Nutrient Content of Human Breast Milk from Overweight and Normal Weight Caucasian Women of Northeast Tennessee

Megan R. Kwon
East Tennessee State University

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Nutrient Content of Human Breast Milk from Overweight and Normal Weight Caucasian Women of Northeast Tennessee

A thesis
presented to
the faculty of the Department of Allied Health Sciences
East Tennessee State University

In partial fulfillment
of the requirements for the degree
Masters of Science in Clinical Nutrition

by
Megan R. Kwon
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W. Andrew Clark, Chair Ph.D, RDN
Eileen Cress EdD, RDN
Ron Hamdy, M.D.

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ABSTRACT

Nutrient Content of Human Breast Milk from Overweight and Normal Weight Caucasian Women of Northeast Tennessee

by

Megan R. Kwon

Many factors influence the nutrient composition of breast milk (BRM) particularly within the fatty acids composition. In this study BRM between 2-14 weeks of lactation was collected from 44 Caucasian women (24 normal weight (NW, BMI 18.5-24.9kg/m$^2$) and 20 overweight (OW, BMI >25.0kg/m$^2$). BRM was subjected to proximate analysis and participants completed food frequency questionnaires (FFQ) to estimate fruit, vegetable, and fat intake. BMI differed between NW and OW groups, 22.03 vs 33.86kg/m$^2$ (p<.0001). No significant differences (p>0.10) were identified for total calories (5,581.8 vs 5562.9cal/g), %fat (39.6 vs 43.9), or %protein (9.8 vs 8.9) for BRM in NW versus OW women as determined by proximal analysis. Gas chromatography of fatty acid methyl esters demonstrated NW BRM contained higher levels of omega-3, mono-unsaturated, and less palmitic acid fatty acids (p>0.10). The increased palmitic acid level seen in OW BRM may support increased de novo synthesis of fatty acids.
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CHAPTER 1

INTRODUCTION

Background

Breastfeeding has long been considered the gold standard for feeding neonates. However, there is a large amount of variation in the composition of different mothers’ breast milk. These differences exist particularly within the omega-6 fatty acids and omega-3 fatty acids, specifically eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). The proposed fatty acid ratio of human breast milk is 30% mothers’ fatty acid intake, 70% fatty acid stores, and minimal amounts derived from de novo synthesis.¹ Tian et al. determined that modern-day hunter-gathers consume a diet high in long-chain polyunsaturated fatty acids with omega-6 to omega-3 fatty acids ratio of 1-2:1.² Currently, the average American diet has an average omega-6 to omega-3 fatty acid ratio of 15-30:1.²,³ The current recommendation for the optimal omega-6 to omega-3 fatty acid ratio for fetal and neonate development is between 5-10 to 1.¹ This demonstrates that the current American diet is not providing enough omega-3 fatty acids to support optimum breast milk for neonates. Most studies on lactation and fatty acids have been conducted in Europe and Central/South America with few to no significant studies taking place in the United States.

The type of fats neonates consume is critically important, especially DHA for its role in neurological development. Retinal development is a good indicator of neurological progress and the retina requires large amounts of DHA.⁴ Neonates who consume higher amounts of DHA also have enhanced motor development, as shown by their significantly higher scores in the eye-hand coordination test given at four months of
Approximately 50-60g of omega-3 fatty acids are transferred from the mother to the fetus during the third trimester of pregnancy for optimal neurological development. If the mother is deficient in omega-3 fatty acids during pregnancy or lactation, neurological damage may occur.

Fatty acid intake by neonates affects their short and long-term weight gains. Throughout pregnancy and lactation, adipocytes are being created by the fetus and neonate from the fatty acids supplied by the mother either via the placenta or breast milk. This process is involved in determining the infant's body fat composition. An increased omega-6 to omega-3 fatty acid ratio is linked to increased adiposity of the child at 3 years of age. The drastic increase in the consumption of omega-6 fatty acid by Americans over the past few decades has been theorized to have had negative effects on fetal and neonate fat cell composition and increased the likelihood of childhood obesity. By providing mothers the optimal omega-6 to omega-3 fatty acid ratio during pregnancy and early childhood, the risk of obesity can be reduced for the following generation. Neonates supplemented with omega-3 fatty acids have lower fat mass compared to those of un-supplemented neonates.

With the significant influence omega-6 and omega-3 fatty acid intake has on the lifelong effects on growth and development of infants, it is vital to understand the current omega-3 fatty acid intake of the maternal population. The purpose of this research was to assess factors related to maternal breast milk composition by infants in East Tennessee.
Purpose

The purpose of this study was to determine if differences in breast milk fatty acid composition are correlated to maternal weight, dietary intake, or ethnicity. Identifying key factors that influence the DHA content of breast milk will allow medical professionals to identify mothers and neonates who are at risk for insufficient DHA intake during pregnancy and lactation.

Research Questions

1. Is there a difference in the nutrient composition of breast milk after parturition between women who have a pre-gravid normal body mass index (BMI 18.5 to 24.9 kg/m²) and women who are overweight (BMI >25.0 kg/m²)?

2. Does maternal fatty acid dietary intake affect the fatty acid type and concentration of breast milk?

Hypotheses

Hypothesis One: There will be a significant difference in the fatty acid profile (greater saturated, omega-6 fatty acids and less omega-3 fatty acids) in overweight/obese women compared to women who are of normal body weight.
CHAPTER 2
REVIEW OF LITERATURE

Introduction

Breast milk is universally seen as the optimal source of nutrition for newborns and infants. However, breast milk composition varies between women affecting the overall quality and nutrition of the milk. One area of recent concern is the amount of long-chain polyunsaturated fatty acids (LCPUFA), specifically DHA, that breast milk provides to the infant. Areas heavily researched are the concentration of DHA in breast milk and its correlation to improved neurological development and reduced risk of childhood obesity.

In vitro studies show that the placenta transfers fatty acids from the mother to the fetus with the highest priority on DHA, followed by arachidonic acid (AA), alpha-linolenic acid (ALA), and lastly linoleic acid (LA). This shows the increased demand of DHA for fetal development and necessitates that maternal DHA intakes be adequate. The fatty acid profile in breast milk is comprised of 70% long-term maternal fat stores, 30% percent current maternal fat intake with minimal amounts derived from de novo synthesis. This is supported by Scholtens et al. who found that fatty acid composition mainly reflects the long term dietary intake, however current intake can influence breast milk composition. The major sources of LCPUFA and DHA in the human diet include marine foods, flax seeds, flax seed oils, and omega-3 fish oil supplements. Based on epidemiological studies, most women are not consuming sufficient omega-3 fatty acids from their diet due to low availability of fish, the high cost of purchasing fish, and a preference for not eating fish. In Canada, there has been an eighty percent
decline in omega-3 fatty acid intake over the past century.\textsuperscript{28} Women of lower socioeconomic status are at the greatest risk for LCPUFA and DHA deficiency.\textsuperscript{17} The purpose of this review is to analyze current research on how maternal weight, dietary intake, and ethnicity effects the fatty acid profile in human breast milk.

**Role of Maternal BMI on Breast Milk Composition**

Since maternal fat stores comprise a significant part of the fatty acids in breast milk, what role does maternal weight play in the different types of fatty acids expressed in breast milk? Do overweight or obese mothers have different fatty acids profiles in their breast milk than mothers of normal weight?

Antonakou et al. found a positive correlation between weight gain during pregnancy and increased amount of saturated fat in breast milk through six months of lactation (p>0.01).\textsuperscript{29} They also found a negative association between maternal weight gain and amount of monounsaturated fatty acids in the breast milk through three months of lactation (p>0.01).\textsuperscript{29} Much et al. found that BMI was negatively correlated with the concentration of omega-6 arachidonic fatty acid and DHA expressed in breast milk.\textsuperscript{30} Makela et al. examined the fatty acid differences in human breast milk from mothers in Finland and found that overweight mothers with a BMI $\geq 25.0$ kg/m$^2$ had a higher concentration (46.3\%) of saturated fatty acids in their breast milk, compared to saturated fatty acids (43.6\%) from normal weight mothers with a BMI $<25.0$ kg/m$^2$ (p=0.012).\textsuperscript{31} Overweight mothers had a higher ratio (5.7:1) of omega-6 to omega-3 fatty acids than that of normal weight mothers (4.9:1) (p=0.031).\textsuperscript{31}
Effect of Diet on Breast Milk Composition

Food choices and access to seafood greatly influence the type of fatty acids the mother consumes and what is expressed in the breast milk and deposited in fat stores. Antonakou et al. found the amount of DHA can range from 0.05 to 0.10% of the total fat in breast milk in women who do not normally consume marine food. DHA can be greater than 1.00% in women who consume marine foods at least once a week or who consume fish oil supplements. In Greek women who traditionally consume high amounts of seafood and monounsaturated fatty acids, researchers consistently found that DHA was around 0.50% of the fatty acids expressed in breast milk which is representative of their DHA rich diet. Overall, fish eating populations have higher concentrations of DHA breast milk than land locked populations due to their higher intake of marine foods.

Peng et al. looked at long-term fatty acid consumption and the fatty acid concentration of breast milk of women in two rural villages in China. The researchers presumed that the mother’s current dietary intake reflected their long-term dietary intake and thus their fatty acid stores. The first village was located inland and the women’s’ diet consisted of rice, meat, eggs, and lake fish. The women in the coastal village had a diet largely consisting of seafood. Maternal polyunsaturated fatty acid intake was higher by 6.43% in the inland village (p=0.021). The differences in total fat, saturated fat, and monounsaturated fat intake between the women in the coastal and inland rural villages were non-significant. Breast milk from the coastal village had 6.27% (p=0.0001) higher concentrations of saturated fatty acids by and 1.91% (p=0.038) higher monounsaturated fatty acids. Breast milk from the inland village had 8.19%
higher concentrations of LCPUFA, 7.85% (p=0.0001) omega-6 fatty acids, and 0.38% (p=0.004) omega-3 fatty acids. The higher maternal dietary intake of LCPUFA in the inland village and the higher LCPUFA in the mothers’ breast milk indicate that the fatty acids consumed in the diet are reflected in the fatty acids of breast milk.

Even though breast milk fatty acids are mainly composed of fatty acids that have deposited in body fat reserves, the mother’s dietary intake can also influence the breast milk’s fatty acid composition. Diets rich in marine fish and seafood results in increased intake of omega-3 fatty acids, EPA, and DHA and increased concentrations of those fatty acids expressed in the breast milk. Increased amounts of omega-3 fatty acids and DHA in breast milk are linked to improved neurological development and a reduced risk of obese children.

Influence of Ethnicity on Breast Milk Composition

Examining the influence of ethnicity on breast milk composition is difficult because ethnicity contains many variables including genes, lifestyle, and cultural diet. Antonakou et al. investigated the breast milk fatty acid composition from women living in Greece, Spain, Italy, Sweden, China, and Germany. They found significant differences in DHA, AA, EPA, omega-3, omega-6, saturated fatty acids, MUFA, and PUFA in the breast milk across the ethnicities (p<0.01). Although maternal diet plays a significant role on the nutrient composition of breast milk, it was hard to isolate ethnicity as the cause for differences when the ethnic diets varied greatly from each other.

