COGNITIVE AND GROWTH COMPARISON BETWEEN BREASTFED AND FORMULA FED INFANTS AT SIX AND NINE MONTHS OF AGE

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Bachelor of Science in Nutritional Sciences

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Bartlesville, Oklahoma

2010

Submitted to the Faculty of the
Graduate College of the
Oklahoma State University
in partial fulfillment of
the requirements for
the Degree of
MASTER OF SCIENCE
May, 2012

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CHAPTER I

INTRODUCTION

There is ample evidence supporting health benefits associated with breastfeeding. The composition of human milk, specifically colostrum and epidermal growth factor allow for a strengthened immune response that is less susceptible to infection and entry of antigens (Leung & Sauve, 1995). Obesity prevention, through improved self-regulation and delayed introduction of solid foods, also provides health benefits thought to carry over into adulthood (American Academy of Pediatrics (AAP), 2011; Armstrong & Reilly, 2002). Yet another benefit is the lowered risk of insulin-dependent diabetes. Studies have noted a relationship between early introduction of cow's milk and beta cell destruction in predisposed individuals through bovine serum albumin cross-reacting with beta cell surface proteins (Leung & Suave, 1995; Scott, 1995).

Differences in growth have also been seen with the different infant feeding approaches. Kathryn Dewey (1998) reviewed the literature and concluded that formula fed infants gain more weight in the first twelve months than do breast-fed infants. This claim is further supported by a study conducted by de Onis, Garza, Onyango, and Borghi (2007) comparing CDC growth standards to WHO growth standards. The Centers for Disease Control and Prevention (CDC) growth charts represents a national population of predominantly formula fed infants, while the World Health Organization (WHO) growth charts, as of 2006, represent predominantly breastfed infants. The study found U.S. children to have a higher weight-for-age and BMI-for-age than seen in the WHO growth standards; thus supporting formula fed infants gain weight more rapidly than their breastfed infants. Some correlate this difference in growth with individual control of satiety, which is noted as likely to be different between methods of feedings (Kavanagh et al., 2008). This difference in self-regulation has even been attributed to problems with weight control later seen in children (Kavanagh et al., 2008).

As previously mentioned, in 2006 the WHO introduced new growth guidelines. After review of the updated guidelines, the CDC has since supported professional use of these in children up to two years of age. The WHO is known to provide a growth standard for children in environments providing optimal conditions. Data included in the WHO growth charts represent locations around the world that met criteria to suggest optimal growth. The CDC growth charts are better known as a growth reference; they suggest the growth of a child from a particular region (U.S.) and time period (CDC, 2010). Data in the CDC growth charts through two years of age reflects a small sample size of children from the United States and lacks pertinent data, such as infant weight before three months of age. It reflects typical growth seen in children instead of optimal growth. Typical growth includes growth data from infant populations that may not be growing under optimal conditions, thus leading to potential misclassifications for the infant being compared.

Improved cognitive development is also associated with breastfeeding (Fergusson, Beautrais & Silva 1982; Rogan & Gladen, 1993). A meta-analysis assessing cognitive function in both breast and formula fed infants found not only was general cognitive function enhanced but breastfed children also had improved vision and enhanced motor skills at an earlier age (Anderson, Johnstone, & Remley, 1999). Cognitive development was assessed by a variety of standard cognitive tests. Some of the commonly used tests included the Bayley Mental Development Index, McCarthy Scales of Children's Abilities General Cognitive Index, Stanford-Binet Intelligence Scale, Wechsler Intelligence Scale, and Peabody Picture Vocabulary Test (Anderson et al., 1999). However, literature reviewing the effects of breastfeeding on cognitive development through nine months of age is limited and the available data are conflicting. This limitation is a reflection of inconsistency in both focus and purpose of the studies, and targeted age groups. An example of this is noted in an extensive review that focused on optimal duration of exclusive breastfeeding (Kramer & Kakuma, 2009). The objective was to "assess the effects on child health, growth, and development" of infants having been exclusively breastfed for six months compared to infants that incorporated mixed feeding after having been exclusively breastfed for three to four months (p. 4). The review focused primarily on the methodological quality and inclusion criteria. After having gathered close to 3,500 citations using specified search terms, 22 studies met the selection criteria of infants having been exclusively breastfed for at least three months prior to the introduction of mixed feeding. Part of the conflict found in literature comparing breast fed infants to formula fed infants stems from varying definitions used to classify mode of feeding along with varying durations of exclusive breastfeeding. As noted by Kramer and Kakuma (2009), "few studies strictly adhered to the World Health Organization's definition" of exclusive breastfeeding which excludes any use of supplemental foods or liquids other than human milk, vitamins, and medications (p. 4). In fact, some studies used the term so loosely as to include juices, teas, and infant formula. Some of the divergence of results may stem from the variation and interpretation of feeding categorization, but also in elapsed time between testing and initial data collection. For example, a study by Rogan and Gladen (1993) identified four groups: short, medium, long, and very long to denote breastfeeding duration. Each of the four groups used two categorical definitions in terms of weeks: mostly breast-fed and age at weaning. Cognitive assessment began at six months of age and continued until subjects turned five years. Fergusson, Beautrais, and Silva (1982) used three groups to classify breastfeeding duration: breast-fed more than four months, breast-fed less than four months, and bottle-fed. Assessment of cognitive development did not occur until age three and then again at ages five and seven. The principles found in each of the studies mirror one another. However, small changes such as the terminology used to define *"predominantly breastfed"* along with differences in time elapsed before cognitive assessment occurs may complicate interpretation of results.

As noted by Rose, Feldman, & Jankowski (2004a), there are several ways cognitive development can be assessed in infancy. Attention, described as a "precondition for any cognitive activity," can be assessed through look duration and changes in heart rate (p. 247; Richards, 1997). The shorter the look duration, the more sophisticated level of attention is present (Rose, Feldman, & Jankowski, 2004a). Interpretation of the novelty quotient also assists in understanding and targeting the development process. The calculated percentage reflects how much time was spent focusing on what is referred to as the novel stimulus versus the familiar stimulus. A higher percentage suggests greater time spent focusing on the novel stimulus because the familiar stimulus is locked in the memory; thus, attention can be placed on a new stimulus. For purposes of this study look duration, and novelty preference were used to assess cognitive development.

Research Question and Hypothesis

The general purpose of this study is to identify whether infants who are breastfed perform better cognitively than previously breastfed infants who begin formula feeding between three and nine months of age. Predominantly breastfed at three months is defined as receiving less than four fluid ounces of formula a day. At six and nine months, infants were classified as breastfed if women reported supplementing less than eight ounces of formula a day and breastfeeding more than four times a day.

The primary hypothesis of this study is that between three and nine months of age, breastfed babies will have improved growth and cognitive development compared to those that are formula fed. Additional hypotheses include:

I. Infants who are predominantly breastfed at nine months of age will have lower six and nine month BMI than infants who are no longer breast fed at both six and nine months of age.

II. Infants who are predominately breastfed at nine months of age will have a greater novelty quotient percentage and shorter looking times than infants who were no longer breast fed at both six and nine months of age.

The design of the study is cross-sectional and observational. The 130 infants included in the study were recruited from a rural Oklahoma community and its surrounding area. Inclusion criteria consisted of full-term, singleton birth, healthy, three-month-old infants predominately breastfed, having received less that four ounces of formula a day.

Growth was assessed using four recognizable anthropometric measures assessed at each infant appointment. Height was measured on a stadiometer to an accuracy of within 0.1 centimeters. Head circumference was measured using a non-stretchable plastic feed-through measuring tape placed around the thickest part of the head. Weight was measured on an infant digital scale designed to give an automatic average of multiples measures to an accuracy of within 0.02 kilograms (Seca, Columbia, MD, accuracy to 0.002 kg). Following anthropometric data collection, BMI is then calculated. All measures were converted into z-scores to compare between genders and categorized into one of three feeding groups for use in the mixed model ANOVA statistical analysis.

Cognitive development was assessed through analysis of the visual habituation procedure looking at the novelty quotient and duration on look time on a stimulus. The novelty quotient represents the average amount of time spent focusing on the novel stimulus. To calculate, it is the summation of total time spent looking at the novel stimulus in both trials divided by the sum of the duration of both trials (Colombo, Richman, Shaddy, Greenhoot, and Maikranz 2001). A shorter look is thought to reflecting a more mature level of development (Colombo et al., 2001).

Summary

Breastfeeding, the recommended nutrient source in early infancy, is widely known for the benefits it provides in terms of health, nutrition, and development. In a general sense, much of the data available that have addressed the effects of breastfeeding on growth and development trend in the same direction. However, few studies look at duration of breastfeeding in comparison to both growth and cognitive development longitudinally in early infancy.

CHAPTER II

LITERATURE REVIEW

Section 1 – Infant Feeding Recommendations

Exclusive breastfeeding is recommended through the first six months of life to provide the necessary nutrients for optimal growth and development in a healthy infant population (American Academy of Pediatrics (AAP), 2005; World Health Organization [WHO], 2011). The WHO defines exclusive breastfeeding as nothing, water included, but breast milk, aside from vitamin and mineral supplementation in the first six months (WHO, 2011). In the early stages of breastfeeding, the AAP (2005) suggests eight to 12 feedings per 24 hours. This number will fluctuate with time, especially in growth spurts. Intake of human milk will decline as complementary foods, rich in iron, are gradually introduced beginning around six months. Continuation of breastfeeding is recommended through the first year, keeping in mind that the AAP has no set upper limits on the duration.

Health professionals consider exclusive breast-feeding with supplements of Vitamin D, iron, and fluoride to provide a complete balanced diet and be the standard of feeding practices for infants up to at least six months of age (Centers for Disease Control and Prevention [CDC], 2010; Fomon et al., 1979). In 2010 the AAP released iron recommendations of supplementing one mg/kg/day in healthy term breastfed infants around four months of age to reduce the risk of iron deficiency prior to the introduction of iron-fortified complementary foods. Prior to four months, human milk is thought to provide infants with adequate iron. This iron recommendation increases to 11 mg/d for infants between six and 12 months of age. In cases where iron recommendations fail to be met through complementary foods, the AAP approves use of liquid iron supplementation. Formula-fed infants do not require an additional iron supplement as this is generally included in the formula and accounted for when complementary foods are incorporated into the diet (AAP, 2010). The vitamin D requirement has been controversial through the years and is included in the revision of the AAP guidelines. As of 2008, the AAP has upped its recommendation to 400 IU/mL of supplemental vitamin D daily. The typical 25 IU/L or less of vitamin D found in human milk alone does not contain the recommended amount for breastfed infants (AAP, 2008; CDC, 2010;).

