MATERNAL DIETARY FAT INTAKE DURING PREGNANCY AND INFANT BODY COMPOSITION AT BIRTH

By

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List of Abbreviations

AA	Arachidonic acid
ADP	Air displacement plethysmography
ALA	Alpha linolenic acid
ApoB-100	Apolipoprotein B-100
BMI	Body mass index
CDC	Centers for Disease Control and Prevention
CTRC	Clinical and Translational Research Center
DOHad	Developmental Origins of Health and Disease
DGLA	Dihomo-gamma-linolenic acid
DHA	Docosahexaenoic acid
EPA	Eicosapentaenoic acid
FFQ	Food frequency questionnaire
HIPAA	Health Insurance Portability and Accountability Act
HDL	High-density lipoprotein
ITG	Information Technology Group
IRB	Institutional Review Board
kcal	Kilocalorie
LA	Linoleic acid
LDL	Low-density lipoprotein
MTP	Microsomal triglyceride transfer protein
MUFAs	Monounsaturated fatty acids
n6:n3	Omega 6 to omega 3 ratio
NHANES	National Health and Nutrition Examination Survey
NEFAs	Non-esterified fatty acids
OHSU	Oregon Health & Science University
LPL	Placental lipoprotein lipase
PUFAs	Polyunsaturated fatty acids
PI	Principal investigator
US	United States
USDA	United States Department of Agriculture
WHO	World Health Organization

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Abstract

Recent evidence links infant body composition at birth to an increased risk of adult chronic disease. However, few human studies have explored the association between maternal dietary fat intake and infant body composition. Specific dietary recommendations for pregnant women for quantity and quality of dietary fat intake are lacking. Our goal was to investigate the association between maternal dietary fat intake during pregnancy and infant body composition at birth. We hypothesized that increased infant adiposity at birth is positively correlated with high maternal dietary total fat intake.

This was a secondary analysis of a cross-sectional study of 79 healthy pregnant women with a singleton gestation who were enrolled at 12 to 16 weeks gestation. The 2005 Block Food Frequency questionnaire was used to assess dietary intake in 3-month intervals across pregnancy at 12-16 weeks, 24-28 weeks, and 37 weeks gestation. Infant anthropometry and flank skinfold measurements were taken within 24 hours of birth, then the Catalano equation was used to calculate infant fat mass. Associations between maternal dietary fat intake and infant anthropometrics were assessed by Pearson's correlation and linear regression analyses.

Infant body fat percentage was weakly correlated with average total daily dietary fat and unsaturated fat intake during pregnancy after adjusting for total gestational weight gain, pre-pregnancy body mass index (BMI), gestational age at birth, and infant birth weight (r=0.233 p=0.045; r =0.241 p=0.037). After adjusting for the same confounding variables, infant body fat percent was associated with

maternal total daily dietary fat, saturated fat, and unsaturated fat during the second trimester of pregnancy (r =0.252 p=0.029; r =0.231 p=0.046; r =0.250 p=0.031). When this analysis was repeated with maternal fat intake as a percent of calories, no relationships persisted after adjustment for confounding variables for total fat, saturated fat, or unsaturated fat and all markers of infant body composition.

Infant body fat percent at birth was significantly associated with average intake of total daily dietary fat and unsaturated fat during pregnancy, and all types of dietary fat in the second trimester were associated with infant adiposity. Our findings suggest that the second trimester may be a key time period for nutritional intervention and fetal metabolic programming. These results expand our current knowledge of maternal dietary intake and infant body composition and will help inform the optimal maternal diet for beneficial birth outcomes.

Chapter 1

Introduction and Specific Aims

An optimal maternal diet during pregnancy promotes appropriate weight gain in the mother and supports fetal growth, thus increasing the likelihood of healthy birth outcomes. Increasing evidence links infant birth weight and development throughout the first two years of life to later risk of chronic disease. Relatively few studies have investigated the relationship between infant body composition and health outcomes, with some studies suggesting that infants with a higher percent body fat at birth are pre-disposed to insulin resistance later in life. The impact of infant body composition on lifetime risk of chronic diseases such as hypertension, hyperlipidemia, stroke, and cardiovascular disease is unknown but is an area of intense research investigation. According to the Centers for Disease Control and Prevention (CDC), in 2010, seven of the top ten causes of death in the United States (US) were chronic diseases and more than one third of the US population was obese. If altering maternal diet during pregnancy could impact infant body composition and lower lifetime risk for chronic disease, as suggested by the developmental origins of health and disease hypothesis, then improving dietary intake of pregnant mothers could be a tool to prevent chronic disease at the earliest of stages of life.

Maternal dietary fat intake affects the amount of fatty acids that are available to transfer from mother to fetus via the placenta. The placenta is an organ through which nutrients, including fatty acids, transfer from the mother to the fetus. Animal studies have shown that high-fat maternal diets during pregnancy can result in

altered fetal body composition and metabolic syndrome in the offspring later in life, characterized by insulin resistance, hypertension, hyperlipidemia, and increased adiposity. However, there is a lack of evidence in human studies connecting maternal dietary fat intake to infant body composition. There is also a gap in research supporting specific dietary recommendations for pregnant women regarding both quantity and quality of dietary fat intake. Current recommendations for macronutrient diet composition during pregnancy are the same as general recommendations for all women of childbearing age. The Academy of Nutrition and Dietetics recommends that 20% to 35% total calories come from dietary fat during pregnancy. The 2015-2020 Dietary Guidelines for Americans recommends this exact same dietary fat intake for all females and males ages 19 years and older.

The goal of this investigation was to evaluate the relationship between maternal dietary fat intake and infant body composition. This project fills a gap in current research by correlating levels of maternal fat intake with infant adiposity. This project is one of very few studies in human infants, rather than animals, examining the relationship between maternal dietary fat quality and infant body composition. The results of this study expand our current knowledge about the role of maternal dietary intake on fetal body composition and are clinically relevant in order to determine the optimal maternal diet for beneficial birth outcomes.

This project was a secondary analysis of a cross-sectional study at Oregon Health and Science University (OHSU) in Portland, Oregon. Nicole Marshall, MD, MCR, was the principle investigator and recruited pregnant women into the study. This project analyzed Block food frequency questionnaires (FFQ) from each

trimester during pregnancy, and infant body composition measurements within 24 hours of birth. The specific aims of this project were:

Aim 1: To determine the relationship between maternal dietary fat intake during the 1st, 2nd, and 3rd trimesters of pregnancy and infant adiposity at birth.

We hypothesize that maternal total fat intake will be positively correlated with infant adiposity at birth.

Aim 2: To evaluate if intake of maternal saturated fat, or unsaturated fat, is associated with infant adiposity at birth.

We hypothesize that maternal saturated fat intake will have the strongest association with infant adiposity at birth.

The primary goal of this investigation was to examine the relationship between maternal dietary fat intake and infant adiposity to provide evidence to support future health recommendations for pregnant women. The results of this project will provide important evidence for optimizing maternal nutrition during pregnancy in order to increase healthy birth outcomes and potentially prevent chronic disease.

Chapter 2. Background

Birth Outcomes

Healthy People 2020 has 72 objectives related to improving the health of women, infants, and children, thus indicating that healthy pregnancies and birth outcomes are important public health goals for the US.[1] Pregnancy plays a key role in achieving the Healthy People objectives because it is an optimal time for nutritional interventions that may improve birth outcomes. Increasing evidence links infant birth weight and development throughout the first two years of life to risk of chronic disease later in life. In 2015, 8.1% of all infants born in the US were low birth weight (defined as less than 2500 grams), which is associated with increased risk of chronic disease later in life.[1] Birth weight is just one of many birth outcomes that has the potential to affect future disease risk. Relatively fewer studies have investigated infant body composition, but some studies suggest that infants with a higher percent body fat at birth are pre-disposed to insulin resistance later in life.[2] The obesity and chronic disease epidemic also remains an important focus of the Healthy People 2020 objectives since over 2/3 of US adults age 20 years and over were overweight and obese from 2013 to 2014; these numbers only continue to grow.[3] The rising rates of obesity in the US demand intervention at key time periods even as early as infancy. However, there are currently no criteria to assess excess adiposity in infants or reference curves to describe infant body composition.[4] Although the World Health Organization (WHO) has published BMI standards for infants, the current US anthropometric standard is weight-for-length.[4] Therefore, investigating factors related to infant body composition and growth trajectories

remains vital to fighting the obesity epidemic, preventing chronic disease, and improving birth outcomes. The role of infant body composition and lifetime risk of chronic diseases such as hypertension, hyperlipidemia, stroke, and cardiovascular disease is unknown but is an area of intense research investigation.

Developmental Origins of Health and Disease

The developmental origins of health and disease (DOHaD) theory suggests that the environment during the prenatal phase of development is associated with lifestyle-related chronic diseases in adulthood.[5] This theory was hypothesized by Dr. David Barker, who observed that low birth weight offspring of British women who were pregnant during the Second World War developed cardiovascular and metabolic disease as adults.[6] During this time period, there were drastic changes in diet due to restricted resources leading to malnourished women.[6] Barker reported that rates of cardiovascular disease in adults were twice as high in poorer areas of Britain with lower socioeconomic status.[6] These two disease prevalence patterns generated the DOHaD hypothesis that adverse conditions in-utero affect disease outcomes later in life. A restricted nutrient environment in-utero is thought to affect development by causing permanent changes to the body's structure, function, and metabolism, resulting in increased risk for future chronic disease.[5] It's important to note that the environmental changes that affect development do not result in birth defects or malformations, but rather are functional changes including altered gene expression that may lead to increased risk for disease, earlier onset of disease, or increased severity of disease.[7] The time period that functional changes occur is during the development of each organ that may be affected, and considering that

different organs develop at different times during pregnancy, there are several windows of opportunity for the environment to affect fetal growth.

One mechanism through which DOHaD is believed to occur is by epigenetic modification of the genome. Two epigenetic modifications that can control gene expression are DNA methylation near gene promoter regions and histone modifications.[7] During pregnancy, the fetal tissues and epigenome are very susceptible to environmental modifications that can predispose cells and tissues to disease and malfunction later in life.[7] Different tissues are developed at different times during pregnancy, which would be the most critical period for environmental epigenetic changes. The DOHad theory has been implemented in research studies around the globe to investigate the relationship between low birth weight, large for gestational age infants, and rapid catch up growth during infancy and risk for disease later in life. Evidence behind the DOHaD hypothesis demonstrates the connection between the in-utero environment and infant development, thus supporting the relationship between diet during pregnancy and infant composition at birth.

Infant Body Composition

Adipose tissue development. The development of fetal adipose tissue has been shown to occur between 14 and 16 weeks of gestation, and classical fat deposit areas are detected by the beginning of the third trimester.[8] However, the exact time period that adipocytes develop has varied between studies suggesting that development could occur through the 23rd week of gestation.[9] Prior to differentiation of fat cells in the 14th week of gestation, fat exists as loose connective

tissue that then aggregates to form masses of mesenchymal cells.[9] At this time, vessels proliferate to develop capillary networks around the mesenchymal cells.[9] The mesenchymal cells then differentiate into preadipocytes that eventually accumulate lipid droplets to form adipocytes.[9] Infants are born with an approximate number of adipocytes at birth, however the sizes of the cells are constantly growing.[8] Body fat percentage in infants can range from 14% to 20% during the first year of life due to changes in adipocyte size.[8] Crume et al. assessed body fat in 1040 US infants using air displacement plethysmography (ADP) and the mean body fat percent at birth was 9.13%.[10] The mean infant body fat percentage from a cohort of 743 Irish infants at birth was 10.8%. [11] Carberry et al. also used ADP for 45 Australian infants and the mean body fat percent at birth was 9.72%.[12] At six weeks follow-up the mean infant body fat percent increased to 19.1% for females and 21.8% for males demonstrating the dramatic increase in adiposity in the months following birth.[12]

*Macrosomia and infant adiposity***.** An infant born at term weighing greater than 4000 grams is considered macrosomic, or large for gestational age, and about 9% of all infants meet macrosomia criteria.[13] Maternal and infant risks associated with macrosomia include shoulder dystocia, maternal anal sphincter injury, neonatal hypoglycemia, post-partum hemorrhage, instrumental vaginal delivery and cesarean section.[14] Maternal factors that increase the risk of infant macrosomia include obesity, diabetes, excess gestational weight gain, and history of giving birth to a macrosomic infant. [13] Maternal diet during pregnancy remains an important opportunity for nutritional interventions to promote both healthy maternal weight

gain during pregnancy and optimal fetal birth outcomes. The relationship between maternal diet and macrosomic birth outcomes will be discussed further.