In the United States, average monthly consumption of DHA and EPA varies greatly among different ethnicities (p<0.05). Caucasians have the lowest monthly
consumption of DHA at $0.93 \pm 0.36g$, Hispanics at $1.64 \pm 0.27g$, mixed race at $2.54 \pm 1.13$, and African Americans at $2.79 \pm 0.51g$. The recommended intake of DHA and EPA is $9g$ per month. Americans, regardless of their race, are far under-consuming DHA and EPA in their diets by $10.3\%$ to $31\%$. Since long-term and current dietary intake affect the fatty acid composition of breast milk one could expect to find that ethnicity influences the DHA in breast milk. However, there are no recent or significant studies that investigated the levels of DHA in breast milk among different ethnicities in the United States.

**Influence of Omega-3 Fatty Acids on the Child’s Weight**

To understand the association between early fatty acid intake and weight, it is vital to know the physiology of fat cell development. Fetal fat cells are rapidly created during gestation especially in the third trimester of pregnancy. Fat cells continue to divide rapidly and increase in size throughout the first year of life. New fat cell creations cease after puberty and adolescence. The type of fat consumed by the mother throughout her pregnancy and the type of fat consumed by the infant in the first year of life determines the composition of the fat cells. If a mother consumes high amounts of long-chain polyunsaturated fatty acids (LCPUFA) throughout pregnancy and lactation, then the infant’s fat cells will be mainly composed of LCPUFA. Standl et al. proposed that increased omega-6 to omega-3 fatty acid ratio may promote increased adipose tissue development compared to a lower omega-6 to omega-3 fatty acid ratio. Omega-6 fatty acids are a “precursor of eicosanoids, such as prostacyclin, [and] prostacyclin enhances the differentiation of adipose cells into adipocytes” whereas, omega-3 fatty acids, such as DHA, have been shown to inhibit adipose tissue
Therefore, by consuming a higher ratio of omega-6 to omega-3 fatty acid, an infant will create more fat cells than an infant who has a lower ratio of omega-6 to omega-3 fatty acid. Since the number of fat cells created as an infant will never decrease, they are at a greater risk to develop obesity later in life.\textsuperscript{20}

Standl et al. compared the omega-3 fatty acid content of the cord blood to the BMI of the child at 2, 6, and 10 years of age.\textsuperscript{36} Overall, there was no significant correlation between the amount of omega-3 fatty acids in cord blood and the child’s BMI.\textsuperscript{36} They found that BMI was negatively correlated with cord blood and omega-6 and omega-3 fatty acid concentration at 2 years of age, neutrally correlated at 6 years of age, and positively correlated at 10 years of age (p=0.0415).\textsuperscript{36}

Standl et al. reported than an infant who consumed an omega-6 to omega-3 fatty acid ratio of 2:1 would have a higher BMI at age two during the period of rapid fat cell development.\textsuperscript{36} BMI would even out as the child reached the age of 6. When the child began the second fat cell development stage during adolescence and puberty the BMI would be lower than that of a child with a higher omega-6 to omega-3 fatty acid ratio due to the adipocyte developmental characteristics of omega-6 fatty acids discussed earlier.\textsuperscript{36}

Other researchers have had mixed results possibly due to shorter follow up times. For example, Scholtens et al. and Haunter et al. found no association between LCPUFA in breast milk and weight gain by 1 year of age, whereas, Donahue et al. found a weak correlation between DHA and EPA cord blood and BMI at 3 years of age (p>0.05).\textsuperscript{1,16,20,37} Kasbi-Chadli et al. studied rats and found no correlation between
omega-3 fatty acid supplementation or high fat diet on the weight of the adult rat offspring.  

In three studies of omega-3 LCPUFA and gestation length of humans, researchers found an increase in human gestation period between 1.6 and 4.5 days. Since most LCPUFA are transferred to the fetus in the third trimester each additional day of gestation is another day that the fetus can receive LCPUFA directly from the mother, thus increasing LCPUFA fat transfer.

Overall, there are mixed results of the influence of LCPUFA on the weight. In vitro and animal studies show a strong correlation between LCPUFA consumption and reduced weight of the offspring. Longer-term human studies found positive correlations between lower BMI in the infant and increased consumption of LCPUFA during the neonate period.

Influence of Omega-3 Fatty Acids on Child’s Neurological Development

Neurological development primarily occurs during gestation and the first two to three years of life. Omega-3 fatty acids are necessary for neurological development as dry weight brain tissue is composed of 60% fat, composed primarily of DHA and AA. Using in vitro and pig studies, researchers found that DHA specifically accumulates in the hippocampus and frontal lobes which are responsible for attention, working memory, and inhibitory control. If a mother is deficient in DHA during pregnancy or the infant does not consume enough DHA through breast milk or formula, neurological development will be suboptimal. During gestation and the first month of life, fatty acids accumulate in the renal cortex and retinal membrane synapses. Omega-3 fatty acids aid in neurological development through the formation and
maintenance of neuronal plasma membranes.\textsuperscript{12,14,43} Higher concentrations of omega-6 fatty acids inhibit neurological development by reducing the formation of new neurons through the brain-derived neurotrophic factor.\textsuperscript{13} Higher saturated fat intake causes oxidative stress and damage to neurons, whereas omega-3 fatty acids reduce oxidative stress and have anti-inflammatory properties.\textsuperscript{12,13,45}

Tian et al., studying rats, found that an increased maternal omega-6 to omega-3 fatty acid ratio intake of 1:1 to 2:1 promotes the development of neurons and brain development.\textsuperscript{43} Baym et al., examined omega-3 fatty acid and saturated fatty acid intake and their effects on neurological development in children 7 to 9 years of age.\textsuperscript{41} They found that saturated fat is negatively correlated with relational memory and item accuracy and omega-3 fatty acids are positively correlated with both (p<0.05).\textsuperscript{41} This shows the importance of higher omega-3 fatty acid and lower saturated fat intake on neurological development.\textsuperscript{41}

Jiao et al. conducted a meta-analysis on the role of omega-3 fatty acids in cognitive function from infancy to elderly and discovered that omega-3 fatty acids improve cognitive functioning in infants but not in children, adults, or elderly.\textsuperscript{23} Because infancy is a critical time for neurological development, the presence of omega-3 fatty acids are vital for that developmental period.\textsuperscript{23} Boucher et al. found similar results by supplementing pregnant mothers with DHA during pregnancy and lactation.\textsuperscript{46} They found positive effects on cognitive development in term and preterm infants (p<0.10).\textsuperscript{46} Contraindicating this, Willatts et al. tested a group of 235 infants who were fed either formula without LCPUFA, formula with LCPUFA, or breast milk.\textsuperscript{47} By age of 6 there was no difference in intelligence quotient (IQ) between the groups, however, children who
consumed the formula with LCPUFA had a faster processing speed \( (p=0.015) \).\(^9,47\)

The need for omega-3 fatty acids during development is especially apparent in premature infants. Since most of the LCPUFAs are transferred in the third trimester of pregnancy, premature infants have less time to receive those vital fatty acids.\(^48\) Fu et al. studied the effect of omega-3 fatty acids supplementation and rate of retinopathy in premature infants.\(^48\) Retinopathy is commonly seen in premature infants because of inadequate retinal vascularization related to premature birth.\(^48\) They found that supplementing premature infants with DHA and AA reduces the incidence of retinopathy, demonstrating the importance of DHA and AA on retinal development in infants \( (p<0.05) \).\(^48\)

Despite \textit{in vitro} and observational study results that support the influence of omega-3 fatty acids on neurological development, the results of randomized control trials are inconsistent.\(^13,40\) Giuseppe et al., Gould et al., and Campoy et al. did not find a consistent correlation in human randomized control trials for omega-3 fatty acid supplementation and neurological development.\(^13,40,49,50\) Neurological development studies included a variety of tests and measurements such as eye and hand coordination, eye movements, and cognitive exams.\(^13\) Frensham et al. proposed that the varied results were due to the type of intelligence tested.\(^21\) For example, measuring nonverbal/ fluid intelligence provides more positive results, whereas, measuring verbal/ crystallized intelligence provides results that tend to be neutral.\(^21\) Since LCPUFA and DHA tend to accumulate in the hippocampus and frontal lobes which are responsible for attention, working memory, and inhibitory control, the type of neurological test administered might not target those specific functions and thus
produce no significant findings.\textsuperscript{40-43} This could be the reason behind the mixed results from many studies of omega-3 fatty acids intake and neurological development.\textsuperscript{21} If researchers only measured verbal/crystallized intelligence, the results might be inaccurate simply due to the type of test or measurement used. Despite the mixed results, since most women are omega-3 fatty acids and DHA deficient it is important to increase intake or supplement up to recommended levels to ensure proper neurological development.

The strongest evidence for omega-3 fatty acid supplementation for improved neurological development exists from research prior to the 1990s, when formula was not supplemented with DHA.\textsuperscript{51} Researchers at that time consistently found that breastfed infants had a higher IQ than formula fed infants, leading formula manufacturers to fortify formula with DHA.\textsuperscript{52,53}

\textbf{Risk of Fish Contaminates}

A concern for pregnant and lactating women consuming fish and seafood is that they are exposed to contaminants in the process of consuming foods rich in omega-3 fatty acids. Contaminates from fish, such as polychlorinated biphenyls, mercury, and lead have been shown to inhibit neurological development in infants.\textsuperscript{46} There is a tight correlation between DHA and polychlorinated biphenyls from cord blood (p=0.05).\textsuperscript{46} They found that high intake of fish contaminants led to a decline in cognitive functioning in infants (p<0.05).\textsuperscript{46} Women who consume fish for the DHA content to improve their child's neurological development may experience a decline in their child's neurological development due to contaminants within the fish. However, Binnington et al. found that eliminating fish from one diet did not reduce contaminant exposure during prenatal,
postnatal, and childhood periods due to the long half-life of the contaminants which remain in the human body.⁵⁴ A women would have to eliminate fish from her diet for one to five years prior to giving birth for it to be effective.⁵⁴ They concluded that the fish advisory to pregnant mothers may not be effective in preventing exposure to fish contamintates, and instead medical professionals should educate patients to avoid fish prone with high contamination prior to conception.⁵⁴ The amount of contaminants from two servings of fish per week or less than 340 grams per week is within the safe range for pregnant women.³²,⁵⁵ However, complete avoidance of seafood could be detrimental to the infant's development because seafood is a good source of DHA.³²

Hormones in Breast Milk

An area of recent research is the effect of hormones produced by the mother and transferred to the infant through breastfeeding of the neonate. The previous belief was that hormones present in breast milk were degraded in the stomach and did not affect the neonate, however, in more recent studies, researchers have found evidence that infants do not degrade the hormones from breast milk because of reduced acidity of the stomach and limited gastric proteolysis.⁵⁶ Current research is being conducted to look at the long-term effects of hormone intake from breast milk on the neonate.

