed neonatal body composition soon after birth using ADP, which avoided the potential influence of the postnatal environment on neonatal body composition, such as feeding patterns.

There are also limitations to our study. First, we had a small sample size (n=40). Second, this was a cross-sectional study. We did not have repeated measures on maternal body composition and did not capture the changes in maternal body composition during pregnancy so we could not investigate if the components of body weight or the components of GWG relate differently to neonatal body composition. Future studies are needed to clarify these relationships. Third, because of our sample size, we correlated each of our dependent and independent variables in separate models. We ran 20 regression models in total using the same data, which increased our type I error probability.

In conclusion, this is the first study to assess the relationship between maternal body composition during pregnancy and neonatal body composition soon after birth. Maternal body composition late in pregnancy was related to neonatal body composition at birth. Maternal FFM and TBW were related to neonatal birth weight and FFM. Maternal FM was not significantly related to neonatal FM but at a borderline significance. Studies with larger sample size across BMI ranges are needed to validate our results and to clarify the relationships between component of GWG and neonatal body composition.

Table 3.1 Pearson correlations between all variables

	Maternal TBW (L)	Maternal FFM (kg)	Maternal FM (kg)	Maternal body fat (%)	Maternal BMC (kg)	Maternal age (years)	Gestational age (weeks)	Infant age at test (weeks)	Infant gender (male)	Birth weight (g)	Infant body fat (%)	Infant FM (g)	Infant FFM (g)
Maternal TBW (L)	1	0.980**	0.564**	0.196	0.486**	0.204	0.247	0.239	0.099	0.476**	0.156	0.242	0.430**
Maternal FFM (kg)		1	0.583**	0.217	0.501**	0.211	0.214	0.242	0.091	0.448**	0.128	0.219	0.414**
Maternal FM (kg)			1	0.907**	0.385*	0.207	-0.016	0.222	0.210	0.275	0.228	0.276	0.128
Maternal body fat (%)				1	0.216	0.130	-0.124	0.128	0.183	0.113	0.225	0.237	-0.041
Maternal BMC (kg)					1	0.022	0.156	0.060	0.220	0.182	0.028	0.078	0.203
Maternal age (years)						1	0.285	-0.058	-0.017	0.411**	-0.017	0.116	0.384*
Gestational ages (weeks)							1	0.005	0.185	0.429**	-0.356*	-0.179	0.635**
Infant age at test (weeks)								1	-0.203	0.082	0.097	0.073	0.000
Infant gender (male)									1	-0.097	0.071	0.026	-0.134
Birth weight (g)										1	0.408**	0.628**	0.915**
Infant body fat (%)											1	0.957**	0.065
Infant FM (g)												1	0.324*
Infant FFM (g)													1

*$P \leq 0.05$; **$P \leq 0.01$

TBW, total body water; FFM, fat free mass; FM, fat mass; BMC, bone mineral content;

Table 3.2 Maternal characteristics for the total sample and each pre-pregnancy BMI group

	Total (n=40)	Normal weight (n=22)	Overweight/Obese (n=18)
Age (years)	29.28 ± 4.58	28.82 ± 4.61	29.85 ± 4.60
Height (m)	1.65 ± 0.07	1.65 ± 0.07	1.64 ± 0.07
Pre-pregnancy weight (kg)	68.97 ± 14.86	59.02 ± 5.30	81.14 ± 13.67
Pre-pregnancy BMI (kg/m^2)	25.43 ± 5.06	21.76 ± 1.69	29.91 ± 4.06
GWG (kg)	16.66 ± 7.40	18.00 ± 6.35	15.02 ± 8.40
Gestational age at test (weeks)	36.58 ± 1.08	36.55 ± 0.96	36.61 ± 1.24
Body weight at test (kg)	83.34 ± 15.04	74.19 ± 7.99	94.53 ± 14.11
Body fat (%)	34.89 ± 5.70	31.79 ± 3.79	38.67 ± 5.42
FM (kg)	29.65 ± 9.59	23.72 ± 4.62	36.89 ± 9.16
FFM (kg)	53.69 ± 7.27	50.46 ± 4.94	57.64 ± 7.81
TBW (L)	40.42 ± 5.55	38.18 ± 3.96	43.17 ± 6.07
BMC (kg)	2.45 ± 0.46	2.37 ± 0.27	2.56 ± 0.62
Races (n)			
Caucasian	24 (60%)	17 (77%)	7 (39%)
African-American	9 (22.5%)	2 (9%)	7 (39%)
Hispanic	5 (15%)	3 (14%)	2 (11%)
Other	2 (5%)	0 (0%)	2 (11%)

All values presented as mean ± standard deviation.

BMI, Body mass index; GWG, gestational weight gain; FM, fat mass, FFM, fat free mass, TBW, total body water; BMC, bone mineral content.

Table 3.3 Neonatal characteristics for the total sample and each pre-pregnancy BMI group

	Total (n=40)	Normal weight (n=22)	Overweight/Obese (n=18)
Age at test (weeks)	0.30 ± 0.09	0.27 ± 0.09	0.33 ± 0.08
Gestational age (weeks)	39.58 ± 0.90	39.72 ± 0.84	39.41 ± 0.96
Infant gender (males)	23	12	11
Birth weight (g)	3459.00 ± 406.41	3410.91 ± 354.78	3517.78 ± 465.60
Birth length (cm)	50.95 ± 3.05	50.22 ± 2.49	51.83 ± 3.48
Body mass (g)	3233.55 ± 378.22	3202.20 ± 324.96	3271.86 ± 441.47
FM (g)	362.42 ± 157.60	340.76 ± 116.69	388.89 ± 197.01
FFM (g)	2871.13 ± 296.53	2861.44 ± 274.60	2882.96 ± 329.09
Body fat (%)	11.01 ± 3.93	10.54 ± 3.04	11.58 ± 4.82

All values presented as mean ± standard deviation.

BMI, Body mass index; FM, fat mass, FFM, fat free mass.

Table 3.4 Multiple linear regression model using maternal body composition to predict neonatal birth weight (kg) [a]

	r^2	β	p
Maternal TBW (L) [b]	0.294	0.029	0.007
Maternal FFM (kg) [b]	0.280	0.021	0.011
Maternal FM (kg) [b]	0.224	0.012	0.053
Maternal body fat (%) [b]	0.169	0.012	0.259
Maternal BMC (kg) [c]	0.231	0.028	0.383

[a] All maternal body composition predictors were run as separate models.

[b] Adjusted for neonatal gestational age.

[c] Adjusted for neonatal gestational age and maternal age.

TBW, total body water; FFM, fat free mass; FM, fat mass; BMC, bone mineral content.

Table 3.5 Multiple linear regression model using maternal body composition to predict neonatal percentage body fat (%) [a]

	r^2	β	p
Maternal TBW (L)	0.146	0.184	0.097
Maternal FFM (kg)	0.126	0.116	0.171
Maternal FM (kg)	0.131	0.091	0.145
Maternal body fat (%)	0.115	0.127	0.234
Maternal BMC (kg)	0.087	0.722	0.584

[a] All maternal body composition predictors were run as separate models.

All models adjusted for neonatal gestational age.

TBW, total body water; FFM, fat free mass; FM, fat mass; BMC, bone mineral content.

Table 3.6 Multiple linear regression model using maternal body composition to predict neonatal FM (g) [a]

	r^2	β	p
Maternal TBW (L)	0.034	6.863	0.133
Maternal FFM (kg)	0.023	4.758	0.174
Maternal FM (kg)	0.052	4.533	0.085
Maternal body fat (%)	0.031	6.557	0.140
Maternal BMC (kg)	- 0.020	26.274	0.634

[a] All maternal body composition predictors were run as separate models.

TBW, total body water; FFM, fat free mass; FM, fat mass; BMC, bone mineral content.

Table 3.7 Multiple linear regression model using maternal body composition to predict neonatal FFM (g) [a]

	r^2	β	p
Maternal TBW (L) [b]	0.519	16.471	0.011
Maternal FFM (kg) [b]	0.521	12.505	0.011
Maternal FM (kg) [b]	0.466	6.287	0.098
Maternal body fat (%) [b]	0.433	4.998	0.173
Maternal BMC (kg) [c]	0.478	105.835	0.164

[a] All maternal body composition predictors were run as separate models.

