Exploring the associations of maternal red blood cell fatty acids, infant body composition, and quality of infant growth

By

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Abstract

Background: Rates of childhood overweight and obesity have increased and are related to earlier development of chronic health diseases. Maternal fat consumption influences fetal adipose tissue development and may program future levels of adiposity. Research has shown relationships between maternal levels of omega-3 (n-3) and omega-6 (n-6) fatty acids and infant body weight. Vaccenic acid is a natural trans fatty acid shown to have health benefits in both immune function and body composition. No studies have related maternal fatty acids and infant body composition (percentage body fat (%fat), fat mass (FM), and fat free mass (FFM)) at birth and during early infancy.

Purpose: The purpose of this study was to explore the relationship between maternal fatty acids, with emphasis on vaccenic acid, and infant body composition at birth and during early infancy.

Methods: Seventy four mother-infant pairs were included in this analysis. Mothers meeting the criteria of a singleton pregnancy, healthy, BMI 18.5 – 40 kg/m^2, completed a blood draw late in pregnancy, and with infants who completed a birth and/or 3-4 month visit to assess body composition were included. Maternal red blood cell (RBC) fatty acids were measured by adsorption chromatography and transmethylation techniques. Infant body composition was measured by air displacement plethysmography. Correlation matrices assessed the relationship between maternal fatty acids and infant body composition at birth and the change in body composition during early infancy. Multiple linear regression assessed the relationship between maternal vaccenic acid values and infant body composition at birth and change in body composition in early infancy. Significance was determined at a level of $p < 0.05$.

Results: Average maternal age was 29.43 ± 4.81 years; pre-pregnancy BMI was 25.49 ± 5.44 kg/m^2; average gestational weight gain was 16.07 ± 6.14 kg. Average infant gestational age was 39.61 ± 0.82 weeks; average birth weight was 3.503.47 ± 420.22 g. Arachidonic acid was negatively related to infant FFM while heneicosanoic acid was positively related to infant FM at birth. Vaccenic acid was negatively related to all measures of change in infant body composition at 3-4 months. Linolelaidic acid was negatively related to change in infant FFM, while linoleic and eicosapentaenoic acids were positively related to change in FFM. Heneicosanoic acid was negatively related to changes in %fat and FM. Multiple linear regression showed that vaccenic acid negatively predicted changes in infant FM and FFM.

Conclusion: Relationships exist between late-pregnancy maternal RBC fatty acids and infant body composition at birth and change at 3-4 months. Vaccenic acid predicted decreased changes in FM and FFM at 3-4 months. Future research is needed to better define these relationships.

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Table of Contents

List of Tables & Figures

Tables

Figures

Chapter 1: Introduction

Rates of overweight and obesity prevalence are impacting the majority the US population directly, and indirectly impacting nearly all parts of the country through consequences of medical care costs. The declining health status of many American adults that in the past has taken years to manifest, is being seen at younger ages (1). The rates of childhood overweight and obesity have increased creating a greater likelihood of these children becoming overweight or obese adults (2, 3) and developing chronic health diseases such as type 2 diabetes, cardiovascular disease, and cancer (4). The question of why overweight and obesity is more prevalent in today's society has led researchers to investigate similar trends in other populations to make links connecting potential causes and outcome.

The maternal population has seen similar increases in obesity rates and excessive gestational weight gain (GWG) (5-7), thus highlighting the maternal influence on fetal development and growth (8). The maternal diet is one of the most influential factors involved in the health of a developing fetus (8), and research has shown the potential relationship between maternal fat intake and the development and growth of adipose tissue in the fetus and growing infant (9, 10). Individual fatty acids cross the placental barrier in a selective way (11), leading to brain, eye, and immune system formation along with the foundation of adipose tissue and future growth and gene expression (12).

Research currently has studied associations between essential fatty acids such as the omega-3 (n-3) fatty acids docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA), and omega-6 (n-6) fatty acid arachidonic acid (AA) in maternal blood with infant body weight (8-10, 13-15). However, research is lacking in the investigation of other individual fatty acids and their

potential associations with birth weight and also body composition (%fat, FM, and FFM) at birth and early infancy (16). In particular, vaccenic acid (18:1n-7t) is a natural trans fatty acid produced in ruminant animals through bacterial action (17). This fatty acid serves as a precursor to conjugated linoleic acid (CLA) which has shown positive effects in both immune function and body composition (18). Therefore, research is needed to further explore the contribution of maternal fatty acids to infant %fat, FM and FFM. With this research, more knowledge can be learned in regards to the maternal fatty acid composition and its role in fetal environment and potential influences in the quality of growth in the early months of life.

Statement of purpose

The purpose study was to explore the relationship of maternal fatty acids, with emphasis on vaccenic acid, and infant body composition at birth and early infancy.

Research question

What is the relationship between maternal fatty acids measured late in pregnancy and infant body composition, measured as %fat, FM, and FFM at birth and the change in body composition during early infancy? A secondary research question was to explore the relationship between maternal vaccenic acid levels and infant body composition at birth and the change in body composition during early infancy.

Specific aims and hypotheses

Aim 1: To determine if maternal fatty acids are related to infant body composition at birth.

- Hypothesis 1.1: Maternal fatty acids will be positively related to infant %fat.
- Hypothesis 1.2: Maternal fatty acids will be positively related to infant FM.
- Hypothesis 1.3: Maternal fatty acids will be positively related to infant FFM.

Aim 2: To determine if maternal fatty acids are related to the change in infant body composition (birth to 3 to 4 months).

- Hypothesis 2.1: Maternal fatty acids will be positively related to the change in infant %fat.
- Hypothesis 2.2: Maternal fatty acids will be positively related to the change in infant FM.
- Hypothesis 2.3: Maternal fatty acids will be positively related to the change in infant FFM.

Aim 3: To determine if maternal vaccenic acid values are related to infant body composition at birth.

- Hypothesis 3.1: Maternal fatty acids will be positively related to infant %fat.
- Hypothesis 3.2: Maternal fatty acids will be positively related to infant FM.
- Hypothesis 3.3: Maternal fatty acids will be positively related to infant FFM.

Aim 4: To determine if maternal vaccenic acid values are related to the change in infant body composition (birth to 3 to 4 months).

- Hypothesis 2.1: Maternal vaccenic acid values will be positively related to the change in infant %fat.
- Hypothesis 2.2: Maternal vaccenic acid values will be positively related to the change in infant FM.
- Hypothesis 2.3: Maternal vaccenic acid values will be positively related to the change in infant FFM.

Chapter 2: Literature Review

Obesity prevalence

The nation-wide epidemic of overweight and obesity is plaguing millions of individuals causing life-threatening effects such as type 2 diabetes, cancer, and heart disease (19), and decreased quality of life (20, 21). These issues largely impact the adult population, but are also prevalent in the younger populations (22). Diseases once thought to take years to manifest are now showing up in young adults, teenagers, and even children. Despite local and national efforts to combat the rising trends of the overweight and obesity over a decade ago, these rates have continued to persist in our country and significantly impact a majority of the population (23). While recent data have shown that rates have remained constant in the past year, lingering effects such as increased obesity-related deaths may be an indirect source of the continued burdens of this epidemic (24).

Adult and child rates

The trends of overweight and obesity have grown to afflict a larger proportion of individuals than ever experienced before. Among adults (greater than 20 years old), 68.8% are overweight and 35.7% are obese (25). Amid the child population (ages 2-19), roughly 31.8% are overweight and 16.9% are considered obese (26). It is estimated that 85% of these children who are obese grow to become obese adults (2, 3). Further, 9.7% of toddlers are overweight and 7.5% of infants are born macrosomic (4,500 g or greater, as defined by the American College of Obstetricians and Gynecologists) (26, 27). These rates are a threat to human health both in the short and long term and they drastically increase the economic cost of health care in the country.