Determinants of body composition. There is some evidence to support that infant adiposity is determined by maternal pre-pregnancy BMI and gestational weight gain.[8] Neonatal body composition can also be affected by dietary restrictions, low-protein intake, high total daily dietary fat intake, or high dietary saturated fat intake at any period of time during pre-conception, pregnancy, or lactation.[15] Research has shown significant associations regarding protein to carbohydrate ratio, percent of energy as polyunsaturated fatty acids (PUFAs), percent of energy as saturated fat, and fetal mid-thigh lean area and fetal abdominal visceral area.[15] There's also data to support that maternal macronutrient intake may affect infant adiposity greater in females than in males.[16] Other maternal factors that have been shown to influence fetal body composition are low maternal Vitamin D status, short duration of breastfeeding, and smoking during pregnancy.[17]

Infant body composition and later in life outcomes. One area of intense research is how infant body composition at birth affects chronic disease outcomes later in life. Male infants with reduced growth during the first year of life have been shown to have significantly increased waist to hip ratio as adults indicating greater abdominal adiposity.[18] This data suggests that reduced fetal growth may also be associated with chronic diseases related to abdominal adiposity such as obesity, hypertension, Type 2 Diabetes, and cardiovascular disease. The "thrifty phenotype" hypothesis may also play a role in later life outcomes suggesting that if infants are nutrient restricted in utero, all energy is utilized for vital processes such as the heart

and brain, and other organs such as the pancreas or kidneys are restricted from energy. This can have deleterious effects in adult life due to compromised nephron and pancreatic beta cell function, thus contributing to the development of diabetes and kidney disease.[19] There is evidence to suggest that infants who are nutrient restricted as well as infants with over-nutrition have increased risk for chronic disease later in life. It has been proposed that infants with rapid weight gain in the first few months after birth have an increased risk for obesity, abnormal pancreatic beta cell function, and insulin resistance later in life.[20] This is most pronounced for infants who were growth restricted at birth and subsequently experience "rapid catch up growth" during the first years of life. Therefore, infant body composition patterns at birth and subsequent growth trajectories during the infancy period may impact risk for chronic disease in adulthood.

Infant growth. Growth charts have traditionally been used as the standard for measuring infant growth and identifying individuals at-risk for inadequate growth and nutrition. The CDC recommends that healthcare providers use the WHO growth charts for infants less than 24 months to plot weight-for-age, length-for-age, weight-for-length, and body mass index (BMI).[21] Values that are two standard deviations below or above the median, or the 2.3rd and 97.7th percentiles, are indicative of adverse health conditions.[21] Although the WHO growth charts reflect optimal growth, many infants do not experience the same environmental factors that were taken into consideration when designing the WHO criteria, and clinicians should recognize this when assessing infant growth. The American Academy of Family Physicians recommends that failure to thrive can be identified in infants with

weight-for-age, weight-for-length, or BMI-for-age below the 5th percentile or if weight-for age or weight-for-length falls by two major percentiles.[21] Conversely, there are not currently any infant body composition standards to categorize an infant with excess fat mass; however, growth charts can be used as a tool to recognize an infant's rate of weight gain and tendency toward obesity.[21] Not only are clinicians limited in their ability to categorize obesity in infancy, but there are also no evidence-based guidelines for treating overweight infants.[21] In addition to infants with excess fat mass, there is evidence that infants with low fat mass or low weight at birth may be at risk for adverse outcomes later in life depending on the growth trajectory that follows after birth.

Thin infant growth trajectories. Although there is no widely accepted definition for thinness during infancy, thin infants have previously been described as infants born at less than the tenth percentile for body fat percentage.[22] These customized centiles are study-specific and therefore decrease some of these studies' external validity. A study investigating infant growth trajectories concluded that thin infants (defined in this case as infants in the lowest sex-specific third of BMI distribution at birth) had significantly larger increases in body BMI in the first six months of life, although their BMI maintained a stable level in the second year of life.[23] Compared with infants in the highest 66% of BMI distribution, thin infants remained shorter, thinner, and lighter at six months of age and throughout childhood. [23] Generally, the goal for all infants is to maintain the same path on the growth curve from where they started at birth, however some infants experience crossing percentile lines or rapid catch-up growth.

Low birth weight infant growth trajectories. Infants born at low birth weight, less than 2500 grams, followed by high BMI after infancy are considered at high risk for developing hypertension, coronary artery disease, and glucose intolerance. [24] Recent publications have shown that for low birth weight infants, rapid catch-up growth after birth has been associated with increased risk of adult obesity and cardiovascular disease.[23] A prospective cohort study with 559 children showed that rapid increases in postnatal weight for length in first six months of life was significantly associated with risk of obesity by three years of age.[25] Furthermore, a systematic review including 24 studies concluded that infants at the highest end of distribution for weight, or those who grow rapidly during infancy are at significantly increased risk of later obesity.[26]

Macrosomic infant growth trajectories. Neonates that meet macrosomia criteria during infancy may also have adverse health outcomes. Specifically, infants at the highest distribution for weight-for-height are more likely to be obese at ages five to seven years old (odds ratios ranging 1.50-9.38).[26] A review including support from 11 studies concluded that infants who are heavier during infancy are more likely to develop obesity in childhood, adolescence, and adulthood.[26] One common theme across research is that individual studies define infant obesity differently by using either weight for height or infant BMI. Adult obesity is typically defined as a BMI of greater than or equal to 30, however infant measurements are not as consistent.[26] Infant weight-for-length greater than the 95th percentile can be categorized as overweight, however there are no cutoffs for obesity under age two.[27] Research supporting the relationship between infant body composition at birth and

later in life health outcomes demonstrates the importance of investigating modifiable factors that can affect body composition at birth including maternal dietary intake.

Placental Transport

Fetal Nutrition. Nutrition during fetal growth is vital to laying the foundation for body composition at birth, and is dependent on maternal diet and stores during pregnancy. During the fetal period, growth is primarily separated into two phases: histiotrophic and hemotrophic. During the histiotrophic growth phase, the embryo depends on exogenous nutrients found in secretions and tissue debris that transfer through the yolk sac.[28] The mechanisms involved in nutrient transfer to the embryo are still under investigation, however animal studies suggest that the yolk sac plays an important role. Research suggests that the method of nutrient delivery switches from histiotrophic to hemotrophic around twelve weeks of gestation.[28] After the embryonic period, the fetus utilizes hemotrophic nutrition in which nutrients are exchanged from the mother's blood through the placenta to the fetus.[28]

Mother to placenta. The placenta is an organ through which nutrients, including fatty acids, transfer from the mother to the fetus. Although placental regulation of this transfer is not fully understood, this is a major focus of current research. The placenta contains trophoblast cells that are responsible for regulating appropriate bidirectional transport of nutrients and waste for proper growth and development of the fetus.[29] The trophoblast cells have two membranes: microvillus membrane facing the maternal bloodstream and the basal membrane facing the fetal bloodstream.[30] The maternal diet, liver metabolism, and adipose tissue stores

determine the fatty acid composition of the maternal triglycerides in plasma that are exposed to the placenta.[29] The placental trophoblast cells synthesize leptin and secrete it into both maternal and fetal circulation.[31] In maternal circulation, leptin signals for maternal fat stores to be mobilized for increased fat transfer to the fetus.[31] The difference in fatty acid concentrations in the maternal and fetal bloodstream creates a concentration gradient allowing for fatty acids to diffuse into the placenta.[30] Placental leptin secretion increases throughout pregnancy as the fetal to placenta weight ratio increases, thus increasing fatty acid uptake as fetal needs increase.[31] Placental lipoprotein lipase (LPL) and endothelial lipase are enzymes that are present on the maternal-facing membrane of the placental trophoblast cells. These enzymes release fatty acids from triglyceride-rich chylomicrons in the maternal plasma to allow the placenta to uptake non-esterified fatty acids (NEFAs).[31] Placental LPL activity also increases during the third trimester of pregnancy to account for increasing fetal energy requirements. Fatty acids are released from the chylomicrons by hydrolyzing the triglycerides then releasing the fatty acids into the placenta.[29]

Placenta to infant. Some studies have shown that the placenta has selectivity for transfer of PUFAs, specifically docosahexaenoic acid (DHA).[29,31] NEFAs enter the placenta by passive diffusion or by carrier proteins such as fatty acid translocase, fatty acid transport proteins, plasma membrane fatty acid binding protein, or placental plasma membrane fatty acid binding protein.[31] Fatty acid transporters, binding proteins, and lipolytic enzymes on the placenta facilitate transport through the placenta.[32] Trans fatty acids and PUFAs compete for the same binding sites on

the placenta, therefore trans fats often interfere with transport of PUFAs.[29] However, placental membrane binding proteins favor PUFAS over non-essential fatty acids.[33]

Recent evidence from biopsies of human placenta has shown that human placenta expresses microsomal triglyceride transfer protein (MTP) and Apolipoprotein B (ApoB) 100 protein.[34] This allows the placenta to synthesize lipoproteins for fetal circulation, however regulation of this pathway is still uncertain. After fatty acids are in fetal circulation, they are transported to the fetal liver where they bind to alpha-fetoproteins and then enter the liver.[32,35] After the fatty acids enter the fetal liver, they are esterified and then secreted in lipoproteins.[35] At this point in lipid metabolism in the fetus, lipoproteins deliver triglycerides to adipose and muscle tissue through the same mechanisms as adult humans to create adipose deposits. Placenta fatty acid metabolism demonstrates that maternal diet intake during pregnancy may have an effect on infant adipose stores, however recommendations for optimal maternal diet composition to promote healthy birth outcomes remain unclear.

Maternal Dietary Recommendations

The 2015-2020 Dietary Guidelines for Americans states that energy needs during the first trimester of pregnancy are the same as energy needs for non-pregnant women.[36] During the second and third trimester, extra calorie needs are 340 calories and 452 calories per day, respectively.[36] Increased energy is required during pregnancy to account for development of maternal and fetal adipose and protein deposits, the placenta, amniotic fluid, increased blood volume, and increase

in basal metabolic rate.[37] Current recommendations for macronutrient diet composition during pregnancy are the same as general recommendations for all women of childbearing age. The 2015-2020 Dietary Guidelines for Americans recommends this exact same dietary fat intake for all females and males ages 19 years and older.[36] The Academy of Nutrition and Dietetics recommends that during pregnancy 20% to 35% total calories come from dietary fat. This dietary fat intake recommendation also remains the same for male and female adults. It is clear that more research is needed to investigate the role of dietary fat during pregnancy and to develop specific recommendations for saturated fat and unsaturated fat intake for pregnant women.

Dietary Fat

Digestion and absorption of dietary fat begins in the small intestine where it is packaged into micelles, then absorbed into enterocytes and further packaged for recirculation in the body. Circulating maternal fatty acids are then transported across the placenta to the fetus during pregnancy. Stores of adipose tissue are imperative during pregnancy because they are mobilized to provide the most high-quality energy source for the body to use for fetal growth. Both Omega 3 and Omega 6 unsaturated fatty acids have been shown to be involved in fetal programming and important to fetal growth.[32] During the prenatal and postnatal stages, fatty acids play an important role in regulating cellular responses between metabolic and neuroendocrine environments.[32] Fatty acids are also able to act as metabolic sensors by regulating genes involved in energy oxidation and storage.[32] Maternal plasma concentrations of trans fatty acids, arachidonic acid (AA), DHA, linoleic acid

(LA), and alpha linolenic acid (ALA) are significantly and positively associated with infant plasma concentrations of the same fatty acids.[29] This evidence suggests that excess fatty acid intake during pregnancy may affect infant plasma fatty acid concentrations and therefore infant metabolic programming.

Unsaturated fat. It has been shown that low consumption of fish during pregnancy is associated with higher infant risk for cognitive and behavioral problems.[32] The three main Omega 3 PUFAs are DHA, ALA, and eicosapentaenoic acid (EPA). Adults are able to synthesize some DHA from EPA and ALA; however, the fetus has a decreased ability to synthesize DHA due to lack of fetal enzyme activity in the placenta.[32,38] The delta-5 and delta-6 desaturase enzymes are responsible for synthesizing DHA and EPA in the fetus.[33] Although these enzymes are present in the fetal liver during gestation, the activity of these enzymes is low until birth.[33] Therefore, it is imperative that pregnant women supplement Omega 3 fatty acids in their diet because they provide the main source of DHA and EPA for the fetus, which are essential for infant neurological development. Although DHA and APA have been shown to be vital to infant development, there are currently no recommendations for a specific amount of maternal unsaturated fat during pregnancy to promote healthy birth outcomes.