[b] Models adjusted for neonatal gestational age and gender.

[c] Model adjusted for neonatal gestational age, gender and maternal age.

TBW, total body water; FFM, fat free mass; FM, fat mass; BMC, bone mineral content.

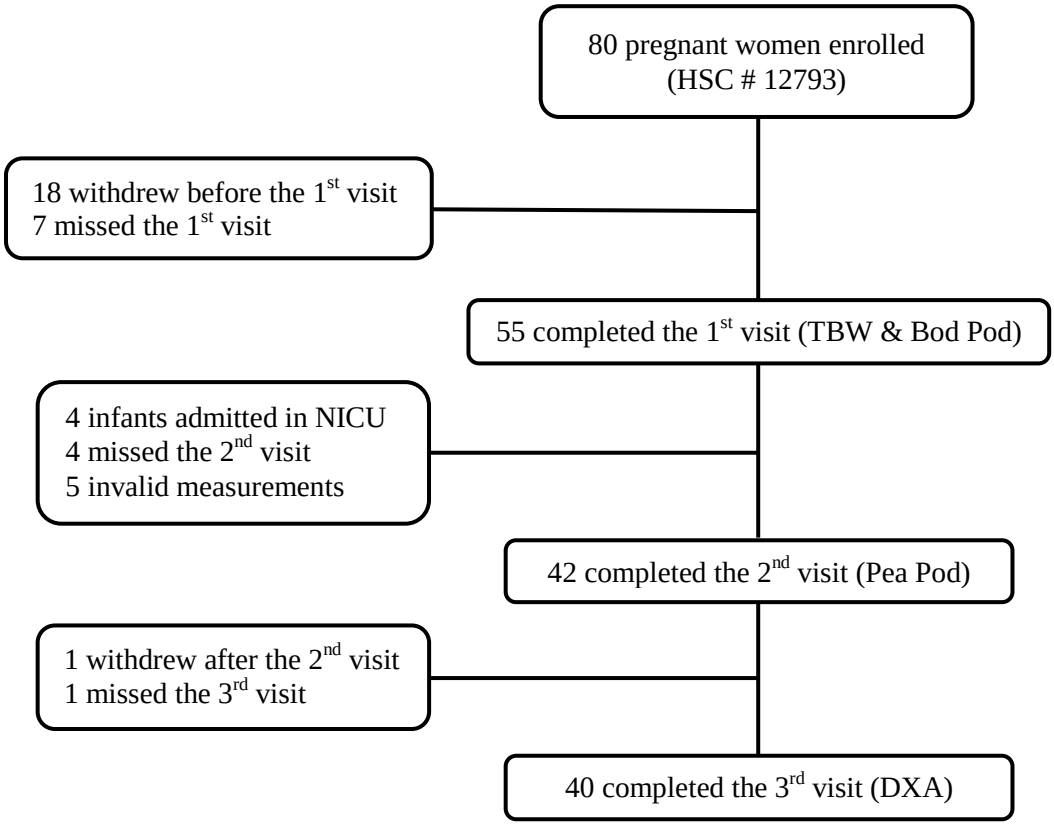

Figure 3.1 Consort diagram for subject enrollment

CHAPTER 4

TRIMESTER GESTATIONAL WEIGHT GAIN AND NEONATAL BODY COMPOSITION AT BIRTH

4.1. Abstract

Background: The relationship between the timing of gestational weight gain (GWG) and neonatal body fat is not clear.

Objective: To explore the association between timing of maternal GWG and neonatal percentage of body fat (%fat), fat mass (FM) and fat free mass (FFM) at birth.

Methods: Maternal trimester-specific GWG was calculated as the 1st (0 to 13 weeks), 2nd (14 to 28 weeks) and 3rd (29 weeks to delivery) trimester using extracted weights from medical records and self-reported pre-pregnancy weight from 48 mother-infant pairs. Neonatal body composition (% fat, absolute weight (g) of FM, and FFM) were measured using air displacement plethysmography (Pea Pod®) within 72 hours after birth. Multiple linear regression models that adjusted for the influential variables (maternal pre-pregnancy BMI, neonatal age at test, gender and gestational age) were used to assess the relationship between maternal GWG in each trimester and neonatal body composition. The interaction between trimester GWG and maternal pre-pregnancy BMI was explored using simple linear regression models. The significance level was $p \leq 0.05$.

Results: Total GWG was related to neonatal % fat ($r^2 = 0.182$, $p = 0.006$) and FM ($r^2 = 0.172$, $p = 0.007$). In women had an obese BMI before pregnancy, GWG in the 1st and 2nd trimesters was related to neonatal FM ($r^2 = 0.443$, $p = 0.021$; $r^2 = 0.352$, $p = 0.041$) and % fat ($r^2 = 0.398$, $p = 0.030$; $r^2 = 0.384$, $p = 0.033$). In women had an overweight BMI, trimester GWG was not related to neonatal % fat, FM or FFM. In women had a normal weight pre-pregnancy BMI, GWG in the 3rd trimester was related to neonatal FM ($r^2 = 0.167$, $p = 0.020$) and % fat ($r^2 = 0.203$, $p = 0.011$). Trimester GWG was not related to neonatal FFM.

Conclusions: Total GWG and GWG in different trimester of pregnancy related to neonatal body fatness.

4.2. Introduction

The Institute of Medicine (IOM) has long recognized the importance of maternal weight gain during pregnancy in relation to fetal growth and development. Maternal inadequate gestational weight gain (GWG) is associated with stillbirth (205), preterm birth (206), infant mortality (205) and a low birth weight (159) while excessive maternal GWG is associated with higher risk of cesarean section delivery (161) and a high birth weight (159). Without intervention, about 50% of women gain more than recommended, with about 70% of overweight and obese women gaining excessively. In 2009, the IOM updated the GWG guidelines (**Table 4.1**) (207). Pregnancy is recognized as an ideal time to intervene. Behavioral interventions have shown success in getting women to gain appropriately (208).

One noticeable outcome of the 2009 IOM report was the dearth of information regarding how the maternal environment influences neonatal body composition (207). There is a direct link between the level of fatness at birth to the level of fatness later in life. Research have shown a weak but direct relationship between neonatal body fat and childhood body fat (128). Children who are obese at age 5 are more likely to be an obese adult (55, 209) with the associated comorbid conditions (55, 210). Therefore, determining maternal factors that influence or program body composition at birth is of great interest.

Both maternal pre-pregnancy body mass index (BMI) and GWG are related to infant body composition (125, 128, 131, 132, 155). A positive relationship between maternal pre-pregnancy BMI and infant FM has been shown (125, 131, 155). Infants born to obese women have greater body fat when compared to infants born to normal weight women (125, 131). Further, maternal GWG is directly related to infant fat mass (FM) at birth (128, 132) and child FM at 6 years old (128). Women who gain excessively or outside of the IOM GWG recommendations have infants with greater FM (128) and this relationship varies by pre-pregnancy BMI category (132). Hull *et al.* (132) found offspring born to overweight women who gained in excess of the IOM recommendation had greater FM than offspring born to overweight

woman who gained appropriately. This difference was not found in women who were normal weight or obese at the start of pregnancy.

Several studies (170, 171, 211-214) have shown trimester-specific GWG relates to neonatal birth weight. GWG in the second trimester was more strongly associated to infant birth weight than GWG in the first and third trimesters. Only one study investigated the effect of the timing of GWG on infant fatness (215); however, GWG was divided as early or late weight gain by 20 weeks of gestation. No study has investigated trimester-specific GWG in relation to neonatal body fatness. And no study has correlated weight status at pregnancy and trimester specific GWG to neonatal body composition at birth. The aim of this study was to describe the relationship between total and trimester-specific GWG and neonatal body composition at birth in women by pre-pregnancy weight status.

