THE INFLUENCE OF EXCLUSIVE BREASTFEEDING ON GROWTH
TRAJECTORY FROM BIRTH TO YEAR 5 IN A WIC COHORT

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ABSTRACT

The Influence of Exclusive Breastfeeding on Growth Trajectory from Birth to Year 5 in a WIC Cohort

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OBJECTIVE. The impact of exclusively breastfeeding (EBF) on child body mass index (BMI) from birth through 60 months of age was investigated.

METHODS. 60,190 mothers and children attending Women, Infant and Children (WIC) clinics in the Santa Barbara and San Luis Obispo counties of California provided data on breastfeeding duration, maternal pre-pregnancy BMI, and child weight-for-length (WL) was measured at 3 month intervals until 5 years of age. Missing time points were interpolated in determining child BMI/WL z-score trajectories plotted on the Center of Disease Control (CDC) infant and child growth curves. Multivariate analysis of variance was used to contrast the impact of notbeing EBF against being EBF from 0-3 months, and being EBF from 0-6 months. In subsets of the population the relationship was examined in White Hispanic (n=43,360) versus all others race/ethnicities of (n=16,830) children. All models controlled for maternal pre-pregnancy maternal BMI and income.

RESULTS. Duration ofEBF from 0-3 months and 0-6 months of age was protective against child BMI z-scores being above the average growth trajectory ($P=0.0001$).

A significant inverse association between EBF and overall BMI/WL z-scores for both males and females ($P<0.0001$) was found. The impact of EBF on child growth trajectory began to weaken at 15 months for females and 18 months for males. Children who were not EBF had higher mean BMI/WL z-scores at every time point than those who were EBF. Those who were EBF for 0-3 months had a lower BMI; those who were EBF for 0-

6months had even lower BMI/WL z-scores by 5 years of age. Hispanic children had a higher BMI growth curve than non-Hispanic children even after adjusting for EBF, pre-pregnancy BMI, and income. Mothers with a higher pre-pregnancy BMI tended to have children with higher BMI/WL z-scores ($P<0.0001$).

CONCLUSION. This study supports the public health efforts to encourage EBF for at least 3 months and optimally for 6 months as a method to protect against childhood obesity.

Keywords: breastfeeding, exclusive breastfeeding, growth trajectory, infant and childhood obesity.

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TABLE OF CONTENTS

	Page
LIST OF TABLES.....	x
LIST OF FIGURES	xi
CHAPTER 1: Review of Literature.....	1
Introduction.....	1
Childhood Obesity Prevention -- A Clear Challenge to Healthcare	2
Breastfeeding and Infant Feeding Practices.....	5
Components of Breast Milk	7
Stages of Lactation.....	10
Benefits of Breastfeeding.....	12
Maternal Benefits.....	13
Infant Benefits.....	14
Growth Standards	15
Z-Scores versus Percentiles	19
Relationship between Infant Feeding and Adipose Development.....	21
Potential Mechanisms Relating Adipose to Infant Feeding Practices	23
High Early Protein Intake Hypothesis	24
Growth Acceleration Hypothesis.....	26
Feeding Practices	28
Breast Milk Energy Balance Regulation	31
Breastfeeding and Adiposity Development	34

Breastfeeding, Race/Ethnicity and Childhood Obesity	36
Breastfeeding, Pre-pregnancy BMI, and Childhood Obesity	39
Pre-pregnancy BMI, Hispanic and Obesity	41
Purpose of This Study	42
Hypotheses	42
CHAPTER 2: Methods	44
Purpose	44
Subjects	44
Statistical Analyses	46
CHAPTER 3: Results	51
CHAPTER 4: Discussion	61
CHAPTER 5: Conclusion	70
REFERENCES	71
APPENDICES	
Appendix A: Figure 4-1A Data for the Mean BMI Z-scores for Males based on duration of breastfeeding (N=16,321).	77
Appendix B: Figure 4-1B Data for Mean BMI Z-scores for Females based on duration of breastfeeding (N=15,884).	78
Appendix C: Figure 4-2A Data for Mean BMI Z-scores for Hispanic males compared to all other race/ethnicities based on duration of breastfeeding (N=16,225).	79
Appendix D: Figure 4-2B Data for Mean BMI Z-scores for Hispanic females	

compared to all other race/ethnicities based on duration of breastfeeding. (N=15,705).....	80
Appendix E: Figure 4-3 Data for the Comparison of mean BMI *Z-scores for children Exclusively Breast Fed 0-6 months based on mothers’ pre-pregnancy BMI (N=2,989).	81
Appendix F: Table 1. Mean (SD) or proportion of maternal and offspring characteristics of San Luis Obispo and Santa Barbara County, CA WIC participants, 2007 (Total N=60190).....	82
Appendix G: Table 2a. Percent obese by selected characteristics from birth to 57 months of age among children participating in San Luis Obispo and Santa Barbara County WIC program, 2005-2009 (Total N=60190).	84
Appendix H: CDC Growth Chart for Birth to 36 months for Boys, Length-for-age and Weight-for-age percentiles.	86
Appendix I: CDC Growth Chart for Birth to 36 months for Girls, Length-for-age and Weight-for-age percentiles.....	87
Appendix J: CDC Growth Chart for 2 to 20 years for Boys, Body mass index-for-age percentiles.	88
Appendix K: CDC Growth Chart for 2 to 20 years for Girls, Body mass index-for-age percentiles.	89

LIST OF TABLES

Table	Page
Table 1-1. Changes in composition of breast milk during the first month of lactation for full-term and preterm infants (Adapted from Riordan, 1999).	12
Table 2-1. Maternal and Offspring Characteristics for WIC data set (N=60,190).	46
Table 3-1. Mean (SD) growth z-scores based on gender for the interpolated data (N=60,190) and p-value for T-test of mean gender differences ($p<0.05$).	52
Table 3-2. Mean (SD) growth z-scores based on gender for the original data set before interpolation and T-test of mean gender differences ($p<0.05$).	53
Table 4-1. Regression Analysis of Missing Visit Data as Predicted by Race, Income, Pre-pregnancy BMI, Birth Weight and Breastfeeding Status.	67

LIST OF FIGURES

Figure	Page
Figure 1-1. Trends in obesity among children and adolescents aged 2-19 years old, by sex: United States, selected years 1971-1974 through 2011-2012.....	3
Figure 1-2. Milk protein concentrations, comparing milk from mothers who delivered preterm and term, by gestational age at delivery and weeks postpartum (From Ballard and Morrow, 2013).	13
Figure 1-3. Left: Proportion (%) of children exceeding the 90th percentile of the BMI reference values, depending on feeding mode in infancy. Right: Proportion (%) of children exceeding the 97th percentile of the BMI reference values, depending on feeding mode in infancy. **P<0.01; ***P<0.001 (From Bergmann et al., 2003).....	29
Figure 1-4. Observed BMI raw score mean growth curve (From Guerrero et al., 2015).	38
Figure 1-5. Prevalence of childhood overweight (BMI $\geq$ 95th percentile) by maternal pre-pregnancy BMI and breastfeeding for the sample (A: 2 to 14 years of age) and by three age groups (B: 2 to 6 years of age; C: 7 to 10 years of age; D: 11 to 14 years of age) (From Li et al., 2005).	41
Figure 3-1A. Mean BMI Z-scores for Males based on duration of breastfeeding (N=16,321).....	55

Figure 3-1B. Mean BMI Z-scores for Females based on duration of breastfeeding (N=15,884).....	56
Figure 3-2A. Mean BMI Z-scores for Hispanic males compared to all other race/ethnicities based on duration of breastfeeding (N=16,225)	58
Figure 3-2B. Mean BMI Z-scores for Hispanic females compared to all other race/ethnicities based on duration of breastfeeding (N=15,705)	58
Figure 3-3. Comparison of mean BMI *Z-scores for children Exclusively Breast Fed 0-6 months based on mothers' pre-pregnancy BMI (N=2,989)	59

CHAPTER 1: REVIEW OF LITERATURE

Introduction

The rapid growth of childhood obesity represents a public crisis with far reaching impacts on all health care systems (Hauner, Brunner and Amann-Gassner et al., 2013). With the rise in childhood obesity, there is a rise in the incidence of comorbidities in obese children, which then increases healthcare utilization and expenditures (Trasande and Chatterjee, 2012). Three co-morbidities that are high among children with obesity include diabetes, gallbladder disease, and obstructive sleep apnea. In addition, mental health has been significantly impacted in children who are obese (Trasande and Chatterjee, 2012). Hampl, Carroll, Simon and Sharma (2007), compared the healthcare utilization and expenditures between healthy-weight patients, overweight patients, and patients with diagnosed and undiagnosed obesity. Hampl et al. (2012) found that pediatric patients diagnosed with obesity at their Well-Child visit had \$172 higher annual healthcare expenditures compared to children of normal bodyweight. The early development of obesity can cause a wide array of serious health complications, persisting from childhood to adulthood, impacting the risk of developing chronic diseases and premature death (Hauner et al., 2013). Children who develop obesity at a young age are likely to maintain their overweight status into adulthood, placing them at higher risk for developing chronic diseases including hypertension, dyslipidemia, type 2 diabetes, heart disease, stroke, gallbladder disease, osteoarthritis, sleep apnea, respiratory problems, and certain cancers (Wang & Lim, 2012). Recent studies have attributed the obesity epidemic to a constellation of factors including system factors such as policy and economic

systems, food supply, and marketing as well as individual factors such as genetic makeup and perinatal influences (Hauner et al., 2013).

Genetics and other non-genetic factors all contribute to the equation that results in obesity (Han, Lawlor and Kimm, 2010). Some other possible contributing factors include genetic variations, epigenetics, endocrine disease, central nervous system pathology, intrauterine exposure to gestational diabetes, intrauterine exposure to high maternal adiposity, diet, energy expenditure, television viewing, microbial infection, education, and socioeconomic status (Han, Lawlor and Kimm, 2010).

To prevent obesity new approaches need to be developed to identify and address the onset of obesity. One current area of research is the critical window of influence on early infant growth as related to both the mother's pregnancy and to lactation duration (Hauner et al., 2013). Research has shown that the early life phases of the embryo during pregnancy and the early postpartum neonate contribute to the susceptibility for later adiposity development (Hauner et al., 2013).

Childhood Obesity Prevention -- A Clear Challenge to Healthcare

The prevalence of childhood obesity for children between the ages of 2 to 18 years of age in the United States has increased approximately 3 to 4 times in the last decade (Boonpleng, Park & Gallo, 2012). The prevalence of obesity varies by race/ethnicity and has become more prevalent among racial/ethnic minority children (Boonpleng, Park & Gallo, 2012). Studies have found that approximately 20% of school age children (5-17 years old) in European countries are overweight and 5% are obese. In North America, 30% of school age children are overweight and 15% are obese. The

World Health Organization (WHO) estimated that 155 million, or 1 in 10 school aged children, are overweight or obese worldwide (Wang & Lim, 2012).

According to the Center for Disease Control and Prevention (CDC), as shown in Figure 1-1, despite decline in prevalence of obesity in recent years, childhood obesity rates still remain high (CDC, 2015). The prevalence of obesity has remained fairly stable at about 17%, affecting approximately 12.7 million children and adolescents aged 2-19 years of age. The prevalence of obesity among 2 to 5 years olds has decreased from 13.9% in 2003-2004 to 8.4% in 2011-2012. In 2011 to 2012, the prevalence of obesity for ages 2-19 years of age was higher among Hispanics (22.4%) and non-Hispanic blacks than among non-Hispanic whites (14.1%) (CDC, 2015).

Figure 1-1. Trends in obesity among children and adolescents aged 2-19 years old, by sex: United States, selected years 1971-1974 through 2011-2012.

The development of obesity is very complex and has multiple contributing factors. Debate still persists as to whether infant feeding practices such as breastfeeding are protective against obesity. However many studies have shown that early dietary

habits have an influence on BMI development and also on the timing of the adiposity rebound (Pedersen, Lauritzen, Brasholt, Buhl & Bisgard, 2012). One way to predict obesity is the timing of the adiposity rebound (AR). The AR is defined by being the lowest point at which the BMI drops on the child growth curve, which occurs when the child is at their maximal leanness and/or their minimum BMI (Whitaker et al., 1998). The AR is a critical period during early childhood for the development of adiposity that persists to later in life (Boonpleng, Park & Gallo, 2012).

Aside from the adiposity rebound there are multiple factors that contribute to the critical period of development in children that could potentially explain the onset of obesity later in life. In a review about early markers for adult obesity, Brisbois, Farmer & McCargar (2012) categorized these early markers into two types, possible and probable. Their review identified possible early markers of obesity such as maternal smoking and maternal weight gain during pregnancy, and probable markers included maternal body mass index, childhood growth patterns (i.e. early rapid growth and early AR), childhood obesity and socioeconomic status. In addition, early life experiences in utero and post-natal influences were seen to potentially induce permanent changes in physiologic function that 'programs' the infant's long-term energy balance regulatory pathways. Recent research suggests that preventative measures should be initiated at preconception, during pregnancy, and throughout early childhood to educate mothers, fathers and children on lifestyle modifications that can be effective in preventing later obesity in their infants (Brisbois et al., 2012).

The first two years of life are the most critical time periods within a child's life in order to ensure the child is receiving all the essentials needed for proper growth, health,

and development (World Health Organization, 2009). Poor nutrition in these early years of life increases the risk of illness, and is responsible either directly or indirectly for approximately one-third of the deaths of children ages 5 or less (World Health Organization, 2009). Nourishment during these years may also have long-term impacts on energy regulation and obesity development. The U.S. Department of Agriculture developed the Supplemental Nutrition Program for Women, Infant, and Children (WIC), which helps to address the concern of proper nutrition in the early years of life for low-income families.

WIC is a federal food and nutrition education program for pregnant, breastfeeding and postpartum women, infants, and children under 60 months of age whom are low-income (i.e. up to 185% of the federal poverty level). WIC recognizes the early years of a child's life to be the most critical in influencing their nutrition and health behaviors for the remainder of their lifetime. According to Whitaker (2004), almost half of U.S. children are enrolled in WIC at some point during their infancy, and approximately 1 in 4 children are enrolled between 2 to 4 years of age. WIC provides education on health eating, and breastfeeding promotion and support to low-income families who are at the highest nutritional risk.

Breastfeeding and Infant Feeding Practices

For optimal feeding, the WHO recommends that infants should be exclusively breastfed for 6 months (or 111 days) and then begin complementary feeding at 6 months until the first 2 years of life or beyond (World Health Organization, 2009). The definition of exclusive breastfeeding used by the World Health Organization (2009) is that infants

would be receiving breast milk as their only source of nutrition. The infant should not require any other liquids including water or solids. The only exception to breast milk not meeting the infant's needs is for oral rehydration solution, medicine, or drops or syrups containing vitamins and minerals. After the first 6 months of life, a mother is recommended to introduce complementary feeding, which is needed because the breast milk is no longer meeting 100% of the infants' needs. Complementary feeding is defined as breastfeeding the infant while concurrently incorporating other solids into the infant's diet (World Health Organization, 2009).