Omega 6 and Omega 3 fatty acids also impact other areas of fetal growth including adipocyte formation and tissue development. [38] Linoleic acid is an Omega 6 fatty acid that is then converted to dihomo-gamma-linolenic acid (DGLA) and AA.[30] Eicosanoids derived from AA have been shown to be pro-inflammatory and have the ability to increase blood flow, cytokines, edema, pain, and fever.[31] Eicosanoids

derived from Omega 6 fatty acids, such as AA, have been shown to play a role in fatty acid uptake and pre-adipocyte differentiation during the early growth phase of adipose tissue in the fetus. On the other hand, eicosanoids derived from Omega 3 fatty acids (DHA and EPA) have been shown to decrease growth of adipose tissue.[38] It's also hypothesized that the ratio of Omega 6 to Omega 3 (n6:n3 ratio) fatty acids in the maternal diet during pregnancy may affect adipose tissue development in the fetus.[39]

Maternal diets low in EPA and high in AA are associated with reduced fetal growth and 40-50% increased risk of small for gestational age infant.[40] Infants born with high levels of DHA and AA have been shown to maintain increased levels for several weeks after birth.[35] This evidence suggests that fatty acids that are stored in fetal adipose tissue prenatally are then released after birth to provide continued benefits for the infant outside of the womb. Further research is needed to explore the adipogenic mechanism of PUFAs. High maternal intake of monounsaturated fatty acids (MUFAs) has been shown to be protective against low birth weight in animal studies.[29] MUFAs have been hypothesized to be able to stimulate thermogenic capacity and changes in liver metabolism.[29] Therefore optimizing composition of maternal diets during pregnancy has potential to change fatty acid levels of the infant at birth to promote adequate growth and neurodevelopment.

Saturated fat. Saturated fatty acids also have the ability to affect fetal growth. Saturated fats may influence gene expression by acting on transcription factors or elevating levels of messenger RNA.[41] The genes affected by saturated fat include those involved in the synthesis and metabolism of cholesterol, fatty acids,

triglycerides, phospholipids, and genes for fatty acid synthase and the low-density lipoprotein (LDL) receptor.[41] Some animal studies have shown that rat offspring of mothers fed a high saturated fat diet present with increased body mass, visceral fat, adipocyte hypertrophy, and insulin resistance.[29] Insulin resistance and increased leptin levels, as a result from adipocytes, increases oxidative stress thus interfering with cell-signaling pathways involved in fetal development.[29]

Trans fat. It is well known that trans fatty acids affect lipid metabolism, endothelial function, risk for developing cardiovascular disease, and insulin resistance.[29] The main trans fatty acid in the diet is elaidic acid, which can inhibit the conversion of ALA to DHA and EPA.[40] Trans fatty acids can increase oxidative stress and inflammatory cytokines, thus affecting metabolic programming.[32] Trans fatty acids can also increase LDL cholesterol, and decrease high-density lipoprotein (HDL) cholesterol.[29] Levels of trans fatty acids in infants have been inversely associated with levels of AA, DHA, and birth weight.[35] This phenomenon can be explained by the ability of trans fatty acids to interfere with Omega 3 and Omega 6 fatty acid metabolism, or by maternal diets high in trans fatty acids.[35] Trans fatty acids can also metabolize Omega 3 and Omega 6 fatty acids into unusual fatty acid isomers that disrupt membrane functions and eicosanoid pathways.[42]

Evidence for Maternal Dietary Fat and Infant Adiposity

Animal studies. Several animal studies have concluded that maternal over-nutrition, including high dietary fat intake, is associated with changes to offspring body composition. It has been shown that after exposure to a maternal diet rich in animal lard, offspring mice exhibit hyperglycemia, hyperinsulinemia, reduced

pancreatic beta cell function, and whole body insulin resistance.[43] However, for offspring from mothers with primarily high PUFA fat intake, glucose tolerance has been shown to be normal.[43] This may suggest that saturated fat or trans fat may be the contributing factor to offspring adiposity. A multi-generational study followed mice for three pregnancies to compare maternal diets with 60% and 10% of calories from fat. This study showed increased fat mass and less lean body mass in offspring from mothers with high fat diets.[44] During the first pregnancy, the high fat diet had not yet induced maternal obesity, yet the pups from the high fat diet mothers had significantly more fat mass then their low fat counterparts.[44] In the following two pregnancies, body fat increased dramatically for each pregnancy in both males and females.[44] This suggests that as the maternal mice became more obese from the high fat diet, the body composition results were exaggerated in the offspring. The lack of maternal obesity during the first pregnancy suggests that the high fat diet may have played a role in the offspring adiposity. It is known that maternal obesity is also a confounder for increased infant adiposity and it's important to consider these highly correlated variables when discussing birth outcomes. It's interesting to note that all of these effects on animals represent that of criteria for metabolic syndrome in adult humans.

Human studies. Several studies have also been completed in humans in effort to examine the relationship between maternal dietary fat intake and infant body composition. However, results are limited in that there are very few randomized controlled trials, and studies are lacking in sample size, study design, and depth and breadth of type of dietary fat investigated. A large prospective cohort study

including 1040 mother-offspring pairs concluded that every 100 calorie increase in total fat, saturated fat, and unsaturated fat was significantly associated with 4.2 grams, 11.1 grams, and 5.9 grams increase in neonatal fat mass, respectively.[45] This study adjusted for nine different covariates providing an even stronger indication that the increase in fat mass is significantly associated with maternal dietary fat intake. Some studies have observed different adiposity association in males compared to females. For example, a prospective cohort study concluded that increased maternal total daily dietary fat intake was associated with higher total abdominal fat and all abdominal fat subtypes in males only.[16]

In addition to fat mass, total abdominal fat, percent body fat, and neonatal waist circumference to length ratio are other accurate measures of neonatal adiposity. One of the only randomized controlled trials in this area of research concluded that increased neonatal waist circumference to length ratio is associated with increased maternal saturated fat intake during the third trimester of pregnancy.[14] More specifically, abdominal adiposity was also positively associated with maternal saturated fat intake during the first and second trimester.[14] This randomized controlled trial is a key study in providing evidence for the relationship between maternal dietary fat intake and infant adiposity.

Offspring fat mass has also been shown to increase as result of the maternal n6:n3 ratio and total unsaturated fat. One particular study showed that each unit increase of estimated maternal n6:n3 ratio intake was significantly associated with a 21 gram increase in female infant fat mass.[38] Very few quality studies have investigated the specific relationship between maternal n6:n3 ratio and infant body

composition. However, there has been some evidence to support the relationship between maternal n6:n3 ratio, body composition during the first year, and body composition later in life.[39,46,47] Abdominal fat mass in infants has been significantly negatively associated with umbilical cord levels of LA, AA, EPA, and DHA.[48] However, it's unclear how these circulating fatty acid levels are related to the mother's dietary fat intake. A small prospective cohort study concluded that for every one percent isoenergetic decrease in maternal saturated fat intake, mid-thigh subcutaneous fat area decreased by 0.27%, and for every one percent isoenergetic increase in maternal PUFA, mid-thigh subcutaneous fat area decreased by 0.48%. [15] However, this study did not report any significant differences with abdominal visceral fat and maternal dietary fat intake.

Chapter 3. Methods

Study Design

This was a sub-study of a cross-sectional study of healthy pregnant women with a singleton gestation. The primary study, investigating how maternal body composition regulates placental function and fetal growth, was approved by the OHSU institutional review board (IRB) (eIRB #1175) and the principal investigator was Nicole Marshall, MD, MCR. This sub-study was a secondary analysis of data from mother-baby pairs in part one of the primary study who were enrolled at 12 to 16 weeks gestation. For this sub-study, the mother-baby pairs were followed for the remainder of pregnancy and until infant anthropometrics were measured at birth. This sub-study included a total of 4 visits, which occurred at each of the following times: 12 to 16 weeks gestation, 24 to 28 weeks, 37 weeks, and at delivery. Women completed Block food frequency questionnaires (FFQ) at the first three study visits to assess maternal dietary fat intake during each trimester of pregnancy. The infant skinfold test took place within 24 hours of birth to assess infant body composition.

Study Population

This sub-study included 79 mother-baby pairs from the primary study. Women were studied to assess the relationship between maternal dietary fat intake during pregnancy and infant body composition at birth. This study was performed in OHSU outpatient clinics, in the Clinical and Translational Research Center (CTRC), Labor and Delivery, OHSU laboratories, and study personnel offices.

Recruitment

Subjects for this sub-study were analyzed from part one of the primary study who were already enrolled and delivered by March 23, 2018. Subjects in the primary study were recruited from obstetric providers at OHSU including obstetricians, perinatologists, residents, nurse midwives, and family medicine physicians. The primary study was advertised in flyers at OHSU, included in relevant presentations of current OHSU research studies, presented at OHSU's birth classes and advertised online in OHSU's Center for Women's Health recruitment website. Mothers who obtained prenatal care at OHSU clinics and planned to deliver at OHSU were identified via the OHSU electronic medical record. Those who appeared to meet the inclusion and exclusion criteria (Table 1) were sent a letter detailing the study, or approached for participation by study staff by phone or at a routine prenatal clinic visit to determine the patient's interest in the study and to schedule a study visit.

Consent

If subjects expressed interest, they were scheduled for a study visit where full informed consent took place before any study activities. The study was described in detail, and subjects were provided a current version of the OHSU IRB-approved consent form to review. The purpose of the study, procedures, risks, benefits, and alternatives were discussed with the subject. Subjects were given adequate time to ask questions prior to signing. The study staff collected and stored the signed consent forms in the research records, and the subjects received a copy. Documentation of the consent process was maintained in the research record and

patient's medical record. A flag was placed in the subject's electronic medical record stating that the patient was enrolled in the study and that the study staff on call was to be notified when the subject is admitted for delivery.

Table 1. Inclusion and Exclusion Criteria for Study Participant Recruitment

Inclusion Criteria	Exclusion Criteria
1. Maternal age of at least 18 years. 2. Viable pregnancy as confirmed by ultrasound/cardiac activity 3. Able to understand and provide an informed consent. 4. Able to understand English or Spanish. 5. Babies (considered viable within the first 30 days of life) born to mothers enrolled during this study	1. Multiple gestation (i.e., carrying >1fetus) 2. Maternal active hepatitis or HIV infection. Hepatitis and HIV testing are conducted as part of routine screening prenatal blood tests. 3. Documented fetal congenital anomalies 4. Current maternal history of heroin, cocaine, crack, LSD, or methamphetamines. "Current history" is defined as during pregnancy. 5. Current history of alcohol abuse. "Current history" is defined as during pregnancy. If subject drinks more than 1 drink per day (1 drink is equivalent to 8 ounces of beer or wine, or 2 ounces of hard liquor), the subject is excluded. 6. Known maternal rheumatological or chronic inflammatory state (i.e. Lupus erythematosis), per investigator discretion 7. Chronic illness, including hypertension, which requires regular use of medication (i.e. prednisone), per investigator discretion. Subjects who use anti-coagulants and/or anti-depressants (i.e. aspirin, lovenox) will be allowed in the study. 8. Known chorioamnionitis 9. Any significant medical complication, as deemed by investigator 10. Non-English speaking subjects (except Spanish) are excluded from this study. The standardized surveys are not validated in any other languages. 11. Neonates of uncertain viability and non-viable neonates

Vulnerable Populations

Pregnant women. Pregnant women, ages 18 years and older, were included in this study, as the scientific question concerns the effects of maternal diet during

pregnancy and infant body composition at birth. Each maternal subject was fully informed of the study procedures, risks, and alternatives.

Infants. Babies born to mothers enrolled during this study and who met inclusion and exclusion criteria were included as subjects in this study. Neonates of uncertain viability and non-viable neonates were not used in this research.

Non-English speaking subjects. For those Spanish-speaking subjects, a Spanish short form was used. A Spanish translator was present for the initial consent process and subsequent study visits. Lastly, the 2005 Block FFQ was available in English and Spanish.

Study Protocol

The primary study included seven study visits for women enrolled in part one of the study. This sub-study only included data from the first four study visits of part one of the primary study. Study protocol regarding the remaining study visits, including those for women enrolled in part two of the primary study, can be found in the primary study protocol. The following study visits include details that are relevant to this sub-study, full details for the primary study can be found in the primary study protocol.