4.3. Material and Methods

4.3.1. Study population

Pregnant women (18-45 years old) without known infectious disease or illness (e.g. autoimmune disease) were recruited between 28 and 39 weeks gestation. The subjects included women who were normal weight (BMI 18.5-24.99 kg/m^2) overweight (BMI 25-29.99 kg/m^2) and obese (BMI 30-40 kg/m^2) before pregnancy. The pre-pregnancy BMI was calculated using self-reported maternal pre-pregnancy body weight and measured height at the study visit. Women with an underweight pre-pregnancy BMI (< 18.5 kg/m^2) or a pre-pregnancy BMI over 40 kg/m^2 were excluded. Women who were diagnosed with preeclampsia or GDM, self-reported smoking and illicit drug use during their pregnancy and women who do not speak English were also excluded. Women's demographics and baseline information were obtained at enrollment. Women were recruited between January 2012 and March 2013 at their prenatal care visits to the clinics of University of Kansas Hospital in Kansas City from 2 study cohorts. All subjects provided written

informed consent. Both studies were approved by the University of Kansas Medical Center (KUMC) Institutional Review Board (IRB) (#12793 and # 13126).

4.3.2. Measures of pregnancy weight gain

Maternal weight was obtained at each scheduled clinic visits. Total maternal GWG was the difference between weight at delivery and pre-pregnancy weight. Maternal trimester-specific GWG was calculated for the 1st (0 to 13 weeks), 2nd (14 to 28 weeks) and 3rd (29 weeks to delivery) trimesters. Because not all women were weighed in the same gestational week within each trimester, the recorded weight gain within each trimester was divided by the weeks within each trimester to calculate a weekly weight gain for each trimester.

4.3.3. Neonatal body composition measurement

Neonatal body composition was measured with 72 h following birth. The Pea Pod® Body Composition System (ADP) (CosMed, Concord, CA) assessed body volume which was used to calculate body density by equation: body weight (kg)/body volume (L). The infant was naked during body volume measurement. Neonatal crown to heel length was measured to the nearest 0.1 cm using a calibrated length board (Shorr Productions, Olney, MD) and neonatal body weight was measured to the nearest 0.01 kg using the Pea Pod® neonatal scale. The Pea Pod software estimates thoracic gas volume for neonates (190, 191). FM was calculated by the equation $BM/BD = FM/D_{fm} + FFM/D_{ffm}$ (194) (BM is body weight, BD is body density, D_{fm} is FM density, and D_{ffm} is fat free mass (FFM) density). D_{fm} is constant throughout life as 0.9007 g/ml (194). D_{ffm} changes during growth (195) so age and gender specific D_{ffm} values are used in calculating percentage of body fat (% fat) (112).Infant % fat was calculated by equation: (FM/BM)*100% (193).

4.3.4. Statistical analyses

Multiple linear regression models were used to assess the relationship between maternal GWG during each trimester and neonatal body composition. To determine which maternal and

infant confounding variables to include in the regression models, bivariate correlations were performed between infant body composition (%fat, FM, FFM) and the following maternal and infant variables known in literature to be related to infant body composition: maternal age, pre-pregnancy BMI, maternal ethnicity, parity, smoking history, neonatal age at test, gender and gestational age. Correlations between variables that were significant were identified as collineated. Pearson correlations between all variables were shown in **Table 4.2**. Stepwise hierarchical regression was performed to remove the least significant collineated variable. The final models included maternal pre-pregnancy BMI, neonatal age at test, gender and gestational age as covariables. We also analyzed the interaction between trimester-specific GWG and each maternal pre-pregnancy BMI category. Since our sample was small, no covariates were included in the models that assessed interactions. Analyses were performed using SPSS v20.0 (SPSS Inc., Chicago, IL). $P \leq 0.05$ was considered statistically significant.

4.4. Results

Eighty women were enrolled in the study and 45 mother-infant pairs were completed the study (**Figure 4.1**). Maternal characteristics are presented in **Table 4.3**, and infant characteristics are presented in **Table 4.4**.

Women who were overweight before pregnancy gained the most weight during gestation and women who were obese before pregnancy gained the least during gestation. Total GWG for overweight women was significantly higher compared to the total GWG for normal weight (p = 0.042) or obese women (p = 0.005). The total weight gain for normal weight and obese women was not different. For trimester GWG, GWG during the 1st and 3rd trimesters were not different among pre-pregnancy BMI groups (p = 0.252 and p = 0.176, respectively). The weight gain during the 2nd trimester for overweight women was significantly higher than weight gain for obese women (p = 0.015). GWG during the 2nd trimester between normal weight women and obese women (p = 0.101) and normal weight and overweight women (p = 0.295) were not

different (p = 0.236) (**Table 4.3**). Infant gestational age, birth weight, and body composition (FM, FFM, % fat) were not different among groups (**Table 4.4**).

4.4.1. Trimester specific GWG related to neonatal %fat

In the group as whole, maternal total GWG was correlated to neonatal % fat (r^2 = 0.182, p = 0.006). When trimester-specific GWG was determined for each maternal pre-pregnancy BMI category, an interaction was found between trimester-specific GWG and neonatal % fat (**Figure 4.2**). GWG during the 1st (r^2 = 0.398, β = 16.862, p = 0.030) and 2nd (r^2 = 0.384, β = 18.052, p = 0.033) trimesters was positively related to neonatal % fat in the obese group, and GWG during the 3rd trimester was positively related to neonatal % fat in normal weight group (r^2 = 0.203, β = 9.415, p = 0.011). No relationship was found between trimester-specific GWG and neonatal % fat in the overweight group. Because we found interactions between maternal pre-pregnancy BMI and GWG on neonatal % fat, main effect of trimester GWG on neonatal % fat was not reported.

4.4.2. Total and trimester specific GWG related to neonatal FM

In the group as a whole, maternal total GWG was positively related to neonatal FM (r^2 = 0.172, p = 0.007). When trimester-specific GWG was determined for each maternal pre-pregnancy BMI category, an interaction was found relating the trimester-specific GWG to neonatal FM (**Figure 4.3**). GWG during the 1st trimester had a positive relationship with neonatal FM in the obese group (r^2 = 0.443, β = 807.644, p = 0.021) and the relationship between GWG during the 1st trimester and neonatal FM in overweight group approached significance (r^2 = 0.363, β = - 682.776, p = 0.067). GWG during the 2nd trimester was positively related to neonatal FM in obese group (r^2 = 0.352, β = 803.540, p = 0.041). GWG during the 3rd trimester was positively related to neonatal FM in normal weight group (r^2 = 0.167, β = 331.613, p = 0.020). Because we found interactions between maternal pre-pregnancy BMI and GWG on neonatal FM, main effect of trimester GWG on neonatal FM was not reported.

4.4.3. Total and trimester specific GWG related to neonatal FFM

No significant relationship was found between either maternal total GWG or trimester-specific GWG and neonatal FFM in the group as a whole. When trimester-specific GWG was determined for each maternal pre-pregnancy BMI category, the interaction between trimester-specific GWG and neonatal FFM was not significant (**Figure 4.4**).

4.5. Discussion

This is the first study to assess the relationship between trimester-specific GWG and neonatal body composition. We found significant relationships between maternal total GWG as well as trimester-specific GWG and neonatal body fat (% fat and FM). Neonatal % fat and FM were related to maternal GWG during the 2nd and 3rd trimesters but not the 1st trimester. Neonatal FFM was not related to either total GWG or GWG during any trimester.

We also explored if the timing of GWG when predicting neonatal body composition differed by pre-pregnancy BMI category. Late weight gain (3rd trimester) in normal weight group predicted infant body fat, while early weight gain (1st and 2nd trimesters) predicted infant body fat in the obese group.

Our data are consistent with published research exploring total GWG and infant body composition. Crozier *et al.* (128) reported a positive association between weight gain during pregnancy and neonatal FM measured at one month ($\beta = 0.10$, $p = 0.0004$) using a cohort of 566 mother-infant pairs in Southhampton Women's Survey Study. They suggested that excessive GWG predicted a higher infant body fat. Hull *et al.* (132) expanded this work in 306 mother-infant pairs and found the effect of excessive GWG on infant body fat varied by pre-pregnancy BMI category. Infants born to overweight women that gained excessively had greater body fat than offspring born overweight woman that gained appropriately (13.7% vs. 9.2%, $p = 0.001$). This difference was not found in any other pre-pregnancy BMI group. However, neither of these studies investigated how the timing of weight gain or trimester-specific GWG related to neonatal

body composition, which was reported by our study. We also found an interaction between trimester-specific GWG and maternal pre-pregnancy BMI category when predicting neonatal body fat.