One of the hormones in breast milk that is being researched is leptin. Leptin is a hormone that targets the hypothalamus and signals an individual to reduce food intake and to increase energy expenditure.⁵⁷,⁵⁸ If leptin levels are elevated during pregnancy and lactation, this can lead to permanent changes in the hypothalamus formation and life-long energy balance.⁵⁷ Leptin levels in breast milk are positively correlated with maternal adiposity, higher body mass index, and leptin serum levels.⁵⁷-⁵⁹ Fields et al.
determined that leptin is correlated to total fat mass; obese mothers tend to have increased levels of serum leptin, and therefore, increased levels of leptin in their breast milk, which results in the infant having increased levels of leptin through intake of the breast milk. When the infant had a high intake of leptin, the body produced less leptin to compensate. When weaned from breast milk the infant would produce less leptin causing them to be more likely to develop severe obesity. Higher leptin intake from the mother may decrease leptin sensitivity due to overloading leptin receptors and it impact the lifelong energy balance of that child. However, the signs of childhood obesity are not typically seen until after two-years of age, which could be related to the cessation of breast feeding and reduced leptin intake from breast milk. As leptin levels in the breast milk increase there is a negative correlation to the infants’ body weight and BMI z-score (p=0.03). Regardless of maternal weight, mothers who have high levels of physical activity have decreased leptin levels. This shows that exercise may be a preventative against high levels of leptin even in overweight mothers.

High concentrations of insulin, another hormone in breast milk being researched, can program the child for insulin resistance at an early age. Insulin is negatively associated with infant weight (p=0.06), BMI z-score (p=0.02), weight for length z-score (p=0.05), and lean mass (p=0.03). A good example of the effect of insulin in breast milk is evident in a comparison of mothers with diabetes to mothers without diabetes. Breast milk from mothers with diabetes has twice the concentration of insulin than that of mothers with normal glycemic levels. With a significant increase in insulin, the offspring of mothers with diabetes had 2.47 times greater risk of being overweight at two years of age than children of mothers who were not diabetic. This indicates that the
level of insulin the infant consumes through breast milk may influence the child’s weight at two years of age.

Adiponectin is a hormone produced by adipose tissue and is positively correlated with adiposity. Adiponectin present in human breast milk is mainly produced by the fat tissue in the breast and is positively correlated with adiposity. Heavier mothers will produce breast milk with increased concentrations of adiponectin. Another factor that influences adiponectin concentration is ethnicity. At one month of lactation researchers found that United States non-Hispanic white mothers’ breast milk contained 19.8 ng/mL of adiponectin compared to that of Hispanic mothers from Mexico who had 11.7 ng/mL (p=0.0005). Also, the mother’s post pregnancy BMI was the best predicative measurement of increased adiponectin levels in that mother’s breast milk (p<0.0001). Martin et al. found an 8.33% increase in adiponectin concentration with each unit increased post pregnancy maternal BMI.

Fields et al. found several correlations between hormones in breast milk and other nutritional factors. They found that the concentration of insulin, TFN-α, and IL-6 in breast milk were negatively associated with milk glucose (p<0.06). The concentration of insulin in breast milk was positively associated with milk leptin and TNF-α (p<0.04). TFN-α and IL-6 were positively correlated to each other (p=0.06). A major factor that influences the hormones of breast milk was pre-gravid BMI, which was positively correlated to leptin (p<0.0001), infant fat mass (p=0.01), and lean mass (p=0.02). Hormones present during pregnancy and lactation play a significant role in the metabolism development of the child.
**Conclusion**

Many factors influence the nutrient composition of breast milk, especially maternal weight, weight gain though pregnancy, maternal diet, and ethnicity. Even though there are many studies that show the benefits of omega-3 fatty acid and DHA supplementation, there are a comparable amount of studies showing a neutral effect on BMI and neurological development of the child. Although literature is inconclusive on the benefits of omega-3 fatty acids, research has shown there is an increased demand of DHA by the neonate. This is demonstrated with higher concentrations of DHA in the placental and cord blood over maternal DHA levels in the blood.\(^{32}\) Since most women are deficient in omega-3 fatty acids and DHA, it is recommended that women increase their intake of low contaminate fatty fish to two servings per week, 200 mg DHA supplement, or 0.2-0.5% DHA per dry weight of formula.\(^{32,55}\) There is no conclusive evidence to support supplementation over recommended levels, but intake of 1 gram of DHA per day or 2.7 grams of omega-3 fatty acids per day is considered safe.
CHAPTER 3
MATERIALS AND METHODS

Participants

Forty-four participants were recruited from the BABE Breastfeeding Coalition of Tri-Cities Facebook support group page. Participants were of Caucasian descent and were between the 2\textsuperscript{nd} and 14\textsuperscript{th} weeks of lactation to ensure standardization of mature milk samples. Women were excluded from the study if they had or were being treated for mastitis or any other breastfeeding disorder. The forty-four Caucasian participants were divided into two groups, one group with normal pre-gravid BMI between 18.5 and 24.9 kg/m\textsuperscript{2} and the other group with overweight or obese pre-gravid BMI greater than 25.0 kg/m\textsuperscript{2}. Participation in the study was voluntary and informed consents were collected from each participant. Each participant completed a demographic and health survey (Appendix A), Block Fruit-Vegetable-Fiber Screener (Appendix B), and Block Dietary Fat Screener (Appendix C). They also provided approximately 4 ounces of expressed breast milk. Participants were compensated with a $20.00 Wal-Mart gift card. This study was approved by the ETSU Institutional Review Board (IRB) on September 21, 2015; study number 0915.8s-ETSU (Appendix D).

Self-Reported Demographics

Each participant completed a demographic and health survey compromised of 35 questions. It contained six questions on demographic information, two questions on health care services used, four questions on tobacco exposure, six questions on physical activity, seven questions on pregnancy, four questions on body weight, and six questions on dietary patterns. Current height and pre-gravid weight was obtained from
the survey and used to calculate pre-gravid BMI using the following formula: \( \text{BMI} = \frac{\text{pre-gravid weight (kg)}}{[\text{current height (m)}]^2} \)

**Food Frequency Questionnaires**

Each participant filled out two food frequency questionnaires; Block Dietary Fruit-Vegetable-Fiber Screener and Block Dietary Fat Screener. The Block Dietary Fruit-Vegetable-Fiber Screener contained ten questions and provided a Fruit-Vegetable Score and a Fruit-Vegetable-Bean Score. From these scores fruit/vegetable servings, vitamin C, magnesium, and dietary fiber intake were calculated. The Block Dietary Fat Screener contained seventeen questions and gives a Meat/Snack Score. This score was used to calculate total fat, saturated fat, and dietary cholesterol intake as well as percent fat and percent saturated fat. The equations (developed by Block et al.) for the calculations are listed below.\(^{61}\)

**Prediction equations for Block Dietary Fruit-Vegetable-Fiber Screener:**

- Fruit/Vegetable servings = -0.23 + [0.37 * (Fruit/vegetable score)] – (0.55 * Sex)
- Vitamin C (mg) = 56.5 + [6.6 * (Fruit/Veg/Beans score)] – (26.7 * Sex) – (0.45 * Age)
- Magnesium (mg) = 272 + [11.6 * (Fruit/Veg/Beans score)] – (92.3 * Sex) – (1.7 * Age)
- Potassium (mg) = 2348 + [114.8 * (Fruit/Veg/Beans score)] – (759 * Sex) – (13.8 * Age)
- Dietary fiber (gm) = 7.9 + [0.74 * (Fruit/Veg/Beans score)] – (4.5 * Sex)

**Prediction equations for Block Dietary Fat Screener:**

- Total fat (gm) = 32.7 + [2.4 * (Meat/snack score)] + (11.2 * Sex)
- Saturated fat (gm) = 9.4 + [0.88 * (Meat/snack score)] – (3.5 * Sex)
- Percent fat (%) = 19.8 + [0.6 * (Meat/snack score)] + (2.3 * Sex)
Dietary cholesterol (gm) = 120 + [7.8 * (Meat/snack score)] – (54.65 * Sex) + (36.6 * Race)

Laboratory Methods

Breast Milk Collection

Participants expressed breast milk into a container using either an automated breast milk pump or hand expression that the researcher transferred to a cooler for transportation to the Human Nutrition and Dietetics Research Lab at ETSU Valleybrook Campus. At the lab, the breast milk was frozen in a -30 degrees Celsius freezer until analysis.

Freeze-Drying

A 600 mL LABCONCO freeze dry flask and lid was weighed. Liquid breast milk was poured into the freeze dry flask, sealed tightly and reweighed to determine the liquid breast milk weight. The flask was placed on LABCONCO FreeZone 2.5 freeze dryer using stainless steel adapters. The samples ran for at least 24 hours on 0.077 mBar at -50 degrees Celsius. The flask was removed from the freeze dryer and weighed to determine the weight of water lost. The percent dry weight was determined with flask weight before and after using the formula below: Percent dry weight = dry weight of breast milk / liquid weight of breastmilk x 100.

Bomb Calorimetry

The tare combustion capsule was zeroed and 1.0 gram of freeze dried breast milk was placed in the combustion capsule. The combustion capsule was placed into the ringer holder of the bomb vessel with the ignition thread completing the circuit between the bomb vessel and the dried breast milk sample. The bomb vessel was
assembled with the valve closed and the vessel was charged with oxygen. Two liters of deionized distilled water were placed into the bucket, followed by the bomb vessel with ignition wires attached. The breast milk weight was entered into the Parr 6200 Calorimeter computer and the combustion sequence started. The breast milk sample combusted and the calorimeter calculated calories per gram of breast milk. The bomb vessel was removed from the bucket and pressure was released. The bomb housing and combustion capsule were rinsed with deionized distilled water into a 250-milliliter beaker. Several drops of methyl red indicator were added to the beaker. The solution was titrated back with sodium carbonate solution (0.0709 Nitrogen solution) until color change was sustained to measure nitric acid formed during combustion. The formula was used to determine total digestible calories: Total digestible calories = total calories – mL of sodium carbonate.