Breastfeeding initiation in the United States met the *Healthy People 2010* objective of 75% of new mothers in the U.S. starting out breastfeeding (CDC, 2010). Therefore, the issue of low breastfeeding percentages with a duration of 12 months of age in the U.S. is not from lack of interest as reflected by a high initiation rate from mothers. The *CDC Breastfeeding Report Card*, a compilation of state data collected for Healthy People 2010 objective analysis, provides the following statistics in regard to breastfeeding practices analyzed at both the national and state level (CDC, 2010). For purposes of this study, Oklahoma is the only state included below. Additional reference data is available for all other states.

Table 1: CDC U.S. 2010 Breastfeeding Report Card

	Nationally (%)	**Oklahoma (%)**
Ever breastfed	75	65
Breastfeeding at 6 months	43	27.4
Breastfeeding at 12 months	22.4	12.2
Exclusively breastfeeding at 3 months	33	22.6
Exclusively breastfeeding at 6 months	13.3	6.7

(CDC, 2010)

According to the CDC 2010 *Breastfeeding Report Card*, less than thirty percent of Oklahoma babies are breastfed at six months of age (accounting for children born up to 2007) (CDC, 2010).

Breastfeeding rates drop off at three months, with an even greater drop off at six and nine months. Such statistics are indicative of the country not providing adequate support for mothers over time. As complications or questions concerning breastfeeding arise, support is crucial for the continuation of feeding. Another plausible cause for such stagnant percentages starting around three months of age and continuing on a pattern of decline, is likely from lack of knowledge pertaining to increased benefits associated with breast-feeding: nutrition, immunity, weight control, and cognition, all increased with duration of breastfeeding. The CDC does report increasing breastfeeding duration through increased support initiatives as the next stage of objectives to increase breastfeeding percentages nationally (CDC, 2010).

Infant formula has made significant advances toward mimicking the composition of human milk. For example, prebiotics and probiotics were recently added to formula compositions serving to promote gut flora production similar to that of breastfed infants (Morrow, 2011). Despite these efforts towards similarity, there are continued differences between the two. It all comes down to human milk being the natural form of what formula strives to be. Unlike formula, the composition of breast milk, including lipid and protein, changes parallel to infant maturation (Huërou-Luron, Blat, & Boudry, 2010). It has been suggested that with the exception of vitamins D and K, human milk is comprised of the proper amounts of macro and micronutrients for an infant to reach optimal growth until six months of age (Leung & Sauve, 2005). Formula is designed using a specific list of nutrients, recognized as beneficial for optimal growth and development of the infant (Koletzko et al., 2005). It is formulated to reflect the composition of breast milk seen at an advanced gestational age (Huërou-Luron, Blat, & Boudry, 2010).

Caloric intake is yet another likely difference between the two feeding methods. Dewey and Lönnerdal (1986) suggest the infant controls energy intake through instinctive self-regulation as described by “the wide range in breast milk volume in relatively well-nourished populations appear[ing] to be due more to infant ‘demand’ than to adequacy of milk production by mothers” (p.897). Little interference or forceful feeding takes place in such a setting. Dewey, Heinig, Nommsen, Peerson, and Lönnerdal (1992) observed that between six and nine months of age, breastfed infants eat a smaller portion of food offered to them, indicating breastfed infants are able to self-regulate not only breast milk but solid foods. Overfeeding on the other hand becomes a risk when using formula, as caregivers may interfere with the infant's ability to self-regulate (Kavanagh, Cohen, Heinig, & Dewey, 2008). This can be through distraction, not wanting to waste formula, or by simply not picking up on the infant's cue to stop feeding. It becomes problematic when done repeatedly, "caus[ing] them [the infant] to lose the ability to precisely self-regulate energy intake, which would explain differences in intake between breastfed and formula-fed infants" (p. 245). Kavanagh et al. (2008) observed evidence suggesting formula-fed infants are 20% to 30% more likely be to overweight as children and adolescents.

Kavanagh et al. conducted a study in 2008 that examined the effect of interceding with education to change the caregiver's feeding practices. This double-blind, randomized trial included a sample size of 38 (18% of the original sample size) Women, Infants, and Children (WIC) participants who were divided into either a control or intervention group. After having met the inclusion criteria, baseline anthropometry measures and behavioral practices were collected, as well as a 48-hour record of formula intake. Through one 45 to 60 minute session, both groups received general infant feeding guidelines, but the intervention group received more specified guidelines (Kavanagh et al., 2008).

The study did not support the hypothesis of increasing caregiver education to alter feeding methods. In fact, the intervention group's infants had significantly increased weight and length gain compared to that of the control group (Kavanagh et al., 2008). As this was the first study to look at the effects of an educational intervention in relation to behavioral change in infant feeding practices, there were some limitations that may have attributed to such results. The study ultimately neglected to incorporate a key and final component of the nutrition care process:

monitoring and evaluation. Kavanagh et al (2008), suggests results of the study stem from improper presentation that resulted in lack of comprehension of key points linked with change.

Section 2 – Assessment of Growth

Anthropometric measures that reflect nutritional status are not limited to just one measure, but rather include a group of measures. Assessment of weight reflects more recent changes in nutritional status, and linear stature depicts more long-term changes in nutritional status (Mascarenhas, Zemel, & Stallings, 1998). Coupled measures, weight-for-height for example, are used not only to identify growth trends, but also to detect possible signs of malnutrition as identified through wasting. Head circumference is also included in the standard growth assessment and typically continues through three years age because of how quickly the brain is developing during this time (Mascarenhas et al., 1998). Record of these measures allow for early intervention at any sign of unusual patterns.

Gokhale and Kirschner (2003) discuss what to expect in terms of phase dependent growth, highlighting the importance of consecutive measures rather than single measures for data interpretation. Although growth is by no means a fixed process, there are some general trends to look for in terms of growth assessment throughout infancy. Healthy infants commonly "double their birth weight by five months of age and triple their weight by one year of age" (p. 154). The mean increase in length is said to be about 25 cm in the first year of life. A less sensitive measure of growth, head circumference, while averaging around 35 cm at birth, will increase by 12 cm in the first year of life (Gokhale & Kirschner, 2003).

Nelson, Rogers, Ziegler, and Fomon (1989) conducted a study looking at growth in early infancy, specifically throughout the first three and a half months of life. The study included both breast (n= 419) and formula fed (n=720) infants. A generally even distribution of males and females in both groups between the years 1965 and 1987 was represented. Study compliance included seven periods of measurement distributed over the 112 days post birth. Breastfeeding was defined as less than 240 mL/day of milk-based formula. Results of the study found males to be significantly heavier and longer than females. As for mode of feeding, formula-fed males were significantly larger in overall size (both weight and length) than breast-fed males. With exception to the Darling Study, which finds no difference between gender when comparing breastfed to formula fed, such findings are consistent with results of previous studies suggesting males gain weight earlier than females and breast fed infants typically weigh less than formula-fed infants (Dewey et al., 1992; Nelson, Rogers, Ziegler, & Fomon, 1989). A possible explanation for the Darling study may come from changes in formula composition over time.

New growth charts released by the WHO in 2006 prove a reliable measure of growth zero to 24 months due to thorough attention paid to methods when collecting data (de Onis, Garza, Onyango, & Borghi, 2007). The WHO data sample represents six countries, the United States included, of predominantly breastfed children. Feeding guidelines for those included in the sample followed WHO and AAP feeding recommendations; these include breastfeeding for at least four months, introducing complementary foods by six months, and breastfeeding continued through at least 12 months of age. The CDC growth chart is based on a smaller sample and came from a series of nationally based surveys. Breastfeeding was not a requirement for inclusion and in fact few reported breastfeeding longer than a few months. Unlike the CDC standards, which also lack growth data in the first two months, the WHO collected growth data 21 times during the two-year collection period. This allowed for documentation of the significant growth changes that occur in early infancy. When comparing growth charts and looking at z-score differences in weight-for-age, length-for-age, weight-for-length, weight-for-height- and BMI, de Onis et al. (2007) found U.S. children were significantly heavier than reflected in the WHO sample. The variance between charts is likely a result of the differences in design between growth charts. Some of these differences include feeding practices and overall precision of data collected. Understanding of such differences is key to providing useful interpretation of growth in children. For example, the WHO growth chart will likely show a decreased presence of malnutrition, but an increased estimation of overweight and obesity (Grummer-Strawn, Reinold, & Krebs, 2010).

In an article released by the CDC in 2010 that reviewed the use of WHO and CDC growth charts, they suggested use of modified WHO growth charts for U.S. children zero to 24 months (Grummer-Strawn et al., 2010).

Assessment of growth, which occurs at an exponential rate in infancy, is essential when tracking nutritional problems. Growth is recorded not only in weight and length, but head circumference as well due to brain development during this time. Identification of skewed growth patterns allows for further evaluation resulting in interventions to correct problems before long-term complications arise. Growth charts are available to assist in evaluating an infant's rate of growth in relation to growth of other infants. When using growth charts it is helpful to understand the primary differences between WHO and CDC growth charts. The CDC growth chart offers a growth reference for children based on a particular time period for children in the U.S. The WHO growth chart provides a growth standard for children worldwide living in environments that allow for optimal growth (Grummer-Strawn et al., 2010).

Section 3 – Assessment of Development

A more general form used to assess development, The Bayley Scale of Infant Development (BSID), is a recognized standard used to assess the current development level of young children from birth to three years of age (Nellis & Gridley, 1994). It is a scale frequently used in studies addressing issues of cognitive development early in life. The scale assesses multiple components, thereby allowing for a comprehensive evaluation and interpretation of a child's progress. The latest version of the scale includes five divisions recognized in for their involvement in the developmental process; they include: cognitive, language, motor, social-environment, and adaptive behavior (Albers, & Grieve, 2006). Providing a developmental standard allows for increased functionality through the recognition of accomplished areas of development, pinpointing areas of developmental delay, and identifying interventions appropriate for the targeted delay (Nellis & Gridley, 1994). Despite revisions made to improve the scale, it has continued with the original purpose, providing a standard resource representing the common level of development in children of this age range (Nellis and Gridley, 1994).

As described by Rose, Feldman, and Jankowski (2004b), infant visual recognition reflects cognitive development through its role in memory and novelty preference. Two major assessment techniques include visual paired comparison (VPC) and habituation. The VPC technique is conducted by presenting a stimulus (or pair of stimuli), recognized as the familiarized stimulus, followed by presentation of a new stimulus to test the infant's recognition ability based off of an infant's preference for a novel stimulus. Novelty preference over the familiarized stimulus comes from the "observation that attention wanes with the repetition of an event and recovers when the event changes" (p. 76). Habituation, the second major technique described, presents the same stimulus until attention becomes disengaged. This disengagement is defined differently throughout studies, but typically includes a decline in time spent looking at the stimulus; at which point a new stimulus is presented. Regained attention, now focused on the new stimulus, is how memory is then measured. The time spent processing is not static; it is said to decrease, as infants get older. More specifically, recognition memory is thought to systematically increase between three and 12 months of age (Rose et al., 2004b).