One study assessed relationship between timing of excessive GWG and neonatal adiposity (215). Davenport et al. reported neonates born to women gained excessive weight during the first half of gestation (16–20 weeks) had higher body fat (17.5 ± 3.1%) compared to neonates born to women gained appropriate weight during pregnancy (13.2 ± 4.1%, $p < 0.01$) or excessive weight late in gestation (14.7 ± 3.3%, $p < 0.01$). They also indicated that neonates born to women who gained excessive weight during first half of pregnancy had an increased risk of elevated body fat at birth (odds ratio (OR) = 2.64, 95% CI: 1.35–5.17) compared to neonates born to women had total excessive weight gain during the pregnancy (OR = 1.49, 95% CI: 0.80–2.79). These results were inconsistent with our results since we found maternal weight gain later in pregnancy (14 to 28 weeks and 29 to delivery) was related to neonatal body fat at birth. When divided our samples by maternal pre-pregnancy BMI category, only results for obese group were consistent with findings reported by Davenport et al. However, they did not examine the interaction between GWG and maternal pre-pregnancy BMI category and they only separated the weight gain as early and late in pregnancy and neonatal body fat was estimated by skinfold thickness. Our study builds on the findings by Davenport et al. and reported the relationship between trimester-specific GWG and neonatal body composition at birth measured by air-displacement, which is a more accurate way to measure neonatal body composition. But we did not compare the body composition of infant born to women who gained appropriate weight during gestation to infant born to women who gained excessive weight according to the IOM recommendations because our small sample size. Most of our subjects, especially those who were overweight or obese before pregnancy, gained in excess of weight according to the 2009 IOM recommendations of GWG.

The potential mechanism underlying the interactive effects between GWG and pre-pregnancy BMI category has not yet been elucidated. Weight gain during different windows during pregnancy may have different outcomes. Data suggests that weight gain during early pregnancy reflects an accumulation of maternal fat stores and weight gained late in pregnancy reflects fetal growth (85). The Dutch Hunger Famine Studies showed altered nutrition during critical period in pregnancy had different influences on offspring birth weight (62, 216). Our overall results were consistent with this theory. However, when our sample was divided by pre-pregnancy BMI category, only normal weight women were following this pattern. In the overweight and obese women, weight gain during earlier pregnancy was related to neonatal body fat accumulation.

The changes in the compartments of GWG might be one reason to explain the interaction between the trimester-specific GWG and pre-pregnancy BMI on neonatal body composition. To our knowledge, only a few studies have investigated the changes in the compartments of GWG. Lederman *et al.* (173) assessed the changes of maternal weight gain in different pre-pregnancy BMI categories in 196 women using the four-compartment model during gestation at 14 and 37 weeks. They found maternal fat gain between 14 to 37 weeks was negatively correlated with pre-pregnancy BMI category (r = - 0.25, $p < 0.0005$) and positively correlated with GWG (r = 0.81, $p < 0.0001$). Women with an obese pre-pregnancy BMI had a smaller change in FM than women in other pre-pregnancy BMI categories. They also found women who gained above the amount recommended by IOM (1990) had the highest fat gain. In contrast to Lederman *et al.* (173), Butte *et al.* (135) reported a higher FM gain in high BMI group (pre-pregnancy BMI > 26 kg/m^2). However, all of the women in her high BMI group gained above the recommendations (IOM 1990), which might lead to a higher fat gain. Butte *et al.* (135) reported the changes in maternal body composition for each trimester for different pre-pregnancy BMI categories. Changes in maternal TBW, protein and FFM (p = 0.001) were found differed by trimester but not by pre-pregnancy BMI category, while trimester changes in maternal FM differed by pre-pregnancy

BMI category (p = 0.01). In our study, we did not evaluated the changes of compartments of GWG but most of our participants gained excessive weight during gestation according to 2009 IOM guidelines so they may have higher fat gain based on Lederman *et al.* (173). Because maternal fat gain was varied by pre-pregnancy BMI according to Butte *et al.* (135), this may underlie the interaction between trimester-specific GWG and pre-pregnancy BMI on neonatal body composition. Future study is needed to clarify this relationship.

Our study has several strengths. First, we assessed neonatal body composition in a few days after birth using air-displacement techniques, which avoided the potential influence of postnatal environment on neonatal body composition, such as feeding patterns. Second, we analyzed maternal weight gain using a trimester-specific model and related that to neonatal body composition at birth, which has never been done. We found the relationships of GWG during each trimester to neonatal body composition was different and these relationships varied by pre-pregnancy BMI category. This suggests that the best windows to intervene pregnant women on weight gain were different depend on their pre-pregnancy BMI to get most beneficial outcomes in offspring. For normal weight women, the best window might be late in pregnancy while for overweight and obese women, intervening during earlier pregnancy might be better.

Our study also has limitations. First, our sample size is small, we only have 45 mother-infant pairs in the study and most of them were in normal weight pre-pregnancy BMI category. The sample size in overweight and obese groups is small. However, for GWG during each trimester, a sample size of 40 provides sufficient power to detect a correlation of at least r = 0.5 at the 0.05 level of significance. But we do not have enough power to adjust for any covariates when we analyzed the interaction between GWG and pre-BMI category. Further, we cannot assess if excessive weight gain during each trimester acted differently compared to appropriate weight gain on neonatal body composition and if this varied by pre-pregnancy BMI group. Future studies with larger sample sizes are needed to answer these questions. At last, we correlated each

of our dependent and independent variables in separate models. We ran 12 models using the same data for this study, which increased our type I error probability.

In conclusion, we found a significant relationship between total GWG and GWG in the various trimesters and neonatal body composition. GWG during the 2nd and 3rd trimesters were positively related to neonatal body fat. Neonatal FFM was not correlated with either total or trimester-specific GWG. The relationship between GWG and neonatal body composition varied by maternal pre-pregnancy BMI category. In normal weight group, neonatal % fat and FM were associated with late GWG, while in obese group, neonatal % fat and FM were associated with GWG during the 1st and 2nd trimesters. Future studies are need in a larger sample size to clarify these relationships.

Table 4.1 Institute of Medicine gestational weight gain recommendations, 2009

Pre-pregnancy body mass index, kg/m^2	Gestational weight gain, kg (lbs)
Underweight (<18.5)	12.5 – 18 (28 – 40)
Normal weight (18.5-24.99)	11.5 – 15.9 (25 – 35)
Overweight (25-29.99)	7 – 11.5 (15 – 25)
Obese (>30)	5 – 9 (11 – 20)

Table 4.2 Pearson correlation between all variables

	Maternal pre-pregnancy BMI (kg/m^2)	Total GWG (kg)	GWG during the 1st trimester (kg/week)	GWG during the 2nd trimester (kg/week)	GWG during the 3rd trimester (kg/week)	Gestational age (weeks)	Infant age at test (weeks)	Infant gender (males)	Infant FM (g)	Infant FFM (g)	Infant body fat (%)
Maternal pre-pregnancy BMI (kg/m^2)	1	-0.222	-0.134	-0.285	0.028	0.020	0.275	-0.083	0.147	0.162	0.095
Total GWG (kg)		1	0.353*	0.755**	0.718**	0.265	-0.057	0.194	0.337*	0.237	0.321*
1st trimester GWG (kg/week)			1	0.001	-0.104	-0.148	-0.348*	0.026	0.166	-0.016	0.157
2nd trimester GWG (kg/week)				1	0.376*	0.314*	0.035	-0.079	0.183	0.278	0.155
3rd trimester GWG (kg/week)					1	0.167	0.207	0.344*	0.236	0.019	0.262
Gestational age (weeks)						1	-0.156	0.168	-0.031	0.555**	-0.151
Infant age at test (weeks)							1	-0.102	-0.168	-0.253	-0.138
Infant gender (males)								1	0.183	-0.035	0.192
Infant FM (g)									1	0.470**	0.964**
Infant FFM (g)										1	0.259
Infant body fat (%)											1

*$P \leq 0.05$; **$P \leq 0.01$

BMI, body mass index; GWG, gestational weight gain; FM, fat mass; FFM, fat free mass (Ctrl)

Table 4.3 Maternal characteristics for the total sample and each pre-pregnancy BMI group