**Kjeldhal Digestion**

After the breast milk was freeze dried, samples were prepared for kjeldhal digestion. The following was added to Kjeldahl flasks: 100 mg of freeze dried breast milk sample, 1.9 g of K$_2$SO$_4$ (Fisher Scientific Potassium Sulfate), 80 mg of HgO (Fisher Scientific red mercuric oxide), 2 mL of H$_2$SO$_4$ (Fisher Scientific concentrated sulfuric acid), and 2 Fisher porous boiling chips. The kjeldhal flask was placed on a LABCONCO digestion unit with the neck of the flask inserted into the glass manifold. Air was turned on and the heater was set to 3. The sample was refluxed for 8 to 12 hours then cooled. Once cooled, 10 mL of deionized distilled water was added to the kjeldhal flask. The sample was filtered using Kroger #2 cone coffee filters into Erlenmeyer flasks. The distillation process begun by placing an Erlenmeyer flask with 5 mL of 4% boric acid solution with
several drops of kjeldhal indicator at the bottom of the LABCONCO rapid distillation unit to collect the ammonium from the sample. The filtered samples were poured into the top of the distillation unit, followed by 10 mL of NaOH/Na₂O₃S₂ (sodium thiosulfate solution). This mixture was distilled until the total volume of boric acid and ammonium solution reaches 20-30 mL. The solution was titrated back with 0.1 M dilute HCl until the indicator color change is complete. The ml of HCl used to titrate the solution back and grams of the sample was entered into the following equations to calculate percent protein: Nitrogen per Kilogram= [(mL HCl – mL blank) x normality x 14.01] / weight of dried breast milk (grams). Percent Protein= Nitrogen per kilogram x 6.38 / 10.

**Soxhlet Extraction**

After the breast milk was freeze dried, samples were prepared for soxhlet extraction. 2.5 g of the freeze-dried breast milk sample was combined with 2.5 g of NaSO₄ (Fisher Scientific Sodium Anhydrous) in mortar. Using a mortar and pestle, the sample was mixed with NaSO₄ until it become a homogenous mixture. Cellulose extraction thimbles were labeled with the sample ID and 2 g of the homogenous mixture was measured into each thimbles with a small piece of glass wool on top. The soxhlet apparatus was assembled starting with the round bottom flask on the bottom with 175 ml of PET (Fisher Scientific pethroleum ether) with 2 Fisher porous boiling chips and placed on the mantel heater followed by the soxhlet extraction chamber with the thimbles containing the breast milk sample. The last part of the apparatus assembled is the condenser with tubing connecting each condenser. Cool water was turned on to slowly run through each condenser. The mantle heaters were turned on to 3 and the sample reflexed for 8-12 hours. The heat and water were turned off after refluxing and allowed to cool. The
PET liquid was drained from the thimbles and the thimbles were placed in a 60-degree Celsius oven for 24 hours to ensure the samples are dry, then reweighed to determine the amount of sample remaining in the PET. The formula below was used to determine percent of fat: \[ \text{Percent Fat} = 100 - \left( \frac{\text{weight of thimble after soxhlet} - \text{thimble weight} - 1 \text{ g of NaSO}_4}{\text{weight of thimble after soxhlet}} \right) \times 100. \]

**Ashing**

The bottom of each crucible was heavily marked with the sample ID using a pencil. Crucibles were weighed, then 4 grams of sample were added to each crucible. Crucibles were placed in the Thermolyne ashing oven and heated at 700 degrees Celsius for 5 hours. Crucibles were weighed following ashing to determine percent inorganics with the following formula: \[ \text{Percent inorganics} = \frac{\text{weight of inorganics}}{4 \text{ grams}} \times 100. \]

**Fatty Acid Analysis**

Freeze-dried breast milk was methylated to using boron trifluoride in methanol to form fatty acid methyl esters. Thirty mg of dried breast milk was combined with 3 mL of hexane (Fisher Scientific) and 3 mL of BF$_3$ (Fish Scientific Boron trifluoride methanol) in a screw top glass vial. Samples were vortexed before being placed in an Isotemp heating block (Fisher Scientific). The Isotemp heating block was heated to 100 degrees Celsius and samples were heated for one hour. After heated samples were cooled and 1.5 mL of deionized distilled water were added to the vial, they were vortexed. The vial was placed in Sorvall Biofuge Primo Centrifuge (Thermo Scientific) and spun at 4,000 rpm for 5 minutes. The supernatant was removed and placed in another glass vial. The vial of supernatant was placed back on the Isotemp heating block at 60 degrees Celsius.
and dried under Nitrogen gas. The sample was re-suspended in 275 μL of Hexane. The sample was transferred to the gas chromatograph auto sample vial with a 300 μL glass insert. An internal standard of 5 μL of C17 was added to the vial.

Freeze dried breast milk was analyzed for fatty acids through flame ionization gas chromatography (Shimadzu GC-2010; Shimadzu Corporation, Kyoto Japan) using a capillary column (Zebron ZB-WAX, 30 m length, 0.25 mm i.d., 0.25 μm film thickness; Phenomenex, Torrance, CA, USA). Column conditions included the carrier gas (helium) flow rate of 30 mL/min and a temperature program of a constant temperature ramp (2°C/min) at an initial temperature of 160°C, held for 5 min; 170°C held for 8 minutes; 180°C held for 10 minutes; 190°C held for 15 minutes; 200°C held for 15 minutes; and final oven temperature of 210°C held for 20 minutes. Additional instrument conditions included: total run time of 100 min; auto sampler injection volume of 1 μL; flame ionization detector (FID) temperature of 255°C; injector port temperature of 250°C; hydrogen flow rate of 40 mL/min; and air flow rate of 400 mL/min. Individual peaks were identified and compared with known standards. The fatty acids were quantified as a percent of total fat by using the percent of total area under the peak. The known peaks of the hexane solution and internal standard of C17 were included in each run and subtracted from analysis. The different fatty acid peaks were averaged between the duplicates and compared to individual fatty acids using standards.

**Statistical Analysis**

Descriptive statistics as well as all subsequent analyses were performed in SPSS version 22 (IBM Corp., Armonk, NY, USA). General linear models (GLM) were constructed for all statistical analyses. The data from the demographic and health
survey (Appendix A), Block Dietary Fruit-Vegetable-Fiber Screener (Appendix B), Block Dietary Fat Screener (Appendix C), and laboratory results were analyzed. General linear models were constructed for the statistical analyses using group status as a factor/treatment effect to determine significance between normal weight and overweight participants. Differences between the values in the general linear models were considered statistically significant if $p < 0.100$. 
CHAPTER 4

RESULTS

Participants

Forty-four participants filled out the demographic and health survey (Appendix A), two food frequency questionnaires (Appendix B and C), and provided at least four ounces of expressed breast milk. The participants were divided into two groups, 24 women with normal pre-gravid BMI (NW) and 20 women overweight pre-gravid BMI (OW). Normal pre-gravid BMI is defined by a BMI between 18.5 to 24.9 m/kg². Overweight pre-gravid BMI is defined by a BMI greater than 25.0m/kg².

Demographic and Health Information

Demographics

NW and OW participants had a mean age of 28.8 ± 5.04 and 28.26 ± 4.31 years, respectively. All the NW and OW participants were female and of Caucasian, Non-Hispanic White descent. The marital status of participants of NW were as follows: 22 (92%) were married, one (4%) was never married, and one (4%) was living with a partner. Of the OW participants, 18 (90%) were married and two (10%) were living with a partner.

The highest education achieved of participants of NW were as follows: 16 (67%) had a bachelors degree or above, seven (29%) had some college or associates degree, and one (4%) had a high school diploma. Of the OW participants, nine (45%) had a bachelors degree or above, seven (35%) had some college or associates degree, and four (20%) had a high school diploma.
The employment status of participants of NW was 13 (54%) were full-time homemakers, nine (38%) worked full-time, one (4%) was self-employed, and one (4%) worked part-time. In the OW group, four (20%) were full-time homemakers, seven (35%) worked full-time, one (5%) was self-employed, six (30%) worked part-time, and two (10%) were temporarily unemployed.

The annual household income for the NW participants was two (8%) earned an income between $20,000-$24,999, two (8%) earned $25,000-$34,999, three (12%) earned $35,000-$44,999, one (4%) earned $45,000-$54,999, seven (29%) earned $55,000-$64,999, three (13%) earned $65,000-$74,999, three (12%) earned $75,000-$99,999, and three (12%) earned $100,000 or more. In the OW group two (10%) earned between $5,000-$9,999, one (5%) earned $15,000-$19,999, two (10%) earned $20,000-$24,999, two (10%) earned $25,000-$34,999, two (10%) earned $35,000-$44,999, two (10%) earned $45,000-$54,999, one (5%) earned $55,000-$64,999, two (10%) earned $65,000-$74,999, four (20%) earned $75,000-$99,999, and two (10%) earned $100,000 or more. The annual household income is displayed in Figure 1.
Figure 1: Annual Household Income

Health-Care Services Utilization

Of the NW participants, 21 (88%) received prenatal care from an obstetrician, two (8%) from a midwife or nurse midwife, and one (4%) from a family doctor, general practitioner, internist, or other physician. Of the OW participants, 19 (95%) received prenatal care from an obstetrician and one (5%) from a midwife or nurse midwife.

During labor and delivery, 21 NW participants (88%) were attended by an obstetrician, two (8%) by a midwife or nurse midwife, and one (4%) by a family doctor, general practitioner, internist, or other physician. Of the OW participants, 18 (90%) were attended by an obstetrician and one (5%) by a midwife or nurse midwife, and one (5%) by another type of health care provider.
**Tobacco Smoke Exposure**

All NW participants (100%) did not smoke the year of the pregnancy, whereas 18 OW participants (90%) did not smoke, one OW participant (5%) smoked but stopped before pregnancy began, and one OW participant (5%) smoked throughout the year of pregnancy. The one OW participant who smoked throughout pregnancy, smoked six cigarettes per day. All NW participants (100%) lived in smoke-free homes, whereas 19 OW participants (95%) lived in smoke-free homes and one OW participant (5%) lived in a home with one smoker. During pregnancy 15 NW participants (63%) never had contact with smoke, eight (33%) sometimes had contact, and one (4%) often had contact. Of the OW participants, seven (35%) never had contact with tobacco smoke, 11 (55%) sometimes had contact, one (5%) often had contact, and one (5%) always had contact.

**Physical Activity**

Participants in both groups engaged in moderate-intensity physical activity; 18 NW participants (75%) participated in moderate physical activity and six (25%) reported they did not participate. Of the OW participants, 14 (70%) participated in moderate-intensity physical activity and six (30%) reported they did not participate. Moderate intensity physical activity was defined in the demographic and health survey (Appendix A) as an “activity that causes small increases in breathing or heart rate such as brisk walking or carrying light loads for at least 10 minutes continuously.” The average number of days per week that NW and OW participants participated in moderate-intensity activity was $3.38 \pm 2.45$ and $2.57 \pm 2.22$, respectively ($p=0.276$). The average
number of hours per day that NW and OW participants participated in moderate-intensity activity was 2.02 ± 3.02 and 2.51 ± 3.41, respectively (p=0.618).