Attention, found in any component of cognitive development, and a key factor needed for execution of visual recognition is prominently described through changes in heart rate coupled with looking at the stimulus (Rose et al., 2004b). Those described as short lookers, who fall below the median of look duration in a group, typically have shorter look duration but an increased number of shift gazes on the stimulus. Decreased looking time is also reflected in greater memory. Long lookers, those with reported a look time above the group median, typically spend more time processing, with fewer shift gazes. As Rose et al. (2004b) describes, "their looks tend to be more narrowly distributed" (p. 89). Longer look durations and slower processing speeds have been associated with lower IQ and language cognitive abilities seen later in childhood (Rose et al., 2004b).

Visual habituation in infancy, assessed through varying procedures, is thought to reflect one of many components involved in the complex process "for learning and cognition upon which higher-order functions are constructed across early childhood" (Colombo & Mitchell, 2009, p 230). One way to test this is through paired-comparison trials as was done by Colombo, Richman, Shaddy, Greenhoot, and Maikranz (2001). This study tested 72 four-month-old infants with the purpose of assessing the relationship between recognition performance with look duration. Seated in a car seat with electrodes strategically placed for electrocardiogram measure, infants were presented with a face as the stimulus in both the pretest and familiarization period. The face remained on the screen until the infant accrued 20 seconds of looking at the stimulus, as the primary measure in testing was peak look duration. Assuming successful completion of familiarization period, the infant was then presented with two stimuli, the now familiarized stimuli plus a novel stimuli, placed parallel to one another for the choice trial section of testing that was conducted twice. Infants were randomly given either a five or 15-second look duration in the initial choice trial before stimuli were repositioned for the second trial, again using the same allotted look time as the first trial. To calculate novelty preference, which is the percentage of time spent looking at the novel stimulus versus the familiar stimulus, they summed the total time spent looking at the novel stimulus in both trials and divided it by the sum of the duration of both trials. Results of the study reported 55.2% as the mean novelty preference; suggesting recognition was not by chance. Those with a shorter look time were more likely to show recognition of the novel stimulus than their counterparts of longer lookers. In keeping with the hypothesis, researchers found no supporting evidence in trial length to increase performance for long-looking infants, as infants in both the five-second and 15-second choice trials were able to show preference for the novel stimulus. Thus, indicating "the response to the stimulus novelty within the paired-comparison paradigm [to be] initiated over a relatively rapid time course" (p. 1613). This supports the evidence of previous work suggesting shorter look durations to be equivalent with higher levels of cognitive performance outcomes (Colombo et al., 2001).

Assessment of development allows for a variety of age-appropriate techniques. As a child ages it is expected that time spent in initial cognitive processes will decrease, thus reflecting an increased processing speed. Many of the procedures used to assess development allow for modification dependent upon available environment and equipment. While many studies use a computer screen to present the stimuli another study used a make-shift sliding door to present and observe testing of visual information processing (Colombo & Mitchell, 2009; Rose, Jankowski, & Feldman, 2002; Kennedy et al., 2008).

Section 4 – Growth Related to Feeding

In the DARLING (Davis Area Research on Lactation, Infant, Nutrition, and Growth) study, Dewey et al. (1992) examined the difference in growth patterns of infants breast-fed (n=46) having followed the AAP feeding recommendations to infants predominantly formula-fed (n=41). They found the weight of formula fed infants to be significantly greater for both males and females than seen in breast-fed infants. Formula-fed males were significantly longer than breast-fed males ($p < 0.05$). No difference was found in the length of females. Head circumference throughout the 18-month time span was relatively constant between groups. Z score analysis showed the mean weight-for-length score to be noticeably less than seen in formula-fed infants; further supporting the known difference in weight, but not length noted in the different feeding practices. Between group weight differences was not evident until the six-month mark, when solid foods are introduced (Dewey et al., 1992). This is likely linked to differences in how breast-fed babies and formula-fed babies self-regulate consumption. The article concludes that the lack of rapid growth found in breast-fed infants is nothing of concern, but rather is likely a normal pattern of growth. The authors go on to suggest the findings of the study to be consistent with the findings of studies using similar socioeconomic populations.

Dewey (1998) reviews 19 studies, done after 1980, focused on the comparison of growth in breast-fed and formula-fed infants of affluent populations in their first 12 months of life. Each feeding group had a minimum of 20 subjects. Some studies included infants in their breastfeeding groups if any amount of breast milk was consumed, with no regard to formula supplementation. Others followed a more stringent classification process and considered an infant to be included in a breastfeeding group if the infant was exclusively breastfed or breastfed for at least a portion of the measured time. Although little difference in linear growth between different modes of feeding was observed (seven of 17 included studies), a substantial difference in weight gain was seen. In fact, “nearly all of the 19 studies indicate that formula-fed infants gain more weight than breastfed infants during the first year of life,” even after the introduction of solid foods which happens around four to six months of age (Dewey, 1998, p. 99). This difference was typically more prominent after four months, and specifically between the six to 12 month age marks. Of the 19 articles, the seven that reported on head circumference found no consistent difference between mode of feeding and head size. Review of the 19 studies alludes to a distinct difference in growth between breastfed and formula-fed infants, with particular emphasis on "a longer duration of breastfeeding [to be] associated with greater decline in weight-for-age z-scores during the first 12 months" (1998, p. 100). Results should be interpreted with caution, as the definition of breastfeeding was noticeably different between studies.

An observational cohort study completed by Kramer et al. (2004) studied the effects of varying feeding practices on infant growth throughout the first year of life. This large study of close to 17,000 healthy infants, included infants recruited from medical facilities in the surrounding republic of Belarus. Methods came from the PROBIT (Promotion of Breastfeeding Intervention Trial) study, and included an experimental (exposure to a breastfeeding intervention) and control (followed standard clinic practices) group. Infant data was collected at six different visits over the 12-month time period. During these visits, assessments of feeding practices, onset of illnesses, and anthropometric measures were collected. The experimental group followed infant feeding recommendations supported by the WHO and CDC. This includes exclusive breastfeeding for the first four to six months while continuing on with partial feedings through at least the first year, and longer if desired. Results of the study found the weight-for-length, length-for-age z scores, and head circumference of formula fed infants to be significantly greater than breastfed infants around the six-month mark. Head circumference, a measure typically of inconsistent or insignificant findings, was reported as significantly smaller between the nine and 12 month age period in infants receiving formula and milk sources outside of breast milk. The study supports results of previous studies, which suggests formula fed infants grow at an increased rate, predominantly after three-months of age, than is seen in breastfed infants. Although further longitudinal data is needed to support such inferences, the study suggests breastfed babies may be at a reduced risk for obesity later in life. They attribute this to the early growth differences found in breast and formula fed babies. The authors proposed three explanations for this difference in growth between groups: level of appetite control, differences in rate of energy metabolism, followed by rate of adipocyte proliferation, a likely result the two former explanations (Kramer et al., 2004).

A study conducted in the United Kingdom interested in further analysis of the effects of infant feeding practices in childhood, used the Millennium Cohort Study (MCS) to assess 10,533 healthy, predominantly white, singleton birth infants (Griffiths, Smeeth, Hawkins, Cole, & Dezateux, 2009). The study collected data in two separate settings. At approximately nine months of age the initial home interview began with collection of feeding practices data and infant birth weight. The second interview, at three years of age, included anthropometric measure. To classify feeding practices, they followed the WHO recommendations for exclusive breastfeeding, or were classified as predominantly breastfed if, having not reported formula use, breastfed with inclusion of clear sugar-free liquids prior to recommendation. Despite more than half having reported breastfeeding initiation (68%), the majority did not continue breastfeeding past four months (60%) (Griffiths et al., 2009). The study also reports introduction of solid foods was initiated early for about two-fifths of the sample included. Results of the study found a small but significant difference of 0.22 kg in weight gain between those breastfed a minimum of four months and those breastfed less than four months. The difference was even smaller (0.16 kg) between those breastfed a minimum of four months and those never breastfed. Having adjusting for height, early introduction of solid foods did not report significance in weight gain that controlled for gender, age, and birth weight, through three years. Griffiths and colleagues go on to explain this suggests infants are “heavier but not fatter at 3 years of age” (p. 579-580). At three years of age, those given supplemental feedings gained more weight rapidly than those breastfed a minimum of four months. Such findings support claims of breastfeeding impacting growth beyond infancy. Although the reported individual differences are small, the article suggests such findings are important at a population level considering it could be a modifiable risk factor in the future, but after looking at additional evidence from future longitudinal studies (Griffiths et al., 2009).

Substantial evidence suggests a difference in growth patterns between breast and formula fed infants. The majority of studies report formula fed infants gain weight more rapidly than breast fed infants. Studies comparing sex differences typically find there to be a greater difference in males than females (Dewey et al., 1992). Few studies report a difference in head circumference between feeding patterns although one study did find formula fed infants between three and six months to have a marginally larger head circumference. However, this minor increase diminished between nine and 12 months when formula fed infants reported a head circumference smaller than breastfed infants (Kramer et al., 2004). This difference may be related to breastfed infants being better able to self-regulate energy consumption, although there is still a need for further evidence to support such assumption. The difference in weight gain has highlighted concerns related to childhood obesity, but again further evidence is needed.

Section 5 – Development Related to Feeding

In 1993, Rogan and Gladen completed a longitudinal study that examined infant feeding mode in relation to later cognitive performance of 383 primarily white middle-class subjects already enrolled in a prospective study between 1978 and 1982. Participants were categorized into five different groups based on the mother’s reported feeding mode and duration (if breastfed). Development was measured using the Bayley Scales, McCarthy Scales, and school report cards of third grade and later. Multiple regression technique was used to examine the relationship based on each of the cognitive test scores to duration of breastfeeding.

The results of the study showed an overall trend toward greater scores in those that were breastfed in each of the varying subscales incorporated into the study. The psychomotor development section of the Bayley scales of infant development indicated those mostly breastfed for 20 plus weeks exceeded those mostly breastfed less than or equal to four weeks by two to four points. The McCarthy scale showed overall "trends towards higher scores with increasing length of breastfeeding, but the relationship was weakest for motor scale" (p. 186). Grade card analysis found breastfed subjects to show a grade increase for both English and math. The increase in math grades was of no statistical significance but was of a marginal level for English (Rogan & Gladen, 1993).