First Study Visit. The first study visit occurred between 12 and 16 weeks gestation. The visit began with the informed consent process. Potential subjects were asked to arrive at the visit in a fasted state. Those subjects interested in participating in the study were provided a signed informed consent and health insurance portability and accountability act (HIPAA) authorization prior to performing any other study-related procedures. Following consent, study personnel

reviewed inclusion and exclusion criteria. Study personnel collected the following standard of care: pre-pregnancy weight, maternal age, parity, smoking status, height, gestational type (singlet, twins, triplets), first trimester weight (as measured in the clinic at the patient's first prenatal appointment). Maternal BMI was calculated from first trimester height and weight measurements, which have been shown to change little throughout the first trimester, and more accurately reflect pre-pregnancy BMI than self-reported pre-pregnancy weight. Subjects were also provided a wallet card to give to OHSU labor and delivery staff on the day of delivery, with instructions for contacting study staff for sample collection. The Block FFQ was also completed during the 12-16 weeks gestational period and covered intake for the 3 months prior to their study visit. The majority of participants completed the Block FFQ during their initial study visit or online. Participants who chose not to complete it during their first study visit were emailed a link with instructions and access to the Block FFQ. If the surveys were not received as requested, study personnel followed-up with the subject and conducted the surveys by phone or at the time of a routine prenatal visit. Following completion of the survey, subjects were provided a $40 gift card for their time completing the visit.

Second study visit. The second study visit occurred between 24 and 28 weeks gestation. During this time period, subjects were e-mailed the instructions and access to complete the Block FFQ at home when they were within the survey window. If the subjects' survey responses were not received, the same protocol followed as defined in the first study visit. Following completion of the survey, subjects were provided a $10 gift card for their time completing the visit. Subjects

also had a routine prenatal visit with their personal provider between 24 and 28 weeks gestation at which time their personal provider obtained the following standard of care information: second trimester weight, results of standard of care prenatal testing (i.e. glucose tolerance testing). For study purposes, the information from this visit was collected through chart review at a later time.

Third study visit. The third study visit occurred at or beyond 37 weeks gestation. The majority of subjects completed their Block FFQ at the time of their in-person visit. Those who chose to complete it at a later time received a link via e-mail and followed the same protocol as previously mentioned in study visits one and two. After completion of the survey, subjects were provided a $40 gift card for their time completing the visit.

Fourth study visit. The fourth study visit occurred following delivery of the infant. Study personnel collected the following standard of care information about the maternal subject: gestational age at delivery, total gestational weight gain, medical information of delivery (i.e. type of delivery, complications during delivery, medications given during delivery), and medical information of complications during pregnancy (i.e. diabetes, hypertension). Study personnel collected the following standard care information about the infant subject: body composition at delivery (i.e. length, weight, abdominal circumference, and head circumference) and body composition changes prior to discharge from hospital. Flank skin fold thickness measurements of the infant were completed within 24 hours of birth per Catalano et al. procedures as described later in the methods.

Study Procedures

Sharing of results. Subjects could request the results of their body composition analysis, which were available as non-diagnostic studies. However, other study results were not shared with subjects or their providers because the research was still in an early phase and the reliability of the results was unknown.

Table 2. Schedule of Events

Event	Study Visit #1 12-16 Weeks	Study Visit #2 24-28 Weeks	Study Visit #3 ≥37 Weeks	Study Visit #4 Delivery ± 6 hrs
Informed consent	✓			
Inclusion and Exclusion	✓			
Maternal Characteristic Information	✓			
Block FFQ Survey	✓	✓	✓	
Infant skin-fold				✓
Infant Characteristic Information				✓
Compensation	✓	✓	✓	

Neonatal measurements. Following delivery, neonatal weight, length, abdominal circumference, head circumference, and sex were recorded by the PI or collected from the infant's electronic medical record. Length measurements were conducted using Grafco paper tape measurers on the labor and delivery unit of OHSU to the nearest half centimeter. Length was measured by flattening the infant's knees, straightening both legs, and marking the paper underneath the infant. The markings on the paper were the measured and recorded as the infant length. Infants were weighed using the scale inside the General Electric Panda iRES Bedded

Warmer in the delivery rooms at OHSU. Flank skin fold thickness measurements of the infant were completed within 24 hours of birth using Lange skinfold calipers. The flank skinfold was measured in the midaxillary line just above the crest of the ileum.[49] For each measurement, the skin was lifted with the thumb and index finger without including any underlying tissue. Each skinfold measurement was repeated three times and then averaged. Neonatal fat mass was calculated per Catalano et al. Neonatal fat mass (kg) = 0.39055 (Birth weight in kg) + 0.0453 (Flank skinfold in mm) - 0.03237 (Length in cm) + 0.54657.

Dietary assessment. The 2005 Block FFQ survey was used to collect information regarding dietary fat intake and diet composition over three month time periods. The Block FFQ was developed at the United States National Cancer Institute in 1984, and has been widely used in nutritional epidemiology.[50] The 2005 Block FFQ contains approximately 110 food items and was designed to estimate usual and customary intake of a wide array of nutrients and food groups. The food list included in this questionnaire was developed from the National Health and Nutrition Examination Survey (NHANES) 1999-2002 dietary recall data and the nutrient database was developed from the US Department of Agriculture (USDA) Food and Nutrient Database for Dietary Studies, version 1.0. The Block FFQ contains adjustment questions to provide increased accuracy when assessing fat and carbohydrate intake. For each food in the questionnaire, individual portion size is asked and pictures are provided in effort to increase accuracy of diet composition results. For this sub-study, the survey was conducted during the gestational time

periods of study visits one, two, and three to recall the prior three month period either in clinic or online through an access link provided by the study coordinator.

Statistical Analysis

Descriptive statistics were collected and summarized as the mean, standard deviation, and range for each numerical variable, or as frequency and percent for each categorical variable (Table 3). Maternal variables of interest included age, height, pre-pregnancy weight, pre-pregnancy BMI, parity, race, ethnicity, tobacco smoking status, total gestational weight gain, gestational diabetes status, average energy intake, average raw dietary fat intake, and average dietary fat intake as a percent of energy. Infant characteristics at birth included gestational age, sex, weight, head circumference, length, abdominal circumference, flank skinfolds, fat mass (per Catalano equation), body fat percentage, ponderal index, and waist to length ratio. Standardized reference curves do not exist for infant abdominal circumference, ponderal index, body fat percentage, or fat mass so all subjects were compared across their distributions.

The primary outcome for the first aim of this sub-study was to determine the relationship between intake of maternal total daily dietary fat and infant adiposity at birth. For each subject, total daily dietary fat intake (grams/day) was calculated for each trimester, and then trimester intakes were averaged together to calculate an average daily total daily dietary fat intake over the total pregnancy. The variables being analyzed were all numerical continuous variables, so correlation analysis was the most appropriate method to test for a relationship between maternal dietary fat intake and infant adiposity at birth. Pearson's correlation coefficients (r) were used

to quantify the linear association between total daily dietary fat intake and each of the following variables: abdominal circumference, waist to length ratio, ponderal index, fat mass, and body fat percentage. Crude correlation results can be found in Tables 4 through 7. For those results that were statistically significant, multiple linear regression analysis was used to adjust for confounding variables (Tables 4, 5, 6, and 7).

For each outcome of interest, a multiple linear regression model was fit that included seven explanatory variables: maternal age, maternal total gestational weight gain, maternal pre-pregnancy BMI, maternal parity, infant gestational age at birth, infant birth weight, and maternal average total energy intake. A partial *F*-test was used to test this initial model against a simpler one that only adjusted for four variables of the initial seven: total gestational weight gain, pre-pregnancy BMI, gestational age at birth, and infant birth weight. This partial *F*-test assessed whether the ensemble of omitted terms (in this case maternal age, maternal parity, and total energy intake) provides useful information beyond those four explanatory terms included in the smaller, simpler model. The *F*-statistics ranged from 0.30 to 1.74 (p values from 0.17 to 0.80) demonstrating that for each response of interest, the model including four variables was not significantly worse than the model with seven variables. In effort to not over fit the regression model, the latter model was used for all adjusted correlation analyses. Partial correlations (r_{part}) adjusting for the four potential confounding variables are found in Tables 4, 5, 6, and 7. These partial correlations quantify the linear association between the two variables of interest, after any joint influence from the four variables (total gestation weight gain, pre-

pregnancy BMI, gestational age at birth, and infant birth weight) was removed from each. Average energy intake was not selected for the adjustment model because it is highly correlated with fat intake (r =0.942) and would have produced collinearity within the model. For relationships that remained significant after adjustment, a partial correlation was also calculated with energy intake as the independent variable and the body composition marker of interest as the dependent variable (Table 11). These partial correlations were compared to the partial correlation with maternal dietary fat as the independent variable to determine if the proposed relationship was independent of energy intake. To further address energy intake, each of the correlation analyses and multiple regression analyses were repeated with total fat intake as a proportion of total energy intake (% of kilocalorie [kcal] from fat) as opposed to raw fat intake (grams/day). Any correlation with a significant result ($p<0.05$), was further analyzed with the multiple regression model, described above, to calculate a partial correlation (Table 5).

The second aim of this sub-study was to evaluate if different types of dietary fats are separately associated with infant adiposity at birth. This analysis tested the relationship between raw maternal total saturated fat intake, total unsaturated fat intake, and n6:n3 ratio and each of the infant adiposity markers mentioned above. Correlation and linear regression analysis was completed for raw maternal total saturated fat intake (Table 6), total unsaturated fat intake (Table 8), and n6:n3 ratio (Table 10) using the same method previously mentioned for the first study aim. This analysis was repeated investigating maternal fat intake as a proportion of energy

and markers of infant adiposity for saturated fat intake (Table 7) and unsaturated fat intake (Table 9). All statistical analysis was performed using STATA 15 software.

Figures showing the crude correlations with raw maternal fat intake are represented by scatterplots of the variables shown in Figures 2A, 2B, 3A, 3B, 4A, and 4B and were created in STATA 15. Although our study contained multiple comparisons, statistical adjustment for multiple comparisons was deemed unnecessary for this investigation, as the goal of this cross-sectional study was to evaluate relationships between variables and generate hypotheses for future research.

Chapter 4. Results

Participant Characteristics

Maternal Characteristics. Maternal FFQs and infant body composition measurements were complete for 79 mother-baby pairs included in the analysis (Figure 1). Twenty subjects were excluded for missing infant body composition data, missing maternal dietary data, or both. Two infants, weighing 2.1 kg and 2.285 kg, were excluded from the analysis because they produced a negative fat mass result after analysis with the Catalano equation, which is explained in detail in the discussion. Maternal participant characteristics are given in Table 3. The mean pre-pregnancy BMI of mothers was 27.4 kg/m^2, ranging from underweight to class

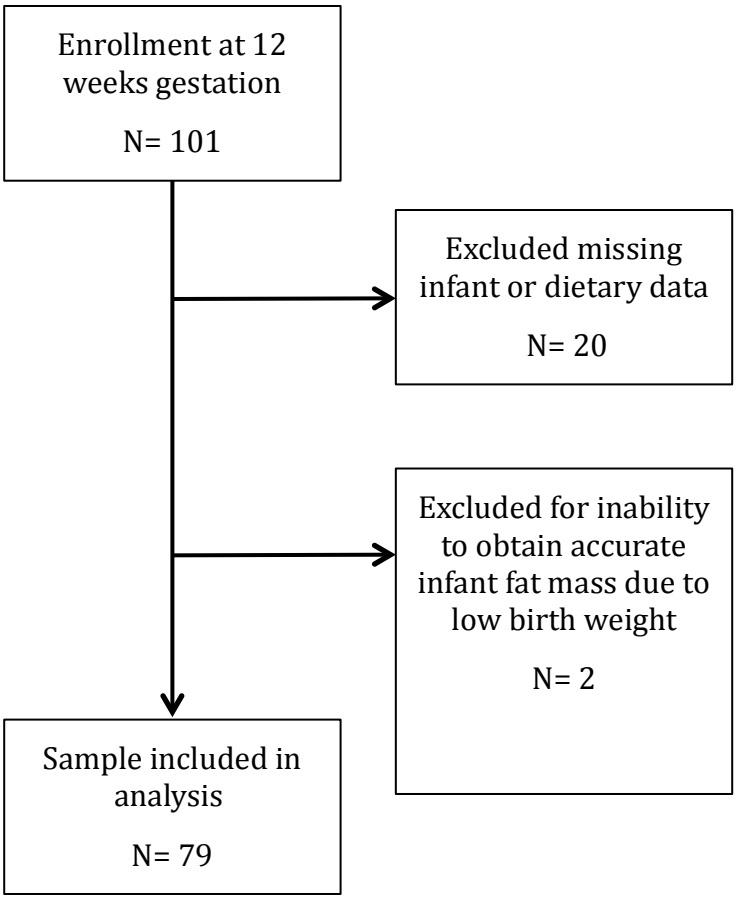

Figure 1. Participant Flowchart

three obesity. Gestational diabetes mellitus (GDM) affected 8.9% of the pregnancies in this study, which is congruent with national prevalence estimates for GDM. Four women lost weight during their pregnancy, and about 58% of the mother participants gained below or within the Institute of Medicine gestational weight gain guidelines for their pre-pregnancy BMI category. The mean percent of calories from fat was 40.8%, and the current Dietary Guidelines for Americans recommends 20-35% of calories from fat for adult females. [36]

Infant Characteristics. This analysis included almost equal proportions of male and female infants (Table 3). The mean gestational age at delivery was 39.6 weeks, and there was one preterm infant born at 34.7 weeks gestation. The mean birth weight was 3.45 kg including three low birth weight infants less than 2500 grams. None of the infants were very low birth weight or extremely low birth weight. The mean fat mass was 0.38 kg fat mass, and body fat percent ranged from 2.9% to 20.1%. Eleven infants were considered to have a low ponderal index, or less than one standard deviation below the mean.