Economic cost of obesity

The financial burden of the obesity epidemic targets both direct and indirect medical care costs (28, 29). In the year 2008, medical cost of obesity reached a total of $147 billion dollars (23). These totals have risen from the cost of obesity in 1998 which was $78.5 billion, nearly half of the costs today (23). The cost to treat an obese compared to a normal weight person has also increased. In 1998 the cost difference was $1,145 however the 2006 per capita cost difference was $1,429 (23). The costs associated with obesity are both physical from the monetary viewpoint as well as physical from the health detrimental standpoint. While Medicare, Medicaid, and private payers are struggling to support the economic consequences of the past and present obesity prevalence, countless families and individuals are struggling to cope with the devastating realities not only of obesity, but also the downstream diseases such as type 2 diabetes, cardiovascular disease, cancers, stroke, and many more (30). These health consequences lead health professionals and researchers to ask the question regarding how this nationwide threat to health can be increasing, and more specifically increasing in *both* the adult and pediatric population. Another key factor that has been similarly trending with the population's overweight and obesity rates is the rising proportion of pregnant mothers that are overweight and gaining excessive amounts of gestational weight (5-7). This awareness poses the challenge to investigate associations between maternal factors that may be influencing the health of developing fetuses who are showing the early signs of increased weight gain. Thus, it is necessary to trace back to the origins of development and factors influencing the development of the growing fetus.

Epigenetics – where it starts

Epigenetics ("epi" = on top of, genetics = characteristic expression of genes) is the study of heritable and reversible change related to environmental influences and their interactions with

a person's genes (31, 32) without changing the sequence of the genes (33). The DNA which make up our genes are tightly wrapped around histones, together called chromatin, and are susceptible to epigenetic "marks" (34). These marks can affect the genes by causing the histone coil to be more condensed (turning "off" the gene, i.e. unexpressed) or less condensed (turning "on" the gene, i.e. expressed) (34). One of the most critical times for epigenetic marks to occur is *in utero* due to the plasticity of development and growth (8, 35). Once this alteration becomes permanent, is it termed a "programming change" and has lasting differences in cell structure and function (35).

Fetal programming

Fetal programming explains an alteration in the structure and function of the fetus in response to environmental occurrences, including both beneficial and adverse events (36). Maternal nutrition serves as an important source of an environmental trigger which influences gene expression of the developing fetus (37). This particular trigger is introduced multiple times daily, and can cue the developing fetus to metabolically adapt and prompt a particular phenotype (38). The sensitive fetal development is plastic in regards to its ability to change in response to environmental stimuli (38), whether it is under-nutrition causing the fetus to decrease energy demands (influencing a rebound effect post-delivery), or over-nutrition causing disruptions in fetal hormone signaling and energy storage (39).

Maternal influence on infant health

The long-term health of a newborn baby is substantially influenced by the developing environment around him/her and the various growth promoters and stressors experienced *in utero*. The fetal environment provided by the mother can adversely impact the fetus later in life, yet these contributing mechanisms and pathways are uncertain (40). Certain maternal factors

such as body weight status, physical activity, smoking, and diet are examples well known to influence the development and growth of a fetus, which manifest immediately in life in the form of birth weight and body composition, or later in life in the form of chronic diseases such as obesity, type 2 diabetes, and cardiovascular disease (8, 41). These epigenetic changes expressed in the newborn can be the result of adaptations in response to growth promoters or inhibitors which trigger a triage-type of survival mechanism, prioritizing one organ or organ system at the expense of another (40).

Health of developing fetus and growing infant

Various factors and extents of these factors contribute to the ultimate development of a fetus, yet all play a role in how well or poorly the fetus responds to the current environment and subsequent surroundings *ex utero*. For example, mothers who are overweight or obese are more likely to deliver infants who are large for gestational age (LGA) and be at a greater risk of becoming overweight and developing metabolic syndrome later in life (42). Birth weight is strongly associated with excessive gestational weight gain, and being physically active has been shown to facilitate better oxygen transfer and blood flow to the fetus and allow adaptation of the placenta to increase nutrient delivery in response to fetal demands (43). The widely known severe effects of maternal smoking cause the opposite effects of physical activity, reducing blood flow and oxygen to the fetus resulting in low birth weight (44). Diet is another key factor in fetal development and growth which interacts with the fetus constantly each day. Fetal nutrition is supplied through maternal nutrition, and research has provided evidence that a mother's diet is one of the primary influences in shaping the future health of her fetus (8).

Maternal diet and infant health

It has been hypothesized that there are three governing players involved with appropriate growth of a developing fetus, and those are genetic susceptibility, maternal delivery of nutrients to the fetus, and the ability of the placenta to transmit these nutrients (16). The maternal diet can potentially influence each of these factors, as it can also serve as a vehicle and source of epigenetic markers that alter the preprogrammed phenotype of the fetus (45). The "thrifty phenotype" is a hypothesis that describes how poor intrauterine nutrition inflicts processes of nutritional shift in the developing fetus (35), changing the fetal metabolism (40). There are several mechanisms in which this can occur, yet the greatest risk is apparent when a deprivation of nutrients *in utero* is followed by an abundance after the fetus is born (40). The western diet common to many Americans today enables this abundance of nutrients as evidenced by the recent trends in overweight and obesity. In contrast, over-nutrition *in utero* also has been shown to influence infant body composition in childhood as well as in adulthood (9). Adipose tissue is initiated and promoted through maternal dietary intake and specific components present in the diet (9, 10). Maternal fat intake and specific fatty acid make up may stimulate the development of adipose cells (9) and provide the foundation of further growth and utilization later in life (46).

Fatty acids and fetal growth and development

It is well known that essential long-chain polyunsaturated fatty acids (LCPUFAs) are vital for fetal brain and eye development (9, 47). They can also influence adipose tissue development and growth in the fetus (48). Additionally, adipose tissue development and growth *in utero* may serve as an irreversible template for future adipose storage (49). Fat accretion significantly occurs in the third trimester, as the fetus relies completely on maternal stores for obtaining essential LCPUFAs such as the n-3 DHA and n-6 AA (13, 50). Fatty acids are

transmitted to the fetus through active placental transfer (11), which contributes significantly to the environment and cues certain epigenetic marks that predispose the fetus to long and short term health factors (39).

Placental transfer of fatty acids

Placental transfer of fatty acids includes polyunsaturated (PUFAs) as well as trans fatty acids. Both have been shown to increase in concentration through increased maternal intake (12). There appears to be a competitive nature between certain fatty acids in their transport across the placental barrier (11, 12, 39, 51). Research has shown that trans fatty acids work in opposition to AA and DHA while n-6 and n-3 fatty acids may also compete for enzymes used in sequential fatty acid synthesis (11). Generation of adipose tissue *in utero* can influence adiposity in infancy and further into childhood years (48), and has demonstrated significant effects in studies assessing maternal fat intake and/or status and infant weight (9, 13, 46). The individual fatty acid composition of the maternal diet has been suggested to influence adipose tissue development and deposition, and subsequent risk of the infant becoming overweight or obese (9, 10, 49). Yet, results across studies have been inconsistent with regard to fatty acid influences on infant birth outcomes (16, 52, 53).

Markers of maternal-placental fatty acid transfer

The maternal fatty acid profile is reliably represented by measurement of dietary intake, RBC, or cord blood. Dietary intake of fatty acids is often assessed through use of a validated food frequency questionnaire. Data showed a direct correlation between the corresponding fatty acid concentration in both maternal and cord blood levels for most PUFAs to the food frequency questionnaire estimated value (11). These findings were confirmed by data from Zhang and colleagues which showed a positive correlation between maternal dietary AA, EPA, and DHA

and respective plasma levels, as well as these fatty acids corresponding to cord plasma concentrations (54).