Several studies to date have compared the association of feeding mode to cognitive development throughout childhood (Fergusson et al., 1982). Results sometimes become shadowed when including the multiple number of variables thought to affect such outcomes outside of feeding mode. Here Rogan and Gladen (1993) made a strong attempt to control for the child's age when testing was administered and type of cognitive measures issued. They also controlled for the extensive list of confounding variables typically included in former studies.

A previously mentioned study by Kramer et al. (2004), reported on growth related to feeding practices in the first year of life. Using the same methods and population from the PROBIT study Kramer et al. (2008) conducted another study, this time looking at the relation between duration of breastfeeding and cognitive development later in life. Ethically children could not be randomized on feeding practices, but were instead randomized into experimental and control groups included in the PROBIT study. The experimental group incorporated resources within the hospitals that encouraged continued breastfeeding according to the Baby-Friendly Hospital Initiative created by UNICEF and WHO. Thus, the control group made no modifications to infant feeding practices supported by the hospital. The PROBIT study found the intervention to be successful as the experimental group had greater breastfeeding durations than the control group through to the first year. Participants were then evaluated at six and a half years of age using the age appropriate Wechsler Abbreviated Scales of Intelligence (WASI) cognitive development scale. School subjects were also evaluated from teachers' blind to the study for those 75% enrolled in school. Results of the study found a general trend of higher IQ points in the experimental group in both the WASI and teacher ratings. For example, the experimental group was seven and a half points higher in verbal IQ. As for duration of breastfeeding, results indicated overall higher scores associated with prolonged duration and exclusivity of breastfeeding. Following exclusive breastfeeding from three to greater than or equal to six months identified a 4.7 greater verbal IQ score than for those that exclusively breastfed for less than three months. Not all areas of testing, like that of nonverbal IQ, found a significant difference in scores. In both teacher ratings and the WASI scale, verbal measures reported greater significance than seen in performance measures.

Whether it is the contents of the milk, outside factors, or both associated with the differences of IQ scores between those exclusively breastfed for a longer period of time is still up for debate. This topic is discussed further by Kramer et al. (2008), who suggested enhanced cognitive development may come from interactions of the mother and child during feedings. Another possibility researched is a difference in specific nutrients and hormones found in higher concentrations in breast milk. Two of these include the insulin growth factor I and docosahexaenoic acid (Kramer et al., 2008).

Docosahexaenoic acid (DHA), a natural component of breast milk, has been suggested to be essential for early cognitive development. In a recent study, long chain polyunsaturated fatty acids (LCPUFA), with specific interest in DHA, were assessed for their role in cognitive development in nine-month old infants (Drover, Hoffman, Castañeda, Morale, & Birch, 2009). Researchers used means-end problems solving, which is the ability to complete a task in a sequential manner, to assess infant level of cognition at the nine-month old visit. The means-end problem solving testing procedure has been associated with predicting later IQ and vocabulary skill level around the three-year age mark. Understanding previous studies have found varied results, this study controlled for limitations noted in those previous studies. Here the DHA supplement was closer to the level found in human milk where in some of the previous studies this percentage was much lower. They also assessed the infants in a timely manner relative to DHA supplementation, allowing for less room for error between time of testing and time of supplementation. The 229 healthy, singleton-birth infants included in the study came from three different randomized controlled, double blind trials in surrounding Dallas areas. In each study infants were randomly assigned to either a LCPUFA formula-supplemented or control group. To keep the three studies separate, Dover and colleagues identified them as a 12-month feeding study, a six-week weaning study, and a four to six month weaning study. The last two of the three studies included breastfeeding infants. The six-week weaning study included a breastfeeding period for the first six weeks at which point infants were then transitioned into formula feeding. The second breastfeeding group was breastfed four to six months prior to weaning and transiting over to formula feeding. At nine months of age, infants were tested on their ability to perform a consecutive series of six tasks, three separate times and given a score based on the infant's level of intention for each particular task. Results of the study found no difference between diets for infants in the four to six month weaning study group. The six-week weaning study and 12-month feeding study found those in the LCPUFA formula-supplemented group to have a significantly higher percentage of success in completing all three trials. Specifically, the six-week weaning study found 46% in the LCPUFA group compared to 13% in the control trial to have successful completion in all three trials. The 12-month feeding study found 51% of the LCPUFA group to have successful completion compared to 29% in the control group. The six-week weaning study also found the LCPUFA group to have a significantly higher percentage (35%) of perfect intention scores compared the control (seven percent). In summary, results from the study suggest DHA supplementation of .36% of LCPUFA to have a positive effect on infant cognition. With DHA being a natural component of human milk and the longer duration of breastfeeding group reporting no differences in problem solving between diets, there is consideration of the benefits not being limited to the supplementation form of LCPUFA (Drover et al., 2009).

In a study by Kennedy et al., (2008) using adapted visual information processing (VIP) as an assessment measure; growth was assessed in relation to cognitive abilities of infants from Southern Ethiopia. The study included 69 six to eight month old infants who completed 75% of the VIP trials. Each trial consisted of a familiarization phase plus two test phases, for presentation of the novelty stimulus. The primary variables assessed during testing were duration of look and frequency of shifts. Anthropometric measures were collected using standardized techniques. The study hypothesized poor infant growth would negatively affect VIP trial outcomes. Of the anthropometric measures included, weight-for-age and head circumference showed significant relations with VIP. Longer look duration during familiarization was reported in smaller weight and length-for-age infants. Considering the majority of infants were below the mean weight of children in developed countries, it is possible that such findings relate to immediate nutritional status, as weight is an indication of short rather than long-term status (Kennedy et al., 2008).

Cognitive development continues to show a level of difference between modes of feeding. There is an overall trend of breastfed infants to perform better than formula fed infants. The degree of performance difference is reported higher some studies than in others. However large or small the degree of difference it still favors those that were breast fed over those that were formula fed. The composition of breast milk and its nutrients like DHA, remains of interest in its effect on the brain and hence cognitive development.

Section 6 – Summary

There are multiple positives associated with breastfeeding, but still the U.S. fails to meet infant feeding recommendations as outlined by the WHO and CDC. Growth differences between breast and formula fed infants used to be more difficult to interpret due to growth charts based on the reference data coming from formula fed infants. However, after these charts were updated with a reference population following breastfeeding recommendations these differences continued. Cognitive development too shows differences between those formula fed and those breastfed. The differences in general follow similar patterns, but within group differences still exist and the specific mechanism for the cognitive differences has not been determined.

CHAPTER III

METHODOLOGY

Section 1 – Background and Design

This study examined the relation between duration of breastfeeding, cognitive development, and growth of infants between three and nine months of age. The design of the study was longitudinal and observational. It was part of a larger study approved by the United States Department of Agriculture. The independent variables compared were based on the infant's duration of breastfeeding. They were divided into three groups: breastfed at nine months, stopped breastfeeding between six and nine months, and stopped breastfeeding between three and six months. The outcome variables included anthropometric z-scores (weight-for-age, length-for-age, weight-for-length, body mass index, head circumference), novelty quotient, longest look, number of looks, average duration of looks, and total duration of looks. Potential confounding factors included infant gender, age of mother, and other maternal factors including employment status, race, education, marital status, as well as number of children in the family. The Oklahoma State University Review Board approved the study's methods and procedures.

Section 2 – Sample

Of the 132 infants tested, 111 were included in the study. Inclusion criteria of the study required healthy, full-term, singleton birth infants that were predominantly breastfed for their first three months. Predominantly breastfed was defined as consuming less than 28 fluid ounces of formula a week. The 21 eliminated participants were each accounted for and the reason for exclusion will be discussed in the results section. Generally elimination resulted from incomplete follow-up visits over the period of nine months.

Section 3 – Procedures:

Participation included three visits, one at each three, six, and nine month age mark (+/- 2 weeks). At each visit anthropometric measures and visual information processing procedures were conducted. Aside from written permission, procedures were explained throughout the sessions with clear understanding that any procedure would be stopped at the request of the mother at any point in time. The Adult-Adolescent Parenting Inventory (AAPI) test form and a demographic questionnaire were both administered at the 3-month visit. Multiple responses were taken from these questionnaires for assessment of confounding variables, which include: race, infant gender, marital status, income and education level, number of children, and employment status.

Section 4 – Anthropometry

At the end of each visit infants' growth was assessed using basic anthropometric measures. Length was measured on an infant length board (Shorr Production, Olney MD, accuracy to 0.1 cm). This was done twice to assure accuracy. A third measure was taken if the first two measures were not within one centimeter of one another. The outlier was discarded before the two measures were averaged. Two research assistants were used to properly measure the infant's length and results were then recorded. The measurer assured the infant was flat on the board with his or her head against the base of the board by using one hand to firmly press on the infant's knees to fully extend legs for an accurate measure. The assistant stabilized the infant's head against the top of the board. Head circumference was measured using a non-stretchable plastic feed-through measuring tape placed above the eyebrows and ears around the thickest part of the head. Weight was measured on an infant digital scale designed to give an automatic average of multiple measures (Seca, Columbia, MD, accuracy to 0.002 kg). The infant was weighed in light clothing and a dry diaper. Following anthropometric data collection, BMI and z-scores, using the obtained measurements, were calculated using the WHO Anthro software.

Section 5 – Dietary Assessment

At the three-month visit mothers were asked to complete a 24-hour food recall using the documentation sheet sent home with them following the visit. Food recall instructions were provided and information was collected within a week from the initial visit. All participants were loaned a portable infant scale for mothers to weigh the infant before and after each feeding. Time of feedings and amount consumed if using a formula were also recorded in the recall. A comprehensive semi-quantitative dietary questionnaire regarding changes in infant feeding practices between the previous and current visit was recorded at the six and nine month visits. The questionnaire provides documentation of whether the infant was still exclusively breastfed, infant formula use, estimated time period of solid food introduction, estimated frequency of formula or solid food consumption, and any use of supplements.

Section 6 – Visual Habituation

At the start of each visit visual habituation was assessed. Testing preparation began with infants securely placed in a car seat with their mothers standing directly behind them as an additional precautionary measure. A 22-inch wide computer monitor was placed directly in front of the infant to view the static stimuli. Behind the monitor, a black curtain was hung to eliminate potential distractions and to disguise the video camera used to record the infant's look. Cables connected the video camera to the screen used in the observation viewing room adjacent to the procedure room. Testing procedures followed those outlined by Colombo, Richman, Shaddy, Greenhoot, and Maikranz (2001). To begin infants would first participate in what is known as the familiarization phase. Here they were presented with a single, static stimulus of a randomly selected face in which they would need a look of at least one second before proceeding further. The infant continued in this phase until habituation criterion was met. Habituation was considered two consecutive looks focused on the stimulus, at half the time of the mean of the two longest looks (Colombo et al., 2004). The familiar stimulus was then paired with a novel stimulus for two trials, each requiring 10 seconds of accumulated looking at the three-month visit. This time period was reduced to five seconds of accumulated looking at the six and nine month visits. Throughout testing an experimenter would code the infant's looks toward or away from the stimulus using a computer mouse and the program would then record these times.