	Total (n=45)	Normal weight (n=27)	Overweight (n=8)	Obese (n=10)
Age (years)	28.10 ± 4.64	28.05 ± 4.36	28.89 ± 4.64	27.62 ± 5.71
Height (m)	1.65 ± 0.06	1.66 ± 0.06	1.63 ± 0.08	1.66 ± 0.06
Pre-pregnancy weight (kg)	69.20 ± 14.62	59.99 ± 5.88	72.10 ± 8.77	91.73 ± 7.76
Pre-pregnancy BMI (kg/m^2)	25.36 ± 5.12	21.88 ± 1.81	26.96 ± 1.29	33.49 ± 5.08
Total GWG (kg)	16.36 ± 5.55	16.10 ± 4.26	21.19 ± 7.19	13.18 ± 5.10
GWG in the 1st trimester (kg/week)	0.18 ± 0.20	0.17 ± 0.21	0.28 ± 0.14	0.12 ± 0.21
GWG in the 2nd trimester (kg/week)	0.48 ± 0.21	0.50 ± 0.20	0.62 ± 0.23	0.34 ± 0.20
GWG in the 3rd trimester (kg/week)	0.52 ± 0.23	0.49 ± 0.18	0.66 ± 0.37	0.50 ± 0.20
Races (n)				
Caucasian	32 (71%)	23 (85%)	3 (37.5%)	6 (60%)
African-American	6 (13%)	2 (7%)	2 (25%)	2 (20%)
Hispanic	5 (11%)	1 (4%)	2 (25%)	2 (20%)
Other	2 (4%)	1 (4%)	1 (12.5%)	0 (0%)

All values presented as mean ± standard deviation.

BMI, Body mass index; GWG, gestational weight gain.

Table 4.4 Infant characteristics for the total sample and each pre-pregnancy BMI group

	Total (n=48)	Normal weight (n=30)	Overweight (n=8)	Obese (n=10)
Age at test (weeks)	0.30 ± 0.09	0.27 ± 0.09	0.33 ± 0.08	0.33 ± 0.08
Gestational age (weeks)	39.67 ± 0.82	39.69 ± 0.71	39.43 ± 1.33	39.80 ± 0.60
Infant gender (males)	23	13	5	5
Birth weight (kg)	3.43 ± 0.39	3.36 ± 0.32	3.43 ± 0.43	3.63 ± 0.51
Birth length (cm)	50.80 ± 3.04	50.18 ± 2.35	51.21 ± 3.50	52.22 ± 4.09
Body mass (g)	3200.76 ± 398.30	3136.30 ± 351.77	3232.41 ± 422.74	3349.46 ± 491.12
Fat mass (g)	348.46 ± 165.76	318.93 ± 137.52	391.32 ± 139.83	393.91 ± 240.73
Fat free mass (g)	2852.29 ± 292.59	2817.37 ± 271.84	2841.08 ± 379.16	2955.55 ± 278.60
Body fat (%)	10.59 ± 4.09	9.95 ± 3.60	12.05 ± 4.15	11.15 ± 5.24

All values presented as mean ± standard deviation.

BMI, Body mass index.