Complementary feeding would last from 6 months to 23 months of age or until weaning, which is when all nutrient needs are provided by foods other than human milk. Breastfeeding could persist beyond two years of life (World Health Organization, 2009). It is especially important for complementary foods to be high in nutrient density (Dewey, 2013). Iron and zinc are the two most problematic nutrients during the complementary feeding period due to their concentrations in human milk being relatively low compared to an infant's needs. Expected energy intake from complementary foods should increase from approximately 200 kcals a day at 6-8 months to 300 kcals per day from 9 to 11 months, and 550 kcals per day from 12 to 23 months. With the energy intake from complementary foods increasing with age, research has found that the second 6 months of life presents the greatest challenge for meeting the micronutrient needs of iron and zinc (Dewey, 2013). The recommendations for breastfeeding are based on multiple studies assessing the benefits of breastfeeding and infant health (World Health Organization, 2009).

Components of Breast Milk

Breast milk is composed of fats, carbohydrates, protein, vitamins, minerals, water, and contains all the vital nutrients that the infant requires for the first 6 months of life (Stam, Sauer and Boehm, 2013). Research has proven breast milk to be a superior source of protein for infants (Lonnerdal, 2013). Breast milk contains bioactive factors, which provide protection against infection, while other factors that help with digestion and absorption of nutrients (World Health Organization, 2009). Bioactive proteins play a vital role in human health by contributing to enzyme activities, enhance nutrient absorption, stimulate growth, modulate the immune system, and defend against pathogens (Lonnerdal, 2013). Ingesting bioactive proteins through breast milk is an essential resource for infant health due to the complex role of these protein in being protective against pathogenic bacteria and viruses through their probiotic effects (i.e. stimulation of beneficial microorganisms in the gut), killing of pathogens, inhibition of pathogens by limiting their effects and preventing attachment or invasion by the pathogen into the intestinal mucosa (Lonnerdal, 2013).

Fat provides about one half of the energy content of breast milk with 3.5g of fat per 100 ml of milk (World Health Organization, 2009). Fat is the most variable macronutrient in human milk (Ballard and Marrow, 2013). Human milk is not a uniform body fluid due to the composition changing continuously (Stam, Sauer & Boehm, 2013). The hindmilk, also known as the last milk, contains 2 to 3 times more fat than found in the foremilk, which is the first milk (Ballard and Marrow, 2013). Human milk contains high amounts of palmitic and oleic acids (Ballard and Marrow, 2013). The foremilk

differs from the hindmilk in that the fat content changes with the time of day as well as during the course of lactation (Stam, Sauer & Boehm, 2013).

The fat in breast milk contains long chain polyunsaturated fatty acids (LC-PUFAs) such as docosahexaenoic acid (DHA), a derivative of omega-3 polyunsaturated fatty acids (PUFAs), and arachidonic acid (AA), a derivative of omega-6 PUFAs, which are not available in other milks (World Health Organization, 2009). The fatty acid profiles in breast milk of LC-PUFAs vary based on the maternal diet (Ballard and Marrow, 2013). Over the years diets in Western societies have changed as food companies have developed more processed foods (Pedersen et al., 2012). With the diets of society changing it has affected the LC-PUFA profile of breast milk; with a corresponding increase in omega-6 PUFAs and a decrease in omega-3 PUFAs.

Researchers suggest that the change in LC-PUFA profiles could contribute to an increase in adiposity at a young age. The proposed mechanism being affected is when the process of differentiation of preadipocytes into mature adipocytes influenced by various hormones and growth factors including certain eicosanoids. Omega-6 PUFA derived eicosanoids have previously been shown to promote preadipocyte differentiation. Pedersen et al. (2012) investigated the relationship between the DHA content of breast milk and body composition. A significant association between the DHA content in breast milk and BMI development from 2 to 7 years of age ($P < 0.01$) and body fat from ages 6 to 9 ($P < 0.01$) was seen. The findings were stronger with females who also demonstrated a significantly higher association between breast milk DHA and age at AR ($P < 0.01$). These findings indicate that early intake of DHA may have an effect on body composition and

that dietary habits of the lactating mother could contribute to the increased prevalence of obesity (Pedersen et al., 2012).

The main carbohydrate secreted in breast milk is the disaccharide lactose and breast milk contains 7g of lactose per 100 ml (World Health Organization, 2009). The concentration of lactose in breast milk is the least variable macronutrient (Ballard and Marrow, 2013). Oligosaccharides are the second most important carbohydrate source in human milk contributing to the non-nutritive bioactive factors. Human milk contains approximately 1g/dL of oligosaccharides (Ballard and Marrow, 2013).

The proteins contained in human versus animal milk differ in both quantity and quality. While both milks provide essential amino acids the protein content in breast milk is 0.9g per 100 ml, a concentration that is lower than found in animal milks (World Health Organization, 2009). Human milk contains 2.5g of protein per 1 cup, and animal milk contains 7.9 g of protein per cup. Animal milk contains approximately 80% casein and 20% whey. Breast milk contains proportionally less casein. The casein in breast milk also has a different molecular structure that is softer, forming more digestible curds compared to other milks (World Health Organization, 2009). Human milk also provides whey, alpha-lactalbumin, lactoferrin, secretory immunoglobulin IgA, lysozyme, and serum albumin (Ballard and Marrow, 2013). Human milk contains more alpha-lactalbumin where cow's milk contains beta-lactoglobulin, which is absent in human milk and may help explain why infants can be intolerant to cow's milk (Riordan, 1999).

The protein content of the breast milk from mothers who deliver at preterm is significantly higher compared to mothers who deliver at term (Ballard and Marrow, 2013). For both mothers delivering at term or preterm the breast milk protein levels

decrease over the first 4 to 6 weeks (Ballard and Marrow, 2013). Human milk also provides whey, alpha-lactalbumin, lactoferrin, secretory immunoglobulin IgA, lysozyme and serum albumin (Ballard and Marrow, 2013).

Breast milk also contains all sufficient vitamins for an infant's needs unless the mother is deficient herself (Butte, Lopez-Alaracon & Garza, 2002). Iron and zinc are present in low concentrations, but their bioavailability in breastmilk and absorption by the infant are both high. Infants are usually born with iron stores that support their needs (World Health Organization, 2009).

Stages of Lactation

When an infant is born breast milk production is stimulated. Colostrum is the first breast milk produced after delivery and is distinct in volume, appearance and composition (Ballard and Morrow, 2013). Colostrum is produced for a few days postpartum with content rich in immunologic components including secretory IgA, lactoferrin, leukocytes, and developmental factors such as epidermal growth factors (Ballard and Marrow, 2013). Colostrum provides protective immune factors and prepares the lining of the gut to receive nutrients from milk (World Health Organization, 2009). Colostrum contains relatively low concentrations of lactose, calcium and potassium, and high levels of sodium, chloride, and magnesium (Ballard and Marrow, 2013). The sodium and potassium ratio after a few days postpartum will decline as the lactose ratio increases indicating secretory activation and production of transitional milk. Timing of the secretory activation varies among mothers, but typically occurs during the first few days postpartum (Ballard and Marrow, 2013).

Transitional milk comes in 2-4 days after delivery, and is called transitional milk from day 5 to about two weeks postpartum. The purpose of transitional milk is to support the nutritional and developmental needs of rapidly growing infants. The final change in human milk occurs between the fourth and sixth week postpartum where the milk becomes fully matured. Once breast milk is fully matured the composition remains relatively similar in composition (Ballard and Marrow, 2013).

Breast milk composition varies depending on stage of lactation and gestational age when delivered as shown in Table 1-1. Breast milk composition varies depending on prematurity and postnatal age. The protein content has been shown to be significantly higher in preterm infants compared to term infants, as shown in Table 1-1 and Figure 1-2 (Ballard and Morrow, 2013; Riordan, 1999). Preterm milk is higher in calories, fat, nitrogen, fatty acids, some vitamins and minerals (Riordan, 1999). Preterm milk has also been shown to be higher in immune factors including cells, immunoglobulins, and anti-inflammatory elements compared to term breast milk. Preterm breast milk varies in composition in order to meet the increased needs of the preterm infant (Riordan, 1999).

Table 1-1. Changes in composition of breast milk during the first month of lactation for full-term and preterm infants (Adapted from Riordan, 1999).

	3-5 Days		8-11 Days		15-18 Days		26-29 Days	
	Full Term	Pre Term	Full Term	Pre Term	Full Term	Pre Term	Full Term	Pre Term
Energy								
(kcal/dl)	48	58	59	71	62	71	62	70
Lipid (gm/dl)	1.85	3	2.9	4.14	3.06	4.33	3.05	4.09
Protein (gm/dl)	1.87	2.1	1.7	1.86	1.52	1.71	1.29	1.41
Lactose								
(gm/dl)	5.14	5.04	5.98	5.55	6	5.63	6.51	5.97

Figure 1-2. Milk protein concentrations, comparing milk from mothers who delivered preterm and term, by gestational age at delivery and weeks postpartum (From Ballard and Morrow, 2013).

Benefits of Breastfeeding

Breastfeeding related research has examined the strength of the relationship between breastfeeding and the potential benefits to both the infant and mother. Identified

benefits of breastfeeding include physical health, nutritional, immunological, developmental, psychological, social, economic, and environmental impacts (American Academy of Pediatrics, 2005).

Maternal Benefits

The maternal medical benefits of breastfeeding include decreased postpartum bleeding with more rapid uterine involution attributable to increased concentrations of oxytocin released during breastfeeding, decreased menstrual blood loss; and increased child spacing attributable to lactational amenorrhea; earlier return to pre-pregnancy weight and decreased risk of breast and ovarian cancer (AAP, 2005). Schwarz et al. (2009) examined dose-response relationships between the cumulative months a women breastfed and risk factors for cardiovascular disease (CVD). The study examined 139,681 postmenopausal women, who had at least one live birth and found that women who breastfed for more than 24 months in a lifetime were less likely to develop CVD (HR=0.66, 95% CI [0.50, 0.86] without adjusting for BMI; HR=0.68, 95% CI [0.52, 0.89]). Godfrey and Lawrence (2012) concluded from Schwarz et al. (2009) that mothers who have breastfed for more than 2 years in their lifetime have a 37% lower risk of developing coronary heart disease (CHD). Bentley-Lewis, Levkoff, Stuebe and Seely (2008) discussed the literature on duration of breastfeeding and that the longer a mother breastfeeds, her risk of developing type 2 diabetes is reduced if the mother didn't develop gestational diabetes. In a systematic review and dose-dependent meta-analysis performed by Aune, Norat, Romundstad and Vatten (2014) a 32% reduction in the relative risk of type 2 diabetes was found in mothers with the longest duration of breastfeeding as

compared those who did not breastfeed, RR=0.91, 95% CI [0.86, 0.96] (Aune et al., 2014).

Infant Benefits

Medical benefits breastfeeding provides to the infant that are strongly supported by research evidence include: decreased incidence of bacterial meningitis, bacteremia, diarrhea, respiratory tract infection, necrotizing enterocolitis, otitis media, urinary tract infections, and late onset sepsis in preterm infants (AAP, 2005).

The Infant Feeding Practices Study II included follow-up data collected at 6 years of age, N=1281 children, which was examined to investigate if there were associations between breastfeeding duration and feeding practices (Li et al., 2014a). The study examined the incidence of infections in the past year of children at 6 years of age. Ear, throat, and sinus infections and number of sick visits differed based on breastfeeding duration, exclusivity, and timing of breastfeeding with formula ($P<0.05$). Ear, throat, and sinus infections were lowest among children who were breastfed for longer than 9 months; exclusively breastfed for 6 months; or ever breastfed for more than 6 months without formula supplementation before 6 months of age. Breast milk exclusivity during first 6 months was only significantly associated with prevalence of ear, throat and sinus infections ($P<0.01$). Children who are breastfed for more than 9 months were significantly associated with a prevalence of sinus infections ($P<0.01$) (Li et al., 2014a).

Breastfeeding has also shown a decreased incidence of sudden infant death syndrome (SIDS) within the first year of life, insulin-dependent (type 1) diabetes mellitus, non-insulin dependent (type 2) diabetes mellitus, lymphoma, leukemia, Hodgkin disease, being overweight or obese, hypercholesterolemia, and asthma in older children

and adults (AAP, 2005). Children who are breastfed have also tended to perform slightly better on cognitive development tests such as the IQ test (AAP, 2005). A meta-analysis performed on 13 studies by the World Health Organization (2013) suggests that breastfeeding is associated with increased performance for IQ scores for children and adolescence by 3.5 points on average. When the analysis took into account the confounder of maternal IQ, they found breastfeeding to still be associated with an average increase of 2.19 IQ points (World Health Organization, 2013).

Growth Standards

Growth standards are a reflection of optimal growth in children suggesting that each child has the potential to reach the standard (Wang & Chen, 2012). The standard growth charts created by the Center of Disease Control (CDC) and World Health Organization (WHO) are the standard comparisons used to plot infant and child growth, having been developed using data collected from different countries around the world (De Onis, Garza, Onyango & Borghi, 2007). The CDC and WHO both developed new growth standards in May of 2000 and April of 2006, respectively. Both growth chart standards were created to replace the 1977 National Center for Health Statistics (NCHS) growth reference because the NCHS dataset suffered from multiple drawbacks that made it inappropriate for assessing the growth pattern of children (De Onis et al., 2007).

Concerns regarding the 1977 NCHS growth standards were raised because these standards relied heavily on the Fels data, which was not a nationally representative sample of infants. The Fels data came from a single longitudinal study that consisted mainly of white, middle-class infants in limited geographic areas of southwestern Ohio,

and was collected from 1929 to 1975. The children of the Fels study were observed at birth and 1 month, then at 3-month intervals from 3 to 12 months, and then at 6-month intervals from 12 to 36 months. The timing of these intervals has been considered one of the drawbacks in the Fels data because these intervals would be inadequate to properly identify growth patterns during periods of rapid change. The Fels data, which was used for the 1977 NCHS growth charts for weight and length, may not be a representative of the current growth patterns of combined breastfed and formula fed infants due to the data being based on relatively few infants who were breast-fed for more than a few months. Information from the Fels and NCHS data sets lead to inconsistent percentile estimates from the 1977 charts when the transition from recumbent length to stature takes place between 24 and 36 months of age. A few of the final concerns in these standards included the limited ability to assess size and growth at extremes beyond the 5th and 95th percentiles, the absence of weight-for-stature references, and the inability to assess ages 18 and over (De Onis et al., 2007).