Fewer participants in both groups participated in moderate-intensity fitness or sports. Moderate intensity fitness or sports was defined in the demographic and health survey (Appendix A) as “fitness or recreational activities that cause a small increase in breathing or heart rate such as bicycling, swimming, or golf for at least 10 minutes continuously.” Nine NW (38%) and 11 OW (55%) participants were involved in moderate-intensity fitness or sport, while 15 NW (62%) and nine OW (45%) were not involved. The average number of days per week that NW and OW participants participated in moderate-intensity fitness or sport was 1.17 ± 1.65 and 1.55 ± 1.65, respectively (p=0.451). The average number of hours per day that NW and OW participants participated in moderate-intensity fitness or sport was 0.26 ± 0.37 and 0.53 ± 0.68, respectively (p=0.101). These data are shown in Table 1.
### Table 1: Moderate-Intensity Physical Activity

<table>
<thead>
<tr>
<th></th>
<th>NW (Mean ± SD)</th>
<th>OW (Mean ± SD)</th>
<th>Significance (p)&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Days per week of moderate-intensity activity</td>
<td>3.38 ± 2.45</td>
<td>2.57 ± 2.22</td>
<td>p=0.276</td>
</tr>
<tr>
<td>Hours per day of moderate-intensity activity</td>
<td>2.02 ±3.02</td>
<td>2.51 ± 3.41</td>
<td>p=0.618</td>
</tr>
<tr>
<td>Days per week of moderate-intensity fitness or sport</td>
<td>1.17 ± 1.65</td>
<td>1.55 ± 1.65</td>
<td>p= 0.451</td>
</tr>
<tr>
<td>Hours per day of moderate-intensity fitness or sport</td>
<td>0.26 ± 0.37</td>
<td>0.53 ± 0.68</td>
<td>p=0.101</td>
</tr>
</tbody>
</table>

**Prenatal Questions**

Six NW participants (25%) were pregnant for the first time, 11 (46%) for the second time, and seven (29%) for the third time. Of the OW participants, five (25%) were pregnant for the first time, 10 (50%) for the second time, four (20%) for the third time, and one (5%) had four or more pregnancies. The average number of pregnancies was 2.0 ± 0.8 and 2.1 ± 0.8 for NW and OW participants, respectively (p=0.964).

Nearly all the participants consumed prenatal vitamins during pregnancy, only one OW (5%) did not consume prenatal vitamins. However, during lactation, only 15 NW (63%) and 18 OW (90%) participants still consumed prenatal vitamins. These data are shown in Table 2.
Table 2: Prenatal Vitamin Consumption during Pregnancy and Lactation

<table>
<thead>
<tr>
<th></th>
<th>NW</th>
<th></th>
<th>OW</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Took prenatal vitamins during pregnancy</td>
<td>24</td>
<td>0</td>
<td>19</td>
<td>1</td>
</tr>
<tr>
<td>Took prenatal vitamins at time</td>
<td>15</td>
<td>9</td>
<td>18</td>
<td>2</td>
</tr>
<tr>
<td>the breast milk was collected</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

All participants reported the type and brand of prenatal vitamin they consumed and this allowed the researchers to determine the DHA content of the prenatal vitamin consumed by each participant. The average DHA content of prenatal vitamins from NW and OW participants were 118.75 ± 105.10 and 86.84 ± 91.05, respectively, p=0.301.

Of the NW participants, 23 (96%) had single births and one (4%) had triplets. In the OW group, 19 (95%) had single births and one (5%) had twins. Of the 26 infants of the NW participants 15 (58%) were male and 11 (42%) were females. Of the 21 infants of OW participants, 9 (43%) were male and 12 (57%) were female.

The average birth weight in pounds of the infants from the NW and OW participants was 6.85 ± 2.02 and 6.79 ± 3.01, respectively (p=0.0965). Of NW participants, three infants (12%) weighed between 2 lbs and 2 lbs 15 oz., one (4%) weighed between 3 lbs and 3 lbs 15 oz., one (4%) weighed between 4 lbs and 4 lbs 15 oz., one (4%) weighed between 5 lbs and 5 lbs 15 oz., four (15%) weighed between 6 lbs and 6 lbs 15 oz., seven (27%) weighed between 7 lbs and 7 lbs 15 oz., seven (27%) weighed between 8 lbs and 8 lbs 15 oz., and two (7%) weighed between 9 lbs and 9 lbs 15 oz. Of the OW participants, two infants (9%) weighed between 4 lbs and 4 lbs 15 oz., one (5%) weighed between 5 lbs and 5 lbs 15 oz., one (5%) weighed between 6 lbs and 6 lbs 15 oz., six (28%) weighed between 7 lbs and 7 lbs 15 oz., eight (38%) weighed...
between 8 lbs and 8 lbs 15 oz., one (5%) weighed between 9 lbs and 9 lbs 15 oz., one (5%) weighed between 10 lbs and 10 lbs 15 oz., and one (5%) weighed between 11 lbs and 11 lbs 15 oz. These data are shown in Figure 2.

Figure 2: Birth Weight of Infant

Breast milk samples from NW and OW participants were collected on 58.33 ± 27.83 and 56.42 ± 31.98 days after parturition, respectively (p=0.835). The range of breast milk collection was between two and 14 weeks of lactation. These data are shown in Figure 3.
Figure 3: Week of Lactation Sample

Maternal Body Mass Index

The average height of the NW and OW participants in inches was 65.33 ± 2.24 and 64.5 ± 2.75, respectively. NW and OW participants mean pre-gravid weight in pounds was 133.71 ± 12.27 and 201.89 ± 32.29, respectively (p=0.0001) and mean pre-gravid BMI is 22.03 ± 1.83 and 33.86 ± 5.62, respectively (p=0.0001). NW and OW participants weight in pounds at 18 years of age was 124.08 ± 16.25 and 162.95 ± 25.52, respectively (p=0.0001) and mean BMI at 18 years of age was 20.46 ± 2.62 and 27.34 ± 4.65, respectively (p=0.0001). NW and OW participants highest weight in pounds ever obtained when not in pregnancy was 142.00 ± 15.29 and 213.53 ± 31.34, respectively (p=0.0001) and highest BMI ever obtained when not in pregnancy is 23.39 ± 2.26 and 35.86 ± 7.55, respectively (p=0.0001). These data are shown in Table 3.
Table 3: Weight and Body Mass Index (BMI) History

<table>
<thead>
<tr>
<th></th>
<th>NW</th>
<th>OW</th>
<th>Significance (p&lt;)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Current height (in)</td>
<td>65.33 ± 2.24</td>
<td>64.5 ± 2.75</td>
<td>0.260</td>
</tr>
<tr>
<td>Pre-gravid weight (lbs)</td>
<td>133.71 ± 12.27</td>
<td>201.89 ± 32.29</td>
<td>0.0001</td>
</tr>
<tr>
<td>Pre-gravid BMI (kg/m(^2))</td>
<td>22.03 ± 1.83</td>
<td>33.86 ± 5.62</td>
<td>0.0001</td>
</tr>
<tr>
<td>Weight at 18 years (lbs)</td>
<td>124.08 ± 16.25</td>
<td>162.95 ± 25.52</td>
<td>0.0001</td>
</tr>
<tr>
<td>BMI at 18 years (kg/m(^2))</td>
<td>20.46 ± 2.62</td>
<td>27.34 ± 4.65</td>
<td>0.0001</td>
</tr>
<tr>
<td>Highest weight ever obtained when not pregnant (lbs)</td>
<td>142.00 ± 15.29</td>
<td>213.53 ± 31.34</td>
<td>0.0001</td>
</tr>
<tr>
<td>Highest BMI ever obtained when not pregnant (kg/m(^2))</td>
<td>23.39 ± 2.26</td>
<td>35.86 ± 7.55</td>
<td>0.0001</td>
</tr>
</tbody>
</table>

Dietary Information

Of the NW participants, only six (25%) consumed daily fish or krill oil supplements, whereas only three OW (15%) participants consumed daily fish or krill oil supplements. Eleven NW (46%) and six OW (30%) participants routinely consumed fish. The average number of times NW and OW participants consumed fish per month was 2.23 ± 3.11 and 1.13 ± 2.75, respectively (p=0.321). Twenty-one NW (88%) and 19 OW (95%) participants consumed nuts. The average number of times NW and OW participants consumed nuts per month was 9.58 ± 9.37 and 11.71 ± 11.44, respectively (p=0.506). Even fewer participants, 6 NW (25%) and seven OW (35%) consumed flaxseed or flaxseed oil regularly. Of all the participants, only 7 NW (29%) and four OW
(20%) consumed dietary supplements other than prenatal vitamins. These data are shown in Table 4.

**Table 4: Dietary Information**

<table>
<thead>
<tr>
<th></th>
<th>NW</th>
<th></th>
<th>OW</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Take a daily fish oil/krill oil supplement</td>
<td>6</td>
<td>18</td>
<td>3</td>
<td>17</td>
</tr>
<tr>
<td>Routinely eat fish</td>
<td>11</td>
<td>13</td>
<td>6</td>
<td>14</td>
</tr>
<tr>
<td>Eat nuts</td>
<td>21</td>
<td>3</td>
<td>19</td>
<td>1</td>
</tr>
<tr>
<td>Eat flaxseed or take flaxseed oil</td>
<td>6</td>
<td>18</td>
<td>7</td>
<td>13</td>
</tr>
<tr>
<td>Take dietary supplements (other than prenatal vitamin)</td>
<td>7</td>
<td>17</td>
<td>4</td>
<td>16</td>
</tr>
</tbody>
</table>

**Food Frequency Questionnaires**

Each participant filled out the BLOCK fruit-vegetable-fiber screener (Appendix B).

The mean intake of servings of fruit and vegetables for NW participants was 3.9838 ± 1.6195, vitamin C was 137.5538 mg ± 40.2344, magnesium was 342.8667 mg ± 72.3828, potassium was 3290.9833 mg ± 710.5524, and fiber was 16.9513 gm ± 4.9015. The mean intake of the OW was 4.7885 ± 2.3691 servings of fruit and vegetables, vitamin C was 152.6500 mg ± 59.1906, magnesium was 369.8000 mg ± 104.9127, potassium was 3556.2200 mg ± 1035.1973, and fiber was 18.7595 gm ± 7.0158. There was no significant difference NW and OW groups (p> 0.100) in results. These data are shown in Table 5.
Table 5: Results of B M L O K Dietary Fruit-Vegetable-Fiber Screener

<table>
<thead>
<tr>
<th></th>
<th>NW (Mean ± STD)</th>
<th>OW (Mean ± STD)</th>
<th>Significance (p&lt;)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Servings of Fruits and Vegetables</td>
<td>3.9838 ± 1.6195</td>
<td>4.7885 ± 2.3691</td>
<td>0.190</td>
</tr>
<tr>
<td>Vitamin C (mg)</td>
<td>137.5538 ± 40.2344</td>
<td>152.6500 ± 59.1906</td>
<td>0.322</td>
</tr>
<tr>
<td>Magnesium (mg)</td>
<td>342.8667 ± 72.3828</td>
<td>369.8000 ± 104.9127</td>
<td>0.321</td>
</tr>
<tr>
<td>Potassium (mg)</td>
<td>3290.9833 ± 710.5524</td>
<td>3556.2200 ± 1035.1973</td>
<td>0.321</td>
</tr>
<tr>
<td>Fiber (g)</td>
<td>16.9513 ± 4.9015</td>
<td>18.7595 ± 7.0158</td>
<td>0.321</td>
</tr>
</tbody>
</table>