Correlations between dietary intake and blood values for EPA and DHA are constant throughout pregnancy, with the exception of alpha linolenic acid (ALA) (55). Plasma and RBC concentrations are both successfully used as indicators of fatty acid status. Red blood cell concentration serves as a long-term assessment tool and is not subject to slight changes in the recent diet (56). Fat accretion in the fetus occurs primarily throughout the last trimester of pregnancy (57-59), thus measuring maternal RBCs late in pregnancy serves as a more appropriate indicator of maternal status and fatty acid composition delivered to the fetus during a significant period of adipose accretion.

Evidence of fatty acid associations

Current research has focused primarily on measuring EPA, DHA, and AA and very little on other individual fatty acids. However, variability exists for when in pregnancy fatty acids were measured fluctuating between measuring blood early versus later in pregnancy. N-6 fatty acids appear adipogenic while n-3s show a contrary effect (9). Donahue and colleagues assessed mid-pregnancy maternal intake and plasma concentrations of n-3 fatty acids DHA and EPA, and found the dietary fatty acids to be associated with lower subscapular and triceps skinfolds as well as produced lower odds risk of obesity in the mother's offspring, yet plasma level associations were not significant (10). Smits et al. measured plasma fatty acids early in pregnancy in 1,659 women. They found EPA, DHA, and dihomogamalinolenic acid (DGLA) were positively related to birth weight, while AA was negatively related to birth weight (13). An intervention study by Much and colleagues administering daily 1,200 mg n-3 fatty acid supplements showed that late pregnancy (32 weeks) RBC DHA, total n-3, and total n-6 were positively related to birth weight.

Additionally, DHA, n-3, n-6, and AA were positively related to newborn lean body mass, and total n-3 was positively related to FM at birth, assessed by sum of skin folds (14).

Other studies have included additional fatty acids in their studies. Van Eijsden and colleagues measured total plasma n-3, n-6, DGLA, and eliadic acid (18:1n-9t, the primary industrial trans fatty acid in the diet) early in pregnancy in 3,704 women. After adjusting for confounding variables, they found n-3s and DGLA to be positively related to infant birth weight while AA was negatively associated (15). Additionally, a higher proportion of n-3 fatty acid to total PUFA intake demonstrated increased infant growth, however, only in overweight women (46). Data have been conflicting in regard to n-6:n-3 ratio, producing both positive associations and a lack of associations with infant body composition (9, 48). Further studies using accurate and precise techniques to measure infant body composition at birth are needed to clarify these results. While much research has been devoted to these important essential fatty acids, little has been conducted to learn about the potential effects of other individual fatty acids. Vaccenic acid is one such fatty acid that has recently taken stage as an indicator of health status in both animal and human studies (17).

Vaccenic acid

Vaccenic acid (18:1n-7t) is a natural monoene isomer of oleic acid (18:1) and is the major trans isomer in ruminant fats (17). It is created by the incomplete microbial biohydrogenation of linoleic acid (LA) (18:2 n-6) and ALA (18:3 n-3) through the primary work of *Butyrivibrio fibrisolvens* bacteria (18). Vaccenic acid is also a precursor to cis-9, trans-11 conjugated linoleic acid (c9, t11 CLA) and is converted through a delta-9 desaturation. This can occur in both humans and ruminant animals (17). CLA has been researched for its beneficial health properties including immune function and its impact on body fat (18). Some research

suggests that the beneficial effects of vaccenic acid stem from its conversion to CLA (60), yet others provide data suggesting it confers more direct effects by improving dyslipidemia and immune response (17).

Many studies have been conducted in animal models (17, 61), with a small portion investigating the association of maternal concentrations with infant levels (18, 62). Maternal vaccenic acid positively correlates with infant cord blood (18, 62), although fetal concentrations appear to be half that of maternal blood (62). Interestingly, Rist and colleagues concluded that an organic diet can produce significantly greater levels of vaccenic acid and CLA in breast milk compared to consuming a similar diet of conventional products (18). In this pilot study, women who consumed 50% or greater energy from organic meat and dairy products had roughly 30% higher CLA content in their breast milk at both 4 and 40 days after birth (18). Additional research regarding ruminant fatty acids and infant outcomes has shown that increased n-3 LCPUFAs, CLA, and vaccenic acid in breast milk were associated with a decreased risk of eczema, atopic dermatitis, and IgE sensitization after the first year of life (63). Further statistical analyses revealed the association between these ruminant fatty acids was independent of the n-3 fatty acids (63). These findings of potential health benefits of vaccenic acid substantiate reason to further investigate the association between maternal levels and infant body composition.

Conclusion

The current epidemic of the overweight and obese population has reached rates producing a declining health status as well as enormous economic burden. Chronic health diseases such as type 2 diabetes, cardiovascular disease, cancer, and more are conditions plaguing adults and early signs of these are becoming more prevalent in younger populations. The need to investigate

possible causes of these health conditions is evident, and one potential source may be learned from research in maternal and infant health. The maternal environment, shaped by factors such as pre-conception weight status, physical activity, lifestyle habits, and diet significantly impact the developing fetus and growing infant. This influence is apparent both in the short and long term, as evidenced by birth weight, body composition, and later presence or absence of excess weight and health concerns as children and adults. Fatty acids serve as critical players for *in utero* organ development as well as fat accretion and growth. Research is needed to expand upon what is known about the essential fatty acids, EPA, DHA, and AA, and uncover potential associations between other fatty acids present in the maternal diet, such as vaccenic acid. This thesis project will address these issues and investigate to answer the question of the relationship between maternal fatty acids late in pregnancy and infant body composition at birth and early infancy.

Chapter 3: Methods

Overview

This study evaluated the relationship between maternal fatty acids and infant body composition at birth and quality of growth during early infancy. It used combined samples from two observational studies from which data has already been collected. The EPIC and Thrasher Studies provided mother-baby pairs who had completed a blood draw measurement around 34-38 weeks gestation, as well as a birth and 3-4 month visit in which infant body composition data were collected.

Sample

Subjects from the EPIC and Thrasher Studies were included if they had completed a blood draw around 34-38 weeks gestation and completed a birth and/or 3-4 month post-delivery visit obtaining infant body composition data. Other inclusion criteria consisted of mothers free of known infectious disease or illness, possessing a body mass index (BMI) of 18.5-40 kg/m^2, and those with singleton pregnancies. Exclusion criteria omit participants who had a BMI less than 18.5 or greater than 40 kg/m^2, those with preeclampsia or gestational diabetes, those under the age of 18, and those who did not speak English.

Subjects were approached in the KU Obstetrics and Gynecology clinic upon approval granted by nursing staff. The study and the consent form were explained in detail. Subjects who agreed to participate were encouraged to ask questions and after consenting, registration forms for official inclusion in the study were completed.

The number of mother-baby pair subjects used in this study was 74 from the EPIC Study and an additional 23 pairs were added from the Thrasher and Study.

Setting

The EPIC and Thrasher Studies were each conducted at the University of Kansas Hospital and Medical Center.

Ethics

The Human Subjects Committee (HSC) approved the EPIC Study (HSC #: 12793) and Thrasher Study (HSC #: 13126) and each used an approved consent form to obtain participation from subjects.

Procedures

Maternal blood

Maternal blood was drawn late in pregnancy (34-39 weeks gestation) and by a Registered Nurse. At this visit the participant's arm was sanitized and fixed with a tourniquet 3-4 inches above the location of vein access. Roughly 20 mL of blood were collected into green tubes and appropriately labeled with the participant's name, identification number, date and time of draw, as well as the nurse's initials. Blood was refrigerated for no longer than 24 hours before being processed and separated into RBC, buffy coat, and plasma layers. The blood was centrifuged for 10 minutes at 4ºC at 30,000g-min in an Ependorf 5804 R refrigerated bench-top centrifuge with an A-4-44 swing-bucket rotor. Red blood cells, buffy coat, and plasma were pipetted (Pasteur pipet) into a 1.5 mL, 10.8 x 40.6 mm plastic micro centrifuge tube, flushed with nitrogen gas, capped, and stored at -80ºC. Aliquots were labeled with the subject ID, content of tube, gestational week at which blood was drawn, date of processing, and initials of the person who processed the blood. The complete aliquots were stored in Ependorf boxes labeled with the name of the study, types of sample included, date the box was started, and the initials of who started

the box. Processing of blood was done by trained research staff of the KU Medical Center Dietetics and Nutrition Department.