Since the early 1990s, the WHO has used the WHO Child Growth Standards charts for assessing the growth and development of children birth to five years of age (De Onis et al., 2007). The WHO based their growth standards on data collected from the WHO Multicentre Growth Reference Study (MGRS) The MGRS is a population-based study that included population samples from Brazil, Ghana, India, Norway, Oman, and the United States collected between the years of 1997 to 2003. The MGRS was a longitudinal study that followed up on mother infant pairs with a total of 21 visits at weeks 1, 2, 4 and 6; monthly from 6 to 12 months; and bimonthly in the second year. There was also a cross-sectional component of the study that measured children aged 18

and 71 months of age. The study required multiple inclusion criteria such as no known health or environmental constraints to growth, mothers willing to follow MGRS feeding recommendations, no maternal smoking before or after delivery, single-term birth, and the absence of multiple morbidities. The MGRS feeding recommendations were for the mother to exclusively breastfeed for at least 4 months; introduction of complementary foods by 6 months; and continued breastfeeding to at least 12 months of age. Participants were required to have a minimum of 3 months of exclusive breastfeeding to be included in the study (De Onis et al., 2007).

In 2000, the CDC developed growth standards based on data collected from NHANES II and III. The pertinent variables from the NHANES II dataset are from 6 months of age and in NHANES III from 2 months of age. Due to the missing data at birth, the CDC 2000 growth charts used the United States Vital Statistics birth certificates. While the WHO charts are based solely on breast-fed infants, the current CDC charts, similar to the 1977 NCHS reference, includes relatively few infants who were breast-fed for more than a few months as part of the referent database (De Onis et al., 2007). Approximately half (54.7%) of the NHANES III sample initiated breastfeeding; only 21% exclusively breast-fed for 4 months, 9.8% partially breast-fed (i.e. supplemented with formula, other milk or solids) for >4 months, and 24% had been completely weaned from breastfeeding by 4 months of age.

The prevalence of breastfeeding was lower in earlier surveys; only 27.7% in NHANES I and II and 24.4% in PedNSS of infants had ever been breastfed. De Onis and Onyango (2003) found that there remained notable differences in the growth trajectory of healthy breast-fed infants compared to the new CDC growth charts. Breast-fed infants

grew faster than the CDC reference for weight-for-age in the first 2 months and less rapidly in 3 to 12 months of age (De Onis and Onyango, 2003). De Onis et al. (2007) determined that the difference between the CDC and WHO growth standards provided important implications for advice given to mothers concerning lactation performance and the introduction to complementary foods. De Onis and Onyango (2003) found that despite the drawbacks of the CDC growth charts to be no different than the WHO growth charts in evaluating the growth of breastfed infants. For healthy breastfed infants to be assessed, ideally new reference data is needed that includes infants exclusively breastfed until 6 months of age, then with the introduction of complementary food, and continued breastfeeding thereafter (De Onis and Onyango, 2003).

The adiposity rebound (AR) curve is included in the Center of Disease Control (CDC) Growth Charts (Kuczmarski et al., 2002). These charts are organized into two sets; first there are a set of charts for use from birth to 36 months, and then another set to be used for children and adolescents ages 2 to 20 years of age. The charts for infants look at sex-specific smoothed percentile curves for weight-for-age, recumbent length-for-age, head circumference-for-age, and weight recumbent length. The charts for children and adolescents focus on weight-for-age, stature-for-age, and BMI-for-age curves. The BMI-for-age is a tool that can be used by health professionals to determine whether a child is progressing along a normal trajectory or not (Kuczmarski et al., 2002). The typical AR progression occurs when the body mass index (BMI) rate increases throughout the first year of life, and after the first year of life the rate decreases until the child is 6 years of age when the rate increases again (Chivers et al., 2010). The curve increases in the first year as the child increases in adiposity, and then decreases as the child begins to get

leaner and taller. During the first year of life adipocyte cell size is increasing. When a child is about 6 years of age the child begins to have increase in adiposity again (Chivers et al., 2010). The rebound of the curve can occur between the ages of 4 and 6 (Gunther, Buyken and Kroke, 2006).

One area of active research is investigating whether an earlier rebound is associated with greater adiposity later in life. If transient obesity occurs in early childhood then it could be due to an increase in adipocyte cell size, and in turn persistent obesity could be due to an early adiposity rebound, which then could be associated with an early cell multiplication (Chivers et al., 2010). Understanding the timing of the adiposity rebound and any association with infant feeding practices is important in understanding how these may affect the adiposity rebound curve (Chivers et al., 2010). The hypothesis justification for an early adiposity rebound development of childhood obesity is related to the theory that there is a critical window in the early life phases of children, which may program them to be susceptible to adiposity later in life (Haurer, Brunner & Amann-Gassner, 2013).

Z-Scores versus Percentiles

The WHO and CDC growth standard charts both use anthropometric measures expressed as z-scores and percentiles to assess children's nutritional status and growth (Wang & Chen, 2012). These measures allow for healthcare personnel to determine whether the individual is under nourished (i.e. underweight, stunting, wasting) or over nourished (i.e. overweight, obese) or developing as expected. Z-scores represent the distribution of standard deviations from the mean, when the population is normally distributed. Percentiles are the percentage of observations (or population) that falls below

the value of a variable. The WHO recommends using z-scores in assessing children and percentiles in assessing adolescents. Z-scores are standardized measures recommended because they are based on the distribution of the reference population. A group of z-scores can be subject to summary statistics such as mean and SD, meaning they can be studied as continuous variables, and can quantify the growth status of children outside of percentile ranges. One downfall of z-scores is that they are complicated to explain and may limit the audience to a clinical setting (Wang & Chen, 2012).

Z-scores at times are called “Standard Scores” that are an application of transformation rules (Wang & Chen, 2012). Z-scores are calculated by dividing the difference between individual value (x) and the population mean (μ) by the population SD (σ).

$$Z = (x - \mu) / \sigma$$

The transformed z-scores will have a mean of zero and an SD of one. This process of quantifying z-scores is called the standardizing or normalizing process. The z-score is useful when doing comparisons of relative standards of different measures, such as height versus weight, from distributions with different means and/or SDs.

There is increasing evidence that weight-for-length z-scores should be used up until the child is 2 years old, and then switching to the use of sex-age-specific BMI percentiles as cut-offs for ages 25 months till 18 years of age for assessing overweight and obesity as well as thinness/ underweight (Wang & Chen, 2012). The cut-off points for the sex-age-specific BMI curves that pass through a BMI of 25 for overweight and 30 for obese, respectively, at the age of 18 years old making the classification more biologically meaningful compared to percentiles and z-scores based on distribution. One limitation to

the use of percentiles is the use of the same interval for percentile values corresponds with different ranges to changes in absolute values for different measurements. An example of this limitation is the increments of the percentiles (i.e. 85th to 90th percentile) corresponds to different ranges in sub scapular and in triceps skin fold thickness meaning the same increments at different percentile levels can respond to different changes in both the z-scores and percentiles. This is why it is suggested to use z-scores to assess over time as opposed to percentiles (Wang & Chen, 2012).

Relationship between Infant Feeding and Adipose Development

The first traces of adipose tissue have been detected in the 14th and 16th week of gestation, which slowly form into fat lobules (Hauner et al., 2013). When an infant is born, the numbers of fat lobules remain constant while fat lobules themselves continuously grow in size (Hauner et al., 2013). The infant's body fat accounts for approximately 14% of their total body mass (Hauner et al., 2013). *In vitro* studies have also shown that human adipose tissue stromal adipocyte precursor cells tend to display the highest proliferation and differentiation capacity during the first year of life and again at pre-puberty (Hauner et al., 2013). This supports the concept that there are specifically sensitive periods of adipose tissue growth early in life (Hauner et al., 2013).

Adipose tissue develops as a result of excess energy in comparison to the energy needs of the body. Adipose tissue is composed of adipocytes, an extracellular matrix (ECM), vascular and neural tissues, and other cell types (Kalupahana, Claycombe and Moustaid-Moussa, 2011). The other cells that are in adipose tissue include preadipocytes, fibroblasts, stem cells, and immune cells such as macrophages and T lymphocytes. Adipose tissue also secretes multiple bioactive peptides known as adipokines. Adipokines

are cytokines, which include hormones that are involved in energy and glucose homeostasis. Adipose tissue is considered to be an endocrine organ that plays a major role in energy balance, glucose homeostasis, blood pressure regulation, and immune function (Kalupahana et al., 2013).

Adipocytes become overloaded when there is excess triglyceride causing there to be positive energy balance that leads to adipocyte hypertrophy and a dysregulation of adipokine secretory patterns (Kalupahana et al., 2013). Adipocytes are a source of pro-inflammatory cytokines in obesity, but the stromal vascular fraction (i.e. preadipocytes, macrophages and adipose stem cells) produces even higher levels of cytokines. Cytokines are important when understanding the complications of obesity because they contribute to the onset of inflammation. As a result obesity is typically associated with a chronic low-grade inflammation. Many individuals who appear with metabolic syndrome frequently exhibit pro-inflammatory profiles putting them at an increased risk for developing type II diabetes and cardiovascular disease. It is known that the long-chain polyunsaturated fatty acids (PUFAs), mainly omega-3s EPA and DHA, have anti-inflammatory properties (Kalupahana et al., 2013). There is a significant amount of evidence to prove that breast-milk provides a beneficial amount of n-3 fatty acids (Kalupahana et al., 2013).

Exclusive breastfeeding is in fact associated with a lower BMI later in life, but the exact mechanisms preventing obesity through breastfeeding are unknown. Current thinking is that researchers and healthcare professionals may find it more effective to prevent obesity before the onset (Kalupahana et al., 2013).

Adults who were obese as children can have difficulties losing weight as they have more fat cells compared to lean body components (Efrat, Tepper & Birk, 2013).

Children with higher BMIs are more likely to become obese as adults by a two- to four-fold rate compared to children of a lower BMI, potentially due to the number of adipocytes being determined at a young age. Obese mothers are approximately four times more likely to have obese children with the child's risk increasing ten-fold if the father is obese as well. The risk is mainly driven by excess childhood weight gain, which is partly driven by genetics and behavioral factors as opposed to increased adiposity at birth. The adiposity rebound (AR) has the ability to predict later adiposity due to it reflecting a higher BMI prior to and during the rebound period (Efrat et al., 2013; Gonzalez et al., 2014; Koyama et al., 2014).

Understanding the development of adiposity is essential in trying to determine the root of obesity. There have been multiple mechanisms proposed that are suggested to contribute to the development of fat cells and therefore are potential causes of obesity later in life.

Potential Mechanisms Relating Adipose to Infant Feeding Practices

Researchers propose that early nutrition in infancy potentially 'programs' the individual's health for later life and that breastfeeding's role in early nutrition may be a key to longer-term outcomes. Several mechanisms have been proposed that attempt to explain how the body may become programmed early in life in ways that may determine an infant's risk for developing obesity, which can then lead to multiple related diseases and disorders later in life.

High Early Protein Intake Hypothesis

The early protein intake hypothesis proposes that protein stimulates insulin secretion resulting in adipocyte multiplication and fat deposition (Gunther, Buyken & Kroke, 2006). The combination of protein and carbohydrates are known to cause a synergistic stimulating effect on plasma insulin concentration levels (van Loon et al., 2000). In the presence of certain amino acid combinations, such as arginine-leucine, arginine-phenylalanine and arginine-glutamine, with glucose can result in some of the largest increases in plasma insulin (van Loon et al., 2000).

Human milk has lower protein content than most types of formula, which is approximately 1.4-1.8 times higher (Gunther et al., 2006). When infants are typically weaned from breastfeeding and started on formula they have an increase in protein intake that results in a two to four times higher intake above the estimated energy requirements; a finding that has led to the proposal of the high early protein intake hypothesis. Protein influences the secretion of insulin therefore contributing to higher insulin-like growth factor-1 (IGF-1) levels and suppressing levels of growth hormone (GH). Both IGF-1 and GH have an influence on growth and adverse effects on adiposity development (Gunther et al., 2006). IGF-1 plays a central role in the regulation of fetal growth and metabolism (Joslowskiet al., 2013). The GH-IGF-1 axis plays an important role in cell proliferation and apoptosis. The bioavailability of IGF-1 is determined by the amount of IGF binding protein (IGFBP) present (Joslowski et al., 2013).

The Dortmund Nutritional and Anthropometric Longitudinally Designed (DONALD) Study was a detailed data set containing information on growth, development, nutrition and metabolism of healthy children (Gunther et al., 2006).

Approximately 35-40 new infants entered the study each year and were first examined at the age of 3 (Gunther et al., 2013). The participant was seen for 4 visits in the first year, 2 in the second year and once annually into adulthood thereafter. In continuation study of multiple studies that use DONALD data examined 233 adults (125 females and 108 males) that ranged from 18-37 years of age that had data on breastfeeding during infancy and fasting blood levels for years 2004 to 2012. They investigated the association of breastfeeding with growth hormone (GH), Insulin Growth Like Factor (IGF) axis, insulin sensitivity, body composition and body fat distribution in younger adults 18 to 37 years of age. Gunther et al. (2013), prolonged breastfeeding was associated with a lower mean fat mass index (FMI) ($p<0.001$), fat-free mass index (FFMI) ($p<0.003$) and waist circumference (WC) ($p<0.001$), and the effect persisted after taking into account confounders.

Joslowski et al. (2013) also used data from the ongoing DONALD study to examine whether animal protein intake in early life, or around the time of the adiposity rebound, were inversely related. The study found no association between animal protein intake, around the timing of the adiposity rebound, and IGF-1 levels within the young adulthood group for males and females. The study did find an association between a higher habitual protein intake in early life with lower concentrations of IGF-1 in young adulthood with males ($P<0.03$), but not with females ($P=0.9$). The authors suggest that a higher animal protein intake during early life may be related to a long-term down regulation of the GH-IGF-1 axis (Joslowski et al., 2013).

Weber et al. (2014) took a different approach by doing a double-blind randomized control trial looking at the BMI differences between a low protein formula and a high

protein formula that infants began receiving at birth. The study was a part of the Childhood Obesity Project that included children from Europe for the years 2002 to 2004. Formula-fed infants were randomly assigned to either the high or low protein formula. Breast-fed infants were enrolled in the study for an observational reference group. Children were followed up at 2, 2.5, and 6 years of age to determine energy intake and body composition measurements. Weber et al. (2014) found that BMI in the higher protein group was higher for 3 to 12 months of age, which BMI attenuated from 12 to 24 months and became stable from 24 to 36 months of age. At approximately 42 months of age the BMI in the higher protein group deviated higher towards the upper tails of the BMI distribution. The high protein formula group received on average approximately 6 to 8 g/d of protein during the first year of life. By 6 years of age, the high protein group had a BMI of 0.51kg.cm² higher than the lower protein group. There were no significant differences found between the breastfed group versus the low protein group, but weight and BMI were significantly higher in the high protein versus breastfed group. Weber et al. (2014) suggests that having a lower protein formula would be more closely related to the protein content in breast milk and could help reduce the chances of childhood obesity.