Each participant filled out the B M L O K Dietary Fat Screener (Appendix C). NW participants had a mean intake of cholesterol of 269.1250 mg ± 49.8814, total fat of 106.6000 g ± 15.3481, saturated fat of 28.8900 g ± 5.6277, percent fat of 37.7750% ± 3.8370, and percent saturated fat of 10.2133% ± 1.5753. OW participants had a mean intake of cholesterol of 258.0100 mg ± 50.4293, total fat of 103.1800 g ± 15.5167, saturated fat of 27.6360 g ± 5.6895, percent fat of 36.9200% ± 3.8792, and percent saturated fat of 9.8625% ± 1.6106. There was no significant difference between fat intake of NW and OW participants (p>0.100) in results. These data are shown in Table 6.
Table 6: Results of BLOCK Dietary Fat Screener

<table>
<thead>
<tr>
<th></th>
<th>NW (Mean ± STD)</th>
<th>OW (Mean ± STD)</th>
<th>Significance (p&lt;)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cholesterol (mg)</td>
<td>269.1250 ± 49.8814</td>
<td>258.0100 ± 50.4293</td>
<td>0.468</td>
</tr>
<tr>
<td>Total fat (g)</td>
<td>106.6000 ± 15.3481</td>
<td>103.1800 ± 15.5167</td>
<td>0.468</td>
</tr>
<tr>
<td>Saturated fat (g)</td>
<td>28.8900 ± 5.6277</td>
<td>27.6360 ± 5.6895</td>
<td>0.468</td>
</tr>
<tr>
<td>Percent fat (%)</td>
<td>37.7750 ± 3.8370</td>
<td>36.9200 ± 3.8792</td>
<td>0.468</td>
</tr>
<tr>
<td>Percent saturated fat (%)</td>
<td>10.2133 ± 1.5753</td>
<td>9.8625 ± 1.6106</td>
<td>0.471</td>
</tr>
</tbody>
</table>

**Laboratory Results of Breast Milk Samples**

The calories per gram of freeze-dried breast milk was 5,581.8 ± 301.3 from the NW group versus 5,562.9 ± 403.0 for the OW group (p=0.860). The breast milk of the NW group was 9.80% ± 1.53 protein compared to and 8.93% ± 2.20 protein in the OW group, which was approaching significance (p=0.130). The breast milk the NW group was 39.6% ± 7.6 fat versus 43.9% ± 9.8 fat in the OW group, which was approaching significance (p=0.112). The breast milk of the NW and OW groups was 1.45% ± 0.44 and 1.48% ± 0.34 inorganics, respectively (p=0.824). The percent dry weight was 12.34% ± 1.22 and 12.48% ± 1.08 for the NW and OW groups, respectively (p=0.712). These data are shown in Table 7.
Table 7: Results of Proximate Analysis Procedures for Breast Milk Samples

<table>
<thead>
<tr>
<th>Component</th>
<th>NW (Mean ± STD)</th>
<th>OW (Mean ± STD)</th>
<th>Significance (p&lt;)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Calories (calories/g of DM)</td>
<td>5,581.8 ± 301.3</td>
<td>5,562.9 ± 403.0</td>
<td>0.860</td>
</tr>
<tr>
<td>Percent Protein (%)</td>
<td>9.80 ± 1.53</td>
<td>8.93 ± 2.20</td>
<td>0.130</td>
</tr>
<tr>
<td>Percent Fat (%)</td>
<td>39.6 ± 7.6</td>
<td>43.9% ± 9.8</td>
<td>0.112</td>
</tr>
<tr>
<td>Percent Inorganics (%)</td>
<td>1.45 ± 0.44</td>
<td>1.48 ± 0.34</td>
<td>0.824</td>
</tr>
<tr>
<td>Percent Dry Weight (%)</td>
<td>12.34 ± 1.22</td>
<td>12.48 ± 1.08</td>
<td>0.712</td>
</tr>
</tbody>
</table>

From the gas chromatograph the fatty acids could be combined based on type of fatty acids. The percent of fatty acid is based on the percent area under the curve determined by the gas chromatograph. The mean saturated fatty acids of NW and OW participants was 65.0 ± 4.45 and 65.2 ± 3.35, respectively. Polyunsaturated fatty acids of NW and OW participants was 26.1 ± 4.83 and 25.0 ± 4.66, respectively. Monounsaturated fatty acids from NW and OW participants was 44.0 ± 3.43 and 40.5 ± 2.68, respectively (p=0.011). Omega-3 fatty acids from NW and OW participants was 2.4 ± 0.68 and 2.1 ± 0.66, respectively. Omega-6 fatty acids from NW and OW participants was 22.5 ± 4.35 and 21.7 ± 4.15, respectively. Myristic acid from NW and OW participants was 4.5 ± 1.25 and 5.8 ± 1.82, respectively (p=0.059). Palmitic acid from NW and OW participants was 19.2 ± 2.07 and 21.2 ± 2.40, respectively (p=0.047). Stearic and oleic acid from NW and OW participants is 40.6 ± 3.11 and 37.6 ± 2.4, respectively (p=0.012). EPA from NW and OW participants was 0.097 ± 0.0726 and
0.051 ± 0.0369, respectively (p=0.064). DHA from NW and OW participants was 0.205 ± 0.1674 and 0.087 ± 0.0426, respectively (p=0.027). These data are shown in Table 8.

Table 8: Gas Chromatograph Fatty Acid Analysis of Breast Milk Samples

<table>
<thead>
<tr>
<th>Component as percent of area under the curve as determined by gas chromatography</th>
<th>NW (Mean ± STD)</th>
<th>OW (Mean ± STD)</th>
<th>Significance (p&lt;)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saturated Fat</td>
<td>65.0 ± 4.45</td>
<td>65.2 ± 3.35</td>
<td>0.948</td>
</tr>
<tr>
<td>Polyunsaturated Fat</td>
<td>26.1 ± 4.83</td>
<td>25.0 ± 4.66</td>
<td>0.562</td>
</tr>
<tr>
<td>Monounsaturated Fat</td>
<td>44.0 ± 3.43</td>
<td>40.5 ± 2.68</td>
<td>0.011**</td>
</tr>
<tr>
<td>Omega-3 Fatty Acids</td>
<td>2.4 ± 0.68</td>
<td>2.1 ± 0.66</td>
<td>0.287</td>
</tr>
<tr>
<td>Omega-6 Fatty Acids</td>
<td>22.5 ± 4.35</td>
<td>21.7 ± 4.15</td>
<td>0.649</td>
</tr>
<tr>
<td>14:0 (Myristic Acid)</td>
<td>4.5 ± 1.25</td>
<td>5.8 ± 1.82</td>
<td>0.059*</td>
</tr>
<tr>
<td>16:0 (Palmitic Acid)</td>
<td>19.2 ± 2.07</td>
<td>21.2 ± 2.40</td>
<td>0.047**</td>
</tr>
<tr>
<td>18:0/18:1 Δ9 (Stearic/Oleic)</td>
<td>40.6 ± 3.11</td>
<td>37.6 ± 2.4</td>
<td>0.012**</td>
</tr>
<tr>
<td>20:5 Δ5,8,11,14,17 (EPA)</td>
<td>0.097 ± 0.0726</td>
<td>0.051 ± 0.0369</td>
<td>0.064*</td>
</tr>
<tr>
<td>22:6 Δ4,7,10,13,16,19 (DHA)</td>
<td>0.205 ± 0.1674</td>
<td>0.087 ± 0.0426</td>
<td>0.027**</td>
</tr>
</tbody>
</table>
CHAPTER 5
DISCUSSION

Participants

The study consisted of 44 Caucasian female participants separated into two groups; 24 participants in Normal Weight (NW) with a BMI between 18.5 and 24.9 m/kg\(^2\) and 20 participants in Overweight (OW) with a BMI greater than 25.0 m/kg\(^2\). Each participant completed a demographic and health survey (Appendix A), two food frequency questionnaires (Appendix B and Appendix C), and donated approximately four ounces of expressed breast milk. The purpose of this study was to determine if women with a normal BMI have a different breast milk macronutrients and fatty acid composition than women who are overweight or obese, and to determine if there is a correlation between dietary intake and the nutrient composition of breast milk.

Demographic and Health Information

Demographics

There was no significant difference between the ages of participants in NW (28.79) and OW (28.26) (p=0.718). The majority of the NW and OW participants were married, 92% and 90% respectively. The majority of NW participants (67%) were college graduates or above compared to 45% of OW. The majority (54%) of NW participants are full-time homemakers compared to 20% of the OW participants. Although OW participants had a greater variety in employment status, both OW and NW participants were similar in full-time employment (35% and 38% respectively). The annual household income for NW participants ranged from $20,000 - $24,999 to $100,000 or more with $55,000 - $64,999 being the most common (Figure 1). The
annual household income for OW participants ranged from $5,000 - $9,999 to $100,000 or more with no income range being more represented. There was a greater range of income in OW participants.

Health-Care Services Utilization

The majority of NW and OW participants received prenatal care from an obstetrician, 88% and 95% respectively. The majority of NW and OW participants had an obstetrician as their birth attendant, 88% and 90% respectively. There was no significant difference between medical professional that provided prenatal care and attended birth between NW and OW participants.

Tobacco Smoke Exposure

All NW participants and majority (90%) of OW participants did not smoke or live in a home with anyone who smoked the year of pregnancy before their child was born. The majority (63%) of NW participants never had contact with smoke, compared to OW participants (35%). The majority (55%) of OW participants sometimes had contact with smoke, compared to NW participants (33%). Overall, OW participants were had higher rates of smoking, live with someone who smokes, and have increased contact with those who smoke than NW participants.

Physical Activity

The majority of NW and OW participants engaged in moderate-intensity activity, 75% and 70% respectively. There was no significant difference between NW and OW participants in the number of days per week or hours per day that they participate in moderate-intensity physical activity. The number of NW and OW participants who participated in moderate-intensity fitness or sports decreases to 38% and 55%,
respectively. There was no significant difference in the number of days per week NW and OW participants participated in moderate-intensity fitness or sport. OW participants participated in moderate-intensity fitness or sport about half an hour longer per day than NW participants (p=0.101) (Table 1).

Prenatal Questions

The majority of NW and OW participants reported that this is their second pregnancy, 46% and 50% respectively. All NW participants consumed prenatal vitamins during pregnancy, compared to only one OW participant not consuming a prenatal vitamin during pregnancy. However, the consumption of prenatal vitamins during lactation decreases to 90% of OW participants and 63% of NW still consuming prenatal supplements during lactation. This indicates that the majority of participants consumed a prenatal vitamin during pregnancy but only OW participants had a higher prenatal vitamin consumption during lactation. The average DHA content of prenatal vitamins from NW and OW participants are 118.75 mg ± 105.10 and 86.84 mg ± 91.05, respectively (p=0.301). There is no significant difference in the DHA content of prenatal vitamin content between NW and OW participants.