Study Aim #1. The first aim of this sub-study was to determine the relationship between intake of maternal total daily dietary fat and infant adiposity at birth, the results of which can be seen in Table 4. Infant body fat percentage demonstrated a weak correlation with raw maternal total daily dietary fat intake in the second trimester, third trimester, and average intake throughout the entire pregnancy (r=.2432 p=0.0308; r=.2599 p=.0207; r=0.249 p=0.027, respectively) (Figures 2A, 2B). After adjustment for potential confounding variables, this relationship remained significant for dietary fat intake during the second trimester

Table 3. Participant Characteristics

Characteristic	All Subjects[a,-c] N=79
Maternal	
Age (years)	32.9 ± 4.42 (23-43)
Height (cm)	165.2 ± 6.24 (147.3-182.9)
Pre-pregnancy weight (kg)	75.1 ± 18.50 (46.3-145.2)
Pre-pregnancy BMI (kg/m^2)	27.4 ± 6.16 (17.9-50.1)
Total gestational weight gain	12.1 ± 6.32 (-5.2-23.5)
Energy per day (kcal)	1666.5 ± 502.62 (650-2825)
Fat per day (grams)	75.7 ± 25.44 (31.7-138.2)
Percent calories from fat (%)	40.8 ± 4.64 (28.5-51.6)
Parity	
Nulliparous	44 (56%)
Multiparous	35 (44%)
Race[c]	
White/Caucasian	63 (80%)
Black	5 (6%)
Asian	5 (6%)
Other	10 (13%)
Ethnicity	
Hispanic	7 (9%)
Non-Hispanic	67 (85%)
Unknown/Declined	5 (6%)
Smokes tobacco	
Never smoked	59 (75%)
Previous smoker	20 (25%)
Gestational Diabetes	
Yes	7 (9%)
No	72 (91%)
Infant at Birth	
Gestational age (weeks)	39.6 ± 1.31 (34.7-42.1)
Weight (kg)	3.5 ± 0.51 (2.3-5.0)
Length (cm)	51.4 ± 2.480(45.5-58.0)
Head circumference (cm)	34.6 ± 1.35 (30.5-37.5)
Abdominal circumference (cm)	33.6 ± 2.35 (27.5-39.5)
Waist to Length Ratio (cm)	0.7 ± 0.04 (0.6-0.8)
Ponderal index (kg/cm^3)	25.3 ± 2.74 (18.8-34.9)
Fat mass (kg)	0.4 ± 0.18 (0.1-1.0)
Body Fat Percentage (%)	10.6 ± 3.57 (2.9-20.1)
Sex	
Male	40 (51%)
Female	39 (49%)

Abbreviations: BMI, body mass index; kg, kilogram; cm, centimeter; kcal, kilocalorie

[a] Values expressed as mean ± standard deviation (min-max)

[b] Values expressed as N (percent) -

[c] Subjects reported all groups that apply

and average intake during the pregnancy (r_{part} =0.252, p=0.029; r_{part} =0.233, p =0.045, respectively). Infant fat mass presented a weak correlation with raw maternal total daily dietary fat intake in only the third trimester (*r*=0.245 p=0.03). However, this finding did not remain significant after adjustment for confounding variables. There were no relationships between raw maternal total daily dietary fat intake and infant abdominal circumference, waist to length ratio, or ponderal index for any trimester or throughout pregnancy (Table 4).

The results evaluating the relationship between maternal total dietary fat intake as a proportion of energy and infant body composition are seen in Table 5. Infant ponderal index at birth demonstrated a weak relationship with maternal total dietary fat intake in the first trimester (*r*=0.236, p=0.036), but this association did not persist after adjustment for confounding variables. There were no relationships between maternal total daily dietary fat intake as a proportion of energy and infant abdominal circumference, waist to length ratio, fat mass, or body fat percent for any trimester or throughout pregnancy (Table 5).

Study Aim #2. The second aim of this sub-study was to evaluate if different types of dietary fats are independently associated with infant adiposity at birth (Table 6). A weak correlation was evident between infant body fat percentage and raw maternal saturated fat intake in the second trimester, third trimester, and average intake during pregnancy (*r*=0.245 p=0.030; *r*=0.231 p=.041; *r*=0.250 p=0.026, respectively). The crude relationships for the second trimester and entire pregnancy are shown in Figures 3A and 3B. After adjusting for the same potential confounding variables mentioned earlier, these results stayed significant only for

the second trimester saturated fat intake (r_{part} =0.231 p=0.046). Infant fat mass showed a weak correlation with raw maternal saturated fat intake during the third trimester and average intake throughout pregnancy (r =0.224 p=0.047; r =0.224 p=0.047, respectively). These relationships did not remain significant after adjustment with the above model. There were no relationships between raw maternal saturated fat intake and infant abdominal circumference, waist to length ratio, or ponderal index for any trimester or throughout pregnancy (Table 6).

Maternal saturated fat intake was also evaluated as a proportion of energy (Table 7). Infant ponderal index and waist to length ratio both demonstrated weak relationships with maternal saturated fat intake as a percent of energy in the second trimester of pregnancy (r =0.236 p=0.036; r =0.229 p=0.042, respectively), and neither relationship remained after adjustment for confounding variables. Infant body fat percent was nearly associated with saturated fat intake as a percent of energy in the second trimester of pregnancy (r=0.222, p=0.050). Infant body fat percent was not associated with saturated fat intake as a percent of energy for any other trimester or throughout the entire pregnancy. There were no relationships between maternal saturated fat intake as a proportion of energy and infant abdominal circumference or fat mass for any trimester or throughout pregnancy (Table 7).

The results evaluating the relationship between maternal dietary unsaturated fat intake and infant body composition are seen in Table 8. Infant body fat percent demonstrated a weak relationship with raw maternal unsaturated fat intake during the second trimester, third trimester, and average overall intake

during pregnancy (r =0.227 p=0.045; r =0.269 p=0.017; r =0.236 p=0.036). The crude relationships for the second trimester and entire pregnancy are shown in Figures 4A and 4B. After adjustment for the aforementioned variables, only unsaturated fat intake during the second trimester and average intake over the entire pregnancy were significant (r_{part} =0.250 p=0.031; r_{part} =0.241 p=0.037) (Figures 4A, 4B). Infant fat mass was weakly correlated with raw maternal unsaturated fat intake in the third trimester of pregnancy (r=0.249 p=0.027). However, this relationship did not remain significant after adjustment for confounders. There were no relationships between raw maternal unsaturated fat intake and infant abdominal circumference, waist to length ratio, or ponderal index for any trimester or throughout pregnancy (Table 8).

The relationship between maternal unsaturated fat intake as a proportion of energy is demonstrated by the results in Table 9. There were no significant relationships between maternal unsaturated fat intake as a percent of energy and infant abdominal circumference, ponderal index, waist to length ratio, fat mass, or body fat percent for any trimester or throughout pregnancy (Table 9).

Table 10 illustrates an additional analysis performed to evaluate for any relationship between infant body composition and maternal n6:n3 ratio. There was a weak relationship between average maternal n6:n3 ratio throughout the entire pregnancy and infant waist to length ratio at birth that remained significant after adjusting for confounding variables (r_{part} =0.2760, p=0.017). There were no relationships between maternal n6:n3 ratio and infant abdominal circumference,

ponderal index, fat mass, or body fat percent for any trimester or throughout pregnancy (Table 10).

Due to the highly correlated nature of maternal energy and fat intake, (i.e. women who consume more calories typically also consume more fat) the analysis was repeated to evaluate maternal energy intake across trimester and infant body composition. After adjusting for confounding variables, there was no significant relationship between maternal energy intake during the second trimester or average intake throughout pregnancy and infant body fat percent at birth ($p=0.05$, $p=0.065$ respectively) (Table 11). However, the p-values are near significant and have very similar correlation coefficients to the fat intake variables. This issue was further addressed by the additional analyses with fat intake as a proportion of energy in Tables 5, 7, and 9. Many of the significant relationships with raw fat intake (Tables 4, 5, and 6) were not reflected in results where fat intake was analyzed as a proportion of maternal energy intake (Tables 5, 7, and 9). These results suggest that maternal fat intake may not have a relationship with infant body composition separate from maternal energy intake, and that maternal energy intake may be driving the results.

For all correlation analyses, the magnitude of the adjusted correlated coefficients were very near the crude coefficients suggesting that the variables in the adjustment model were minimal confounders. However, during the third trimester, the adjusted correlation coefficients decreased notably indicating that the variables adjusted for may play a different role in infant body composition during this time period.

Table 4. Maternal Total Daily Dietary Fat Intake (grams/day) and Infant Body Composition

	1st Trimester				2nd Trimester				3rd Trimester				Total Pregnancy			
Variable	Crude[a]		Partial[b]		Crude[a]		Partial[b]		Crude[a]		Partial[b]		Crude[a]		Partial[b]	
	r	*p*[c]	*r*	*p*[c]	*r*	*p*[c]	*r*	*p*[c]	*r*	*p*[c]	*r*	*p*[c]	*r*	*p*[c]	*r*	*p*[c]
Body Fat Percentage(%)	0.163	0.151	-	-	0.243	0.031	0.252	0.029	0.260	0.021	0.159	0.172	0.249	0.027	0.233	0.045
Fat mass (g)	0.130	0.254	-	-	0.211	0.062	-	-	0.245	0.030	0.129	0.269	0.219	0.052	-	-
Abdominal circumference (cm)	-0.056	0.624	-	-	0.001	0.995	-	-	0.160	0.158	-	-	0.043	0.710	-	-
Waist to Length Ratio (cm)	-0.008	0.947	-	-	0.039	0.733	-	-	0.089	0.435	-	-	0.046	0.686	-	-
Ponderal index (kg/cm^3)	0.172	0.130	-	-	0.184	0.105	-	-	0.133	0.242	-	-	0.181	0.111	-	-

Abbreviations: *r*, Pearson's correlation coefficient; *p*, p-value; cm, centimeter; kg, kilogram;g, gram; %, percent

[a] Crude correlation analysis without any adjustment models

[b] Partial correlation analysis adjusting for maternal total gestational weight gain, pre-pregnancy BMI, infant gestational age at birth, and infant birth weight

[c] P-Value <0.05 is considered statistically significant

Table 5. Maternal Total Daily Dietary Fat Intake (% of kcal) and Infant Body Composition

	1st Trimester				2nd Trimester				3rd Trimester				Total Pregnancy			
Variable	Crude[a]		Partial[b]		Crude[a]		Partial[b]		Crude[a]		Partial[b]		Crude[a]		Partial[b]	
	r	*p*[c]	*r*	*p*[c]	*r*	*p*[c]	*r*	*p*[c]	*r*	*p*[c]	*r*	*p*[c]	*r*	*p*[c]	*r*	*p*[c]
Body Fat Percentage(%)	0.124	0.278	-	-	0.194	0.087	-	-	0.148	0.192	-	-	0.186	0.100	-	-
Fat mass (g)	0.115	0.311	-	-	0.182	0.108	-	-	0.163	0.152	-	-	0.185	0.103	-	-
Abdominal circumference (cm)	0.036	0.754	-	-	0.136	0.231	-	-	0.121	0.289	-	-	0.121	0.290	-	-
Waist to Length Ratio (cm)	0.123	0.265	-	-	0.180	0.113	-	-	0.017	0.880	-	-	0.132	0.246	-	-
Ponderal index (kg/cm^3)	0.236	0.036	0.201	0.084	0.176	0.121	-	-	0.005	0.962	-	-	0.170	0.136	-	-

Abbreviations: R, Pearson's correlation coefficient; p, p-value; cm, centimeter; kg, kilogram;g, gram; %, percent; kcal, kilocalorie

[a] Crude correlation analysis without any adjustment models

[b] Partial correlation analysis adjusting for maternal total gestational weight gain, pre-pregnancy BMI, infant gestational age at birth, and infant birth weight