Section 7 - Statistical Analyses

Means, frequencies, and descriptive statistics were assessed for all variables including infant feeding patterns and introduction of solid foods. A mixed model ANOVA was be conducted for each dependent variable included in the analysis of growth and cognitive performance. The three different feeding groups at nine months of age: breastfed, formula fed for six months, and formula fed for three months were the between-subject factors. The comparison at three, six, and nine months was the within-subject factor. A mixed model MANOVA was then used to analyze any relationships between dependent variables without change to the independent variables. After running the variables without covariates, weight-for-age at three months was then included to assess any significance with this included.

CHAPTER IV

RESULTS

In analyzing the results of the study the participants were divided into three feeding groups based upon the mothers' reported breastfeeding responses as reported on the six and nine month dietary questionnaires. Research assistants reviewed the questionnaires to confirm that the responses used met the study's criteria of breastfeeding less than or equal to four ounces per day. The subject infants in the study were classified into one of three feeding groups: breastfed at nine months, stopped breastfeeding between six and nine months, and stopped breastfeeding between three and six months. The study concluded with a comparison of differences in anthropometry and visual habituation measures between the three feeding groups.

The sample consisted of 111 infants, with 132 tested. Twenty-one infants were excluded as a result of failure to complete the longitudinal trial. In comparing those excluded with those left in the sample, there was no difference in maternal race (chi square, $p = 0.787$), education (chi square, $p = 0.194$), income level (chi square, $p = 0.340$), age (t-test, $p = 0.953$), number of children (t-test, $p = 0.475$), or infant gender (chi square, $p = 0.965$).

Section 1 –Maternal Characteristics of the Study Sample

Table 1 below presents the maternal characteristics of this sample. The mean age of women was 28.3 ± standard deviation of 4.4 and a range of 19 to 42 years. The majority of women were white (88.3%), married (90.2%), and well educated (67.5% $\geq$ college graduate). One participant reported separated and was thus placed into the divorced group under marital status.

Table 1: Maternal Characteristics (*n*= 111)

Variable	Number	Percentage
Race		
White	98	88.3%
Hispanic	2	1.8%
Native American	8	7.2%
Asian	2	1.8%
Black	1	0.9%
Education		
Less than High School Diploma	1	0.9%
High School Graduate	4	3.6%
Some College	31	27.9%
College Graduate	29	26.1%
Post Graduate or Above	46	41.4%
Employment		
Employed Full-Time	31	27.9%
Employed Part-Time	18	16.2%
Unemployed	60	54.1%
Retired	2	1.8%
Annual Household Income^		
Under $15,000	13	12%
$15,001 - $25,000	17	15.7%
$25,001 - $40,000	22	20.4%
$40,001 - $60,000	27	25%
Over $60,000	29	26.9%
Marital Status		
Married	101	91%
Unmarried	7	6.3%
Divorced/Separated	3	2.7%

^Data not reported by three subjects

Section 2 – Sample Characteristics

The infants of this sample included 57.7% females and 42.3% males. Tables two through five below describe the characteristics of the study sample. The distribution for number of children per family was skewed so mean and then the frequencies were reported. The mean reflected about two (1.8 ± 0.9) children per family. However, when assessed using frequency the number of children was overwhelmingly closer to one child per family (46.8%). Overall children included in the study were considered healthy (97.3%) as reported by the mother. Only two infants were reported as unhealthy prior to the study, but were still included as they were of good health at the time of study.

Table 2: Characteristics of Sample (*n*=111)

Variable	Number	Percentage
Gender		
Female	64	57.7%
Male	47	42.3%
Number of Children in Family		
1	52	46.8%
2	40	36.0%
3	12	10.8%
4	5	4.5%
5	2	1.8%

Table 3 describes what mothers reported feeding their infant at the time of the visit. The diets of the sample represents 73.9% of the sample were still breast fed at the nine-month visit. The diet questionnaire does not give full representation of the sample at the six months due to four infant visits occurring prior to the time the study began collecting this information. Although not included in Table 3, one subject reported feeding cow's milk at nine months. A small number of mothers (*n* = 4; 3.6%) reported solids had yet to be introduced at nine months. The majority (81%) reported introducing solids between four and six months while about 15% waited until the infant was older to begin offering solid foods. The number of foods reported as being fed at the time of the visit increases substantially between six and nine months. Those eating meat at nine months (n = 57) is significantly greater than was reported at the six-month visit (n = 7). Parent-reported total supplementation, which includes the combination of both vitamins and medications, did increase between the six and nine month period. An extended list of medications and supplements reported can be found in the appendix. The reported number of infants that took a vitamin supplement of some form was about half of the total number reporting supplemental use at six months. However, this number was also smaller at the nine-month visit than the six month. General reporting of supplemental use did not really change over time. The primary vitamin supplement reported was vitamin D.

Table 3: Diet of Sample at Six and Nine Months (*n* = 111)

Food	6 Months^ Frequency (%)	9 Months Frequency (%)
Breastfed	90 (84.1)	82 (73.9)
Formula fed	17 (15.9)	29 (26.1)
Baby Cereal	82 (76.6)	94 (84.7)
Fruit	31 (29)	91 (82)
Vegetable	50 (46.7)	91 (82)
Meat	5 (4.7)	57 (51.4)
Dinners	None	19 (17.1)
Infant Juice	10 (9.3)	37 (33.3)
Desserts	None	11 (9.9)
Table Food	13 (12.1)	72 (64.9)
Regular Supplements*	20 (18)	23 (21)
Vitamin/Multivitamin*	10 (9)	8 (7)

^ Data not reported by four subjects; *Supplementation *n* = 111

A research assistant later categorized all participants into breastfeeding groups as describe in Table 4) that were used for further analyses of differences between groups based on duration of reported breastfeeding. The number of participants reported as predominantly breastfed versus those that switched over to formula feeding does not necessarily match what is reported in the previous table. This is due to the four missing from the six-month dietary questionnaire. Thus, explaining the differences in numbers.

Table 4: Breastfeeding Groups Tested (*n* = 107^)

Breastfeeding Group	N	%
Predominantly Breastfed at 9 Mo	78	73%
Stopped Breastfeeding 6-9 Mo	13	12%
Stopped Breastfeeding 3-6 Mo	16	15%

^ Missing 4 from original *n* = 111 due to visits occurring prior to dietary component

Infant visits as described in Table 5, under age-in-days, occurred within the allotted time period of plus or minus two weeks, three months past the previous visit. Paired samples t-test was used to compare results over time. For example, three-month visits were compared with six or nine month results and six and nine-month results were compared. Significance was found at all three pairings for age in days ($p = 0.000$) As described in Table 5, most measures show expected growth over time (i.e., no change as an infant growing typically would have a z-score of zero at all three time points) with the exception of length, which decreased over time. Length-for-age, though not significant between three and six months ($p = 0.161$), was significant between both six to nine months ($p = 0.001$) and three to nine months ($p = 0.000$). Weight-for-length and BMI both reported significance at all three pairings. Despite both weight and length reported as decreasing over time, when combined as weight-for-length the mean of the sample did gradually increase between the three visits ($p = 0.0017$; $p = 0.001$; $p = 0.000$).

Table 5: Anthropometric Characteristics of Sample (*n* = 111)

Variable	**3 Months** **Mean ± SD**	**6 Months** **Mean ± SD**	**9 Months** **Mean ± SD**
Age in Days	92.84 ± 9.73	181.76 ± 9.16	271.61 ± 11.18
Weight-for-age^z	0.43 ± 0.95	0.13 ± 0.96	0.22 ± 0.96
Length-for-age^z	- 0.11 ± 1.09	- 0.22 ± 1.07	- 0.49 ± 1.07
Weight-for-length^z	0.25 ± 1.10	0.47 ± 1.03	0.70 ± 1.00
BMI^z	0.15 ± 1.04	0.36 ± 1.04	0.67 ± 1.02
Head circumference^z	0.90 ± 1.99	0.92 ± 2.43	1.05 ± 2.34

^{z} indicates z-score used for variable

Table 6: Anthropometric Paired Samples T-Test *P*-values

Variable	**3 to 6 Months** ***P* Value**	**6 to 9 Months** ***P* Value**	**3 to 9 Months** ***P* Value**
Age in Days	0.000	0.000	0.000
Weight-for-age	0.089	0.092	0.016
Length-for-age	0.161	0.001	0.000
Weight-for-length	0.017	0.001	0.000
BMI	0.015	0.000	0.000
Head circumference^	0.961	0.661	0.600

Degrees of Freedom = 110; HC Df = 109 (3-6 Mo; 3-9 Mo), Df = 110 (6-9 Mo.)

Table 7: Visual Habituation Characteristics of Sample (*n* = 111)

Variable	3 Months^^ Mean ± SD	6 Months Mean ± SD	9 Months Mean ± SD
Novelty Quotient^	0.49 ± 0.19	0.56 ± 0.16	0.56 ± 0.11
Longest Look	60.56 ± 88.69	17.06 ± 16.92	11.02 ± 6.81
Total Duration of Looks	134.54 ± 154.32	48.80 ± 34.97	35.51 ± 21.93
Number of Looks	6.68 ± 3.00	7.68 ± 4.52	7.08 ± 3.13
Average Duration of Looks	21.34 ± 25.50	7.30 ± 6.18	5.14 ± 2.17

^At 3 mo. n= 98; 6 mo. n=108; 9 mo. n=109; ^^Excluding novelty quotient, 3 mo. n=110

Table 8: Visual Habituation Paired Samples T-Test *P*-values

Variable	3 to 6 Months *P* Value	6 to 9 Months *P* Value	3 to 9 Months *P* Value
Novelty Quotient	0.005	0.935	0.003
Longest Look	0.000	0.001	0.000
Total Duration of Looks	0.000	0.000	0.000
Number of Look	0.044	0.203	0.354
Average Duration of Looks	0.000	0.000	0.000

Degrees of Freedom = 109 (3-6 Mo. & 3-9 Mo.) Df = 110 (6 -9 Mo.)