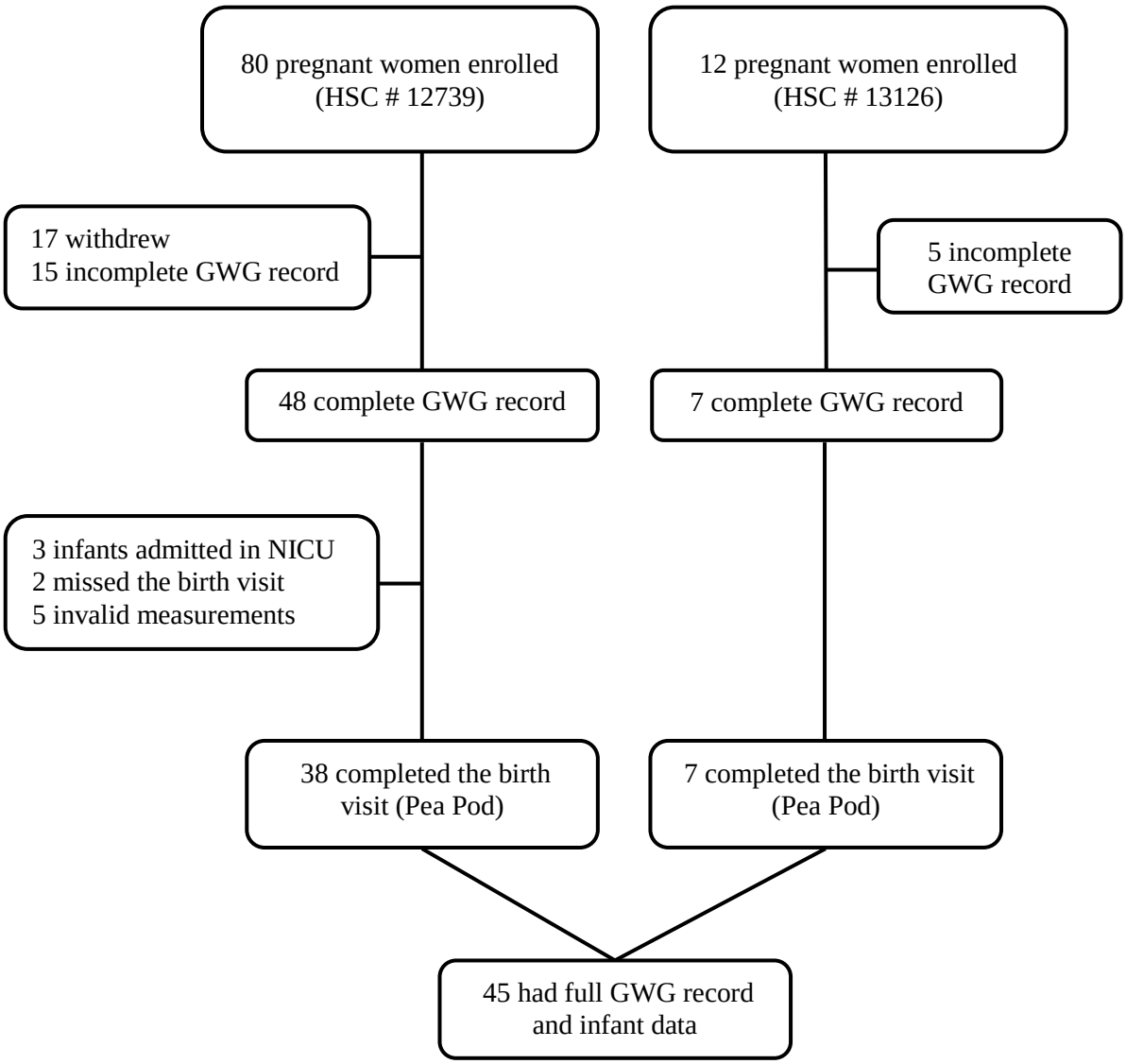

Figure 4.1 Consort diagram for subject enrollment

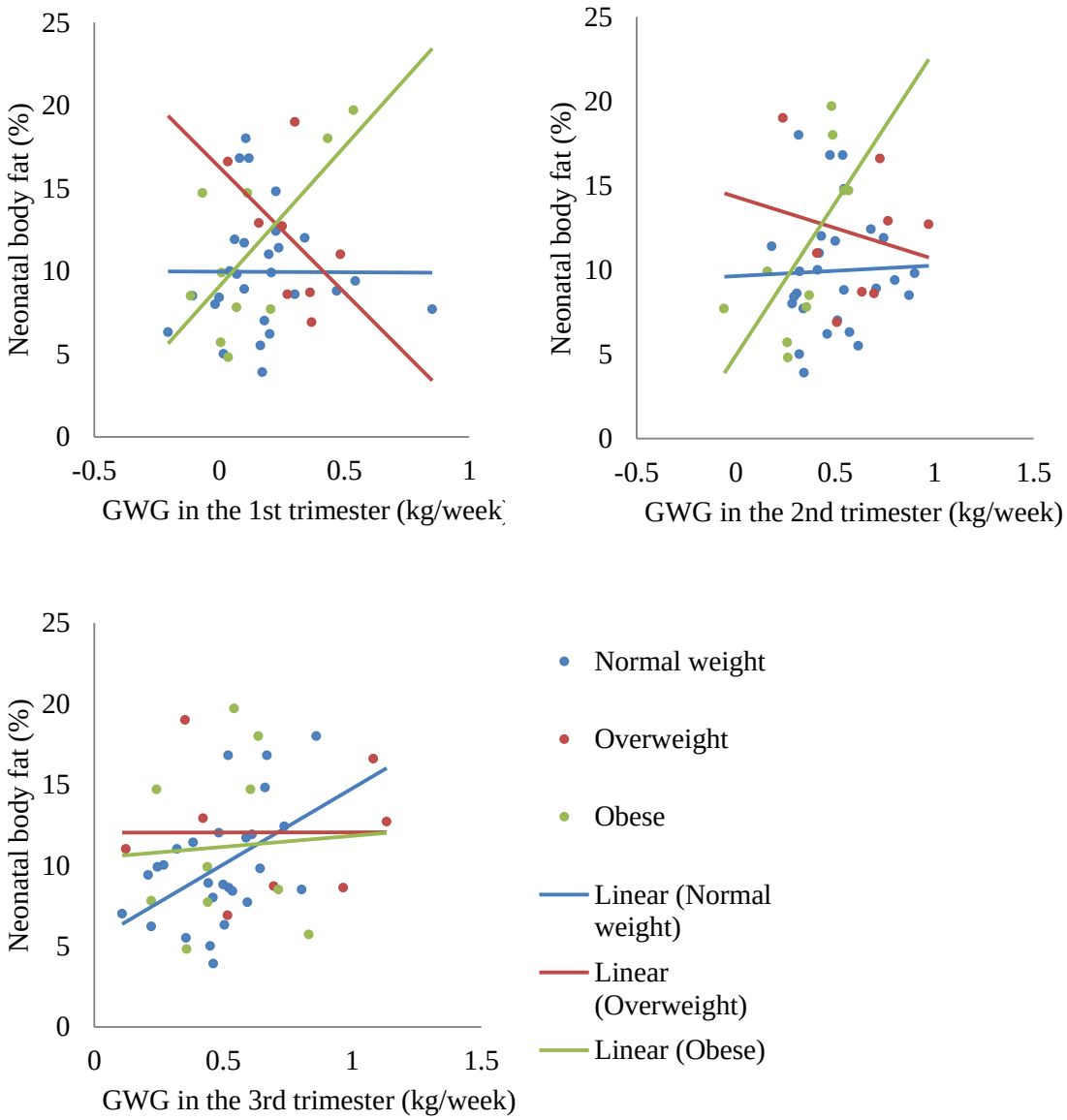

Figure 4.2 Interaction between gestational weight gain (GWG) and pre-pregnancy body mass index (BMI) on neonatal percentage body fat (% fat)

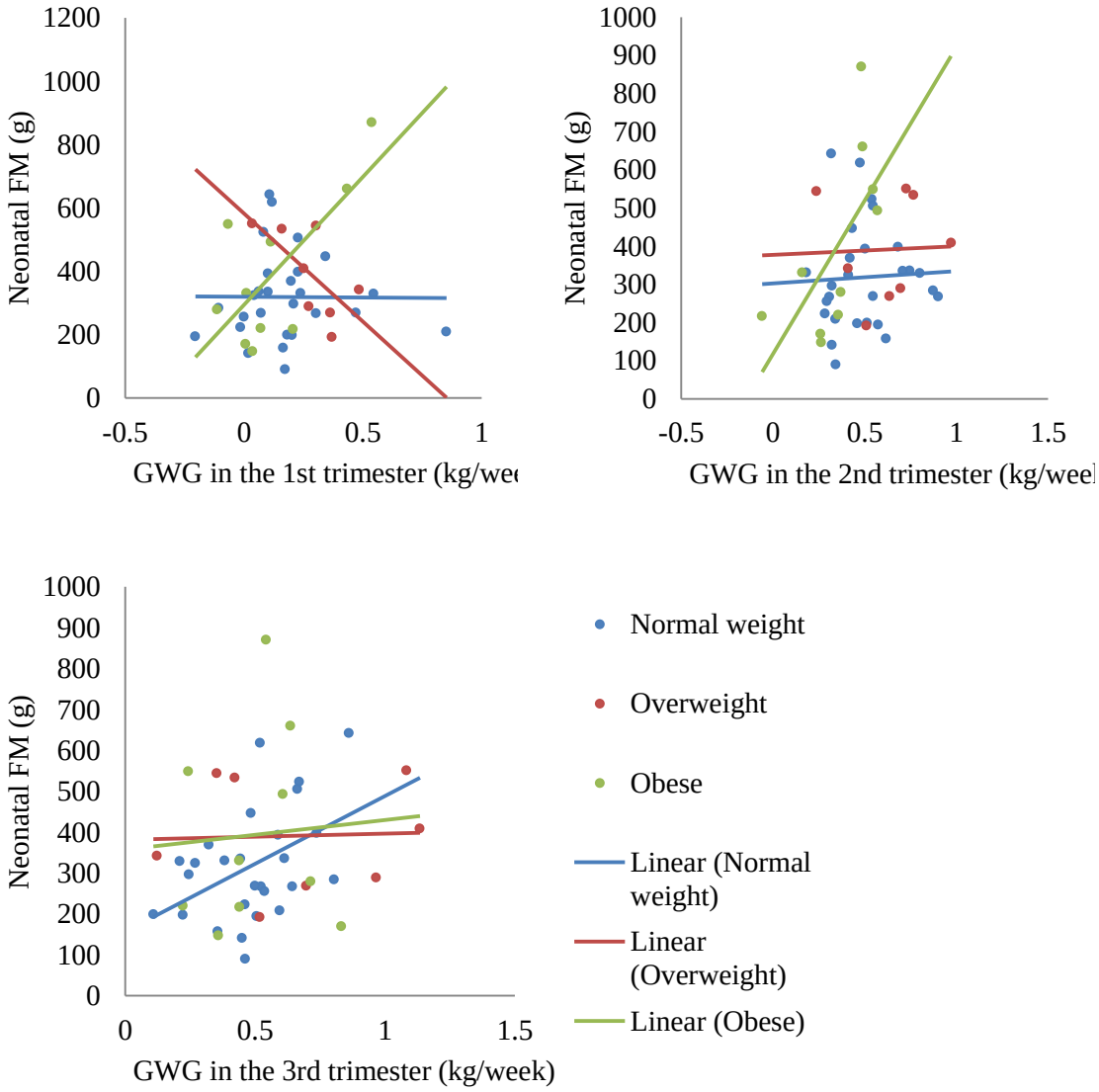

Figure 4.3 Interaction between gestational weight gain (GWG) and pre-pregnancy body mass index (BMI) on neonatal fat mass (FM)