[c] P-Value <0.05 is considered statistically significant

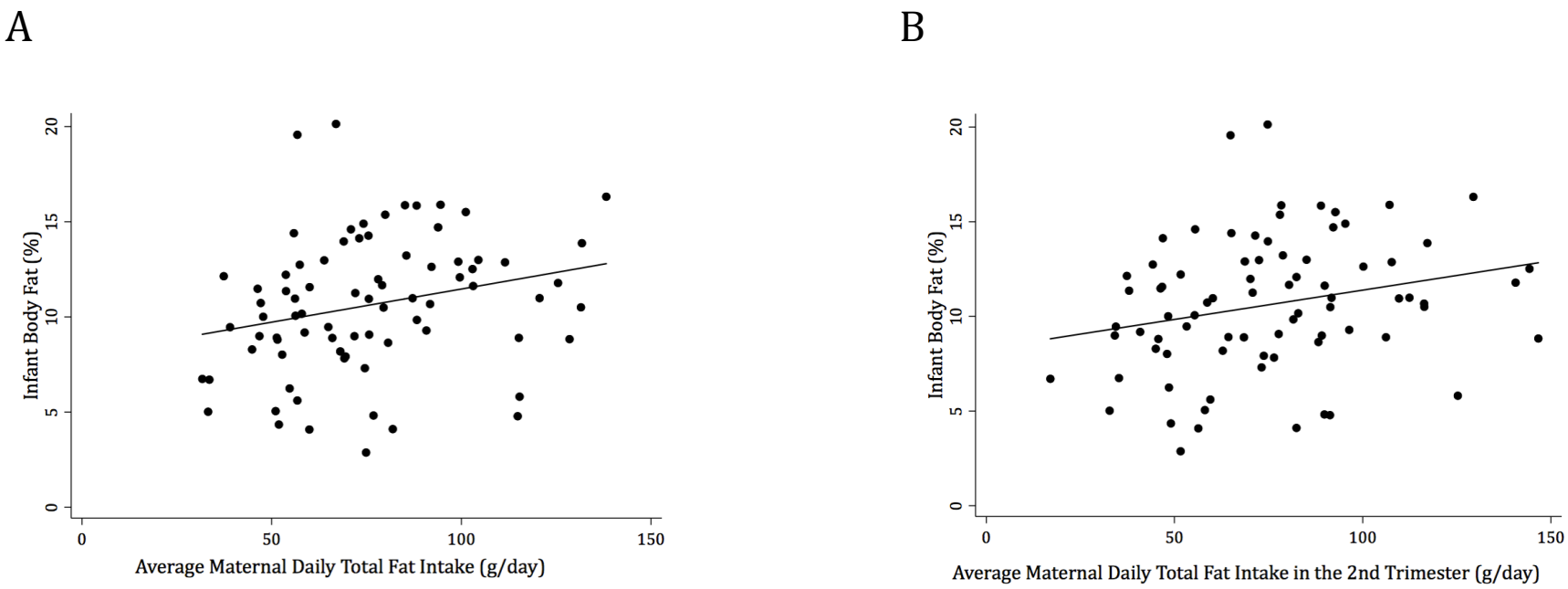

Figure 2. A) Crude correlation between maternal average total fat intake during pregnancy and infant body fat percent at birth. r = 0.249 (p=0.027). B) Crude correlation between maternal average total fat intake during the 2nd trimester and infant body fat percent at birth r =0.243 (p=0.031). Individual points are marked with a circle and the line represents the crude correlation between variables of interest, without adjustment for any confounding variables.
r = Pearson's correlation coefficient; p= p value of the correlation

Table 6. Maternal Total Daily Saturated Fat Intake (grams/day) and Infant Body Composition

	1st Trimester				2nd Trimester				3rd Trimester				Total Pregnancy			
Variable	Crude[a]		Partial[b]		Crude[a]		Partial[b]		Crude[a]		Partial[b]		Crude[a]		Partial[b]	
	r	*p*[c]	*r*	*p*[c]	*r*	*p*[c]	*r*	*p*[c]	*r*	*p*[c]	*r*	*p*[c]	*r*	*p*[c]	*r*	*p*[c]
Body Fat Percentage(%)	0.193	0.088	-	-	0.245	0.030*	0.231	0.046	0.231	0.041	0.112	0.339	0.250	0.026	0.201	0.083
Fat mass (g)	0.161	0.156	-	-	0.213	0.060	-	-	0.224	0.047	0.082	0.486	0.224	0.047	0.162	0.164
Abdominal circumference (cm)	0.019	0.866	-	-	0.036	0.756	-	-	0.203	0.073	-	-	0.100	0.379	-	-
Waist to Length Ratio (cm)	0.045	0.696	-	-	0.082	0.475	-	-	0.133	0.241	-	-	0.099	0.388	-	-
Ponderal index (kg/cm^3)	0.187	0.099	-	-	0.207	0.067	-	-	0.116	0.308	-	-	0.188	0.096	-	-

Abbreviations: R, Pearson's correlation coefficient; p, p-value; cm, centimeter; kg, kilogram;g, gram; %, percent

[a] Crude correlation analysis without any adjustment models

[b] Partial correlation analysis adjusting for maternal total gestational weight gain, pre-pregnancy BMI, infant gestational age at birth, and infant birth weight

[c] P-Value <0.05 is considered statistically significant

Table 7. Maternal Total Daily Saturated Fat Intake (% of kcal) and Infant Body Composition

	1st Trimester				2nd Trimester				3rd Trimester				Total Pregnancy			
Variable	Crude[a]		Partial[b]		Crude[a]		Partial[b]		Crude[a]		Partial[b]		Crude[a]		Partial[b]	
	r	*p*[c]	*r*	*p*[c]	*r*	*p*[c]	*r*	*p*[c]	*r*	*p*[c]	*r*	*p*[c]	*r*	*p*[c]	*r*	*p*[c]
Body Fat Percentage(%)	0.158	0.164	-	-	0.222	0.050	-	-	0.121	0.286	-	-	0.189	0.095	-	-
Fat mass (g)	0.150	0.189	-	-	0.216	0.056	-	-	0.147	0.197	-	-	0.195	0.086	-	-
Abdominal circumference (cm)	0.141	0.214	-	-	0.206	0.070	-	-	0.215	0.057	-	-	0.214	0.058	-	-
Waist to Length Ratio (cm)	0.162	0.153	-	-	0.236	0.036	0.133	0.255	0.134	0.239	-	-	0.207	0.067	-	-
Ponderal index (kg/cm^3)	0.197	0.082	-	-	0.229	0.042	0.083	0.477	0.014	0.900	-	-	0.176	0.121	-	-

Abbreviations: R, Pearson's correlation coefficient; p, p-value; cm, centimeter; kg, kilogram;g, gram; %, percent; kcal, kilocalorie

[a] Crude correlation analysis without any adjustment models

[b] Partial correlation analysis adjusting for maternal total gestational weight gain, pre-pregnancy BMI, infant gestational age at birth, and infant birth weight

[c] P-Value <0.05 is considered statistically significant

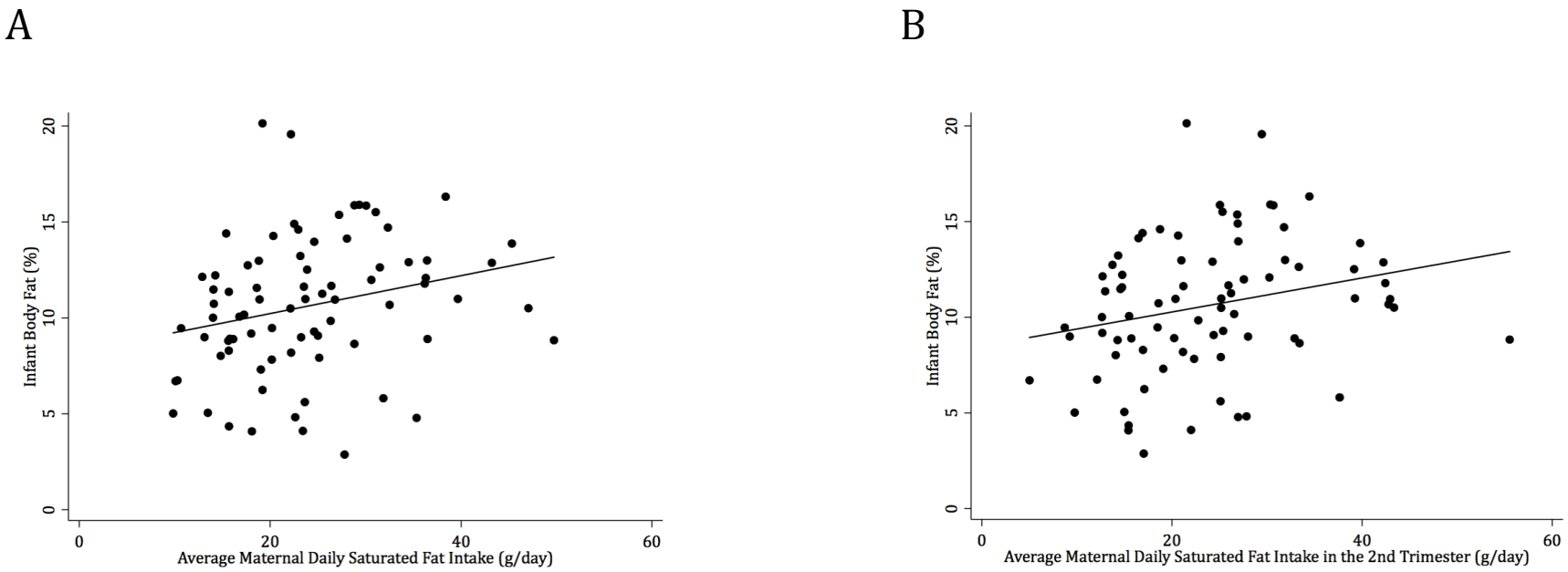

Figure 3. A) Crude correlation between maternal average saturated fat intake during pregnancy and infant body fat percent at birth. r = 0.250 (p=0.026). B) Crude correlation between maternal average saturated fat intake during the 2nd trimester and infant body fat percent at birth r =0.245 (p=0.030). Individual points are marked with a circle and the line represents the crude correlation between variables of interest, without adjustment for any confounding variables.
r = Pearson's correlation coefficient; p= p value of the correlation

Table 8. Maternal Total Daily Unsaturated Fat Intake (grams/day) and Infant Body Composition

	1st Trimester				2nd Trimester				3rd Trimester				Total Pregnancy			
Variable	Crude[a]		Partial[b]		Crude[a]		Partial[b]		Crude[a]		Partial[b]		Crude[a]		Partial[b]	
	r	*p*[c]	*r*	*p*[c]	*r*	*p*[c]	*r*	*p*[c]	*r*	*p*[c]	*r*	*p*[c]	*r*	*p*[c]	*r*	*p*[c]
Body Fat Percentage (%)	0.139	0.223	-	-	0.227	0.045	0.250	0.031	0.269	0.017	0.184	0.114	0.236	0.036	0.241	0.037
Fat mass (g)	0.106	0.353	-	-	0.197	0.081	-	-	0.249	0.027	0.156	0.183	0.206	0.069	-	-
Abdominal circumference (cm)	-0.093	0.414	-	-	-0.017	0.885	-	-	0.124	0.275	-	-	0.008	0.945	-	-
Waist to Length Ratio (cm)	-0.034	0.764	-	-	0.016	0.890	-	-	0.054	0.634	-	-	0.014	0.901	-	-
Ponderal index (kg/cm^3)	0.155	0.173	-	-	0.158	0.165	-	-	0.138	0.224	-	-	0.167	0.142	-	-

Abbreviations: R, Pearson's correlation coefficient; p, p-value; cm, centimeter; kg, kilogram;g, gram; %, percent

[a] Crude correlation analysis without any adjustment models

[b] Partial correlation analysis adjusting for maternal total gestational weight gain, pre-pregnancy BMI, infant gestational age at birth, and infant birth weight

[c] P-Value <0.05 is considered statistically significant

Table 9. Maternal Total Daily Unsaturated Fat Intake (% of kcal) and Infant Body Composition

	1st Trimester				2nd Trimester				3rd Trimester				Total Pregnancy			
Variable	Crude[a]		Partial[b]		Crude[a]		Partial[b]		Crude[a]		Partial[b]		Crude[a]		Partial[b]	
	r	*p*[c]	*r*	*p*[c]	*r*	*p*[c]	*r*	*p*[c]	*r*	*p*[c]	*r*	*p*[c]	*r*	*p*[c]	*r*	*p*[c]
Body Fat Percentage (%)	0.067	0.555	-	-	0.106	0.352	-	-	0.142	0.213	-	-	0.127	0.263	-	-
Fat mass (g)	0.060	0.597	-	-	0.094	0.410	-	-	0.141	0.216	-	-	0.119	0.295	-	-
Abdominal circumference (cm)	-0.042	0.713	-	-	0.036	0.750	-	-	0.013	0.910	-	-	0.007	0.950	-	-
Waist to Length Ratio (cm)	0.065	0.569	-	-	0.077	0.500	-	-	-0.078	0.497	-	-	0.029	0.799	-	-
Ponderal index (kg/cm^3)	0.189	0.096	-	-	0.074	0.519	-	-	0.009	0.937	-	-	0.109	0.340	-	-