Paired samples t tests were computed for each visual habituation variable to assess any differences between each of the three visits. The novelty quotient variable found a significant difference between three and six months (p = 0.005) and between three and nine months (p = 0.003). However, no significance was found between six and nine months (p = 0.935). As for the log transformed longest look variable, significance was reported between each of the three pairings: three to six months (p = 0.000), three to nine months (p = 0.000), and six to nine months (p = 0.001). Total duration of look and average look also reported significance at all three pairings (p = 0.000). The only significance found for number of looks was between three and six months (p = 0.044). Visual habituation characteristics results describe a decreased number of looks and time spent looking to have decreased over time. The drop in number of looks and time spent looking drops at a greater rate between three and six months as opposed to the difference between six and nine months.

Section 3 – Breastfeeding Groups and Relation to Demographic Variables

To test any potential confounding variables effect on duration of breastfeeding, Pearson's Chi Square and Oneway ANOVA statistical analyses were tested. As noted in Table 9, no significance was reported for any of the listed variables.

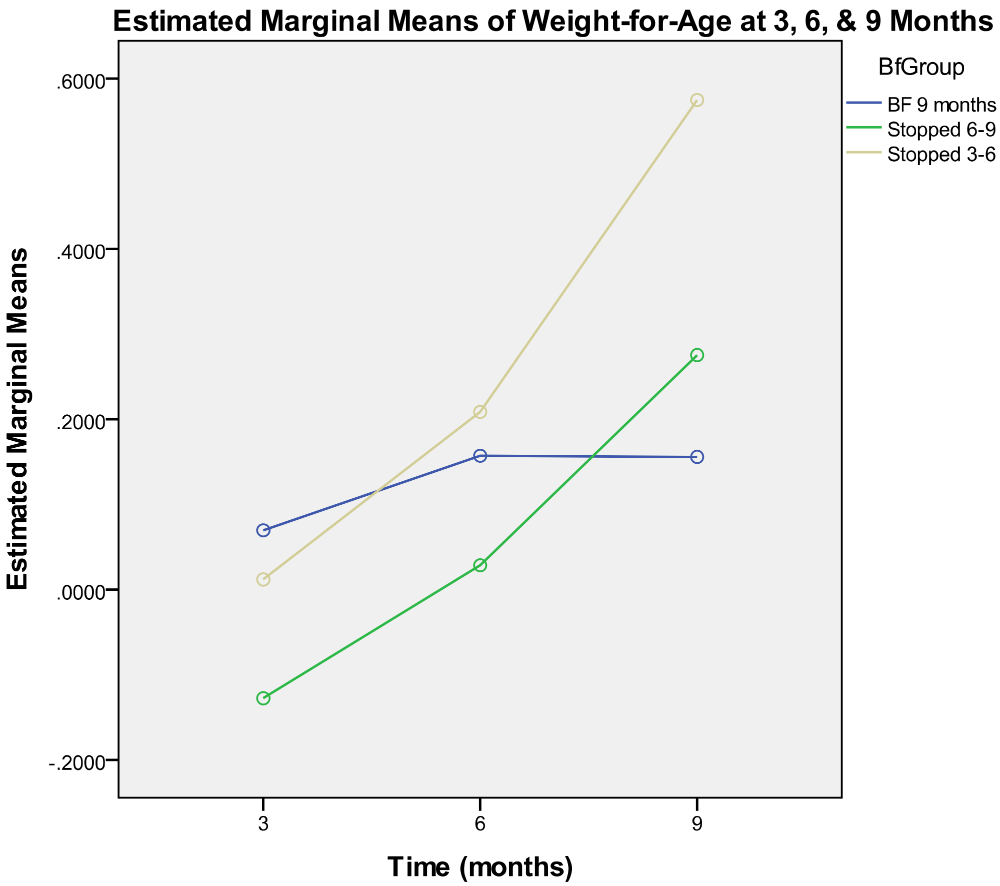
Table 9: Breastfeeding Groups in Relation to Potential Confounding Factors

Variables	**Pearson Chi Square *p* value**
Breastfeeding group*RaceAAPI	0.823
Breastfeeding group*EducationAAPI	0.677
Breastfeeding group*Income Level	0.200
Breastfeeding group*Marital Status	0.940
Breastfeeding group*Gender	0.361
	Oneway ANOVA *p* value
Number of Children: Between Groups	0.247
Mom Age when Taken: Between Groups	0.847
Income Level: Between Groups	0.119

Section 4 – General Linear Model Results for Anthropometry and Visual Habituation

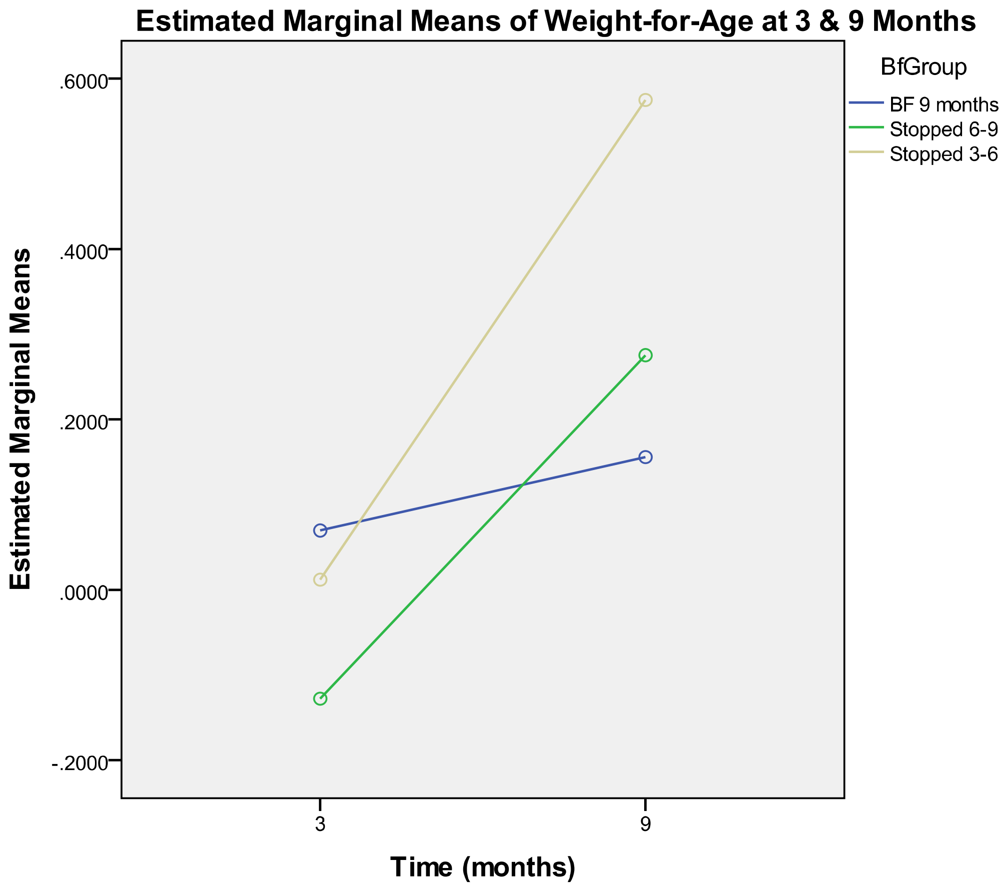
We used general linear modeling to examine if there was a change in anthropometric and visual habituation variables over time (three to nine months of age) in relation to breastfeeding group using the Wilk's Lambda test for significance. Using weight-for-age as the dependent variable, there was a significant time effect as weight for age increased ($p = 0.002$) and the interaction between breastfeeding group and time approached significance ($p = 0.079$).

Figure 1: Estimated Marginal Means of Weight-For-Age at 3, 6, & 9 Months



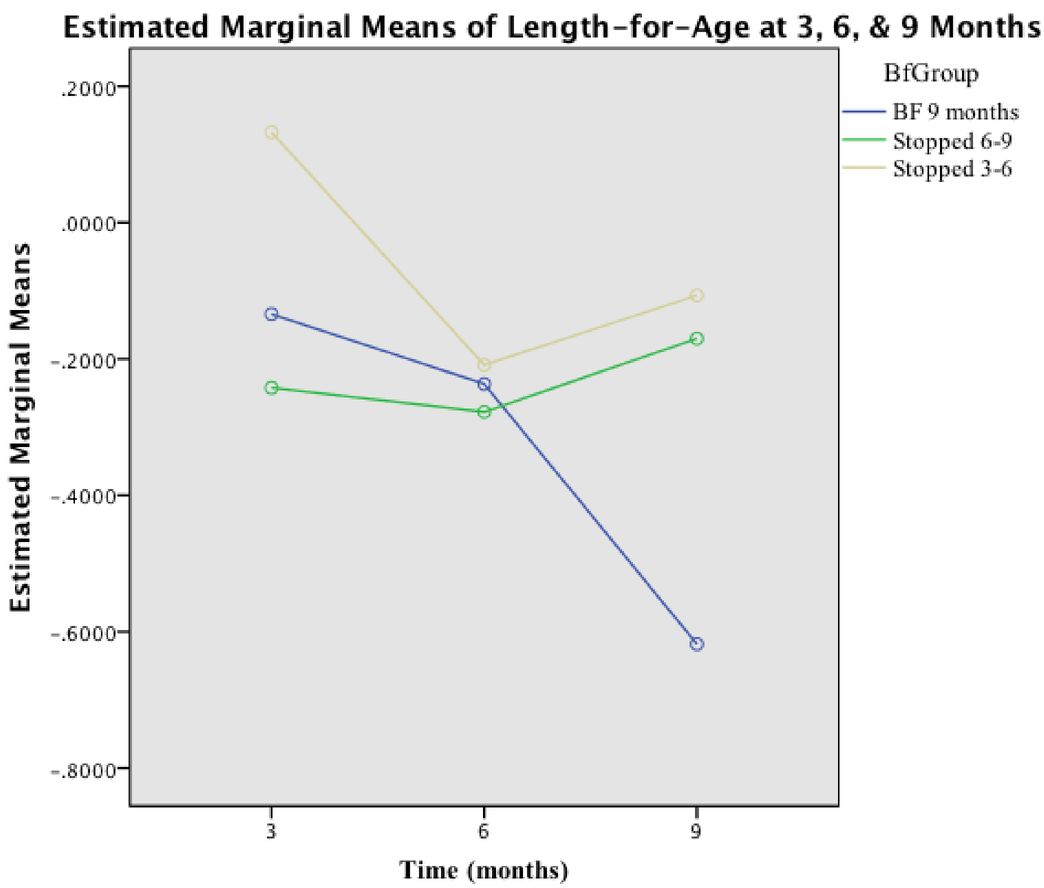
To further explore the relation we redid the analysis with gender as a covariate but neither the main effect of time (p = 0.092) nor the interactions effects of time*gender (p = 0.274) or time*breastfeeding group (p = 0.093) were significant. However, when the analysis was redone with just two time points, three and nine months both the main effect of time (p = 0.001) and the interaction (p = 0.045) were significant. Post Hoc Analysis using least significant difference found those that were breastfed for the entire nine months showed less change in weight-for-age z score ($m_{\text{weight change}}$ = -0.0014 $\pm$ 0.5336) than those that stopped breastfeeding three to six months ($m_{\text{weight change}}$ = 0.3663 $\pm$ 0.3970, p = 0.012) and those that stopped six to nine months ($m_{\text{weight change}}$ = 0.2469 $\pm$ 0.5897, p = 0.116).