Red blood cell analysis

Red blood cell phospholipids were used to determine individual fatty acid composition of the blood. Lipids were extracted by using a modified method from Folch et al (64) including processes of total phospholipid separation and transmethylation. To prepare materials needed for separation, 4mL of methanol were added to clean extraction tubes followed by 500µL of RBCs and were immediately mixed by vortex. One hundred microliters of an internal standard (17:0) were subsequently added and mixed (vortex) and left to rest for 15 minutes after 8mL of chloroform were mixed into the solution. Contents were transferred through a funnel fitted with filter paper (Whatman #1) into a clean extraction tube. KCl (1.6mL, 0.5 M) was used to wash the extract under vortex and was further centrifuged for 5 minutes (at 750 r.p.m.), then separated with a clean Pasteur pipet. The upper phase was appropriately discarded and the lower phase was evaporated under nitrogen in a 35ºC water bath. While evaporation was occurring (roughly 30-45 minutes), a dry bath was turned on and 1mL of boron-trifluoride (BF3) was pipetted into new extraction tubes and placed on ice.

Once the extract was entirely dry, 100µL of dichloromethane were used to dissolve the extract. Silica plates were heated for at least 20 minutes, allowed to cool, and then spotted with all 100µl of the sample. Spotted plates were immediately placed in a thin layer chromatography (TLC) chamber containing 80:20:1 of hexane:ether:acetic acid (adequate space for two plates per chamber). This process enabled the distinguishing of triacylglycerol from phospholipids. Solvent was allowed to travel up to the top of the silica plate and then was removed from the TLC chamber for drying. The band generated from the phospholipids was identified and collected

from the plate with a single-edged blade and placed in another clean extraction tube containing 1mL (BF_3).

Transmethylation of samples was completed by layering each tube with nitrogen (placed on ice until all samples were finished spotting), and placed in a 100ºC dry bath for 10 minutes. Tube caps were checked for tightness after 2 minutes of heating to ensure caps were not coming loose. After heating, tubes were immediately placed on ice and cooled; 1mL of water and 2mL of pentane were added once samples were chilled. Fatty acid methyl esters (FAMEs) were extracted from the pentane phase after 1-2 minutes of vortex, and further centrifuged for 5 minutes at 800 r.p.m. The upper pentane phase was separated with a Pasteur pipet into a Varian 2mL vile (transmethylation tubes then appropriately discarded). A stream of nitrogen was used to concentrate the FAMEs and once dry were combined with 70µL of dichloromethane.

Each tube was swirled to capture all traces of FAMEs in the tube and then transferred to a sleeve using a Hamilton syringe. The sleeve was then placed into a 2 mL vial and secured with a Teflon-lined cap. Samples were analyzed in groups along with one weighted standard sample in a Varian 3900 gas liquid chromatography with CP 8400 autoanalyser. FAMEs were detected through evaluation of their relative retention periods matched to pure standard blends. Peak travel of each fatty acid was measured and divided by the total area of all combined fatty acids to calculate the percentage of each single fatty acid present in the sample. Clues of oxidation or mishandling of the sample was evidence to repeat the test for improved quality of results.

Infant body composition

Infant body composition was measured within the first 72 hours of life and at 3-4 months of age by an instrument called a PeaPod® using air displacement plethysmography (ADP).

Multiple studies have validated this measure of body composition and confirmed its ease of use (65). The PeaPod® system is a non-invasive and appropriate instrument to use when calculating body density from body volume and weight (66). Body density enables the estimation of %fat with the use of established densities of FM and FFM with an insignificant difference in measurements compared to a four-compartment model (66).

First, length was measured to the nearest 0.1 cm using a Shorr board designed for infants (Shorr Productions) and weight was measured with an integrated PeaPod® scale to the nearest 0.0001kg. Upon proper calibration of the instrument, the infant was fitted with a standard compression cap and placed nude in a supine position into the plastic test chamber. Once the chamber door closes, the infant remained inside for 2 minutes to estimate the infant's occupied volume. The Fomon equation was used to estimate the infant's body density, %fat, FM, and FFM (67). The infant PeaPod® measurements were conducted by trained research staff of the KU Medical Center Dietetics and Nutrition Department.

Analysis of data

Means and standard deviations were calculated for all variables of interest. Data checks and visual inspection of data was performed to determine if there were any outliers or errors in the data. Specific data analysis was performed for each aim of the study. For analyses that included confounding variables, inter-relatedness of the confounding variables was explored before inclusion in the model. Variables that are known to be related to infant body composition were included in the model but only significant variables were retained in the final model. Due to the sample size, the number of confounding variables was limited. These are the following maternal variables that were explored: age, parity, pre-pregnancy BMI, gestational weight gain,

and median household income. The following infant variables were explored: age at test, gestational age, and gender. The significance level was $P<0.05$.

Aim 1: To determine if maternal fatty acids are related to infant body composition at birth. *Statistical analysis:* A correlation matrix was run to determine the relationships between the measured maternal fatty acids and infant %fat, FM and FFM at birth.

Aim 2: To determine if maternal fatty acids are related to change in infant body composition in early infancy. *Statistical analysis:* A correlation matrix was run to determine the relationships between the measured maternal fatty acids and infant %fat, FM, and FFM in early infancy.

Aim 3: To determine if maternal vaccenic acid values are related to infant body composition at birth. *Statistical analysis:* Multiple linear regression was completed to assess the relationship between maternal vaccenic acid values and infant body composition at birth. Maternal vaccenic acid was the independent variable and the respective infant body composition parameter was the dependent variable.

Aim 4: To determine if maternal vaccenic acid values are related to change in infant body composition in early infancy. *Statistical analysis:* Multiple linear regression was completed to assess the relationship between maternal vaccenic acid levels and change in %fat, FM, and FFM in early infancy. Maternal vaccenic acid was the independent variable and the respective infant body composition parameter was the dependent variable.

Chapter 4. Results

The purpose of this research was to explore the relationship of maternal RBC fatty acids, with an emphasis on vaccenic acid, and infant body composition at birth and in early infancy. Maternal RBC fatty acids were collected late in pregnancy and correlated with infant body composition including %fat, FM, and FFM at birth and the change in infant body composition from birth to 3-4 months of age.

Maternal characteristics

Seventy four mother-infant pairs were available for analysis for the birth visit from the EPIC and Thrasher studies as shown in Table 1. Of this sample, the average age was 29.42 ± 4.81 years. The average pre-pregnancy BMI was 25.49 ± 5.44 kg/m^2. The majority (62%) of mothers had normal pre-pregnancy BMIs (18.0-24.9 kg/m^2), with 14% having overweight BMIs (25.0-29.9 kg/m^2), and 24% obese BMIs (≥ 30.0 kg/m^2) (Figure 1). Total GWG was 16.07 ± 6.14 kg. The median income per household was calculated based on self-reported zip code of residence. Median income data was extracted from 2009 values using Census Bureau information from the Mid-America Regional Council database (68). Median income was $52,954.51 ± $17,791.57. Seventy four percent of mothers were of Caucasian ethnicity, followed by 16% Asian, 7% Hispanic, and 3% other (Figure 1).