Growth Acceleration Hypothesis

The growth acceleration hypothesis proposes that a fast post-natal growth (i.e. switching from a lower to a higher percentile) programs the infant's health for later life (Singhal & Lanigan, 2007). Once the body is programmed then it puts the body at risk for developing several components related to metabolic syndrome including insulin resistance, higher low-density lipoprotein (LDL) cholesterol levels, and higher blood

pressure. Acceleration of both weight and length in the first 2 weeks of life has been associated with endothelial dysfunction, which is consistent with this hypothesis (Singhal & Lanigan, 2007). Rapid growth during infancy and early childhood can put a child at risk for an early adiposity rebound, intersecting two growth patterns that are strongly associated with the development for adult obesity (Brisbois, Farmer & McCargar, 2012). These two growth patterns have been suggested to accelerate fat cell growth in childhood that persists into adulthood. Researchers have proposed that fat cell growth in early childhood may explain a critical period influencing future body composition and fat deposition. The growth acceleration hypothesis is also referred to as rapid growth, increased growth trajectory, increased growth velocity and earlier BMI rebound or adiposity rebound (Brisbois et al., 2012).

Singal and Lanigan (2007) suggested that an earlier age at AR could be a key factor in determining later adiposity because of the ability of AR to identify children whose BMI percentile is high and/or crossing upwards. The timing of AR allows health care professionals to detect if there is a fast rapid growth for age (Singhal & Lanigan, 2007).

In a review performed by Brisbois, Farmer and McCargar (2012) 16 studies were reviewed that reported on growth periods that were faster at an earlier age than normal. Fifteen of the 16 studies found an association between a rapid period of growth in early childhood and adult obesity. The studies that assessed change in BMI found that rapid change in BMI between the time frames of age of 0 to 8 days; 0 to 4 months; 0 to 5 months; 0 to 1 year; and 0 to 2 years; 2 to 5 years; and 3 to 7 years were all associated with increased adult overweight and obesity. The studies included in the analysis

followed up into adulthood between the ages of 20 to 35 years of age. In the studies reviewed, those that focused on early adiposity rebound (i.e. AR occurring at less than 5 years of age) found there to be an increased risk for development of adult overweight or obesity (Brisbois et al., 2012). In each of the 16 individual studies to determine the association of rapid growth during infancy and early adiposity rebound, all detected a significant impact (p-value of 0.05 or less). Therefore understanding what factors influence the timing of the AR are important to target in addressing the onset of obesity.

Feeding Practices

One of the known benefits of breastfeeding is that it lowers children's risk of developing obesity later in life but the exact mechanism is still unknown (Bergmann et al., 2003). Bergmann et al. (2003) investigated the relationship between breastfeeding and adiposity, by observing the anthropometrics of two different groups defined by feeding status. The first group was classified as 'bottle-fed' (BO) if they were bottle-fed from birth or breastfed less than 3 months. The second group was called the 'breast-fed' (BR) group if the infant was breastfed longer than 3 months. The study found that by 3 months of age the BO group had significantly higher BMIs and thicker skin folds than the BR group. The BO group compared to the BR group, had a consistently higher in proportion exceeding the 90th and 95th percentile for BMI and for skin-fold values from the age of 3 months and moving forward. The prevalence of obesity nearly doubled between BO kids aged 4 to 5 years old, and tripled for kids who were 6 years old. In the BO group AR occurs sooner compared to the BR group (Bergmann et al., 2003). See Figure 1-3.

Figure 1-3. Left: Proportion (%) of children exceeding the 90th percentile of the BMI reference values, depending on feeding mode in infancy. Right: Proportion (%) of children exceeding the 97th percentile of the BMI reference values, depending on feeding mode in infancy. ** $P < 0.01$; *** $P < 0.001$ (From Bergmann et al., 2003).

Bergmann et al. (2003) suggested that breastfed infants are able to regulate their food intake better compared to infants who are bottle-fed because bottle-fed infants tend to empty the bottle due to the encouragement to do so. When infants are breast-fed, they control the milk production of their mother based on their satiety. When infants are encouraged to finish the bottle continuously it causes the infant to not develop control over their intake compared to breast-fed infants. Bottle-fed infants are typically consuming solely formula, which is also more concentrated in energy and nutrients (Bergmann et al., 2003). Bergmann et al. (2003) findings support those of Gunther et al. (2006) that a higher early habitual protein intake is associated with an earlier AR.

Li et al. (2012) investigated the mechanisms relating breastfeeding and childhood obesity by assessing the association of weight gain with the mode of milk delivery separately from the type of milk given. The study used the Infant Feeding Practices Study II data set, which is a longitudinal study examining new mothers and their infants. The data were collected from May 2005 to June 2007. Women were recruited in their third

trimester from a consumer opinion panel that was sent to approximately 500,000 homes in the United States. Weight measurements were reported on the 3, 5, 7 and 12-month surveys. The surveys asked the infants weight and other measurements and the date of their last doctor visit. The study then calculated the z-scores based off of the child's weight and length measurements. Mothers completed a postpartum survey at 1, 2, 3, 4, 5, 6, 7, 9, 10, and 12 months of age, and responded to questions regarding the 3 main exposures: type of milk; percentage of milk feedings given at breast; and percentage of milk feedings by the bottle. The sample consisted of 1899 mothers who completed all of their surveys. A multilevel analysis was performed to assess weight gain and any association with feeding mode. Li et al. (2012) found that infants fed by the bottle only gained 71 or 89g more per month when fed non-human milk only ($P<0.001$) or human milk only ($P=0.02$) compared to infants fed only by the breast. Infants gained approximately 37g per month when they were fed both expressed breast milk and non-human milk ($P=0.08$). Infants fed at the breast and by bottles gained similar to infants fed only at the breast. Infants fed at the breast and by bottle of nonhuman milk gained approximately 45g more per month ($P<0.001$). Infants fed human milk by bottle only and nonhuman milk by bottle only gained more weight than infants fed by breast only. There were no bottle effects seen in infants consuming human and nonhuman milk by the bottle. Li et al. (2012), suggests that it may be due to those infants (i.e. human and nonhuman milk by bottle group) being fed at the breast previously developing better self-regulation of milk intake, which may be carried over when transitioning from the breast to a bottle as well as mothers better recognizing their infants hunger and satiety cues. Li

et al. (2012b) suggests that infant weight gain may not solely be associated with type of milk, but also related to mode of milk delivery.

Researchers have recognized the importance of breastfeeding and the influence of BF on decreased incidence of obesity later in life. Bergmann et al. (2003) shows that infants who are breast-fed longer than 3 months tend to have a later AR compared to infants who are generally bottle-fed or breast-fed less than 3 months. The idea of exclusive breastfeeding for 6 months being protective against obesity seems to be logical when taking into consideration the results of Bergmann et al. (2003). When further looking into the research it also appears logical that a higher habitual protein intake early in life is associated with an earlier development of adiposity and an earlier shift in the AR. Aside from the inconclusive research on determining if the timing of the AR has an effect on obesity later in life, the recommendations to exclusively breastfeed for at least 6 months still remains based on the finding that infants who are breastfed tend to remain in the normal BMI range throughout life.

Breast Milk Energy Balance Regulation

Additional factors that are currently being researched to contribute to obesity are the levels of satiety and hunger regulating hormones that have been found to be present in breast milk. Studies on the composition of breast milk and obesity have discovered the presence of adipokines, leptin and adiponectin, as well as hormones including, insulin-like-growth-factor-1 (IGF-1), gherlin, obestatin and resistin (Savino, Liguori, Fissore and Oggero, 2009). Research is limited on the availability and the exact function of each of these hormones through the transmission of breast milk. Researchers have based their hypotheses on what is currently known about the function these hormones in the human

body and predict that the levels found in breast milk may contribute to the infants hunger and satiety hormones.

Leptin is an adipocyte-derived hormone that reduces appetite and increases energy expenditure (Savino et al., 2009). Circulating leptin levels within children and adults directly correlate with their fat mass. Leptin levels are detectable from cord blood beginning in the second trimester of pregnancy and have been shown to correlate with adiposity at birth (Savino et al., 2009). The amount of circulating leptin increases dramatically after 34 weeks, contributing to the rapid accumulation of fat mass before delivery (Savino, Liguori and Lupica, 2010). Leptin is present in breast milk being produced and excreted by mammary epithelial cells in milk fat lobules with the secretory epithelial cells transferring leptin from the blood to the milk (Savino et al., 2009). Leptin has been reported to be in breast milk concentration means ranging from 0.2 to 73.22 ng/mL (Savino et al., 2011). Savino et al. (2009) suggests that leptin could pass from breast milk to infant blood and play a role in the short-term regulation of feeding by acting on satiety signals and then could also exert a long-term effect. Savino et al. (2006) evaluated the relationship between serum leptin concentrations in infants and infant maternal BMI. The study consisted of 51 exclusively breastfed infants for at least 4 months and 24 formula-fed infants. The study found there to be a significant positive correlation between infant serum leptin concentration and maternal BMI was observed in breastfed infants ($r = 0.389$, $p = 0.005$) and after adjustment for infant age and infant BMI the relationship remained significant ($\beta = 0.065$, $p = 0.006$). The same relationship did not hold true for formula fed infants. The study concluded that serum leptin concentrations in breast fed infants correlated positively with maternal BMI. Savino et al.

(2006) suggests that a higher maternal BMI might increase leptin levels in breast milk showing that maternal adiposity is involved in infant energy balance.

Adiponectin is also derived from adipose tissue and are seen to be reduced in obesity (Mantzoros et al., 2009). Adiponectin levels are inversely associated with adiposity and positively correlated with insulin sensitivity (Savino, Liguori and Lupica, 2010). Adiponectin has been reported to be present in plasma concentrations ranging from 0.5 to 30 $\mu\text{g/mL}$, which is 1000-fold higher than the concentrations of other hormones (i.e. insulin and leptin) (Savino et al., 2011). In a study performed by Weyermann, Brenner and Rothenbacher (2007) the role of adiponectin and leptin in human milk in overweight children at the age of 2 was assessed. The study examined 674 children, whose mothers breastfed at least 6 weeks postpartum, provided milk samples and attended the two year follow-up. Forty-five percent of the infants were breastfed for 6 months. The study found there to be higher levels of adiponectin in breast milk to be associated with being overweight at two years of age in infants who were breast-fed at least 6 months (Weyermann, Brenner and Rothenbacher, 2007).

In addition to adiponectin and leptin, gherlin also plays a role in appetite regulation by stimulating appetite, decreasing fat utilization and increasing adipose stores (Savino et al., 2009). Gherlin is present in both preterm and term breast milk (Savino et al., 2011). Gherlin has been reported to be in lower concentrations in colostrum ($70.3 \pm 18 \text{ pg/mL}$) and transitional milk ($83.8 \pm 18 \text{ pg/mL}$) compared to mature milk ($97 \pm 13 \text{ pg/mL}$) (Aydin, Aydin, Ozkan and Kumru, 2006).

The research still remains inconclusive but Savino et al. (2009) suggest that breast milk hormones playing a role in appetite may contribute to the causal relationship

between breastfeeding and obesity. The study points out that observationally from the behavioral stand point of breastfeeding that breastfed infants are able to self-control the amount of milk they consume, however it remains unclear that the relationship persists into adulthood. In addition, the study suggests that breastmilk's unique composition may modulate neuroendocrine pathways involved in the regulation of bodyweight (Savino et al., 2009).

Breastfeeding and Adiposity Development

Brisbois, Farmer and McCargar (2012) wrote a systematic review that focused on evaluating factors in early childhood (<5 years of age) that are significant predictors in the development of obesity in adulthood. The systematic review focused on multiple markers from the prenatal period, infancy, and early childhood. Factors included breastfeeding related variables, socio-economic status (SES), and birthplace. The review found that research reporting on breastfeeding findings displayed a variety of findings. A number of studies looked at duration of breastfeeding and high adult BMI with a yes or no association. Out of 7 studies reviewed there was no association between breastfeeding duration and obesity outcomes. Of the 7 studies, two found a protective effect of breastfeeding; babies breastfed for more than 1 month had a decreased prevalence of adult obesity ($P < 0.05$) (both males and females) compared to those breastfed for less than 1 month or not breast fed at all. The second study of the two found that babies who breastfed longer (3-5 months duration) compared to babies who breastfed less than a month had a decreased risk of adult obesity (Brisbois et al., 2012).

A study performed by Gillman et al. (2001) examined the extent at which overweight status among adolescents was associated with the infant feeding patterns (i.e. breast milk or formula) and duration of breastfeeding. The study examined a total of 15,341 participants with 8,186 females and 7,155 males aged 9 to 14 years of age who were participants in the Growing Up Today Study – a nationwide cohort of diet, activity and growth. For the study, they mailed a questionnaire in 1996 to each subject, and in 1997 mailed a supplemental questionnaire to their mothers. The mother's questionnaire contained information regarding the infant feeding status of the infant categorizing it by 0 months, less than 1 month, 1 to 3 months, 4 to 6 months, 7 to 9 months, and greater than 9 months. The questionnaire also questions regarding timing of introduction to solid foods, cow's milk, and infant formula including type of formula. The study found adolescents who were mostly or only breastfed vs. mostly or only fed infant formula in the first 6 months to be associated with approximately a 22% lower risk of being overweight, OR in fully adjusted model, 0.78; 95% CI [0.66, 0.91].

Carling et al. (2015) tested the hypothesis that children with a rising weight-for-length (WFL) z-score and breastfed for a short duration were at a higher risk for obesity. The study recruited 595 women from an obstetric patient population in rural central New York State. Weight gain trajectories for infants' WFL z-scores from 0 to 24 months were identified using maximum likelihood latent class models. The study used a logistic regression to investigate whether there was an association between breastfeeding duration (< 2 months, 2-4 months and > 4 months) and weight gain trajectory across obesity risk groups. The study found that children with rising trajectories were more likely to have mothers who were obese (57.9% vs. 42.1%; $P=0.03$), had less than a high school

education (56.9% vs. 43.1%; $P=0.01$), and had smoked during pregnancy (58.5% vs. 41.5%; $P=0.03$) when compared to children with stable growth trajectories. The study assigned obesity risk scores based on the number of significant risk factors to which they had been exposed. The risk scores were categorized as low (0 risk factors; $n=128$, 29.2% of sample), medium (1 risk factor; $n=181$, 41.3%), or high (2 or 3 risk factors; $n=129$, 29.5%). The study found that children who were at high risk for obesity who were also breastfed <2 months of age were 2.55 times more likely to belong to a rising growth trajectory than high risk children that were breastfed >4 months of age ($P=0.02$). The study found that children with rising trajectories were more likely to have mothers who were obese (57.9% vs. 42.1%; $P=0.03$), had less than a high school education (56.9% vs. 43.1%; $P=0.01$), and had smoked during pregnancy (58.5% vs. 41.5%; $P=0.03$) when compared to children with stable growth trajectories. The study showed that infants who had high levels of obesity risk and were breastfed for shorter durations were more likely to be in a rising weight gain trajectory compared to infants' breastfed for a longer duration (Carling et al., 2015).