The majority of NW and OW participants had single births, 96% and 95% respectively. Of the births from NW participants 58% were male and 42% were female compared to births from OW participants where 43% were male and 57% were female. There was no significance in the average birth weight of NW and OW participants, 6.85 ± 2.02 and 6.79 ± 3.01, respectively (p=0.0965). However, the data in Figure 2 indicates that OW participants tend to give birth to heavier infants compared to NW participants.
The breast milk samples were collected between two and 14 weeks for both NW and OW participants (Figure 3). The breast milk samples from NW and OW participants were collected 58.33 ± 27.83 and 56.42 ± 31.98 days respectively, after parturition (p=0.835). There was an even distribution of breast milk samples within the two to 14 weeks of lactation with similar average day of lactation sample between NW and OW participants.

**Maternal Body Mass Index**

The average pre-gravid BMI of NW and OW participants was 22.03 ± 1.83 and 33.86 ± 5.62, respectively (p=0.0001). Pre-gravid BMI is significant because participants were placed into NW and OW groups based on this factor. BMI at 18 years of age and highest BMI obtained when not pregnant was significant between NW and OW groups, p=0.0001 (Table 3). This shows that participants who were overweight at 18 years of age based on BMI and remained overweight prior to conception.

**Dietary Information**

Twenty nine percent of NW participants consumed dietary supplements other than prenatal vitamins, compared to 20% of OW participants (Table 2). Twenty five percent of NW participants consumed fish or krill oil supplements, compared to 15% of OW participants. Ninety five percent of OW participants consumed nuts, compared to 88% of NW participants. OW participants consumed of nuts more frequently per month than NW participants, 9.58 ± 9.37 and 11.71 ± 11.44 respectively (p=0.50). Thirty five percent of OW participants consumed flaxseed or flaxseed oil (35%), compared to NW participants (25%). Forty six percent of NW participants consumed fish (46%), compared to OW participants (30%). NW participants consumed fish twice as many
times per month as OW participants (2.23 ± 3.11 and 1.13 ± 2.75, respectively), however this was not significant (p=0.321) (Table 4). All participants consumed far below the recommended twice a week fish intake for pregnant women.  

**Food Frequency Questionnaires**

OW participants consumed slightly more servings of fruits and vegetables, milligrams of vitamin C, milligrams of magnesium, milligrams of potassium, and grams of dietary fiber per day compared to NW participants (Table 5). While NW participants had slightly higher intake of milligrams of dietary cholesterol, grams of total fat, grams of saturated fat, percent fat, and percent saturated fat compared to OW participants (Table 6) none of the food frequency values were significantly different between the groups.

**Laboratory Results of Breast Milk Samples**

Although NW participants expressed breast milk that was slightly higher in calories (18.8 calories) than that of OW participants, the difference was not significant, NW 5,581.8 ± 301.3 versus OW 5,562.9 ± 403.0 calories. NW breast milk had higher protein content by 0.9% compared to OW, the p value was approaching significance (p=0.130). OW breast milk had higher fat content by 4.2% compared to NW, with the p value approaching significance (p=0.112). There were no significant differences in the percent of inorganic material or the dry weight of the breast milk (Table 7).

Even though there were no significant differences in the macronutrients of the breast milk, there were significant differences in the fatty acids of the breast milk (Table 8). The proposed fatty acid ratio of human breast milk is 30% mothers’ fatty acid intake, 70% fatty acid stores, and minimal amounts derived from de novo synthesis. There were no significant differences in the saturated fatty acid, polyunsaturated fatty acids,
total omega-3 fatty acids, or total omega-6 fatty acids between NW and OW participants. Myristic acid was higher by 1.3% in OW compared to NW (p=0.059). Monounsaturated fatty acids were higher by 3.5% in NW compared to OW (p=0.011). Palmitic acid was higher in OW participants by 2% compared to NW (p=0.047). The increased palmitic acid concentration in OW participants’ breast milk may support the increased rate of de novo synthesis of fatty acids and is indicative of over consumption of calories. Stearic and Oleic acid was 3% higher in NW compared to OW participants (p=0.012). EPA was almost twice the concentration in NW breast milk compared to OW (p=0.064). A major source of EPA in the diet is fish and marine food.\textsuperscript{16} NW breast milk has twice the concentration of DHA compared to OW participants (p=0.027). Like EPA, DHA content is linked to fish consumption or fish oil consumption.\textsuperscript{29,32} Since NW participants had twice the monthly consumption of fish compared to OW participants, this may suggest the EPA and DHA concentration of breast milk is linked to fish intake. In addition, Much et al. found the women with overweight or obese BMI had decreased DHA concentration in their breast milk.\textsuperscript{30} There was no significant difference in the DHA content of prenatal vitamins or nut intake between NW and OW participants.

**Conclusion**

Women who are overweight at 18 years old tend to remain overweight at the time of conception. There was no significant difference (p>0.100) between the calories, percent protein, or percent fat between NW and OW participants. However, NW participants had higher protein concentration and OW participants had fat higher fat concentration. The major differences in the breast milk of NW and OW participants was in the fatty acid concentration. Breast milk from NW participants had increased
concentrations of EPA and DHA fatty acids that could be correlated to increased fish consumption. Whereas, breast milk from OW participants had increased levels of palmitic acids likely due to increased rate of de novo synthesis and excessive caloric intake. This shows that maternal pre-gravid BMI and the types of fat consumed affect the neonate’s nutrition.

Some limitations of the study were the time of day the breast milk was expressed was not specified and there may have been variations between samples because of the time of day the breast milk was expressed. In addition, the researcher did not observe the breast milk being expressed so there may be variation in how the breast milk was collected and no guarantee that the milk came from the participant.

Further research could address the DHA content of prenatal vitamins and average fish consumption per month compared to the DHA and EPA expressed in breast milk without BMI as a factor. An area for further research is the impact of nutrition education prior to conception or in the first trimester of pregnancy and the impact on the fatty acids expressed in the breast milk.
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APPENDICES

Appendix A

Demographic and Health Survey

Lactation Study
Demographic and Health survey

Date: ________________  Participant PIN: ________________

Demographic Information

1. Date of birth:
   __________/________/________
   Month/Day/Year

2. Race/Hispanic Origin:
   □ 1 Mexican American
   □ 2 Other Hispanic
   □ 3 Non-Hispanic White
   □ 4 Non-Hispanic Black
   □ 5 Other Race – Including Multi-Racial

3. Marital status:
   □ 1 Married
   □ 2 Widowed
   □ 3 Divorced
   □ 4 Separated
   □ 5 Never married
   □ 6 Living with partner
   □ 77 Refused
   □ 99 Don’t know

4. What is the highest grade or level of school you have completed or highest degree received?
   □ 1 Less than 9th grade
   □ 2 9-11th grade (includes 12th grade with no diploma)
   □ 3 High school graduate/GED or equivalent
   □ 4 Some college or AA degree
   □ 5 College graduate or above
   □ 77 Refused
   □ 99 Don’t know

5. Employment Status:
   □ 1 Work for someone else full time
   □ 2 Temporarily unemployed
   □ 3 Self-employed
   □ 4 Work for someone else part-time
   □ 5 Retired, not employed
   □ 6 Student, disabled, etc., not employed
   □ 7 Full time homemaker
   □ 77 Refused
   □ 99 Don’t know
Appendix A Continued

Lactation Study
Demographic and Health survey

Date: ___________________________  Participant PIN: ___________________________

6. What was your total household income for the last completed year, before taxes and deductions?
   □ 1 $0 to $4,999
   □ 2 $5,000 to $9,999
   □ 3 $10,000 to $14,999
   □ 4 $15,000 to $19,999
   □ 5 $20,000 to $24,999
   □ 6 $25,000 to $34,999
   □ 7 $35,000 to $44,999
   □ 8 $45,000 to $54,999
   □ 9 $55,000 to $64,999
   □ 10 $65,000 to $74,999
   □ 11 $75,000 to 99,999
   □ 12 $100,000 or more
   □ 77 Refused
   □ 99 Don’t know

Health Care Services Utilization

7. Who provided your prenatal care?
   □ 1 An obstetrician
   □ 2 A family doctor, general practitioner, internist, or other physician
   □ 3 A midwife or nurse midwife
   □ 4 Another type of health care provider
   □ 5 I was not getting prenatal care from a health professional

8. Which type of health professional was your birth attendant (someone who delivered your baby)?
   □ 1 An obstetrician
   □ 2 A family doctor, general practitioner, internist, or other physician
   □ 3 A midwife or nurse midwife
   □ 4 Another type of health care provider
   □ 5 No health professional was present

Tobacco Smoking

9. Which sentence best describes your smoking pattern during the year before your child was born?
   □ 1 I did not smoke
   □ 2 I smoked but stopped before pregnancy began
   □ 3 I smoked, and stopped during the first 3 months of pregnancy
   □ 4 I smoked through the first 3 months but stopped before my child was born
   □ 5 I smoked throughout the year

10. During the year before your child was born, how frequently did you come in contact with family, friends or co-workers who smoked?
    □ 1 Never
Lactation Study
Demographic and Health survey

Date: ___________________________  Participant PIN: ________________

☐ 2 Sometimes
☐ 3 Often
☐ 4 Always

12. On the average, how many cigarettes do you smoke a day now? (Write in 0 if you do not smoke).
______________ CIGARETTES PER DAY

13. How many people not including yourself smoke inside your home most days? (Include family members, friends, and anyone else.)

☐ 1 0
☐ 2 1
☐ 3 2
☐ 4 3
☐ 5 4 or more

Physical Activity

14. Think about the time you spend doing work. Think of work as the things that you do such as paid or unpaid work, studying or training, household chores, and yard work. Does your work involve moderate-intensity activity that causes small increases in breathing or heart rate such as brisk walking or carrying light loads for at least 10 minutes continuously?

☐ 1 Yes
☐ 2 No (Go to question 17)

15. In a typical week, on how many days do you do moderate-intensity activities as part of your work?

Number of days _______

16. How much time do you spend doing moderate-intensity activities at work on a typical day?

Number of minutes or hours ______

17. In a typical week do you do any moderate-intensity sports, fitness or recreational activities that cause a small increase in breathing or heart rate such as bicycling, swimming, or golf for at least 10 minutes continuously?