Table 1. Maternal Descriptive Statistics (n = 74)

Variable	Mean	Std. Deviation
Age (years)	29.43	4.81
Pre-pregnancy BMI (kg/m^2)	25.49	5.44
Total GWG (kg)	16.07	6.14
Median Income (dollars/year)	$52,954.51	$17,791.57

Figure 1.

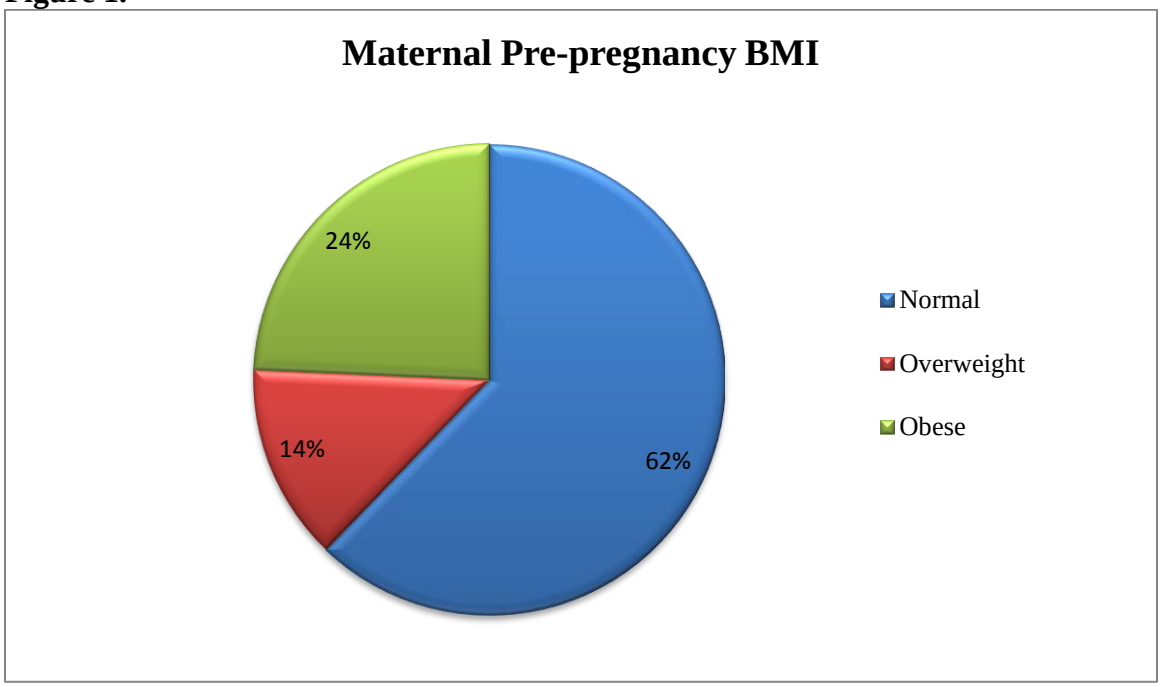
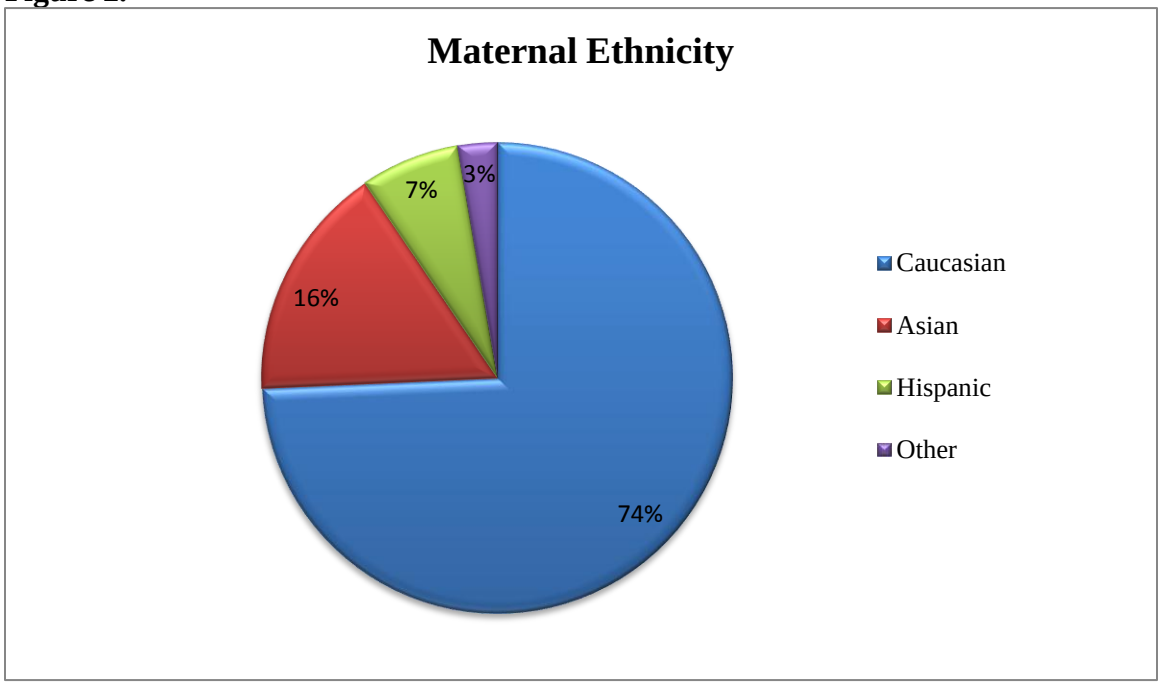


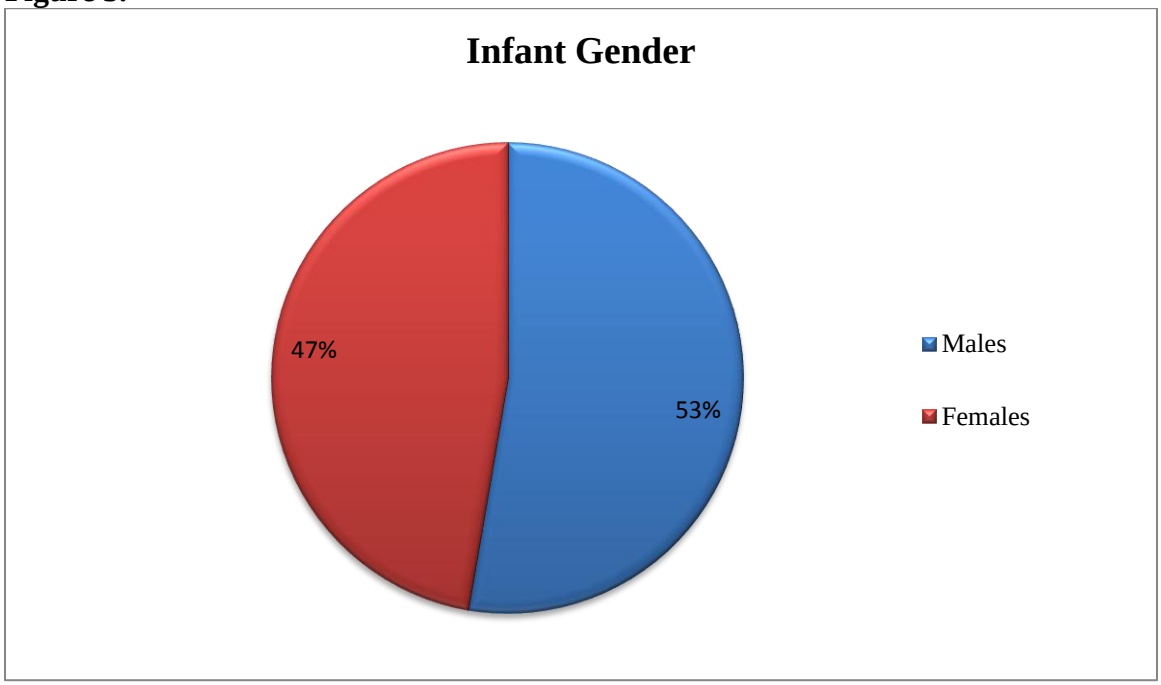
Figure 2.