Breastfeeding, Race/Ethnicity and Childhood Obesity

Approximately 25% of children in the United States between the ages of 2 and 5 years old are overweight (Guerrero et al., 2015). Young Latino children appear to be disproportionately affected by overweight and obesity. A study done by Guerrero et al. (2015) focused on describing the growth trajectories in BMI among the major racial and ethnic groups of U.S. children. In addition, this study looked at predictors of children's BMI trajectories. Guerrero et al. (2012) used data from the Early Childhood Longitudinal

Study-Birth Cohort (ECLS-B) that collected data from birth certificates as well as from home visits made at 9, 24, 48, 60 and 72 months of child's age (N= 15,418). The study contained 3312 White, 1208 African American, 1450 Latino, 798 Asian, and 785 of other race/ethnicities for females. The study also contained 3354 white, 1211 African-American, 1552 Latino, 934 Asian, and 814 other race/ethnicities for males. The home visits consisted of in-depth interviews and direct child assessments of physical, cognitive and social-emotional development. Guerrero et al. (2015) focused on the influence of maternal and home practices on children's early development. The study used a hierarchical linear model (HLM) to fit a variety of mixed linear models to data to examine various statistical influences in the data using different covariance structures.

As shown in Figure 1-4, Spanish speaking Latinos started with a higher BMI raw score trajectory at 4 years of age while maintaining a higher BMI over time. African-American and Latino children maintained high BMI trajectories from an early age as compared to white children. The study found there to be several factors that were significantly associated with a lower BMI trajectory including low birth weight ($P<0.01$), higher maternal education ($P<0.01$), residing in a two-parent household ($P<0.05$), and breastfeeding during infancy ($P<0.01$). The study found all races/ethnicities to be associated with the BMI trajectory level ($P<0.01$) (Guerrero et al., 2015). Guerrero et al. (2015), in their discussion suggest that childhood weight disparities in Latinos compared Whites could be attributed to low maternal education, higher rates of maternal depression, early introduction to solid foods, restrictive feeding practices, physical inactivity, and beverage and fast food consumption patterns.

Figure 1-4. Observed BMI raw score mean growth curve (From Guerrero et al., 2015).

Burdette and Whitaker (2007) tested the hypothesis that the relationship between breastfeeding and later obesity would differ by race/ethnicity. The data for the study were obtained from the Fragile Families and Child Wellbeing Study, which is a birth cohort study that followed children who were born between 1998 and 2000, from 20 large cities within 15 states. For the study, mothers were surveyed at time of delivery and again one year following their delivery to obtain information regarding their delivery. At approximately three years after delivery, information about the children and their mother's height and weights were obtained at their homes. The study found the relationship of breastfeeding and the prevalence of obesity to be significantly different between White (N= 419), Black (N= 1,182), and Hispanic (N= 545) children (likelihood ratio statistic = 8.24, df = 2, P=0.02). These effects remained when breastfeeding was

used as a binary variable (ever/never, $P=0.01$) and as a continuous variable (months, $P=0.03$). Prevalence of obesity was lower in Hispanic children who were ever breastfed compared to those who were never breastfed (23.3% vs. 33.0%, $P=0.01$). The study found there to be a differing relationship between breastfeeding and obesity based on race/ethnicity (Burdette and Whitaker, 2007).

Breastfeeding, Pre-pregnancy BMI, and Childhood Obesity

A systematic review that evaluated early markers of adult obesity focused on evidence of markers that would have occurred during early-life experiences – in utero and post-natal. The goal was to identify which influences might create permanent changes in physiologic function and program the long-term regulation of energy balance (Brisbois, Farmer & McCargar, 2012). In 135 studies reviewed the authors found 14 separate perinatal variables predictive of adult obesity. These predictors included maternal smoking, maternal diabetes, maternal weight gain, maternal weight, maternal height, maternal BMI, pre-eclampsia, maternal dichlorodiphenyldichloroethylene (DDE) and polychlorinated biphenyl (PCB) levels, and or exposure to famine. The study also found paternal diabetes and BMI to contribute to offspring adult obesity as well. The top three markers that impacted the risk of adult obesity were maternal pre-pregnancy BMI, smoking and weight gain during pregnancy. Maternal BMI was a predictor of adult obesity in 17 of the studies reviewed. The weight status of the mother was most likely a combination of multiple factors including genetic, biological, environmental, and behavioral (Brisbois et al., 2012).

Li et al. (2005) performed a study using 2036 children from the 1996 National Longitudinal Survey of Youth, Child and Young Adult data to examine the interactions of maternal pre-pregnancy BMI and breastfeeding on childhood overweight risk. The National Longitudinal Survey of Youth, Child and Young Adult began in 1986 with all children of mothers involved in the survey. Data was collected on physical and behavioral development, and health status. Data was collected biennially through the year 2000. The study found that the prevalence of overweight was lower among children who were ever breast-fed compared to never breastfed at each level of pre-pregnancy BMI as shown in Figure 1-5. The study further examined the additive interactions between maternal pre-pregnancy BMI and breast-feeding on childhood overweight prevalence. The study found that a higher pre-pregnancy BMI was associated with an increased risk of childhood overweight ($P < 0.001$). Li et al. (2005) found that a longer duration of breastfeeding was associated with a reduced risk of childhood overweight ($P = 0.02$). The Wald F test showed there to be an additive interaction between maternal pre-pregnancy BMI and lack of breastfeeding on childhood overweight risk (Wald F , 3.1; df, 4; $p < 0.05$) concluding that children whose mothers were obese before pregnancy and who were never breastfed had a 6-fold increased risk of being overweight during childhood compared with children of mothers with a normal pre-pregnancy BMI and who were breastfed for at least 4 months (Li et al., 2005).

Figure 1-5. Prevalence of childhood overweight (BMI $\geq$ 95th percentile) by maternal pre-pregnancy BMI and breastfeeding for the sample (A: 2 to 14 years of age) and by three age groups (B: 2 to 6 years of age; C: 7 to 10 years of age; D: 11 to 14 years of age) (From Li et al., 2005).

Pre-pregnancy BMI, Hispanic and Obesity

Childhood obesity is influenced by multiple different factors. Pre-pregnancy BMI is one of the factors that are associated with the development of obesity. According to the CDC, an individual is considered to be overweight if they have a BMI of 25 to 29.9 and obese if they have a BMI greater than 30.

A study done by Whitaker (2004) examined whether children whose mothers were obese in early pregnancy were more likely to be obese at 2 to 4 years of age. The study used data for 8494 children who were enrolled in the Women, Infant and Children

(WIC) nutrition program in Ohio. Children were followed from the first trimester of pregnancy until 24 to 59 months of age. The study found evidence that maternal pre-pregnancy BMI rates differ significantly ($P<0.001$) by maternal race/ethnicity (29.4% in Whites, 35.5% in Blacks, and 23.7% in Hispanics). Obesity during the preschool years was found to be strongly associated with the maternal pre-pregnancy BMI ($P<0.001$). Children whose mothers were obese in the first trimester of pregnancy had a prevalence of obesity at ages 2, 3 and 4 years that was 15.1%, 20.6% and 24.1%, respectively. The prevalence rates were 2.4 to 2.7 times the prevalence of obesity among children who were born to mothers of normal pre-pregnancy BMI (Whitaker, 2004).

Purpose of This Study

The objective of this study is to determine whether or not exclusive breastfeeding (EBF) from 0 to 3 months and EBF from 0 to 6 months of age significantly influences the infants' growth trajectory through 60 months of age in a WIC population sample from San Luis Obispo and Santa Barbara counties in California. The secondary objective of this study was to determine if other variables, specifically Hispanic ethnicity or maternal pre-pregnancy BMI influence the effect of EBF in 0-3 and 0-6 month infant growth trajectory through 60 months of age.

Hypotheses

The hypothesis being studied was that EBF has a protective influence on children's BMI at 60 months of age and that additional effects would be seen with additional duration of breastfeeding, i.e. that effects would be stronger in children EBF

from 0-6 than in those EBF 0-3 months. It was anticipated that the protective effects of exclusively breastfeeding would continue to be seen even when controlling for related variables such as pre-pregnancy BMI and race/ethnicity.

CHAPTER 2: METHODS

Purpose

The purpose of this study is to determine the influence of exclusive breastfeeding on BMI at 60 months of age in a diverse set of mothers and infants who were enrolled in the Special Supplemental Nutrition Program for Women, Infants, and Children (WIC) in the central coast of California during the years 2005 to 2009. This study is intended to provide insight into early prevention practices targeting the growing epidemic of childhood obesity. The study also explored factors such as mother pre-pregnancy body mass index, ethnicity, and income that may moderate the impact of exclusive breast feeding on the development of obesity beginning in infancy.

Subjects

Mother/child pairs enrolled in WIC from years 2005 to 2009 were included in the data analysis. Data was obtained from the pairs within Santa Barbara and San Luis Obispo Counties at their regular WIC visits. All WIC clinics in the Santa Barbara and San Luis Obispo County were included. All data was collected according to WIC protocols. The data set contained a total of 60,190 mother/child pairs. WIC visits occurred at diverse time points between birth and up until 60 months of child's age. Data collected at these clinic visits were grouped in 3-month periods to create the analysis variables. For example, any weight and length data collected between birth and 3 months of age were grouped into the first 3-month period. A mother/child pair was not required to be present at all-time points in order to be included in the data analysis. For more information on the WIC data set reference Appendix F & G.

Of the children 50.6% were male and 49.4% were female. 72.04% of the subjects were Hispanic, and 27.96% were all other races/ethnicities. (See Appendix F for breakdown of other races/ethnicities.) The race/ethnicity variable was only composed of mother/infant pairs who indicated their race/ethnicity at their initial visit. A breastfeeding variable was constructed based on the food package that the mother picked up at the time of the visit. WIC gives each mother a food package at her visit based on her feeding status. When developing the breastfeeding variable it was assumed a mother was EBF or not EBF based on the food package that she picked up. Each of the mothers was not directly asked if she EBF or not at the WIC visit.

There were 21,712 infants that had data on their feeding status from 0-3 months of age and 10,551 of those infants were exclusively breastfed for 0-3 months of age (See Table 2.1.). There were 21,656 infants for whom data was collected on how they were they were fed from 0-6 months of age, of those infants 3,860 were exclusively breastfed until 0-6 months of age. There was data for 33,192 infant birth weights (Table 3.1). Differences in the number of measurements were due to the number of responses of the mothers with information regarding their infants. Table 2.1 summarizes the demographics of the original mother/child data set.

Table 2-1. Maternal and Offspring Characteristics for WIC data set (N=60,190).

Maternal Characteristics	N	
Mean Maternal pre-pregnancy BMI*	44,057	27.12 (SD $\pm$ 5.98)
Maternal pre-pregnancy BMI groups	44,057	Percent
<18.5		(%)
18.5-24.9		2.3
24.9-29.9		39.7
30.0+		31.2
Race/ethnicity		26.8
Hispanic		72.04
Other		27.96
Offspring Characteristics	N	Percent
Sex	60,190	(%)
Male		50.6
Female		49.4
Birth weight, kg	33,192	3.36
Feeding in 0-3 months of life	21,712	
EBF	10,551	47.31
BF + Formula		24.91
Formula Only		27.78
Feeding in 0-6 months of life	21,656	
EBF	3,860	22.22
BF + Formula		20.66
Formula Only		19.25
Other, any		33.0

*kg/m2

Statistical Analyses

The dataset consisted of pre-existing WIC data collected for programmatic purposes. Based on the preexisting data set for coding purposes if a mother left the WIC program and then returned, her child would receive a new participant ID, but the mother would use her previous WIC ID number. Researchers went through the list to ensure that each child was only in the dataset once. To alleviate potential duplicates the following

conditions were satisfied for each subject: child's birth date, family ID, and gender, then researchers concluded that the each unique family ID belonged to one child. Weight and length measurements were grouped into 3-month periods beginning with 0 months of age up through to 60 months of age creating a total of 20 3-month intervals used in the analyses. Maternal pre-pregnancy BMI (N=44,057) was recorded during the initial WIC visit. Researchers then further coded the rest of the dataset including income, number of people per household, ethnicity and maternal education.

The WIC dataset variables were examined for outliers and for normal distribution. Pre-pregnancy BMI had 3 BMI observations that had extremely low and implausible BMI scores (i.e. 10.2-11kg/m²). Given that these observations were input by hand it is likely that these BMI values were mistakes. These 3 observations were excluded from the analysis.

Variables were created to define the exclusive breastfeeding categories. The categories of interest were data to define exclusive breastfeeding from 0 to 3 months (EBF 0-3), exclusive breastfeeding 0 to 6 months (EBF 0-6) and no exclusive breastfeeding (No EBF). A child who was exclusively breastfed up to 6 months would appear in both the EBF 0-3 and the EBF 0-6 analyses. There were a small portion of observations that did not exclusively breastfeed for 0 to 3 months, but did for 3 to 6 months (N=192). These observations were excluded from the analysis as the observations would be incorrectly categorized if they were forced into the EBF 0-6 months or No EBF groups.

Lastly, the data was checked to ensure that none of the variables were too heavily skewed and to determine if any transformation was needed. Income was heavily skewed

to the right, lower income. It was determined that after looking at histograms, fitted normal lines and diagnostic plot, the best option for income was to use the square root transformation. Pre-pregnancy BMI was also heavily skewed to the right variable indicated a majority of the maternal population had a higher BMI. It was determined that the natural log transformation was the best option to normalize the data for statistical analyses.

Z-scores for children greater than 2 years of age were determined for BMI and Weight-for-Length percentiles were determined for children from birth to 24 months of age using a growth analyzer program (Growth Analyzer, The Netherlands). The growth analyzer program was created by the Dutch Growth Research Foundation (DGRF), by experts on the growth and development of children. DGRF, over the past 40 years, has developed an extensive knowledge domain on the growth and development of children. According to the Foundation website, the main functions of the society include scientific research, assessment and surveillance of growth hormone treatment, and promotion of knowledge to healthcare professionals (Growth Analyzer, The Netherlands). The analyzer provides tools for healthcare professionals to effectively document, monitor and analyze the growth and development of children and adolescents. The program contains a database of over 200 different growth charts from different geographic regions all over the world as well as standard charts for syndromes with endocrine disorders (Growth Analyzer, The Netherlands).