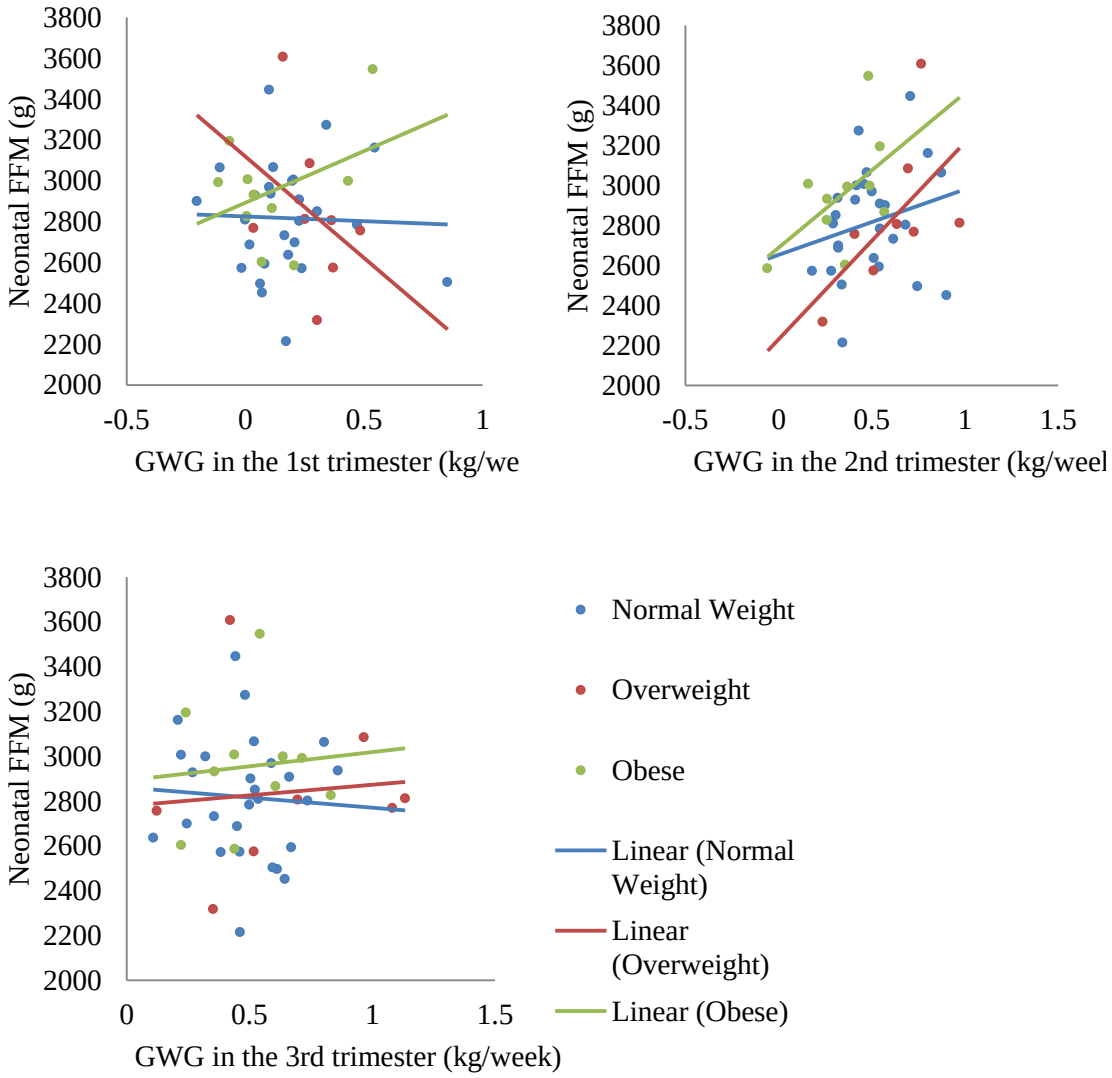

Figure 4.4 Interaction between gestational weight gain (GWG) and pre-pregnancy body mass index (BMI) on neonatal fat free mass (FFM)

CHAPTER 5

DISCUSSION AND CONCLUSION

5.1. Summary of findings

This is the first study to relate maternal body composition during pregnancy to neonatal body composition at birth. This research extends the literature relating maternal factors to neonatal body composition (125, 128, 131, 132, 155, 217, 218). Overall, the presented study provides evidence that maternal body composition and GWG were related to neonatal body composition. The findings aided in our comprehension of how maternal adiposity relates to fetal growth which may relate to the future health of the offspring. The results of this study also added to the body of knowledge on the relationship between maternal body composition, GWG and neonatal body composition. Such results are expected to have an important positive impact, because understanding the factors that influence the development of neonatal body fat are critical as this may relate to later offspring health and identify time periods for intervention.

Chapter 3 Maternal body composition late in pregnancy and neonatal body composition at birth

The purpose of this study was to measure maternal body composition late in pregnancy and offspring body composition 1-3 days following birth to determine how they were related. Our results demonstrated a relationship between maternal body composition and neonatal birth weight and body composition. Maternal FFM and TBW were significantly related to birth weight and maternal TBW and FFM were significantly related to neonatal FFM. Although no significant relationships were found between maternal FM and neonatal FM, the relationship between maternal FM and neonatal FM approached significance. The trend of this relationship indicated a larger sample size may be needed in future studies to clarify the association between these variables.

Chapter 4 Relationship between timing of maternal gestational weight gain and neonatal body composition at birth

While many studies have demonstrated a relationship between total GWG and neonatal birth weight and body composition (128, 132, 151, 165-172, 218), no study has assessed how GWG during different trimesters relates to neonatal body composition at birth. The purpose of this chapter was to describe the relationship between both total and trimester-specific GWG and neonatal body composition at birth using a prospective pregnant cohort. The results of this study provided evidence that the timing of GWG was related to neonatal body composition at birth. A significant interaction was found between maternal pre-pregnancy BMI and GWG on neonatal body fat. Early weight gain (1^{st} and 2^{nd} trimesters) was positively related to neonatal body fat in obese women while late weight gain (3^{rd} trimester) was positively related to neonatal body fat in normal weight women. Main effect between maternal trimester GWG and infant body composition at birth was not reported because we found significant interaction between maternal pre-pregnancy BMI and GWG on neonatal body composition.

5.2. Discussion

5.2.1. Comparison with other studies

Chapter 3 Maternal body composition late in pregnancy and neonatal body composition at birth

Several studies have assessed the relationship between maternal factors and neonatal body composition (125, 131, 155, 217); however, all of these studies used maternal anthropometrics such as maternal pre-pregnancy BMI as variables to predict neonatal body composition. In our study, we used maternal body composition measured by the four-compartment model as a predictor for neonatal body composition at birth. Measuring body composition quantifies fat and lean body mass while BMI is simply a surrogate marker of adiposity that is correlated with FM.

Four previous studies investigated the relationship between maternal pre-pregnancy BMI and infant body composition up to 1 month of age (125, 131, 155, 217). Two studies measured infant body composition using ADP (Pea Pod®) at 2 weeks of age (131, 155), one study used TOBEC (125) within 72 h after birth and one study used MRI (217) from 1 to 28 days of age to assess infant body composition. All studies found positive relationship between maternal pre-pregnancy BMI and infant body fat (125, 131, 155, 217). However, results on FFM were not consistent. Sewell *et al.* (125) found maternal pre-pregnancy BMI did not affect infant FFM while Hull *et al.* (131) found infants born to overweight/obese women had lower FFM compared to infants born to normal weight women. Timing of neonatal body composition assessment might be the reason for the discrepancy. We observed that neither infant FM nor FFM were related to maternal pre-pregnancy BMI. Different findings may be due to our smaller sample size (n = 43) and we separated the overweight and obese groups instead of collapsing the overweight/obese groups together.

We also evaluated the correlation between maternal body composition late in pregnancy to neonatal birth weight. Two studies that analyzed maternal body composition during pregnancy using four-compartment model related that to birth weight (135, 174). Our results partly confirmed their results that maternal TBW and FFM were related to birth weight. However, we found maternal FM was associated with birth weight as well which was inconsistent with earlier studies. Our results indicated that maternal FM may also influence neonatal birth weight, especially in a higher BMI population.

Only one study used a multi-compartment model to measure maternal body composition during pregnancy and related that to infant body composition (135). Infant body composition was measured at 2 weeks of age using DXA. No relationship was found between maternal body composition at 36 weeks and infant body composition at 2 weeks of age. In contrast, we found a positive association between maternal FFM and TBW and neonatal FFM. Maternal FM was related to neonatal FM with a borderline significance. The difference between our results and an

earlier study by Butte *et al.* (135) may due to the timing of infant body composition measurement. We conducted our measurement within 1-3 days after birth while Butte *et al.* (135) did their measurement at 2 weeks of age. A later infant body composition measurement may be influenced by *ex utero* factors including the infant feeding pattern. This is the first study that related maternal body composition during pregnancy measured by a multi-compartment model to neonatal body composition at birth.