Abbreviations: R, Pearson's correlation coefficient; p, p-value; cm, centimeter; kg, kilogram;g, gram; %, percent; kcal, kilocalorie

[a] Crude correlation analysis without any adjustment models

[b] Partial correlation analysis adjusting for maternal total gestational weight gain, pre-pregnancy BMI, infant gestational age at birth, and infant birth weight

[c] P-Value <0.05 is considered statistically significant

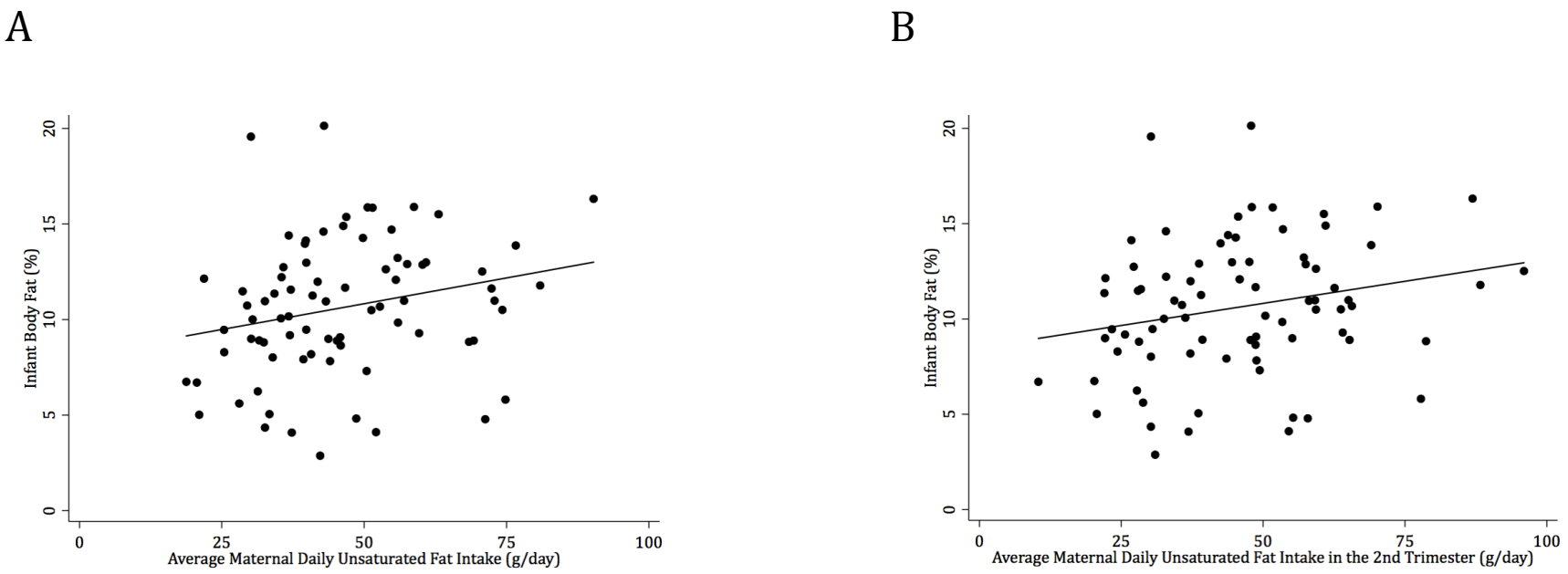

Figure 4. A) Crude correlation between maternal average unsaturated fat intake during pregnancy and infant body fat percent at birth. r = 0.236 (p=0.036). B) Crude correlation between maternal average unsaturated fat intake during the 2nd trimester and infant body fat percent at birth r =0.227 (p=0.045). Individual points are marked with a circle and the line represents the crude correlation between variables of interest, without adjustment for any confounding variables.
r = Pearson's correlation coefficient; p= p value of the correlation

Table 10. Maternal Omega 6 to Omega 3 Ratio and Infant Body Composition

	1st Trimester				2nd Trimester				3rd Trimester				Total Pregnancy			
Variable	Crude[a]		Partial[b]		Crude[a]		Partial[b]		Crude[a]		Partial[b]		Crude[a]		Partial[b]	
	r	*p*[c]	*r*	*p*[c]	*r*	*p*[c]	*r*	*p*[c]	*r*	*p*[c]	*r*	*p*[c]	*r*	*p*[c]	*r*	*p*[c]
Abdominal circumference (cm)	-0.071	0.536	-	-	-0.026	0.823	-	-	0.109	0.337	-	-	0.031	0.789	-	-
Waist to Length Ratio (cm)	0.114	0.316	-	-	0.193	0.089	-	-	0.206	0.068	-	-	0.221	0.050	0.2760	0.017
Ponderal index (kg/cm^3)	0.078	0.495	-	-	0.046	0.690	-	-	0.069	0.546	-	-	0.122	0.283	-	-
Fat mass (g)	-0.100	0.381	-	-	-0.212	0.061	-	-	-0.090	0.429	-	-	-0.089	0.437	-	-
Body Fat Percentage (%)	-0.058	0.610	-	-	-0.190	0.094	-	-	-0.077	0.502	-	-	-0.060	0.597	-	-

Abbreviations: *r*, Pearson's correlation coefficient; *p*, p-value; cm, centimeter; kg, kilogram;g, gram; %, percent

[a] Crude correlation analysis without any adjustment models

[b] Partial correlation analysis adjusting for maternal total gestational weight gain, pre-pregnancy BMI, infant gestational age at birth, and infant birth weight

[c] P-Value <0.05 is considered statistically significant

Table 11. Maternal Energy Intake and Infant Body Composition

	1st Trimester				2nd Trimester				3rd Trimester				Total Pregnancy			
Variable	Crude[a]		Partial[b]		Crude[a]		Partial[b]		Crude[a]		Partial[b]		Crude[a]		Partial[b]	
	r	*p*[c]	*r*	*p*[c]	*r*	*p*[c]	*r*	*p*[c]	*r*	*p*[c]	*r*	*p*[c]	*r*	*p*[c]	*r*	*p*[c]
Body Fat Percentage (%)	0.133	0.242	-	-	0.213	0.059	0.227	0.050	0.221	0.050	0.215	0.065	0.210	0.063	-	-

Abbreviations: *r*, Pearson's correlation coefficient; *p*, p-value; %, percent

[a] Crude correlation analysis without any adjustment models

[b] Partial correlation analysis adjusting for maternal total gestational weight gain, pre-pregnancy BMI, infant gestational age at birth, and infant birth weight

[c] P-Value <0.05 is considered statistically significant

Discussion

Summary. The purpose of this study was two fold: 1) to investigate the association between maternal dietary fat intake during pregnancy and infant body composition at birth, and 2) determine whether maternal dietary fat type relates to infant body composition. Raw maternal average total fat intake during pregnancy and the second trimester were weakly correlated with infant body fat percent at birth. Contrary to our hypothesis, the only evident relationship with raw maternal dietary saturated fat intake and infant body fat percent was during the second trimester of pregnancy. Lastly, raw maternal unsaturated fat intake during the second trimester and average intake throughout pregnancy had a weak correlation with infant body fat percent at birth. The repeated relationships during the second trimester suggest that this may be a key time period for nutritional interventions and fetal body composition development, which will be explored further. Maternal fat intake as a percent of calories was not associated with any marker of infant body composition after adjustment for confounding variables for total fat, saturated fat, or unsaturated fat suggesting that maternal energy intake may be driving the significant relationships in our results.

Maternal total daily dietary fat. Fat intake during the second and third trimesters was more often associated with stronger correlations with infant adiposity than fat intake during the first trimester of pregnancy. Although the overall correlations were weak, this may suggest that dietary intake during the second and third trimesters may be key time points for dietary intervention with pregnant women. There was also a weak relationship between maternal average

daily total fat intake during the entire pregnancy and infant body fat percent at birth. Other studies have found conflicting results and the difference in study methods warrants caution when comparing study results.

Blumfield et al. used two FFQs during pregnancy and found no significant relationship between maternal total dietary fat as a percent of calories and infant adiposity.[15] However, this study used ultrasound cross-sectional area to calculate neonatal mid-thigh and abdominal fat as markers of infant adiposity. A large Singapore cohort study concluded that a high protein and low fat diet was associated with lower neonatal internal adipose tissue.[16] Additionally, this study found no significant relationships between superficial or deep subcutaneous adipose tissue and any maternal macronutrient intake, which is more in line with our study methods. Horan et al. used three-day diet records during each trimester of pregnancy to assess the relationship between maternal fat intake and infant abdominal circumference, waist to length ratio, and sum of four skinfold site measurments.[14] This study did not conclude any relationships between maternal total fat intake and infant adiposity at birth.[14] Horan et al. was limited in their ability to quantify infant adiposity as they did not calculate infant fat mass, and they also did not adjust for total gestational weight gain or infant birth weight.[14] Crume et al. in Colorado used ADP for infant body composition and multiple 24-hour recalls to conclude that each 100 kcal increase in maternal total daily dietary fat intake throughout pregnancy was associated with a 4.2 g increase in neonatal fat mass after adjustment for pre-pregnancy BMI.[10] Our regression analysis showed that every 100 kcal increase in average maternal dietary fat intake during only the third

trimester was associated with a 16.2 g increase in infant fat mass at birth (p=0.030, 95% CI [1.64,30.7]), but this did not persist after adjustment. When our analysis was repeated with maternal fat intake as a percent of calories, these results also did not persist (Table 5). Perhaps the 13-fold increase in sample size and variation in statistical adjustment models and diet assessment method may account for the difference in findings from Crume et al. Our measurements of infant body fat percent take into account infant fat mass as a proportion of birth weight, whereas other studies did not account for infant birth weight.[10,15] Both Blumfield et al. and Crume et al. did not assess intake specific to each trimester making our study unique in its ability to demonstrate repeated relationships between infant body composition and maternal dietary fat intake in the second trimester. Although the conclusion that maternal dietary total fat intake is associated with infant adiposity was congruent among our study and Crume et al., our study suggests that maternal energy intake may be a driving force in this relationship.

Maternal Saturated Fat. In our study, raw maternal dietary saturated fat intake during the second trimester of pregnancy remained associated with infant body fat percent at birth after adjustment for confounding variables. Maternal saturated fat as a proportion of energy intake in the second trimester was also weakly associated with infant ponderal index and waist to length ratio, but did not remain significant after adjustment for confounding variables. Blumfield et al. did not conclude any relationship between maternal saturated fat intake as a percent of calories and infant mid-thigh or abdominal fat.[15] This study used two FFQs during pregnancy so they did not have the ability to determine fat intake solely during the

second trimester unlike our study. Crume et al. demonstrated that each 100 kcal increase in maternal saturated fat intake was associated with a 11.1 g increase in neonatal fat mass after adjustment for pre-pregnancy BMI.[10] We found that every 100 kcal increase in average daily maternal saturated fat intake during pregnancy was associated with a 48.7 g increase in infant body fat mass at birth (p=0.047 95% CI [0.623, 96.7]), however these results did not remain significant after adjustment for confounding variables. Our results also showed a relationship between raw maternal saturated fat intake in the third trimester and throughout pregnancy and both infant fat mass and body fat percent at birth, however none of these persisted after adjustment. Horan et al. concluded a significant positive relationship between infant abdominal circumference at birth and maternal saturated fat intake during the third trimester of pregnancy, which persisted after adjustment for confounding variables.[14] Our study, Horan et al., and Crume et al. suggest that there may be a relationship between saturated fat intake and infant adiposity, however the evidence is not as strong as the support we have for maternal total daily dietary fat intake. Our findings also suggest that maternal saturated fat intake may play a larger role during the second trimester than any other time point during pregnancy. The lack of significant findings in our evaluation of maternal saturated fat intake as a proportion of energy intake suggests that our significant findings with raw fat intake may be driven by maternal energy intake.