In the current analysis which the dataset came from the various WIC clinic visits there are almost no cases in which a mother/child pair contributed data at each of the 20 possible time points used to calculate the weight-by-length and BMI z-scores that

encompass 0 to 5 years of age. To use multivariate statistics there must be no missing values so interpolation was used to account for the missing values. Interpolation is the estimation between two known quantities, or drawing conclusions about missing information. Interpolation is useful when there is missing data that is likely to have a normal trend. The interpolation was done using SAS 9.3 (SAS Institute, Cary, NC). In SAS, the time periods with missing values were flagged and then summed. The sum of the missing values was then used later in the analysis. After the summing of the values, interpolation began by pulling the first known value forward, and the last known value backwards. For example, if time 1 and 2 were missing, but time 3 was known, the value for time 3 would be substituted for time 1 and 2. Fitting a line between 2 known values and using those for missing values imputed the intermediate missing values.

The primary research question was answered using a multivariate analysis on the 20 3-month interval time points for WL/BMI z-scores. The response variables were weight-by-length z-scores for the first 8 time periods (2 years of age) and BMI z-scores for the last twelve time periods (up until 5 years of age). Using these time periods allowed this z-score data to be compared to other growth trajectory datasets. Each time period was equal to a 3-month interval. For example, time period 1 included data collected anytime between 0 and 3 months of age for any mother/child pair.

Due to the imputations done to account for missing values a weight was calculated by subtracting the number of missing values from 20. A MANOVA model was fitted to protect the analysis from type II error and to account for natural structure in the response variables. The MANOVA was run twice, once for each gender. Included in the

multivariate analysis addressing the main effect of EBF were the following covariates: ethnicity (white Hispanic/ other ethnicities), Pre-pregnancy BMI, and income.

Separate models were also run to investigate the secondary research questions regarding the impact of Hispanic versus non-Hispanic ethnicity and of pre-pregnancy BMI on a child's growth curve after adjusting for confounding variables. To better visualize the association, a pre-pregnancy BMI categorical variable was used in the model to categorize pre-pregnancy BMI into a 4 level variable (i.e. Underweight, Normal, Overweight and Obese) based on the CDC BMI categories. For the analysis addressing the impact of EBF by ethnicity, only mother/child pairs who had indicated their race/ethnicity were included. There were 21,964 Hispanic males, 21,396 Hispanic females, 5,366 all other race/ethnicity for males, and 5,144 other race/ethnicity for females available for this analysis. The p-value used to determine statistical significance was $p < 0.05$ for the MANOVA. All analyses were performed using JMP (JMP, Version 10.0. SAS Institute Inc., Cary, NC).

CHAPTER 3: RESULTS

The overall prevalence of overweight/obesity among the children in the raw data, based on all the data collects, was: 13.2%, 27.8% and 40.1% at 12-, 24- and 57-months of age, respectively. Of the 60,190-mother/infant pairs, the data was interpolated for 29,620 female and 30,548 male offspring. T-tests were run to determine the mean z-scores for ages 0-60 months, in each gender, using first the interpolated dataset, then the original dataset. T-tests were run for each gender to test whether there was a statistically significant ($p < 0.05$) difference in z-scores between males and females (see Table 3.1 for the interpolated data and 3.2 for the original data).

At all BMI time points, males and females were statistically significantly different (< 0.001) in mean z-scores for the interpolated data (See Table 3.1). The mean z-scores and sample sizes at each time point for the non-interpolated data between males and females is also shown in Table 4.2. The mean z-scores remained generally statistically significantly different ($p < 0.05$) until about 24 months of age. After 24 months there was a substantial decrease in the number of subjects.

Table 3-1. Mean (SD) growth z-scores based on gender for the interpolated data (N=60,190) and p-value for T-test of mean gender differences (p<0.05).

BMI		Female n=29,631		Male n=30,559		P for Difference
Months of Age		Mean z-score	SD	Mean z-score	SD	
0 to 3		0.43	1.09	0.51	1.13	<0.001*
3 to 6		0.44	1.08	0.53	1.13	<0.001*
6 to 9		0.43	1.03	0.51	1.08	<0.001*
9 to 12		0.41	1.08	0.49	1.12	<0.001*
12 to 15		0.41	1.06	0.50	1.08	<0.001*
15 to 18		0.43	1.10	0.52	1.11	<0.001*
18 to 21		0.45	1.09	0.54	1.09	<0.001*
21 to 24		0.47	1.13	0.54	1.11	<0.001*
24 to 27		0.47	1.09	0.53	1.07	<0.001*
27 to 30		0.48	1.09	0.54	1.08	<0.001*
30 to 33		0.50	1.09	0.56	1.08	<0.001*
33 to 36		0.52	1.10	0.58	1.11	<0.001*
36 to 39		0.54	1.09	0.60	1.12	<0.001*
39 to 42		0.56	1.09	0.61	1.13	<0.001*
42 to 45		0.57	1.08	0.63	1.14	<0.001*
45 to 48		0.58	1.09	0.64	1.15	<0.001*
48 to 51		0.59	1.08	0.65	1.15	<0.001*
51 to 54		0.60	1.08	0.66	1.15	<0.001*
54 to 57		0.60	1.08	0.66	1.15	<0.001*
57 to 60		0.60	1.09	0.66	1.15	<0.001*

*P-Value (<0.001) is statistically significant

Table 3-2. Mean (SD) growth z-scores based on gender for the original data set before interpolation and T-test of mean gender differences ($p < 0.05$).

BMI		Female			Male		P for Difference
Months of age	n=	Mean z-Score	SD	n=	Mean z-Score	SD	
0 to 3	2610	0.27	1.15	2901	0.27	1.12	0.67
3 to 6	15905	0.46	1.06	16542	0.54	1.13	<.0001*
6 to 9	4258	0.35	1.09	4517	0.43	1.17	0.0023*
9 to 12	15282	0.37	1.10	15863	0.45	1.15	<.0001*
12 to 15	4678	0.29	1.18	4754	0.41	1.19	<.0001*
15 to 18	12669	0.39	1.16	12970	0.48	1.14	<.0001*
18 to 21	4708	0.43	1.20	4812	0.51	1.17	0.0009*
21 to 24	11466	0.46	1.22	11761	0.52	1.16	0.0002*
24 to 27	5001	0.45	1.17	4991	0.44	1.07	0.89
27 to 30	9773	0.49	1.14	10084	0.52	1.08	0.0532
30 to 33	5216	0.51	1.20	5364	0.54	1.15	0.24
33 to 36	9053	0.58	1.14	9334	0.61	1.16	0.08
36 to 39	5229	0.60	1.17	5321	0.62	1.20	0.58
39 to 42	8172	0.65	1.12	8346	0.68	1.22	0.11
42 to 45	5040	0.69	1.13	5291	0.71	1.23	0.31
45 to 48	7787	0.73	1.10	7791	0.76	1.26	0.05
48 to 51	4829	0.74	1.12	5101	0.77	1.24	0.18
51 to 54	6861	0.74	1.07	6982	0.82	1.24	0.0001*
54 to 57	4477	0.77	1.10	4475	0.82	1.25	0.03*
57 to 60	1651	0.80	1.12	1705	0.86	1.25	0.13

*P-value (< 0.05) is statistically significant

MANOVA was used to test the primary research question to determine the association between exclusively breastfeeding and child's BMI trajectory from age 0 to 5 years old. The MANOVA considered the whole time frame from 0-5 years of age to determine the impact of breastfeeding as a condition. Breastfeeding was associated with weight-for-length from birth to age 5 (Pillai's Trace=0.034, $F(40, 16960) = 7.39$, $P < 0.0001$). Breastfeeding remained to have an impact for both the 0 to 3 months EBF ($F(20, 8479) = 7.16$, $P < 0.0001$) and 0 to 6 months EBF ($F(20, 8479) = 4.51$,

P<0.0001) groups. Children that were EBF for 0 to 3 months on average had a higher BMI than infants who were EBF for 0 to 6 months, but lower than infants who were not EBF.

The mean BMI z-scores for EBF children 0-6 months of age were statistically significantly lower (<0.001) than in the EBF 0-3 groups. The mean BMI z-scores for EBF 0-6 months of age were statistically significantly lower compared to both non-EBF and EBF 0-3 months groups. For both males and females, there was evidence that non-EBF was different from 0 to 3 months EBF, and 0 to 3 months EBF was different from 0 to 6 months EBF, and 0 to 6 months EBF was different from non-EBF. Our results allow us to conclude that children who were not EBF had, on average, a higher BMI than those who were EBF for 0-3 and 0-6 months of age (shown in Figure 3-1A for males and 3-1B for females). Infants included in figure 3-1A (N=16,321) and 3-2B (N=15,884) were based on the food package picked up by the mother during the EBF 0-3, EBF 0-6 or no EBF 0-6 months of age time periods from the raw dataset. Both males and females followed the same trend in that infants EBF for 0 to 6 months had the lowest BMI consistently throughout the growth trajectory compared to infants who were not EBF consistently who had a higher BMI consistently than either EBF groups.

Figure 3-1A. Mean BMI Z-scores for Males based on duration of breastfeeding (N=16,321).

Figure 3-1B. Mean BMI Z-scores for Females based on duration of breastfeeding (N=15,884).

When looking within the growth trajectories, there was a flattening in the rate of change in the growth trajectory around 15 months of age for males, and 18 months of age for females, see in 3.1A and B, and referenced in appendix A and B, respectively. At this point the dose-dependent relationship between breastfeeding and BMI appears to decrease. However these initial relationships in trajectories based on breastfeeding categories persists with the BMI z-scores remaining consistently lower for those EBF 0-6 through 57 months of age meaning the protectiveness of breastfeeding continued until 57 months of age.

The secondary hypothesis took into account other variables known in the literature to be related to child size such as race/ethnicity, pre-pregnancy BMI, and

income. To test if any of these had an effect on infant growth the F test was used. For both males and females, Hispanic children tended to have a higher BMI growth curve versus non-Hispanic children after adjusting for EBF, pre-pregnancy BMI, and income ($F(20,8479)=3.10$, $P<0.0001$ for males; $F(20,8152)=5.10$, $P<0.0001$ for females). When looking at the growth curves of Hispanic children compared to non-Hispanic children the protective effect of EBF 0-3 and EBF 0-6 was still present even though children of Hispanic race/ethnicity maintained the higher z-scores compared to the other ethnicities. Figure 3-2A and 3-2B, shows Z-scores based on ethnicity and breastfeeding status. In Figure 3-2A and B, there were a total of 16,225 male subjects and 15,705 female subjects included in the graph based on if they had the data for race/ethnicity, breastfeeding status and z-scores. Infants included in the race/ethnicity analysis were only infants whose mothers reported on their race/ethnicity. There were 4473 Hispanic males EBF 0-3 months, 851 infants of other race/ethnicities EBF 0-3 months, 1523 Hispanic infants EBF 0-6 months, 320 infants of other race/ethnicities EBF 0-6 months, 7537 Hispanic infants were not EBF for 0-6 months, and 1521 of other race/ethnicities were not EBF 0-6 months (See Figure 3-2A). There were 4448 Hispanic females EBF 0-3 months, 831 infants of other race/ethnicities EBF 0-3 months, 1629 Hispanic infants EBF 0-6 months, 378 girls of other race/ethnicities EBF 0-6 months, 7001 Hispanic females were not EBF for 0-6 months, and 1418 of other race/ethnicities were not EBF 0-6 months (See Figure 3-2B).

Figure 3-2A. Mean BMI Z-scores for Hispanic males compared to all other race/ethnicities based on duration of breastfeeding (N=16,225).

Figure 3-2B. Mean BMI Z-scores for Hispanic females compared to all other race/ethnicities based on duration of breastfeeding (N=15,705).

Pre-pregnancy BMI, after adjusting for race, EBF and income, demonstrated a statistically significant association with growth curve outcomes ($F(20, 8479) = 19.33$, $P < 0.0001$ for males, $F(20, 8152) = 15.37$, $P < 0.0001$ for females). Further analysis was done among mothers who had reported pre-pregnancy BMI and who had EBF 0-6 ($n=2,989$) to determine the impact of pre-pregnancy BMI on child growth trajectory within this sub-category. Pre-pregnancy BMI was categorized by underweight, normal, overweight and obese BMI categories and used to stratify the mean z-scores of the infants by breastfeeding status from 0-6 months of age (See Figure 3-3). There were 65 infants born to mothers with an underweight pre-pregnancy BMI of less than 18.5; 1232 infants born to mothers with a normal pre-pregnancy BMI between 18.5 and 24.9; 977 infants born to mothers with an overweight pre-pregnancy BMI between 25 and 29.9; and 715 infants born to mothers with an obese BMI above 30.

Figure 3-3. Comparison of mean BMI *Z-scores for children Exclusively Breast Fed 0-6 months based on mothers' pre-pregnancy BMI (N=2,989).

As shown in Figure 3-3, children born to mothers with a higher pre-pregnancy BMI and who were EBF for 0-6 months of age had a higher BMI themselves from 0 to 57 months of age. It remained consistent overtime from 0 to 57 months that mothers with a high pre-pregnancy BMI had children with higher birth weights that persisted to 5 years of age. Mothers in this study who had a higher pre-pregnancy BMI were more likely to have children with higher BMI growth trajectories than mothers with lower pre-pregnancy BMIs even with the protective impact of EBF.

In an analysis looking at the covariate income, after adjusting for race, EBF and pre-pregnancy BMI, there was an association between income of the family and growth curves for males ($F(20, 8479) = 2.27, P < 0.0010$), but not females ($F(20, 8152) = 1.39, P < 0.1157$). The SES for the two counties that comprised this dataset were statistically significantly different from each other ($P < 0.001$), but the number of males or females in both counties was not ($P < 0.07$) (Appendix F).

After adjusting for race/ethnicity, pre-pregnancy BMI and income, there was still significant evidence (< 0.0001) that EBF is protective against obesity through 60 months of age. After including potentially confounding variables into the MANOVA model, EBF for 0-6 months of age was associated with lower BMI/WL z-scores compared to infants who were not EBF. Significance still remained for infants EBF 0-6 months being associated with a lower BMI than infants EBF 0-3, and infants EBF for 0-3 months had a lower BMI than infants that were not EBF.

CHAPTER 4: DISCUSSION

The purpose of this study was to determine whether exclusive breastfeeding from 0-3 and 0-6 months of age was protective against obesity at 60 months of age. The results support the protective effect of exclusive breastfeeding; finding that children who were not EBF had, on average, a higher BMI than those who were EBF for 0 to 3 months or 0 to 6 months of age. Additionally, there was a dose-response effect for protection seen; children who were EBF for 0-3 months had a lower BMI than children not EBF, and those who were EBF for 0-6 months of age had a lower BMI than children EBF for 0-3 months of age.

A secondary focus was to determine if EBF 0-3 or 0-6 months of age were protective against obesity at 60 months of age in those at higher risk, such as being Hispanic, the mother having a higher pre-pregnancy BMI, or with a lower income level. Our study found that Hispanic infants, for both males and females, who were EBF for 0-6 months had a lower BMI than Hispanic infants who were not EBF or were EBF 0-3 months. Hispanic infants EBF 0-6 months had a higher BMI than infants of other race/ethnicities in similar breastfeeding categories. This was true for both genders. This finding was similar to Guerrero et al. (2015), which also found a significantly higher proportion of Hispanic children to have a higher BMI on average compared to Non-Hispanic children.