Figure 2: Estimated Marginal Means of Weight-For-Age at 3 & 9 Months



For the length for age variable, using the previous two time points, the difference between groups approached significance for both the main effect of time (p = 0.066) and the interaction (p = 0.099). However, using all three time points, and again length for age as the dependent variable, there was a marginally a significant interaction between time and breastfeeding groups (p = 0.056) but not for main effect of time (p = 0.169). Additional analysis, using length-for-age at three months as a covariate found a significant effect for time (p= 0.000), and the interaction between time and length was significant (p = 0.000) as was the interaction between length and breastfeeding groups over time was significant (p = 0.026). Post-hoc analysis using least significant difference found those that were breastfed for the entire nine months had less change in length-for-age z score ($m_{length\ change}$ = -0.3815 ± 0.7730) than those that stopped breastfeeding three to six months ($m_{length\ change}$ = 0.1019 ± 0.7131, p = 0.027) and those that stopped six to nine months ($m_{length\ change}$ = 0.1077 ± 0.9461, p = 0.040).

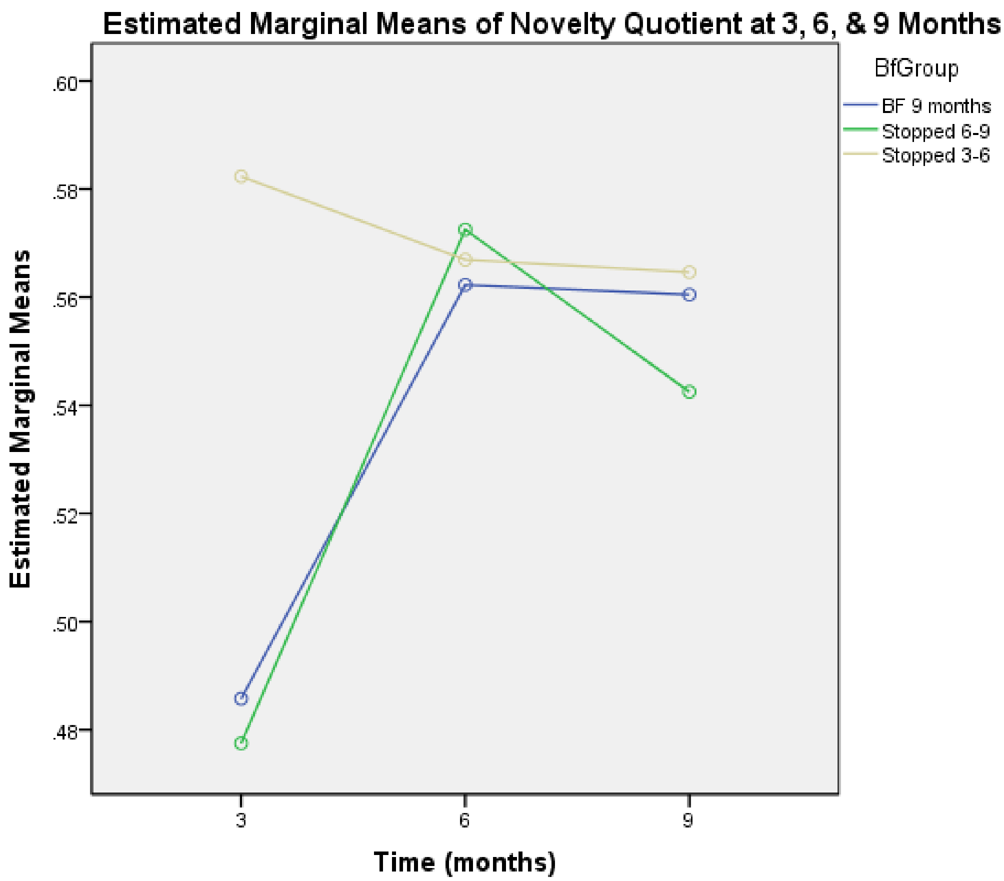
Figure 3: Estimated Marginal Means of Length-For-Age at 3, 6, & 9 Months



Finally when examining BMI for age, there was a significant increase over time (p = 0 .000), but the interaction between time and breastfeeding group (p = 0.537) was not significant and did not change when BMI at three months was used as a covariate. Head circumference did not show any change either over time (p = 0.660) or as an interaction with breastfeeding group (p = 0.423). This variable was skewed, but a natural log transformation did not alter the results.

Using novelty quotient as the dependent variable, there was no significance reported both for change over time (p = 0.292) and the interaction between groups (p = 0.674). A reanalysis using only the three and nine month time points did not find significance for both the main effect of time (p = 0.185) and the interaction (p = 0.374).

Figure 4: Estimated Marginal Means of Novelty Quotient at 3, 6, & 9 Months



To normalize the distribution, total duration of looks, average duration of looks, longest look and number of looks were all log transformed. For both total look duration and the longest look variables significance was reported for both in the main effect of time (p = 0.000). No significance was reported with the group interaction for either total look duration (p = 0.737) or longest look (p = 0.231).

Figure 5: Estimated Marginal Means of Total Look Duration at 3, 6, & 9 Months

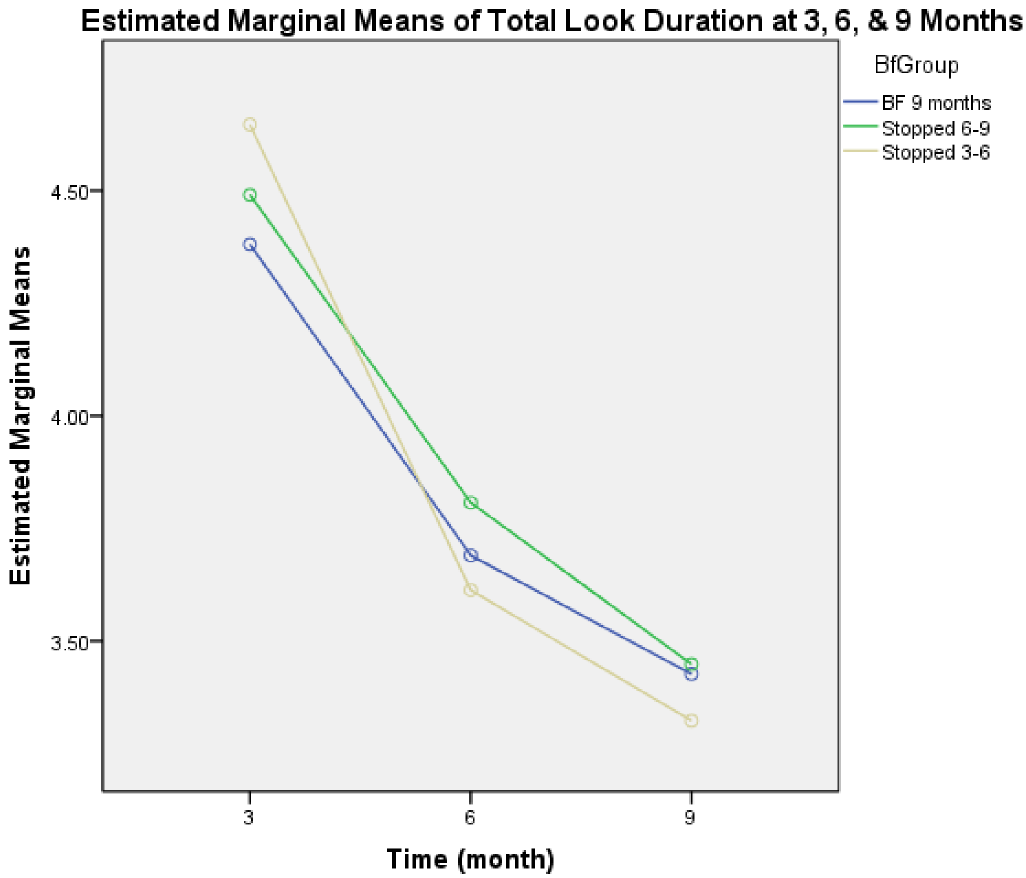
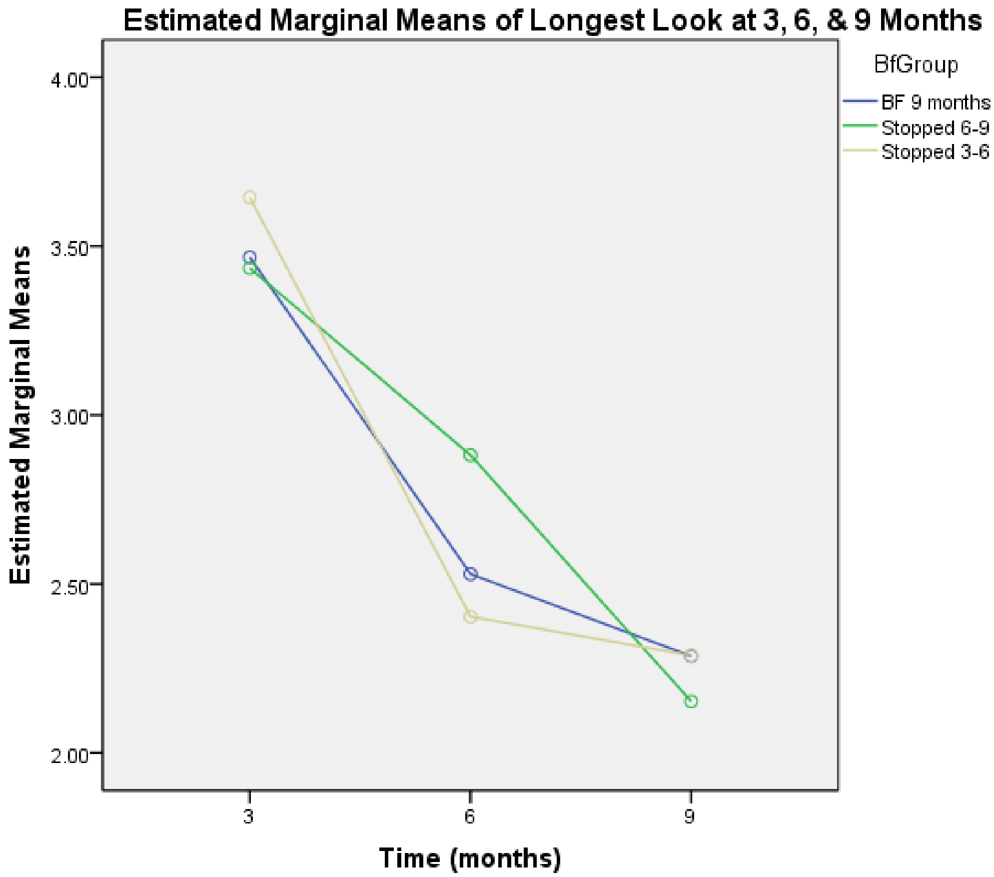


Figure 6: Estimated Marginal Means for Longest Look at 3, 6, & 9 Months



The average duration of looks also had a significant time effect ($p = 0.000$), but again the interaction was not significant ($p = 0.217$). The number of looks did not find significance for both the main effect of time ($p = 0.923$) and the interaction ($p = 0.307$).