☐ 1 Yes
☐ 2 No (Go to question 20)

18. In a typical week, on how many days do you do moderate-intensity sports, fitness or recreational activities?

Number of days ______

19. How much time do you spend doing moderate-intensity sports, fitness or recreational activities on a typical day?

Number of minutes or hours ______
Appendix A Continued

Lactation Study
Demographic and Health survey

Date: ____________________________  Participant PIN: ____________________________

Pregnancy Questions
20. Including this most recent pregnancy, how many times have you been pregnant?
   - [ ] 1
   - [ ] 2
   - [ ] 3
   - [ ] 4 or more

21. Did you take prenatal vitamins?
   - [ ] 1 Yes
   - [ ] 2 No

22. Are you taking prenatal vitamins now?
   - [ ] 1 Yes
   - [ ] 2 No (Go to question 18)

23. Name of your prenatal vitamin ____________________________

24. Birth weight of baby (lbs/oz) ____________________________

25. Sex of baby
   - [ ] 1 Male
   - [ ] 2 Female

26. Lactation sample (number days after giving birth) ____________________________

Weight Questions
27. What was your pre-gravid weight (weight before most recent pregnancy) ____________ lbs

28. Weight at age 18 years:
   - [ ] 1 ____________ lbs
   - [ ] 2 Not applicable

29. Highest weight ever obtained when not pregnant ____________________________ lbs

30. Current Height: ____________________________ ft/inches
Appendix A Continued

Lactation Study
Demographic and Health survey

Date: ______________  Participant PIN: ______________

Dietary Information

31. Do you take a daily fish oil/krill oil supplement?
   □ 1 Yes
   □ 2 No

32. Do you routinely eat fish?
   □ 1 Yes, How frequently? ____________ times per month
   □ 2 No

33. Do you eat nuts?
   □ 1 Yes, How frequently? ____________ times per month
   □ 2 No (Go to Question 29)

34. What types of nuts do you consume? ______________

35. Do you eat flaxseed or take flaxseed oil?
   □ 1 Yes, How frequently? ____________ times per month
   □ 2 No

36. Do you take any dietary supplements?
   □ 1 Yes, Please list ______________
   □ 2 No
Appendix B

Block Dietary Fruit-Vegetable-Fiber Screener

**Dietary Fruit-Vegetable-Fiber Screener**

Name: 
Age: 
Sex:  □ Male  □ Female

Think about your eating habits over the past month. About how often do you eat each of the following foods? Remember breakfast, lunch, dinner, snacks and eating out. Mark one bubble for each food.

<table>
<thead>
<tr>
<th>Fruits and Vegetables and Fiber</th>
<th>(0) Less than 1/WEEK</th>
<th>(1) Once a WEEK</th>
<th>(2) 2-3 times a WEEK</th>
<th>(3) 4-6 times a WEEK</th>
<th>(4) Once a DAY</th>
<th>(5) 2-4 times a DAY</th>
<th>Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>(1) Fruit juice, like orange, apple, grape, fresh, frozen or canned (Not sodas or other drinks.)</td>
<td>□</td>
<td>□</td>
<td>□</td>
<td>□</td>
<td>□</td>
<td>□</td>
<td></td>
</tr>
<tr>
<td>(2) How often do you eat any fruit, fresh or canned? (Not counting juice.)</td>
<td>□</td>
<td>□</td>
<td>□</td>
<td>□</td>
<td>□</td>
<td>□</td>
<td></td>
</tr>
<tr>
<td>(3) Vegetable juice, like tomato juice, V-8, carrot</td>
<td>□</td>
<td>□</td>
<td>□</td>
<td>□</td>
<td>□</td>
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<td></td>
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<tr>
<td>(4) Green salad</td>
<td>□</td>
<td>□</td>
<td>□</td>
<td>□</td>
<td>□</td>
<td>□</td>
<td></td>
</tr>
<tr>
<td>(5) Potatoes, any kind, including baked, mashed or French fried</td>
<td>□</td>
<td>□</td>
<td>□</td>
<td>□</td>
<td>□</td>
<td>□</td>
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</tr>
<tr>
<td>(6) Vegetable soup, or stew with vegetables</td>
<td>□</td>
<td>□</td>
<td>□</td>
<td>□</td>
<td>□</td>
<td>□</td>
<td></td>
</tr>
<tr>
<td>(7) Any other vegetables, including string beans, peas, corn, broccoli or any other kind</td>
<td>□</td>
<td>□</td>
<td>□</td>
<td>□</td>
<td>□</td>
<td>□</td>
<td></td>
</tr>
<tr>
<td>(8) Fiber cereals like Raisin Bran, Shredded Wheat or Fruit-a-Fiber</td>
<td>□</td>
<td>□</td>
<td>□</td>
<td>□</td>
<td>□</td>
<td>□</td>
<td></td>
</tr>
<tr>
<td>(9) Beans such as baked beans, pinto, kidney, or lentils (not green beans)</td>
<td>□</td>
<td>□</td>
<td>□</td>
<td>□</td>
<td>□</td>
<td>□</td>
<td></td>
</tr>
<tr>
<td>(10) Dark bread such as whole wheat or rye</td>
<td>□</td>
<td>□</td>
<td>□</td>
<td>□</td>
<td>□</td>
<td>□</td>
<td></td>
</tr>
</tbody>
</table>

Fruit-Vegetable Score (Sum for items 1-7) = 

Fruit-Veg-Beans Score (Sum of items 1-10) = 

[blockdietarydatasyncs.com](http://www.blockdietarydatasyncs.com)  
(910) 794-8514
Appendix C
Block Dietary Fat Screener

Dietary Fat Screener

Name: ______________________
Age: ______________________
Sex: ○ Male ○ Female

Think about your eating habits over the past month. About how often do you eat each of the following foods? Remember breakfast, lunch, dinner, snacks and eating out. Mark one bubble for each food.

<table>
<thead>
<tr>
<th>Meals and Snacks</th>
<th>(0)</th>
<th>(1)</th>
<th>(2)</th>
<th>(3)</th>
<th>(4)</th>
<th>Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hamburger, ground beef, meat burritos, tacos</td>
<td>○</td>
<td>○</td>
<td>○</td>
<td>○</td>
<td>○</td>
<td></td>
</tr>
<tr>
<td>Beef or pork, such as steaks, roasts, ribs, or in</td>
<td>○</td>
<td>○</td>
<td>○</td>
<td>○</td>
<td>○</td>
<td></td>
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<tr>
<td>sandwiches</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Fried chicken</td>
<td>○</td>
<td>○</td>
<td>○</td>
<td>○</td>
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<td></td>
</tr>
<tr>
<td>Hot dogs, or Polish or Italian sausage</td>
<td>○</td>
<td>○</td>
<td>○</td>
<td>○</td>
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<td></td>
</tr>
<tr>
<td>Cold cuts, lunch meats, ham (not low-fat)</td>
<td>○</td>
<td>○</td>
<td>○</td>
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<tr>
<td>Bacon or breakfast sausage</td>
<td>○</td>
<td>○</td>
<td>○</td>
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<tr>
<td>Salad dressings (not low-fat)</td>
<td>○</td>
<td>○</td>
<td>○</td>
<td>○</td>
<td>○</td>
<td></td>
</tr>
<tr>
<td>Margarine, butter or mayo on bread or potatoes</td>
<td>○</td>
<td>○</td>
<td>○</td>
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<td></td>
</tr>
<tr>
<td>Margarine, butter or oil in cooking</td>
<td>○</td>
<td>○</td>
<td>○</td>
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<tr>
<td>Eggs (not Egg Beaters or just egg whites)</td>
<td>○</td>
<td>○</td>
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<tr>
<td>Pizza</td>
<td>○</td>
<td>○</td>
<td>○</td>
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<td></td>
</tr>
<tr>
<td>Cheese, cheese spread (not low-fat)</td>
<td>○</td>
<td>○</td>
<td>○</td>
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<tr>
<td>Whole milk</td>
<td>○</td>
<td>○</td>
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<tr>
<td>French fries, fried potatoes</td>
<td>○</td>
<td>○</td>
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<tr>
<td>Corn chips, potato chips, popcorn, crackers</td>
<td>○</td>
<td>○</td>
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<tr>
<td>Doughnuts, pastries, cake, cookies (not low-fat)</td>
<td>○</td>
<td>○</td>
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<tr>
<td>Ice cream (not sherbet or non-fat)</td>
<td>○</td>
<td>○</td>
<td>○</td>
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<td>○</td>
<td></td>
</tr>
</tbody>
</table>

Fat Score = ____________

NUTRITIONQUEST / BLOCK DIETARY DATA SYSTEMS
www.nutritionquest.com
510.764.8514
Appendix D

Institutional Review Board

September 22, 2015

Dr. Andrew Clark
Box 70552

Re: Nutrient Content of Human Breast Milk from Overweight and Normal Weight Women of Hispanic and Caucasian Heritage in Northeast Tennessee

IRB#: 0915.8s
ORSPA #: [Blank]

The following items were reviewed and approved by an expedited process:

- new protocol submission xform, CV of PI, informed consent document version 9/4/15
- recruitment flyer, email indicating interest, Dietary Fruit-Vegetable-Fiber Screener in English and Spanish, Demographic and Health Survey, Dietary Fat Screener in English and Spanish, grant

On September 21, 2015, a final approval was granted for a period not to exceed 12 months and will expire on September 20, 2016. The expedited approval of the study will be reported to the convened board on the next agenda.

The following enclosed stamped, approved Informed Consent Documents have been stamped with the approval and expiration date and these documents must be copied and provided to each participant prior to participant enrollment:
- ICD version 09/04/15 stamped 09/21/2015

*The Chair has requested that once the Spanish ICD and demographic survey have been translated, please submit an xform modification for review of those additional documents*.

Federal regulations require that the original copy of the participant’s consent be maintained in the principal investigator’s files and that a copy is given to the subject at the time of consent.

Projects involving Mountain States Health Alliance must also be approved by MSHA following IRB approval prior to initiating the study.
Unanticipated Problems Involving Risks to Subjects or Others must be reported to the IRB (and VA R&D if applicable) within 10 working days.

Proposed changes in approved research cannot be initiated without IRB review and approval. The only exception to this rule is that a change can be made prior to IRB approval when necessary to eliminate apparent immediate hazards to the research subjects [21 CFR 56.108 (a)(4)]. In such a case, the IRB must be promptly informed of the change following its implementation (within 10 working days) on Form 109 (www.etsu.edu/irb). The IRB will review the change to determine that it is consistent with ensuring the subject’s continued welfare.

Sincerely,
George Youngberg, M.D., Chair
ETSU/VA Medical IRB
VITA
MEGAN R KWON

Education: Waukee Community School District, Waukee, Iowa 2011
B.S. Dietetics, Iowa State University, Ames, Iowa 2015
M.S. Clinical Nutrition, East Tennessee State University,
Johnson City, Tennessee 2017

Professional Experience: Graduate Assistant, East Tennessee State University,
2015-2016

Publications: M. Kwon, B. Pond, E. Cress, R. Hamdy, J. Reece, W.A.
Clark. Research and Practice Innovations Poster
Presentation on “Nutrient Content of Breast Milk from
Over and Normal Weight Caucasian Women in
Northeast Tennessee.” October 16, 2016, Boston,
Massachusetts.

Honors and Awards: Thesis Scholarship
Top Senior Cadet of Cyclone Battalion
Distinguished Military Graduate and Top 10% of Class
Iowa State University Dean’s List