Infant characteristics

Fifty three percent of infants were male and 47% were female, as shown in Figure 3. Descriptive statistics at birth including gestational age, birth weight, length, and head circumference were collected from electronic medical records; if the infant was born at a location other than the University of Kansas Hospital, this information was provided by the mother at visit 1 (Table 3). At birth, all infants were born term (>37 weeks), and the average gestational age was 39.61 ± 0.82 weeks. Average birth weight was 3,503.47 ± 420.22 g. Mean birth length was 50.87 ± 3.42 cm, and mean head circumference was 34.46 ± 1.62 cm.

Figure 3.



Infant body composition at birth

At visit 1 (1-3 days after birth), body composition measures were calculated from the infant PeaPod® as described previously in the methods section. Average infant age at visit one was 0.45 ± 0.66 weeks (Table 2; n=74).

Percentage Body Fat: 11.31 ± 4.75%

Fat Mass: 384.79 ± 195.12 g

Fat Free Mass: 2,913.06 ± 307.83 g

Infant body composition at visit 2

Identical measures were taken at visit 2 (3-4 months). Average age at visit 2 was 17.11 ± 3.09 weeks (Table 2; n=58).

Percentage Body Fat: 25.69 ± 4.84%

Fat Mass: 1,726.42 ± 414.87 g

Fat Free Mass: 4,948.35 ± 607.42 g

Change in infant body composition

The change in infant body composition was generated by calculating the difference between visit 1 and 2 measures for %fat, FM, and FFM (i.e. change = visit 2 – visit 1) (Table 2; n=58).

Change in Percentage Body Fat: 14.73 ± 5.66%

Change in Fat Mass: 1,349.97 ± 433.11 g

Change in Fat Free Mass: 2,100.23 ± 846.23 g

Table 2. Infant Descriptive Statistics

Variable	Mean	Std. Deviation
Birth (n = 74)		
Gestational Age (wks)	39.61	0.82
Birth Weight (g)	3,503.47	420.22
Length (cm)	50.87	3.42
Head circumference (cm)	34.46	1.62
Visit 1, 1-3 days (n = 74)		
Age at test (wks)	0.45	0.66
Percentage Body Fat (%fat)	11.31	4.75
Fat Mass (g)	384.79	195.12

Fat Free Mass (g)	2,913.06	307.83
Body Mass (g)	3,297.85	428.31
Visit 2 & Change in Growth, 3-4 months (n = 58)		
Age at test (wks)	17.11	3.09
Percentage Body Fat (%fat)	25.69	4.84
Fat Mass (g)	1,726.42	414.87
Fat Free Mass (g)	4,948.35	607.42
Body Mass (g)	6,674.05	818.12
Percentage Body Fat change (%fat)	14.73	5.66
Fat Mass change (g)	1,349.97	433.11
Fat Free Mass change (g)	2,100.23	846.23
Body Mass change (g)	3,319.80	823.78

Significant fatty acids at visit 1

The correlation matrix revealed two fatty acids that reached significance for measures of infant body composition at visit 1 (Table 3). The n-6 fatty acid, AA (20:4n-6), showed a negative relationship with infant FFM (r = -0.28, p = 0.015) while a positive relationship was found between saturated heneicosanoic acid (21:0) and infant FM (r = 0.23, p = 0.049). Vaccenic acid (18:1n-7t) showed an association that approached significance (presented in italics in Table 3).

Table 3. Correlation Matrix of Significant Fatty Acids for Visit 1

Fatty Acid (%)	Percentage Fat (%fat)	Fat Mass (g)	Fat Free Mass (g)
Vaccenic acid 18:1n-7t	0.19 0.109	0.23 ***0.051***	0.078 0.519
Arachidonic acid 20:4n-6	-0.06 0.623	-0.12 0.319	-0.28 **0.015**
Heneicosanoic acid 21:0	0.18 0.128	0.23 **0.049**	0.19 0.105

Top line: Pearson Correlation (r)
Bottom line: Significance (p value); values < 0.05 were considered significant; italics indicate values approaching significance

Significant fatty acids for growth change

A greater number of fatty acids produced significant relationships, when assessed by simple correlations, with the change in infant growth at 3-4 months of age (Table 5). Two trans isomers were found to be related to measures of growth change. Vaccenic acid (18:1n-7t), a natural trans isomer of CLA, was negatively related to the change in infant FM and FFM (r = -0.35, p = 0.007; r = -0.40, p = 0.002, respectively). The trans isomer of LA, linolelaidic acid (18:2n-6t) was negatively associated to change in infant FFM (r = -0.31, p = 0.018). A positive relationship between the trans isomer of oleic acid, eliadic acid (18:1n-9t), and the change in infant FFM approached significance (p=0.082).

The essential fatty acid, LA (18:3n-6), and EPA (20:5n-3), a derivative of the essential fatty acid ALA, were both positively related to change in FFM (r = 0.58, p = 0.000; r = 0.42, p = 0.001, respectively). Lastly, heneicosanoic acid (21:0) was negatively related to change in %fat as well as FM (r = -0.31, p = 0.019; r = -0.30, p = 0.022, respectively). Relationships between ALA (18:3n-3) and AA (20:0) and %fat and FFM approached significance (presented in italics in Table 4).

Table 4. Correlation Matrix of Significant Fatty Acids for Growth Change

Fatty Acid	Percentage fat (%fat)	Fat Mass (FM) (g)	Fat Free Mass (FFM) (g)
Vaccenic acid 18:1n-7t	-0.29 **0.029**	-0.35 **0.007**	-0.40 **0.002**
Eliadic acid 18:1n-9t	-0.07 0.608	-0.04 0.784	0.23 ***0.082***
Linolelaidic acid 18:2n-6t	0.08 0.564	-0.06 0.683	-0.31 **0.018**
Alpha linolenic acid 18:3n-3	-0.24 ***0.076***	-0.21 0.115	0.01 0.942
Linoleic acid 18:2n-6	0.11 0.413	0.12 0.389	0.58 **0.000**

Arachidic acid 20:0	-0.06 0.646	-0.11 0.403	-0.24 ***0.068***
Eicosapentaenoic acid 20:5n-3	0.18 0.173	0.10 0.445	0.412 **0.001**
Heneicosanoic acid 21:0	-0.31 **0.019**	-0.30 **0.022**	-0.21 0.109

Top line: Pearson Correlation (r)
Bottom line: Significance (p value); values < 0.05 were considered significant; italics indicate values approaching significance

Linear regression models

Linear regression models were conducted to analyze variables of interest that were related to measures of infant body composition at birth and the change in growth at 3-4 months. Variables that were not significantly related to the measure of focus were excluded from the model until only significant variables remained.

Infant body composition at birth

Percentage body fat:

Table 5. Linear Regression Model to Predict Infant Body Fat (%) at Birth (n = 74).		
	β	**Significance**
Infant Gender (0 = male)	2.79	0.010
R^2 = 0.08		

Infant gender (0 = male) was the only variable that was related to infant body fat at birth ($\beta = 2.79$, $p = 0.010$). Thus, females were predicted to be born with 2.79% higher percentage body fat when compared to male infants.

Fat mass:

Table 6. Linear Regression Model to Predict Infant Fat Mass (g) at Birth (n = 74).		
	β	**Significance**
Infant Gender (0 = male)	88.65	0.043
Infant Age at Test (weeks)	79.82	0.017
R^2 = 0.11		

Infant gender (0 = male) and infant age at test (weeks) predicted infant FM at birth (β = 88.65, p = 0.043; β = 79.82, p = 0.017). Thus, females were predicted to be born with 88.64 more grams of FM compared to male newborns; and for every week increase in age at the time of measurement, an increase of 79.82 grams of FM was predicted to accumulate.