Our study found that Hispanic children, regardless of EBF status and maternal pre-pregnancy BMI, had a higher BMI compared to children of other race/ethnicities in this dataset. Guerrero et al. (2015) also found that young children, 48 to 72 months old, of Latino descent had higher BMI growth trajectories compared to White children, and

being of Hispanic/Latino race/ethnicity appears to contribute to children having a higher BMI compared to those of other race/ethnicities. Guerrero et al. (2015), also found that children of Latino households that are primarily Spanish speaking tended to have higher BMI growth trajectories compared to those of English-speaking. Guerrero et al. (2015) proposed that there may be a language barrier that could be preventing mothers of Hispanic race/ethnicity from understanding fully what EBF is. Guerrero et al. (2015) also indicated some dietary behaviors that may contribute to the increased BMI in Hispanic/Latino children, which included fast food and soda consumption. Burdette and Whitaker (2007) found breastfeeding to be associated with a reduced risk of obesity in Hispanic children but not white children. Burdette and Whitaker (2007) suggested the differing relationship between breastfeeding and BMI in race/ethnicity groups might be best explained by other environmental and genetic factors. Future research into the protective effects of exclusive breastfeeding on childhood growth should attempt to model the interaction of factors in all the domains of the social ecological model to determine best practices within cultures for public health advice.

Our study found mothers with an overweight or obese pre-pregnancy BMI were more likely to have infants with a higher BMI z-score regardless if the infant was EBF for 0-3 or 0-6 months of age compared to mothers who had an underweight or normal pre-pregnancy BMI. Li et al. (2005) study, which also found that mothers who had a higher pre-pregnancy BMI demonstrated an increased risk of offspring being overweight.

In the current analysis we found that income was associated with an infant's growth trajectory for males, but not females. After adjusting for race/ethnicity, pre-pregnancy BMI and income, our study found that EBF for 0-3 and 0-6 months of age was

still associated with having a lower BMI, for both males and females, compared to infants who were not EBF. This finding may be related to the demographics of the two counties who form the analysis sample because WIC eligibility is dependent on income this finding is inconsistent with WIC as all subjects have low-income status. County of residence may present some unknown confounding factors. Further studies could identify possible explanation for this finding.

Birth weight for the EBF groups and non-EBF group were not statistically significantly different from each other for males and female strengthening our confidence in the protective aspects of breastfeeding. This study finds that there was a dose-dependent relationship for breastfeeding being protective against BMI up until 15 months of age for males and 18 months of age for females, the z-scores remaining consistent after 15 and 18 months of age for males and females, respectively through 5 years of age. This finding is consistent with other research examining the impact of breastfeeding practices on child growth trajectory. Bergmann et al. (2003) suggested that feeding practices (i.e. breastfed versus bottle-fed) had an influence on BMI. They also found breastfeeding to be protective in a dose-dependent relationship up until 18 months of age for both sexes combined. In our study, infants who were breastfed had a consistently lower BMI z-score than those who were not. Bergmann et al. (2003) noticed that infants who were bottle-fed had a consistently higher prevalence of being overweight or obese compared to those who were breastfed. Similar to our study, Anderson, Hayes and Chock (2013), also found breastfeeding exclusively to six months was protective against overweight and obesity up until 2 years of age ($P=0.0197$) in a WIC population from Hawaii ($n=15,141$) for the years 2005 through 2009 controlling for race/ethnicity in a binomial regression model.

One metabolic mechanism that potentially could explain the risks of childhood obesity by the use of formula versus breast milk is the “early protein intake hypothesis” which proposes that the high protein intake in infant formula could contribute to an early programming of the body for later obesity (Weber et al., 2014). The hypothesis is based human milk supplying less protein (9 and 10 g/d) at ages 3 and 6 months as compared to formula (14 and 18 g/d, respectively). The protein content in human milk versus formula may attenuate the early weight gain and later obesity. Weber et al. (2014) found that breastfed infants tended to have a lower BMI than infants fed a high protein formula, and BMI was not significantly different for the low protein formula versus the breastfed group. While the present study did not identify protein content of formula being used, our results were similar in that the breastfed group showed a significantly lower BMI compared to the higher protein formula group. Currently WIC does not favor one formula over another, and formula recommendations vary by WIC agency. Future research is needed on the comparison of protein content in multiple formulas and their impact on the growth trajectory curves. Weber et al. (2014) suggested that targeting protein intake during infancy could be a valued approach at preventing obesity later in life.

Our study found that infants born to mothers of an overweight or obese pre-pregnancy BMI were more likely to have an increased BMI at 60 months of age compared to infants born to mothers with a normal pre-pregnancy BMI. Bergmann et al. (2003) conducted a longitudinal birth cohort composed of 918 children followed up till 6 years of age. They found that overweight mothers had a significantly increased risk that their child will be overweight or obese by the age of 6 years. The authors noticed the influence suggested there may be a genetic or cultural influence as well. Multiple studies

have consistently shown that maternal BMI status influences their offspring's likelihood of being obese or not. Furthermore one of the factors influencing is obesity, which explains why we consistently see a higher BMI in children who were born to mothers of a high pre-pregnancy BMI. Other research identified children born to mothers of a overweight or obese pre-pregnancy BMI and were exclusively breastfed for 0-6 months were protected against having a higher BMI (Grant, 2014). Our studies finding in relation to pre-pregnancy BMI being associated with a higher BMI in offspring even when the infants are EBF for 0-3 or 0-6 months of age leads us to believe that genetics play a role in the development of obesity.

Our study and the Bergmann (2003) study are similar in that they both collected data to the date of origination; which helps the validity, the time course of the development of obesity is visible (i.e. by percentile for Bergmann (2003) and Z-scores for ours). Bergmann (2003) focused on the feeding style of being bottle-fed versus breast-fed, but did not focus on if the infant was consuming breast milk or formula in the bottle-fed group. In the future, a study would be beneficial that assessed if an infant was breast-fed or bottle-fed, and if bottle-fed then determined if they were consuming breast milk, formula or a combination of both.

While this analysis was able to examine the effects of breastfeeding status, demographic differences within the two counties that composed the data set were not addressed. Some variances between the two counties are seen, such as income (Appendix F). These variances could potentially affect the generalizability of the findings as Santa Barbara County has a higher poverty level compared to San Luis Obispo County and therefore has more WIC enrollees. However the potentially confounding factor of income

was controlled for in the analysis and the protective effect of EBF remained statistically significant. The county differences may potentially explain why income was associated with the growth trajectories for males, but not females. The income and poverty levels were statistically significantly different from each other for the Santa Barbara and San Luis Obispo counties ($P < 0.001$), however the difference in the percent of males and females within the two counties remained insignificantly different from each other ($P = 0.7$). The poverty level and income quartiles within the two counties were associated with an obese weight-for-length at 1 year of age in offspring (Appendix G).

The current studies analysis used MANOVA to infer statistical associations therefore interpolation was used on the entire dataset to create values for missing data points in time. There was no one mother-infant pair that had no missing time points, so every subject had some degree of interpolation. Interpolating the data limits the study in that through this process some natural variability in the data may not be apparent. However the use of interpolated data points has the advantage of a much larger data set than one in which no interpolated data would be used. The results attained from using the larger, more comprehensive dataset can be argued to be more generalizable because the data represents the more typical WIC mothers, mothers who are seldom able to attend every WIC clinics in every 3-month period. To further understand the impact of interpolating the number of missing visits and possible associations with other variables in the study, we conducted an additional regression analysis.

Regression analysis was used to identify the association between missing data and other potentially explanatory variables. The analysis found that none of the subjects ($N = 60,190$) were present for all 20-time points.

Table 4-1. Regression Analysis of Missing Visit Data as Predicted by Race, Income, Pre-pregnancy BMI, Birth Weight and Breastfeeding Status.

Variable	F-Statistic	p-Value
Race	368.69	<0.0001*
Income	501.9	<0.0001*
Pre-pregnancy BMI	97.03	<0.0001*
Birth Weight	18.36	<0.0001*
EBF 0-3	9.38	<0.0022*
EBF 0-6	612.39	<0.0001*

The regression analysis found that Hispanics had on average the lowest number of missing visits, approximately 14.5 missing visits, compared to all other race/ethnicity category ($p<0.0001$). Subjects with a higher income status were associated with less missing time points compared to those of lower income ($p<0.0001$). Subjects with a higher pre-pregnancy BMI (i.e. overweight or obese) were more likely to not miss a time point compared to those of a lower pre-pregnancy BMI (i.e. underweight or normal) ($p<0.0001$). The analysis also found that infants of a lower birthweight were more likely to miss WIC visits compared to infants of a higher birth weight ($p<0.0001$). Infants who were EBF 0-3 ($p<0.0022$) or 0-6 ($p<0.0001$) months of age were more likely to not miss WIC visits as well.

Other statistical models could have been used that would not require interpolated data, but would have required approximately 20 other tests to derive the final conclusion and would have increased the chance of having a type 1 error. Interpolation in our study was a limitation in that we were not using the raw data. But the age of this large data set strengthens confidence in the findings of this study. With the data set being normally distributed and when comparing z-scores for males and females at all time points in the original data set (Table 4-2) we can see that there some difference between the original data and non-interpolated data. Having all mothers present at all time points would raise

the internal validity of the study; however this would not represent how WIC families generally attend WIC clinics. The large data set, inclusive of mothers representing the WIC population increases the generalizability and external validity of these findings. For this dataset, there was no one subject present for all WIC clinic visits. In the future, if the same growth trajectory study was redone using no missing values that would increase the confidence that these results were not due to some unexplained factors. Using the larger data set, by interpolating missing data, increased the statistical power of these findings.

During time period this dataset was collected, 2005-2009, the typical WIC appointment pattern differed between low-risk and high-risk children. Low-risk children could be seen every 3 months, but were only required to be seen twice a year. On the other hand, high-risk children, who were over or underweight or had a relevant medical diagnosis, would be seen every three months at minimum. Based on the registered dietitians discretion, a high-risk child could be seen as often as once a month. WIC assessed mothers and their infants for high risk based on their assessment questions, assessment of growth, weight gain and evaluation of blood work. WIC has a series of diagnoses that are considered to be high risk for infants and/or their mothers located in the WIC handbook. Diagnoses that deemed a child at high-risk had to be made by the physician. With high-risk and low-risk children having different timelines to be seen it could potentially cause our study to be biased towards higher risk children. One deficiency of the data set is that it did not identify, which subjects were considered high- or low-risk which limits our ability to generalize these findings to the entire WIC population. In addition, WIC requires participants to be recertified every 6 months

making it possible for infants to have been removed from WIC due to not meeting eligibility and then re-enrolled later when they meet the eligibility requirements.

Another potentially limiting aspect of this dataset is that it was developed based on questions already asked at clinic visits. The dataset was developed after WIC clinic visits took place therefore, for the purposes of this study we were limited to variables based on the questions asked during the visits. For example, the feeding variable was established based on the feeding package that the mother picked up at the WIC visit. The mother could have picked up an EBF package, but still be offering her child occasional formula from samples she received. One limitation of this study is due to the feeding variable being developed based on the food package picked up leading to the possible mis-categorization bias. This study found that by using data that was configured prior to the study provided multiple limitations to the generalizability of the results. In the future, a WIC study similar to this one would benefit from additional questions regarding total feeding practices at WIC visits so variables such as EBF could be more accurately assessed rather than based on food package being picked up. In addition knowing the type of formula being used would be beneficial, so variables such as protein content could be assessed. The current data set did not distinguish between a combination of breastfeeding and formula, which should be included in future studies. In addition, future studies would benefit by a variable based on a child being high- or low-risk to assess impact of breastfeeding duration based on risk category. The advantage of breastfeeding duration on child BMI over time was demonstrated in a WIC population study however further research would be helpful to better understand additional factors in this relationship.

CHAPTER 5: CONCLUSION

The current study demonstrated the protective influence of exclusive breastfeeding (EBF) at 0-3 and 0-6 months of age on the growth trajectory curve at 60 months of age. Significant associations found EBF to have a influence on BMI at 60 months of age for both males and females. EBF duration of 0-3 and 0-6 months was found to be associated with a lower BMI. This study demonstrated at dose-dependent response on breastfeeding duration and BMI at 60 months of age.

Our study showed EBF for Hispanic infants to be protective against obesity, but found all other race/ethnicities to be more protective. Our study implies the significance of educating Hispanics in particular, however that does not mean all race/ethnicities should be educated on the benefits of breastfeeding till at least 6 months of age.

Our study suggests that breastfeeding for 0-3 and 0-6 months of age are protective against the development of obesity at 60 months of age. Our study supports the importance of educating mothers during pregnancy about the multiple health benefits that breastfeeding provides.

Our study found evidence for mothers of a high pre-pregnancy BMI (i.e. overweight or obese), who EBF their offspring for 0-3 or 0-6 months of age their offspring tended to have a higher BMI at 60 months of age compared to infants with mothers who had a normal or underweight pre-pregnancy BMI. This finding suggests the need for education on maintaining a BMI in normal range prior to pregnancy.

Future studies are needed to further clarify other factors involved in the long-term relationship of breastfeeding's influence on BMI for both males and females.

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APPENDICES

Appendix A: Figure 4-1A Data for the Mean BMI Z-scores for Males based on duration of breastfeeding (N=16,321).

Age (in months)	EBF 0-3 Males N=5249	EBF 0-6 Males N=1846	Non-EBF Males N=9226
0 - 3	0.66	0.7	0.65
3 - 6	0.67	0.71	0.67
6 - 9	0.6	0.62	0.65
9 - 12	0.53	0.51	0.63
12 - 15	0.51	0.47	0.63
15 - 18	0.5	0.44	0.64
18 - 21	0.51	0.44	0.65
21 - 24	0.51	0.43	0.64
24 - 27	0.51	0.44	0.64
27 - 30	0.51	0.45	0.64
30 - 33	0.52	0.45	0.65
33 - 36	0.53	0.45	0.66
36 - 39	0.54	0.45	0.68
39 - 42	0.55	0.46	0.69
42 - 45	0.56	0.46	0.7
45 - 48	0.57	0.46	0.71
48 - 51	0.57	0.46	0.71
51 - 54	0.57	0.46	0.71
54 - 57	0.57	0.46	0.71
57 - 60	0.57	0.46	0.71

Appendix B: Figure 4-1B Data for Mean BMI Z-scores for Females based on duration of breastfeeding (N=15,884).