Maternal Unsaturated Fat. We have evidence to support a weak relationship between raw maternal total unsaturated fat intake in the second trimester and throughout pregnancy and infant body fat percent at birth. We concluded that every

100 kcal increase in maternal unsaturated fat in the second trimester was associated with 0.30 percentage point increase in infant body fat percent (p=0.031 95% CI [0.028, 0.572]).). Likewise, average maternal unsaturated fat intake throughout the entire pregnancy was associated with a 0.326 percentage point increase in infant body fat percent(p=0.037 95% CI [0.020, 0.632]). Our findings are in accord with Crume et al. who demonstrated that every 100 kcal increase in maternal unsaturated fat was associated with a 5.9g increase in infant fat mass, including adjustment for pre-pregnancy BMI.[10] Likewise, our study found that every 100 kcal increase in maternal unsaturated fat in the third trimester was associated with a 27.4 g increase in infant fat mass at birth (p=0.027 95%CI [3.18, 51.6]), but this relationship did not persist after adjustment. Our study and Crume et al. are congruent in their conclusion that increased raw maternal unsaturated fat intake is associated with increased infant adiposity. However, once maternal fat intake is taken into consideration as a proportion of maternal energy intake, these relationships did not persist. Blumfield et al. did not describe total unsaturated fat intake but rather MUFAs and PUFAs in relation to infant body composition.[15] Infant mid-thigh fat was negatively associated with percentage of energy as PUFAS. This finding does not reflect what we found with total unsaturated fat intake, but is limited in its application to our study because we did not separate MUFAs and PUFAs in analysis. Other studies investigated MUFAs and PUFAs, but did not look at the relationship between total unsaturated fat intake and infant body composition.[14,16,38]

Our study demonstrated a weak relationship between average maternal n6:n3 ratio throughout the entire pregnancy and infant waist to length ratio at birth, and this remained significant after adjusting for confounding variables. Pereira da Silva et al. used FFQs to assess n6:n3 ratio across pregnancy and only found significant relationships regarding fat mass and percent body fat in the female infants.[38] Alternatively, Hauner et al. concluded that there is no effect of fish oil supplementation on infant adiposity as an effort to reduce the n6:n3 ratio.[46] Data on the relationship between n6:n3 ratio and fetal fat development remains scarce and poorly understood, however this may be an area for future research.

Infant body composition. In all analyses of total fat, saturated fat, and unsaturated fat, infant body fat percentage at birth consistently demonstrated stronger relationships with raw maternal dietary fat intake compared to all other infant body composition measurements. This suggests that maternal dietary fat intake may have the greatest affect on infant body composition in the form of body fat percent. Infant fat mass and birth weight are highly correlated (r=0.9217, p<0.0001). In addition to including birth weight in the adjustment model, infant body fat percent takes into account fat mass as a proportion of birth weight to remove the affect of this variable. This method allowed us to investigate the relationship with maternal dietary fat intake separate of the effect of birth weight.

Strengths. One strength of this study was that the Block FFQ was used as the diet assessment method, which has been validated in women and for dietary assessment of macronutrients.[50,51] The Block FFQ has the ability to measure average intake over time, a three month window for our study, whereas a 24-hour recall

only gives a brief snapshot into one day of usual dietary intake. When comparing the Block FFQ with two 24-hour recalls in Canadian women, the Block FFQ has been shown to have correlation coefficients ranging from 0.50 to 0.70 for macronutrients and 0.53 to 0.83 for micronutrients which is similar to other studies' estimates of reliability.[50] The Block FFQ was also validated in participants in the Women's Health Trial Feasibility Study, and all nutrient estimates were within 20% of estimates produced by the average of three four-day diet records.[51] These studies provide evidence to suggest that the validity and reliability for the Block FFQ are high, and this method of diet assessment was appropriate for women and dietary fat in this sub-study.

Additionally, the FFQs in this study were collected during each trimester of pregnancy to account for how maternal diet changes over the course of pregnancy. This allows investigation into the impact of maternal intake during each trimester of pregnancy relevant to specific critical developmental windows for organ development, which may have long term DOHaD significance. Another strength of this study was that it investigated maternal dietary fat intake whereas other studies have assessed overall diet patterns and food groups.[52] Lastly, this is one of few studies that has investigated a specific macronutrient in regard to infant body composition, and significantly adds to the literature in this area.

Limitations. Sample size was a limitation in this study considering that 101 women were enrolled, but only 79 had complete dietary and body composition data for analysis. The above discussion highlights that studies with larger sample sizes have varying results compared to our study suggesting that our methods should be

repeated with a larger sample size. A larger sample size would also allow us to adjust for more confounding variables and evaluate more specific relationships with maternal diet and infant body composition.

Neonatal fat assessment is a challenge in all current research as there is no recognized gold standard. A validation study including 95 newborns assessed by ADP and skinfolds at birth, reported a moderate correlation of 0.55.[53] However, the original validation study of the Catalano equation reported a correlation of 0.84 ($p=0.0001$) when comparing estimated neonatal fat mass by total body electric conductivity and the developed equation.[49] This correlation continued to remain significant even after one macrosomic infant was excluded from the dataset ($R=0.82$, $p=0.0001$). Lastly, this validation study reported no significant difference in estimated fat mass between the Catalano equation and the total body electric conductivity method.[49] In 2014, Catalano also cited the correlation between ADP and flank skinfold with neonatal fat mass as $R=0.83$ ($p<0.001$), however this report stated that this data was from an unpublished source.[54]

Other body composition methods have been used in other studies, however they all have individual strengths and limitations that were not appropriate for our study design. The ADP method requires expensive equipment and is less conducive to the time and physical restraints associated with delivery of newborn infant. Dual energy x-ray absorptiometry is another method of fat assessment, but can be seen as unethical to perform on infants due to the radiation exposure. Electrical body conductivity also requires fairly expensive equipment and relies heavily on body water, which quickly changes within hours to days after infant delivery. The flank

skinfold method was appropriate for the setting of this study, considering that flank skinfold measurement has been previously shown to produce similar results to other body composition methods, is quick to perform, can be performed without separating mother and infant, and does not require highly technical equipment. Also, the results of our study can now be easily replicated in large population studies and in various settings using equipment that is inexpensive, does not require electricity, and easy to transport.

The study that originally validated the Catalano equation excluded infants under two kg, infants admitted to the neonatal intensive care unit, and infants with congenital anomalies.[49] That original study cited that the Catalano model may be less predictive of neonatal fat mass in small infants and the study likely included very few, if any, small for gestational age infants.[49] These limitations of the Catalano study most likely contributed to the negative fat mass that resulted for two infants in our study weighing 2.1 kg and 2.285 kg. The equation predicted negative fat masses for these two infants, which is physiologically implausible thus resulting in exclusion from our study. Although infant body fat percent ranged from 2.9% to 20.1% to in our study, these results are likely reasonable as the combination of varying lengths and weights can produce low body fat percent values when calculated with the Catalano equation. Other studies have reported similar mean body fat percent values to our study, however other studies did not report the range of infant body fat percent.[10,15]

This study was also limited in its ability to determine length measurements of infants because the institution uses paper measuring tapes to measure infant

length at birth. The gold standard for measuring infant length is using a length board to measure while two people assist in stretching and stabilizing the infant. Although this study did not use the gold standard to measure length and this could have been a source of error, each baby was assessed in a standardized way during the study suggesting it could have been a potential systematic error. One source of random error in this study was that the length measurement was performed at the time of delivery by the delivery room nurse,, suggesting that there was variation in measuring procedures. However, the skinfold thickness and abdominal circumference measurements were performed by six trained study personnel, which contributed to the repeatability of this study.

Few research studies have investigated the accuracy of different length measurement methods.[55-58]A descriptive study including 32 term infants reported a mean difference of 2.51 centimeters between the paper barrier method and length board.[55] Another study with 25 infants concluded a mean difference of 2.23 centimeters, however these results were not statistically significant.[58] Lastly, a study including 160 children under 2 years old reported a mean difference of 1.3 centimeters between paper barrier and length board methods.[56] Research with larger sample sizes and greater generalizability is needed to better understand the accuracy of different measurement methods. However, repeated measurements and trained practitioners remain positive efforts to improve accuracy.

Although the Block FFQ is a valid diet assessment tool, it was also a limitation to this study because the gold standard for diet assessment is multiple 24-hour recalls. The FFQ in this study also served as an opportunity for recall bias as

women had to remember their approximate food intake during each trimester of pregnancy. Using multiple FFQs that only required subjects to recall the previous three months reduced the effects of recall bias. Additionally, specific groups of women may have underreported their dietary intake thus affecting the accuracy of dietary assessment in this study. Previous research has shown that pregnant women with lower educational status or higher BMI are more likely to underreport their dietary intake.[59] Although this study adjusted for BMI, it was limited in its ability to adjust for educational status or another proxy for socioeconomic status. Lastly, as an observational study, this sub-study was limited in its ability to make causal inferences regarding maternal diet during pregnancy and infant body composition. However, this study provides evidence to support future research in this area and generate hypotheses for future randomized controlled trials.

Implications of research. There are not currently any infant body composition standards to categorize an infant with excess fat mass, however there are a few descriptive studies of infant populations. Carberry et al. described the infant body composition for 45 Australian infants from birth to four and a half months.[12] Hawkes et al. illustrated body composition in the first four days of life for 743 Irish infants.[11] Our study describes body composition of 79 infants to suggest that infant adiposity is weakly associated with raw maternal dietary intake. Other infant measurements like weight, length, and head circumference are categorized as large for gestational age if an infant is greater than the 90th percentile. However, markers of infant body composition including infant abdominal circumference, waist to length ratio, fat mass, and body fat percentage do not have standardized

comparison values. Our study adds to the small body of literature describing infant body composition measurements at birth.

Our study showed that infant body fat percent at birth was consistently related to raw maternal dietary fat intake during the second trimester of pregnancy. Analyses of human fetuses suggest that the initiation of fetal fat formation occurs between 14 and 23 weeks gestation, and after 23 weeks the total number of fat lobules remains constant.[9] Between 23 and 29 weeks gestation, fat lobules increase in size and expand their surrounding capillary network.[9] This time period overlaps with the second trimester ranging from 13 to 27 weeks gestation, which provides biologic plausibility that infant fat and body composition could be programmed during this time. It is known that maternal energy needs increase during the second trimester due to demands of the fetus and changes in maternal metabolism.[37] The second trimester also marks the typical end of first trimester side effects including maternal nausea, vomiting, food aversions, and loss of appetite thus contributing to an increase in maternal dietary intake in the second trimester. Our findings suggest that the second trimester time period is crucial to fetal development and changes in maternal dietary intake thus presenting as an essential opportunity for nutritional intervention. These findings can help support public health programs, prenatal clinics, and healthcare providers focus their interventions on this fundamental time period in effort to improve maternal diet and subsequent birth outcomes.

The results of this study expand our current knowledge about the role of maternal dietary fat intake on infant body composition and are clinically relevant in order to determine the optimal maternal diet and key intervention time periods for

beneficial birth outcomes. Current evidence is lacking regarding specific diet recommendations for women during pregnancy to promote optimal infant body composition, and this study serves to fill the gap in this area of research. Although current body composition reference curves do not exist for infants, this sub-study contributes to the body of literature describing infant body composition. These study results show varying strengths of relationships between maternal dietary fat intake and different markers of infant adiposity. These results could help generate future hypotheses for other potential causes of infant adiposity such as other dietary variables, overall dietary patterns, lifestyle factors, or genetics.

Conclusion. Our results support our hypothesis that increased infant adiposity at birth was positively correlated with raw maternal dietary total fat intake during pregnancy. This relationship remained true for average maternal dietary total fat intake during the entire pregnancy, and for intake during the second trimester. We rejected the hypothesis that maternal saturated fat intake has the strongest association with infant adiposity at birth. Infant body fat percent was only associated with maternal saturated fat intake during the second trimester of pregnancy. Our results support the conclusion that maternal unsaturated fat intake has a stronger association with infant adiposity than maternal saturated fat intake. Infant body fat percent was associated with maternal unsaturated fat intake during the second trimester of pregnancy and average intake throughout the entire pregnancy. Our findings in regard to maternal fat intake as a proportion of energy suggest that maternal energy intake may be driving the significant findings found with raw maternal fat intake. This study demonstrates the relationship between

maternal dietary fat intake during the second trimester and infant body composition at birth thus necessitating the need for a focus on nutrition during this time period.

Future Research. Our results are vital to continuing research on improving birth outcomes. This study demonstrated that unsaturated fat may play a larger role in infant body composition than saturated fat. This could contribute to research regarding placental fatty acid transport and uptake by the fetus, and may indicate that unsaturated fatty acids should be an area of focus. Although this sub-study did not collect information on circulating infant lipids, it could generate hypotheses regarding infant fatty acid metabolism. Although few studies have reported infant body composition measurements, our study contributes to this body of literature and still more research is needed to build reference curves data with adequate external validity. Our results also provide evidence to further explore different dietary, counseling, and support interventions during the second trimester as a time period that is central to the development of the fetus and may subsequently impact risk for chronic disease later in life.

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