Fat free mass:

Table 7. Linear Regression Model to Predict Infant Fat Free Mass (g) at Birth (n = 74).		
	β	**Significance**
Infant Gender (0 = male)	-134.59	0.035
Infant Age at Test (weeks)	139.51	0.004
Parity (0 = first birth)	130.76	0.041
Infant Gestational Age (weeks)	174.60	0.000
R^2 = 0.27		

Four variables were found to predict infant FFM at birth. Infant gender (β = -134.59, p = 0.035), infant age at test (β = 139.51, p = 0.004), parity (0 = first birth) (β = 130.76, p = 0.041), and infant gestational age (weeks) (β = 174.60, p = 0.000) were predictors. From this regression model, females were predicted to have 134.59 grams less FFM when compared to males. Each one week increase in age at test as well as gestational age resulted in an increase of 139.51 and 174.60 grams of FFM, respectively. Lastly, each previous pregnancy (increase in parity) resulted

in 130.76 grams greater FFM prediction at birth compared to infants born to mothers in their first pregnancy.

Change in infant body composition

Percentage body fat:

Table 8. Linear Regression Model to Predict the Change in Infant Percentage Body Fat (%) (n = 58).		
	β	**Significance**
Vaccenic acid (18:1n-7t)	-7.51	*0.056*
R^2 = 0.05		

No variables remained significant in the model for the change in infant %fat; however, vaccenic acid approached significance (β = -7.51, p = 0.056).

Fat mass:

Table 9. Linear Regression Model to Predict the Change in Infant Fat Mass (g) (n = 58).		
	β	**Significance**
Infant Age at Test (weeks)	49.20	0.008
Vaccenic acid (18:1n-7t)	-596.77	0.041
R^2 = 0.20		

Infant age at test and vaccenic acid were the two variables predicting the change in infant FM (β = 49.20, p = 0.008; β = -596.77, p = 0.041, respectively). Each one unit increase in age at test predicted a 49.20 gram increase in FM. Vaccenic acid did not vary greatly between BMI categories in our sample of mothers (normal = 0.95%, overweight = 0.92%, obese = 1.05%, p = 0.220), thus a 1 unit (percentage point) increase was not a realistic example to provide. To capture a more meaningful influence of vaccenic acid to change in infant FM, a linear equation

was calculated to predict the change in FM from a 0.1% difference in vaccenic acid. For each 0.1% increase in vaccenic acid, a 59.68 gram decrease in the change of infant FM was predicted.

Fat free mass:

Table 10. Linear Regression Model to Predict the Change in Infant Fat Free Mass (g) (n = 58).

	β	**Significance**
Infant Age at Test (weeks)	97.47	0.000
Gender (0 = male)	-424.81	0.000
Vaccenic acid (18:1n-7t)	-682.16	0.002
R^2 = 0.69		

Three variables, infant age at test (β = 97.47, p = 0.000), gender (β = -424.81, p = 0.000), and vaccenic acid (β = -682.16, p = 0.002), remained significant in the linear regression model for change in infant FFM. For every week increase in age, a 97.47 gram increase in FFM was predicted to result. Females were predicted to have 424.81 grams less FFM compared to male infants. Lastly, and in a similar fashion to calculating vaccenic acid prediction of change in infant FM, linear equation produced that for every 0.1% increase in maternal vaccenic acid, change in FFM would decrease 68.16 grams.

Chapter 5. Discussion

In this study, maternal RBC fatty acids were analyzed to explore their relationship with infant body composition at birth and change in infant growth at 3-4 months of age. There were a few fatty acids related to measures of infant body composition at birth as measured by %fat, FM, and FFM; however, vaccenic acid proved to be negatively related to two of the three measures of the *change* in infant body composition from birth to 3-4 months. As shown in linear regression models and equations, increases in maternal vaccenic acid concentrations predicted decreased growth in both infant FM and FFM.

Prior studies have assessed maternal fatty acid composition, with emphasis on n-3 and n-6 fatty acids, in relation to adiposity (newborn and childhood) and birth weight (10, 13-15), while few have investigated measured indices for body composition (69-71) and assessed a wide spectrum of fatty acids. To our knowledge, this was the first study to explore maternal RBC fatty acids late in pregnancy and their association with measured parameters of infant body composition at birth and the change in body composition in early infancy.

Fatty acids significant at birth

Our results showed significant relationships between AA and heneicosanoic acid with infant FFM and FM at birth, respectively. AA, a polyunsaturated n-6 fatty acid was negatively related to infant FFM at birth. This fatty acid is present in animal products (meat, fish) and is produced from the desaturation and elongation of the essential fatty acid, LA. AA has been previously studied for its relationship with birth weight and has shown to be associated with decreased birth weight (13, 15).

Smits et al. measured maternal plasma fatty acids of 1,659 women early in pregnancy and found a positive association between EPA, DHA, and DGLA and birth weight, and also found a negative association between AA and birth weight (13). Similarly, van Eijsden et al. found a positive association between EPA and DGLA and birth weight, and a negative association between AA and birth weight. Maternal plasma was taken early in pregnancy and found that the combination of low EPA and DGLA with high AA predicted a 52-57 gram decrease in birth weight (15).

Our results indicated a negative relationship between AA and infant FFM at birth, which agrees with previous data suggesting an overall decrease in birth weight (although our blood samples were measured late in pregnancy and in RBCs rather than early in pregnancy and with plasma). In contrast, an intervention study lasting from 15 weeks gestation through 4 months postpartum using a daily supplement of 1200mg of n-3 fatty acids and nutritional counseling in 208 women found AA to be positively related to infant lean body mass at 1 year of age, assessed by skin fold thickness measurements (14). Our results did not find a significant positive relationship between AA and infant FFM at birth however we do not have measures of lean mass at one year. Heneicosanoic acid is a saturated fatty acid often present in human milk fat, and in our results was positively associated with FM at birth. This provokes the question of whether the high RBC content of heneicosanoic acid could reflect the content present in the mothers' milk, and potentially influence infant body composition after months of breastfeeding. Further, the mean content found in our sample was 0.11%, a rather small amount. To date, heneicosanoic acid has not previously been studied in relation to infant or even human body composition.

Fatty acids significant in change in growth

Results from our simple correlation analysis found multiple fatty acids were related to infant growth from birth to 3-4 months of age when compared to fatty acid's related to infant body composition at birth. In markers of change in infant body composition, %fat, FM, and FFM, vaccenic acid, linolelaidic acid, LA, EPA, and heneicosanoic acid each showed an association with at least one measure of the change in infant body composition. Vaccenic acid was negatively related to all three of the three measures of the change in body composition.

This finding is of importance, as vaccenic acid is a natural trans isomer of oleic acid and precursor to CLA, and has been previously found to confer certain health benefits both inside and outside of its role in forming the health protective CLA (17, 18, 60, 62). As shown by our results, vaccenic acid was negatively associated with the change in infant growth parameters (FM and FFM). Vaccenic acid has not been studied regarding human infants and body composition measures thus far, but has been researched in conjunction with CLA in piglets representing an infant animal model. Corl et al. (61) performed a 2 X 2 factorial design where 24 piglets were fed either a low- or high-fat diet supplemented with or without CLA for 16-17 days beginning days after birth. At the end of the study, body weight did not differ between groups; however, piglets fed the high-fat diet accumulated 50% more body fat than piglets fed the low-fat diet. Further, those supplemented with CLA had reduced body fat accretion regardless of diet, measured by total body grinding and separation to determine water, crude protein, and fat content (61). Additionally, they found that CLA supplementation resulted in a decreased expression of acetyl CoA carboxylase and lipoprotein lipase in adipose tissue, reducing fatty acid synthesis and utilization. This agrees with other data suggesting hypolipidemic effects of vaccenic acid in animals and humans (17, 72-74).