Age (in months)	EBF 0-3 Females N=5302	EBF 0-6 Females N=2014	Non-EBF Females N=8568
0 - 3	0.53	0.52	0.57
3 - 6	0.54	0.54	0.59
6 - 9	0.48	0.45	0.57
9 - 12	0.4	0.34	0.55
12 - 15	0.37	0.3	0.55
15 - 18	0.36	0.25	0.55
18 - 21	0.37	0.26	0.57
21 - 24	0.38	0.27	0.58
24 - 27	0.39	0.27	0.58
27 - 30	0.41	0.29	0.59
30 - 33	0.42	0.29	0.61
33 - 36	0.44	0.3	0.62
36 - 39	0.44	0.3	0.63
39 - 42	0.45	0.31	0.63
42 - 45	0.46	0.31	0.64
45 - 48	0.46	0.31	0.65
48 - 51	0.47	0.31	0.65
51 - 54	0.47	0.31	0.65
54 - 57	0.47	0.31	0.65
57 - 60	0.47	0.31	0.65

Appendix C: Figure 4-2A Data for Mean BMI Z-scores for Hispanic males compared to all other race/ethnicities based on duration of breastfeeding (N=16,225).

Age (in months)	EBF 0-3 Hispanic N= 4473	EBF 0-6 Hispanic N=1523	No EBF Hispanic N=7537	EBF 0-3 Other N=851	EBF 0-6 Other N=320	No EBF Other N=1521
0	0.69	0.75	0.67	0.49	0.48	0.54
12	0.56	0.55	0.64	0.4	0.33	0.57
24	0.53	0.46	0.66	0.38	0.31	0.57
36	0.56	0.48	0.68	0.37	0.31	0.57
48	0.6	0.49	0.73	0.39	0.31	0.58
60	0.6	0.49	0.74	0.4	0.31	0.59

Appendix D: Figure 4-2B Data for Mean BMI Z-scores for Hispanic females compared to all other race/ethnicities based on duration of breastfeeding. (N=15,705).

Age (in months)	EBF 0-3 Hispanic N= 4448	EBF 0-6 Hispanic N=1629	No EBF Hispanic N=7001	EBF 0-3 Other N=831	EBF 0-6 Other N=378	No EBF Other N=1418
0	0.57	0.59	0.61	0.33	0.27	0.43
12	0.44	0.4	0.57	0.23	0.13	0.44
24	0.42	0.32	0.61	0.18	0.05	0.44
36	0.48	0.36	0.65	0.22	0.07	0.46
48	0.51	0.36	0.69	0.22	0.07	0.47
60	0.51	0.37	0.69	0.23	0.07	0.47

Appendix E: Figure 4-3 Data for the Comparison of mean BMI *Z-scores for children
Exclusively Breast Fed 0-6 months based on mothers' pre-pregnancy BMI (N=2,989).

Age (in months)	Underweight N=65	Normal N=1232	Overweight N=977	Obese N=715
0	0.28	0.52	0.71	0.66
12	0.08	0.24	0.49	0.47
27	0.03	0.23	0.46	0.48
42	0.02	0.23	0.49	0.52
57	0.01	0.23	0.49	0.52

Appendix F: Table 1. Mean (SD) or proportion of maternal and offspring characteristics of San Luis Obispo and Santa Barbara County, CA WIC participants, 2007 (Total N=60190).

Table 1. Mean (SD) or proportion of maternal and offspring characteristics of San Luis Obispo and Santa Barbara County, CA WIC participants, 2007. (Total N=60190)

Maternal characteristics	N	SLO County (N=14392)	SB County (N=45798)	Overall (N=60190)	P-value*	US WIC (2007)	CA WIC
Maternal age, years	53870	25.8 (5.8)	26.0 (5.9)	25.9 (5.9)	0.04		
Maternal age group, years	53870	-	-	-	0.001		
≤19		13.1	13.8	13.7	-		7.2
20-29		61.4	59.3	59.8	-		35.8
30-39		23.7	25.0	24.7	-		55.0
40+		1.7	1.8	1.8	-		2.0
Race/ethnicity	53444	-	-	-	<0.001		
White		32.5	9.4	14.8	-	60.3	17.6
Hispanic		62.9	86.7	81.1	-	42.1 [#]	63.9
Black		1.1	1.3	1.2	-	19.6	4.9
Asian		1.7	1.7	1.7	-	3.5 ^{##}	4.7
Native AM/PI/HI/AK		0.4	0.5	0.5	-	11.4	2.2
Multi-racial		1.3	0.4	0.6	-	3.6	10.3
Language preference	60190	-	-	-	<0.001		
English		61.1	40.0	45.1	-		58.3
Spanish		38.2	57.8	53.1	-		39.5
Other		0.64	2.2	1.8	-		2.2
Country of origin	60190	-	-	-	<0.001		
US		45.7	30.9	34.5	-		
Mexico		39.1	56.2	52.1	-		
Other		15.2	12.9	13.4	-		
Number living in household	60183	-	-	-	<0.001		
1-2		11.7	7.7	8.7	-		
3-4		50.7	45.8	47.0	-		
5-6		32.2	38.9	37.1	-		
7+		5.5	7.5	7.0	-		
Monthly income, USD	60190	1692.94 (1042.51)	1661.07 (987.91)	1668.68 (1001.31)	0.04		
Income quartiles	60190	-	-	-	<0.001		
Q1		29.0	27.5	27.9	-		
Q2		21.1	22.6	22.3	-		
Q3		23.1	25.4	24.9	-		
Q4		26.7	24.4	25.0	-		
Poverty status	60190	-	-	-	<0.001		
<100%		58.8	62.7	61.8	-	68.3	
100-149%		25.0	23.4	23.8	-	21.5	
150-185%		11.3	9.6	10.0	-	8.4	
>185%		5.0	4.2	4.4	-	1.7	
Maternal education, years completed	55685	-	-	-	<0.001		
No schooling (0)		0.4	2.4	1.9	-	0.6	
Elem./middle school (1-8)		18.9	32.6	29.3	-	14.3	
High School (9-12)		62.4	52.7	55.0	-	67.6	
>High School (13+)		18.3	12.4	13.8	-	17.6	

*P-values for SLO vs. SB County by chi-squared test for categorical variables and Kruskal-Wallis for continuous variables

[#]Hispanic of any race/ethnicity

^{##}US WIC data for Asian includes Pacific Islander

(cont.)	N	SLO County	SB County	Overall	P-value [*]	US WIC (2007)	CA WIC
Maternal pre-pregnancy BMI, kg/m ²	44057	27.12 (5.98)	27.24 (5.66)	27.21 (5.74)	0.0001		
Maternal pre-pregnancy BMI groups, kg/m ²	44057	-	-	-	<0.001		
<18.5		2.3	1.8	1.9	-		
18.5-24.9		39.7	37.5	38.0	-		
25.0-29.9		31.2	33.9	33.3	-		
30.0+		26.8	26.8	26.8	-		
Offspring characteristics							
Sex	60190	-	-	-	0.7		
Male		50.6	50.8	50.8	-		
Female		49.4	49.2	49.2	-		
Birth weight, kg	33192	3.36 (0.52)	3.32 (0.52)	3.33 (0.52)	0.0001		
Birth length, cm	32539	50.4 (2.7)	50.1 (2.6)	50.2 (2.6)	0.0001		
% Low birth weight (≤ 2.5 kg)	33192	5.32	6.23	6.01	0.003		
% High birth weight (≥ 4.0 kg)	33192	9.33	7.50	7.94	<0.001		
Feeding in 0-3 months of life	21712	-	-	-	<0.001		
EBF		47.31	48.99	48.60	-		
BF + Formula		24.91	20.53	21.55	-		
Formula only		27.78	30.48	29.85	-		
Feeding 0-6 months of life	21656	-	-	-	<0.001		
EBF		22.22	16.49	17.82	-		
BF + Formula		20.66	26.27	24.96	-		
Formula only		19.25	20.20	19.98	-		
Others, any		37.87	37.04	37.23	-		

^{*}P-values for SLO vs. SB County by chi-squared test for categorical variables and Kruskal-Wallis for continuous variables

[#]Hispanic of any race/ethnicity

^{##}US WIC data for Asian includes Pacific Islander

Appendix G: Table 2a. Percent obese by selected characteristics from birth to 57 months of age among children participating in San Luis Obispo and Santa Barbara County WIC program, 2005-2009 (Total N=60190).

Table 2a. Percent obese by selected characteristics from birth to age 57 months among children participating in San Luis Obispo and Santa Barbara County WIC programs, 2005-2009. (Total N=60190)

	N at follow-up	Weight for length			BMI		
		Birth	1 year (10-12 m)	2 years (22-24 m)	3 years (34-36 m)	4 years (46-48 m)	4.75 years (55-57 m)
Sex		31618	31145	23227	18387	15578	8952
Male		3.74	14.29	11.39	16.86	21.54	22.86
Female		5.06	11.96	12.71	16.35	18.76	21.44
	P-value	<0.001	<0.001	0.002	0.3	<0.001	0.1
Race/ethnicity							
White		4.22	12.32	9.06	11.11	13.49	15.05
Hispanic		4.39	13.51	12.59	17.22	21.08	23.42
Other		3.85	9.94	8.32	10.99	13.75	13.68
	P-value	0.6	0.001	<0.001	<0.001	<0.001	<0.001
Language preference							
English		4.26	13.37	10.71	14.43	16.81	18.18
Spanish		4.54	12.90	12.65	17.59	21.64	24.37
Other		2.36	17.24	13.90	16.28	17.54	18.97
	P-value	0.03	0.01	<0.001	<0.001	<0.001	<0.001
Poverty status							
<100%		4.27	13.64	12.18	16.82	20.14	21.94
100-149%		4.56	12.48	12.05	16.53	20.53	22.90
150-185%		4.53	12.44	12.09	15.73	18.52	21.61
>185%		4.69	11.86	9.93	15.85	21.98	22.55
	P-value	0.7	0.01	0.2	0.6	0.3	0.8
Income quartiles							
1 (lowest)		4.23	14.56	12.83	16.61	20.05	20.53
2		4.05	12.96	11.88	17.07	21.20	24.07
3		4.51	12.80	12.16	16.78	19.87	22.28
4 (highest)		4.73	12.32	11.42	16.03	19.60	21.72
	P-value	0.2	<0.001	0.1	0.6	0.3	0.04
Maternal age, years							
≤19		3.09	14.16	13.47	16.29	20.42	20.75
20-29		4.33	13.39	11.84	16.19	19.67	21.68
30-39		4.99	12.58	12.35	17.37	21.47	24.08
40+		5.68	11.21	10.33	17.30	19.93	27.66
	P-value	<0.001	0.04	0.07	0.3	0.1	0.04
Maternal education, years completed							
0		4.69	15.89	12.58	19.78	22.56	31.47
1-8		4.31	13.00	13.00	18.60	23.09	24.93
9-12		4.30	13.31	11.73	15.64	18.54	20.98
13+		4.98	11.82	9.38	12.74	16.30	18.22
	P-value	0.3	0.02	<0.001	<0.001	<0.001	<0.001
Maternal pre-pregnancy BMI, kg/m ²							
<18.5		2.63	7.91	4.53	4.90	7.59	1.22
18.5-24.9		3.13	10.14	8.24	10.90	13.06	13.83
25-29.9		4.54	14.06	13.17	17.33	21.32	22.64
30+		5.74	16.62	16.95	23.85	28.80	31.84
	P-value	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001 [#]

(cont.)	Birth	1 year	2 years	3 years	4 years	4.75 years
# Living in household						
2	4.03	13.27	11.65	16.04	18.06	17.59
3-4	4.44	13.41	11.91	17.12	21.16	23.90
5-6	4.48	12.89	12.25	16.28	19.96	21.85
7+	3.84	12.70	11.90	16.27	18.23	19.61
P-value	0.5	0.6	0.8	0.5	0.03	0.003
Birth weight, kg						
≤ 2.5	0 [#]	6.82	8.14	11.80	15.82	17.34
>2.51-4.0	3.89	12.87	11.54	16.78	21.27	25.71
> 4.0	11.64	22.69	20.13	25.56	31.06	40.42
P-value	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Feeding in 0-3 months of life						
EBF	4.47	11.98	9.55	14.54	19.43	22.55
BF + Formula only	4.45	15.23	14.49	20.61	24.91	20.45
Formula only	4.88	18.52	15.92	21.62	25.09	20.55
P-value	0.5	<0.001	<0.001	<0.001	0.009	0.9 [#]
Feeding 0-6 months of life						
EBF	4.74	10.33	7.32	14.24	22.41	20.00
BF + Formula only	4.20	13.94	12.19	16.91	13.48	23.81
Formula only	4.75	20.30	16.14	16.61	23.81	20.00
Others, any	4.71	14.13	12.92	18.60	22.80	21.13
P-value	0.6	<0.001	<0.001	0.2	0.2	>0.9 [#]
Feeding 0-3 months of life						
EBF	4.47	11.98	9.55	14.54	19.43	22.55
Not	4.70	17.02	15.21	21.09	25.00	20.50
P-value	0.5	<0.001	<0.001	<0.001	0.002	0.6 [*]
Feeding 0-6 months of life						
EBF	4.74	10.33	7.32	14.24	22.41	20.00
Not	4.56	15.37	13.25	18.15	22.43	21.24
P-value	0.7	<0.001	<0.001	0.07	>0.9	>0.9 [#]

Weight for length and BMI z score for age >1.645, equivalent to >95th percentile

P-values by chi² test

[#]Fisher's exact test

^{*}N<1000

^{**}N<100

Birth to 36 months: Boys

Length-for-age and Weight-for-age percentiles

NAME _____

RECORD # _____

Length-for-age percentiles (Top Chart)

Y-axis: Length (cm, in). X-axis: Age (Months).

Weight-for-age percentiles (Bottom Chart)

Y-axis: Weight (kg, lb). X-axis: Age (Months).

Mother's Stature _____			Gestational Age: _____ Weeks		Comment
Date	Age Birth	Weight	Length	Head Circ.	

Published May 30, 2000 (modified 4/20/01).

SOURCE: Developed by the National Center for Health Statistics in collaboration with the National Center for Chronic Disease Prevention and Health Promotion (2000). <http://www.cdc.gov/growthcharts>

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Appendix I: CDC Growth Chart for Birth to 36 months for Girls, Length-for-age and Weight-for-age percentiles.

Published May 30, 2000 (modified 4/20/01).
 SOURCE: Developed by the National Center for Health Statistics in collaboration with
 the National Center for Chronic Disease Prevention and Health Promotion (2000).
<http://www.cdc.gov/growthcharts>

Body mass index-for-age percentiles

Date	Age	Weight	Stature	BMI*	Comments

*To Calculate BMI: Weight (kg) ÷ Stature (cm)² × 10,000
or Weight (lb) ÷ Stature (in)² × 703

This chart displays BMI-for-age percentile curves for girls aged 2 to 20 years. The x-axis represents age in years from 2 to 20. The left y-axis shows BMI values from 12 to 27 kg/m², and the right y-axis shows corresponding BMI values from 12 to 35 kg/m². Curves are plotted for the following percentiles: 97, 95, 90, 85, 75, 50, 25, 10, and 5.

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