Chapter 4 Relationship between timing of maternal gestational weight gain and neonatal body composition at birth

Three studies explored how total GWG affected neonatal body composition (128, 132, 218). All of the studies categorized weight gain using the 2009 IOM guidelines for GWG (see **Table 1**). One study measured neonatal body composition using DXA at 1 month (128), one study used ADP (Pea Pod®) within 3 days after birth (132), and the other study used skinfold thickness within 1-3 days after birth (218). All of the studies found excessive weight gain during pregnancy related to a greater neonatal body fat at birth. However, when data were analyzed by maternal pre-pregnancy BMI groups, only one study found higher body fat in offspring from the normal weight group when the mother gained excessive weight. This relationship was not found in offspring from the overweight and obese groups (218). Other results found a significant interaction between GWG and maternal pre-pregnancy BMI on neonatal body composition. The effect of excessive GWG on neonatal body fat was greatest in overweight women (132). In our study, we observed an association between total GWG and neonatal body fat but not FFM at birth. However, we were unpowered to detect if excessive weight gain affects neonatal body composition since this study was not designed for that aim. Future studies are needed to further illuminate the details between GWG and neonatal body composition.

Studies have investigated how GWG during different trimesters affects birth weight (170-172) but only one study has evaluated the effects of the timing of GWG on neonatal adiposity

(215). GWG during the 1st and 2nd trimesters, especially the 2nd trimester, was more significantly related to birth weight when compared to the 3rd trimester (170-172). For infant adiposity, Davenport *et al.* (215) found if women gained excessive GWG during the first half of gestation (16 to 20 weeks), their infants would have greater body fat compared to infants born to women who gained appropriate GWG or when compared to infants born to women who gained excessive weight late in gestation. Our results were inconsistent with the observations reported. We found a significant relationship between late GWG (2nd and 3rd trimesters) and neonatal body fat. In addition, we found interaction between maternal pre-pregnancy BMI and GWG during the different trimesters on neonatal body fat. Weight gain late in gestation in normal weight women was associated with a greater infant body fat. However in overweight and obese women, weight gained earlier during gestation was associated with greater infant body fat. The interaction might be the reason for the difference between our results and the Davenport *et al.* study. We had 30 normal weight 8 overweight and 10 obese women in our study. This may pull the association between weight gain and neonatal body fat into later gestation.

5.2.2. The clinical implications

5.2.2.1. Maternal body composition

It is known that adequate GWG together with maternal pre-pregnancy BMI are important for optimal pregnancy and infant outcomes. However, the effects of specific components of maternal body weight and weight gain during pregnancy on fetal growth are not delineated clearly. As pregnancy progresses, water, protein, fat and minerals are accreted in the fetus, placenta, and maternal lean and adipose tissues. Variations in the composition of the mother's body may be crucial in determining the association between the maternal environment and infant outcomes. Measuring body composition during pregnancy is difficult because the basic assumptions used for common two-compartment models are not valid in this population. Multi-compartment models are needed to measure body composition during gestation accurately. Our study showed a relationship between maternal FFM and TBW late in pregnancy to neonatal FFM,

as well as a trend for an association between maternal and neonatal body fat. Although the mechanisms underlying these results are not clear yet, placental transportation of protein relating to maternal adiponectin level and maternal glucose production rate during gestation may be involved in explaining the effects of maternal body water or body composition on fetal development. These results are critical because it provides clues for future recommendations for GWG to achieve full benefit with less fat gain.

5.2.2.2. The timing of GWG

The intrauterine environment is essential for fetal growth and thought to affect many aspects of human health throughout the life course. Gestational weight gain may alter the intrauterine environment and inappropriate weight gain could impede fetal growth. Gestational weight gain is associated with both infant birth weight and body composition. However, the timing of overnutrition and resulting excessive GWG on neonatal outcomes at birth is not clear. Current GWG guidelines developed by the IOM provide recommended total and trimester GWG guidelines to prevent adverse maternal and neonatal outcomes based on pre-pregnancy BMI. For weight gain in different trimesters, all women are recommended to gain 0.5-2.0 kg during the 1st trimester and a weekly weight gain during the 2nd and 3rd trimesters was recommended by pre-pregnancy BMI (207).

Weight gain during different trimesters could have different effects on neonatal outcomes. Specific windows to intervene in pregnant women to have the greatest beneficial impact on offspring body composition are unknown. Our data suggest the critical window to intervene varied by pre-pregnancy BMI. For normal weight women, the critical window would be late in pregnancy to promote a healthy weight gain during the 3rd trimester. For overweight and obese women, the critical window would be earlier during gestation to promote a healthy weight gain. The excessive weight gain in our overweight and obese women highlights the need for focused nutritional advice and care during pregnancy to promote appropriate weight gain, which may lead to less body fat in their offspring.

5.2.2.3. Neonatal body composition

Accurate measurement of neonatal body fat is important because it is related to childhood body fat (128). Greater body fat in childhood is related to obesity development in adulthood along with the associated comorbid conditions. Barker *et al*. proposed that an infant phenotype with a high body fat and low muscle mass persists into childhood (55). Many studies used infant birth weight as a surrogate marker of infant adiposity; however, birth weight does not account for the compartment of body weight that is fat or lean body mass. Some studies used skinfold thickness to estimate infant body fat; however, the accuracy of body fat estimated by skinfold thickness depends on the skill and experience of operators. Our study used ADP (Pea Pod®) to measure neonatal body composition, which measured infant body weight and body volume and used a gender and age-specific equation to calculate neonatal body fat. It is an accurate, easy, quick and safe way to assess neonatal body composition. The evaluation of maternal factors related to neonatal body composition may shed light on the issue of fetal programming or the "*fetal origins of adult disease*" hypothesis.

5.2.3. Strengths and limitations

5.2.3.1. Strengths

There are several strengths of our study. First, we measured maternal body composition during pregnancy using a multi-compartment model, which is the only validated method to assess maternal body composition during pregnancy. We measured the components of maternal body weight (FM and FFM) during gestation which reflects the *in utero* environment and is related that to neonatal outcomes. Second, we measured neonatal body composition in a few days after birth to avoid the potential influence of *ex utero* environment, such as mode of feeding (breastfeed vs. formula). Third, we tracked all of our participants' weight records throughout gestation and calculated their weight gain rate (kg/week) during different trimesters.

5.2.3.2. Limitations

Our study has limitations as well. First, our sample size is small (n = 43 for Chapter 3 and n = 48 for Chapter 4). The small sample we had limited our statistical analysis when we want to control for potential confounding variables. Second, this is a cross-sectional study; we did not monitor the changes of maternal body composition from pre-pregnancy to delivery. We don't know if the changes of maternal body composition relate to neonatal outcomes or if these changes have any interaction with pre-pregnancy BMI on neonatal body composition. At last, because of our small sample size, we correlated each dependent variable and independent variable in separated regression models. In total, we ran 20 regression models for Chapter 3 and 12 regression models for Chapter 4 using the same cohort, which increased the type I error probability for our study.

5.3. Future Directions

Future studies are needed to validate our results. First, studies with larger sample size are needed to evaluate if the relationship between maternal and neonatal body composition varies by pre-pregnancy BMI. Second, repeated measures of maternal body composition during pregnancy are needed to see whether the changes of maternal fat and lean body mass influence neonatal body composition at birth. Third, our study only recruited healthy pregnant women and pregnant women with complications such as gestational diabetes mellitus and preeclampsia need to be studied since those complications might have different effects on neonatal body composition compared to healthy women. Fourth, growth rate and follow up measurements in offspring are needed to observe how neonatal body fat at birth related to long-term health. Last, most of the studies assessed the effects of maternal factors on neonatal outcomes were observational studies, behavior interventions are needed to validate the effects of maternal environment on neonatal outcomes.

5.4. Conclusions

This study is innovative because we related neonatal body composition to maternal body composition using the four-compartment model in wide a range of pre-pregnancy BMI. Maternal body volume was measured using ADP late in pregnancy and this has never been published before. Neonatal FM was measured by ADP at birth, which has never been related to maternal body composition before.

We found a relationship between maternal body composition late in pregnancy and neonatal body composition at birth. A positive relationship was found between maternal TBW and FFM and neonatal FFM. The relationship between maternal FM and neonatal FM approached significance. Maternal GWG during the 2^{nd} and 3^{rd} trimesters was related to neonatal body fat. The effects of trimester-specific GWG on neonatal body fat varied by maternal pre-pregnancy BMI. Neonates born to normal weight women had more body fat if women gained weight late in pregnancy, while neonates born to overweight and obese women had more body fat if women gained weight during early gestation.

Future studies are needed to verify our results in a larger sample size with more obese women and to clarify the effects of changes in maternal body composition during gestation on neonatal body composition.

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