Linolelaidic and heneicosanoic acid, trans and saturated fatty acids, respectively, were negatively related to changes in infant growth. Linolelaidic acid is a trans isomer of LA and is found in sources such as hydrogenated vegetables oils as well as quail egg. It was negatively related to the change in infant FFM, while heneicosanoic acid was negatively related to change in %fat and FM. Linolelaidic acid has not been researched previously in isolation; however, dietary trans fatty acids have been shown to be associated with greater infant %fat assessed by PeaPod® ADP (69) as well as related to child adiposity, though not significant (10).

Our results for heneicosanoic acid present a change in relationship as this fatty acid was positively related to FM at birth, yet negatively related to %fat and FM changes at 3-4 months. These findings are unique as no other study has explored the relationship between these fatty acids and growth in early infancy. As heneicosanoic acid is commonly found in breast milk, the negative association found between change in %fat and FM could be related to these infants being exclusively breast fed compared to those also supplemented with formula. The effect of breastfeeding was not explored in this analysis.

Positive relationships were also found in our data. The essential n-6 fatty acid, LA, and EPA (n-3) were both found by simple correlations to be positively related to the change in infant FFM. Therefore greater concentrations of these fatty acids were associated with greater increases in infant FFM from birth to 3-4 months. , Similar findings were found in previous studies assessing the relationship between birth weight and EPA and n-6 fatty acids (13, 15). Van Eijsden et al. (15) found total n-6 was negatively associated with birth weight though after adjustment for covariates, significance was lost. Further, Donahue et al., examined maternal n-3 and n-6 concentrations in the diet (late pregnancy) and plasma (mid-pregnancy) and found that higher dietary n-3 intake and umbilical cord plasma concentrations were associated with lower

child adiposity at age 3, however maternal *plasma* DHA and EPA were not significantly related (10). Much et al. studied how an intervention with daily 1,200mg n-3 supplementation and nutritional counseling from gestational week 15 through 4 months postpartum impacted the relationship between maternal RBC LCPUFAs and infant growth at 1 year of age. Their findings show agreement with ours regarding an increase in tissue growth, indicating n-6 LCPUFAs were positively related to BMI and ponderal index at 1 year, yet none were related to infant FM, measured by skin fold thickness. However, Moon et al. found total n-6 positively predicted FM at 4 and 6 years of age though none were related to lean mass (9). While some data appear to be conflicting, research does support the association between n-3 and n-6 LCPUFAs and birth weight, FM, and FFM at birth and beyond, as we also found in our results.

Vaccenic acid related to infant body composition at birth

Linear regression models showed that vaccenic acid did not predict infant %fat, FM, or FFM at birth. These findings support our previous correlational matrices of fatty acids associated with measures of infant body composition at birth, as vaccenic acid was not related to any measure, yet reached greater significance when analyzing change in infant body composition.

Vaccenic acid and the change in growth

As shown in the correlation matrices for fatty acids related to the change in infant body composition, vaccenic acid predicted the change in infant FM and FFM, and approached significance for predicting the change in %fat ($p = 0.056$). These results indicate that increased vaccenic acid is related to the change in growth of both fat and FFM from birth to 3-4 months of age.

To help explain the negative association found between FM and FFM and vaccenic acid, we explored differences in vaccenic acid based on pre-pregnancy BMI group. We performed a t-test to determine if differences were found between maternal BMI group (normal, overweight, obese) for vaccenic acid concentration. There were no significant differences in vaccenic acid values between BMI groups in our sample. Ode et al. reported on changes in infant body composition from birth to 3 months based on maternal pre-pregnancy BMI group though maternal fatty acids were not measured. They found a deceleration in infant growth from birth to 3 months in measures of weight, length, and FM (measured via ADP) in 97 infants. Each of these measures was significantly lower in infants born to overweight or obese mothers when compared to those born to normal weight mothers (75). Results of these associations were strongest in overweight mothers, who gained excessive gestational weight, and the greatest weight for the entire sample (mean GWG = 16.38 ± 1.34 kg).

While pre-pregnancy BMI may not explain the negative relationship we found between maternal vaccenic acid concentration and change in infant body composition, analysis did reveal the greatest concentration of vaccenic acid to be in obese mothers (non-significant; 1.05%) and may be some influence, though not fully understood, in the change in infant body composition.

Though no relationship between vaccenic acid and body composition has been published, vaccenic acid appears to play a significant role, potentially both alone and as precursor to CLA, in reducing atherosclerotic progression, inhibiting fatty acid uptake, increasing apoptosis and decreasing proliferation (expressing anticancer properties), and enhancing immune function (17, 62). Our results seem to conform to this previous research. Vaccenic acid was associated with a decrease in the growth of FM and FFM. However, the appropriateness of this decrease in newborn infants is questionable. While it is well known how vital fatty acids are in fetal

development *in utero*, they are also crucial in early infancy for optimal growth, function, and well-being. As decreased growth of adipose tissue can be beneficial in the adult population, the same may not be true for infants who are still developing the foundation for which they will build upon in childhood and beyond. Further research is needed to replicate these findings and interpret their impact on long term adiposity and risk of chronic disease due to excess or inadequate FM and FFM.

There are several strengths to this study. First, maternal RBC fatty acids were measured using a sophisticated methodology. Maternal fatty acids were assessed late in pregnancy. However, maternal RBC analysis provides a stable snapshot of fatty acid status over the last 3 months. Second, infant body composition was measured with validated and accurate technology at birth and again at 3-4 months to allow relationships to be assessed in the change in infant body composition. There are also limitations to the analysis. The sample size was small. Further, infant body composition data beyond 3 months were not available to determine if the relationships are present at later ages. We did not control for feeding type (exclusively breast fed, mixed fed, formula fed) which may have played a role in infant body composition. Fatty acid status was measured only in mothers and not infants, thus we can only make associations between the maternal fatty acid composition and infant body composition, and not appropriately predict identical amounts in the infant blood.

Conclusion

In conclusion, we found relationships between maternal fatty acids assessed late in pregnancy in relation to the change in infant body composition. Our results reiterate certain findings of other previous studies demonstrating a positive relationship between n-3 fatty acids,

such as EPA, and birth weight, and a negative relationship between certain n-6s, such as AA, and birth weight. They also introduce new findings regarding the saturated heneicosanoic acid and trans vaccenic acid, each negatively relating to measures of infant body composition change at 3-4 months. While the contribution made by vaccenic acid could not be explained by differences in maternal pre-pregnancy BMI groups, data did provide percent distributions among BMI groups which may give meaningful values that can predict later changes in infant body composition outcomes.

Further questions arise from our results. While vaccenic acid has been researched for its benefits relating to decreased lipid profiles and fat accretion, the appropriateness of this in newborns may not be the same as in adults. Current recommendations by the American Academy of Pediatrics suggest an infant not be transitioned to reduced fat milk until 2-3 years of age (76), thus data suggesting a reduction in both fat and lean tissue would seem to be more detrimental than beneficial in a growing infant's overall health. On the other hand, if less fat and FFM growth at 3-4 months is serving as a template for future growth and suggesting that the infant is more appropriately building to a smaller body mass, it may be viewed as a more positive phenotype. The way these findings translate into long term health requires further research of infants into childhood and beyond, assessing changes in body composition and other indicators of health status such as presence or absence diabetes, heart disease, and obesity. Greater sample numbers, longer assessment periods into childhood and adulthood, and supplementary measures of infant status (i.e. infant RBC fatty acid analyses) can enhance the current knowledge of the influence of maternal nutrition and lead to evidence-based recommendations for maternal fat intake to best support infant development and growth.

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