CHAPTER V

CONCLUSIONS AND DISCUSSION

Section 1: Conclusions

The majority of infants included in the study came from families that were white, well educated, married, mother reported as unemployed, and a household annual income of $25,000 or greater. It was also found that participants excluded from the study did not significantly alter the previously mentioned socioeconomic and demographic factors, nor did it significantly alter the distribution of infant feeding groups. The study found covariates including marital status, race, income level, number of children, infant gender, and level of education to have any effect on the duration of breastfeeding.

Section 2: Relationship of Findings to the Literature

Infant Feeding Recommendations

The majority of mothers reported following infant feeding recommendations as reflected in a study sample with a distribution skewed toward a larger number of participants whom reported continued breastfeeding at nine months. It is assumed that by continuing on with breastfeeding participants will continue, following the recommendation of breastfeeding into the first year of life as described by the AAP and WHO. As part of the infant feeding recommendations the WHO and AAP, solids are to be introduced at about six months of age

(AAP, 2005). Although some did not adhere to the six-month introduction of solid foods recommendation as noted in the nine-month dietary questionnaire, the majority did appear to continue following the breastfeeding duration guidelines. A minimal number of participants followed supplementation, specifically iron and Vitamin D recommendations. Even more surprising was the number of medications, allergy in particular, that mothers reported giving to infants as noted on the 'other' section of the dietary questionnaire for supplements section.

Breastfeeding and Demographics

This study found no association between potential confounding variables and breastfeeding. This does not necessarily support evidence found in some of the literature. For example, Dewey et al. (1992) found growth differences by gender in response to feeding. While females reported no difference in length between feeding groups, males were reported to be significantly longer. Literature support appears to vary by individual confounding factors. In a study by Kavanagh et al. (2008), increasing maternal education was a direct focus in relation to parents changing infant feeding methods. Although it was proposed to have an effect, this was not the case in our study. The lack of significance found in our study is possibly attributed to the population included being very similar in general demographic characteristics. Populations with minimal demographic variations are likely to overlook variables that otherwise may attribute to differences in breastfeeding durations when greater diversity is included.

Assessment of Growth

Overall, infant growth trended towards those in the breastfeeding group to grow at a steady pace, while those in the formula feeding groups showed more drastic increases in growth over time. The only factor reporting significance was weight-for-age at three versus nine months. Length-for-age and BMI were extremely close to significance, however still cannot be reported as truly significant at the .05 level. Our study supports a past study by Dewey et al. (1998), that looked at a large portion of studies meeting similar criteria and found a significant difference in weight gain with less of a difference in length, and no significant difference in head circumference. However, it does not support all literature as some found significant differences in weight-for-length, length-for-age, and head circumferences as early as six months (Kramer et al., 2004). The literature compares infant growth using the different feeding groups into the first year while our study does not have data after nine months.

The lack of support found in the results of our study to the results of previous literature could be associated with differences in sample size. For example, Kramer et al. (2004) used a much larger sample size (n = 17,046) than was used in our study (n = 111). The inconsistency in sample size is also apparent in they way previous studies defined breastfeeding groups. Although our study required infants to have been predominantly breastfed for three months before the use of infant formulas, this was not always the case in other literature that also looked at growth between feeding groups. When looking at how feeding groups were defined, in the meta-analysis by Dewey et al. (1998) eight of the 19 studies defined breastfeeding as exclusive breastfeeding for a minimum of three and sometimes four months. As for those included in the formula fed groups, it was not necessarily a requirement to initiate breastfeeding. Some were never breastfed while others were partially breastfed (Dewey et al, 1992). The variations in both sample size and how authors chose to define their feeding groups may be part of the reason for the results of our study not always supporting the results of previous studies.

It is of particular interest that the largest difference in growth, specifically weight, was seen between three versus nine months. The introduction of solid foods is when the largest difference in weight was noted. Such a finding appears to support Dewey's proposed explanation for these differences being partially attributed to differences in self-regulation between infants that were nursed and those that were formula fed (Dewey et al., 1992).

Assessment of Cognitive Development

Our study found no difference in cognitive development among the three feeding groups as assessed through visual habituation measures. The results of this study do not support the findings of previous studies suggesting differences in cognition in relation to feeding practices (Kramer et al., 2008; Rogan & Gladen, 1993). An important factor to keep in mind is that most studies were also looking at infants at one year of age, allowing them to reach the recommended benchmark for infant feeding practices in the first year. Thus, it could be assumed that lack of significance may stem from our study stopping at nine months when in fact those last three months would allow for a greater difference to develop over a longer period of time.

At both the six and nine month visit, the mean novelty quotient of this study reported as 56% of time spent focusing on the novel stimulus versus the familiar stimulus. These results are very similar to the results found by Colombo et al. (2001). They reported a mean novelty quotient of 55.2%. Considering the similarity of results between studies, it seems reasonable to suggest the conclusion drawn by Colombo and colleagues, that recognition did not happen by chance, is just as applicable in this circumstance.

Section 3: Implications

Feeding Recommendations

Although the study did not find a significant difference between duration of breastfeeding and visual habituation and growth measures, with the exception of weight-for-age, I would advise parents continue to follow national infant feeding recommendations. There are many additional benefits associated with breastfeeding for both the mother and infant's health. These were not necessarily tested in this particular study, but are well supported in literature and by established health organizations. It is possible that results did not find significant growth and cognitive development differences between feeding groups as the study stopped before the 12 month mark in which breastfeeding is recommended. The literature appears to support studies continuing throughout the entire first year of infancy as changes are still occurring that lead to possible differences. Rose et al. (2004b), mentions the cognitive development process, recognition memory, to increase in a steady manner specifically, between three and 12 months. Many of the studies that assessed growth in relation to feeding also support this 12-month notion. In a meta-analysis conducted by Dewey et al. (1998), changes in weight between breast and formula fed infants was noticeable beginning at four months, but even greater between six and 12 months. The study goes on to report an inverse relationship lasting throughout the first year was found in infant breastfeeding and weight-for-age z-scores. Therefore, one can suspect if this study group was tested at a later time, some of the insignificant results reported between breastfeeding groups may later show significance with greater time elapsed.

Growth Differences

It has been mentioned that differences in growth between different feeding groups may stem from differing degrees of self-regulation of intake better in one group than the other. Although no differences in demographics related to feeding practices were found, it is likely many other factors, not included in our study, attribute to these differences in growth and self-regulation. Some may argue one of these differences may come from differences in parenting style and those that breastfeed are more aware or in tune with their child's needs. Another possibility is the level of stress in the home that is carried into mealtimes. Stress at meal times not only affects how an infant responds to mealtimes, but also to how they will then regulate intake and respond to the introduction of new foods. In actuality there are probably combinations of factors rather than just one that explain these differences in growth.

Section 4: Research Questions

The hypothesis of this study was that between three and nine months of age, breastfed babies will have improved growth and cognitive development compared to those that are formula fed. Additional hypotheses include:

I. Infants who are predominantly breastfed at nine months of age will have lower six and nine month BMI than infants who are no longer breast fed at both six and nine months of age.

II. Infants who are predominately breastfed at nine months of age will have a greater novelty quotient percentage and shorter looking times than infants who were no longer breast fed at both six and nine months of age.

Hypothesis Part One:

Although weight-for-age was significantly different between three and nine months, BMI showed no significant between those that were predominantly breastfed at nine months and those no longer breast fed at six and nine months of age. Considering the equation used to calculate BMI: weight (kg) divided by length (meters) squared, a possible reason for the lack of significance may stem from measurement error of length as length-for-age decreased in our study.

Hypothesis Part Two

Those still predominantly breastfed at nine months of age reported no significant difference from both groups that stopped breastfeeding either three to six months or six to nine months.

Section 5: Limitations and Further Research

Sample Distribution and Size

The study sample was strong in the sense that it represented a very similar population, allowing for ease of interpretation, as potential confounding variables did not skew results. However, this could also be viewed as a downfall because it does not reflect a population outside of a predominantly white suburban geographic location. The size of the sample along with an uneven distribution of participants in each of the three feeding groups may also have attributed to a lack of overall significance reported in the study. For future research it would be beneficial to include a more diverse sample population to strengthen results of the study and add greater application for a larger number of individuals.

Growth Measures

There is always the possibility for human error when collecting data as was done in this study. The infants included in this study as a whole reported decreasing in length over time. Human error in this instance is likely. Infants at nine months are much less willing to lie still while being measured then they are at six, and especially three months. However, aside from double-checking data entry little can be done to fix and reassess these measures.

Further Research

Considering the length of previous studies along with the results from this study, a study continuing on through 12 months of age and even longer would be beneficial for many reasons. One reason being it would either add support to significance or no significance. It appears that infants begin to show greater differences in response to cognitive measures, in this case visual habituation as they become older and thus significance may be seen at a later age as opposed to great differences so early in life. Yet another significant contribution in a future study would be to include a more even distribution of feeding groups. It would be unethical to control feeding, but by using a larger sample size with a more diverse population this may even itself out. A more even distribution may allow for differences that were minimal in our study to become more apparent when compared at a larger, more even scaled level.

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APPPENDIX 1
DEMOGRAPHICS FORM

Demographic Information Questionnaire

Child Information

What is your relationship to the baby? Example: mother, father, stepmother.

Mother

Gender of baby 47 Male 64 Female

Birthdate of baby July 5 2009
Month Day Year

Birth weight of baby 7 lbs 69 oz

Date of expected birth (due date) ______________________
Month Day Year

Was the baby born by c-section? YES NO

Maternal Information

Birthdate January 07 1981
Month Day Year

Your marital status (check one)

___ Married, first time
___ Single, separated
___ Single, widowed
___ Other, please specify: ______________________
___ Single, never married
___ Single, divorced
___ Remarried

Your own ethnic group (please check)

9 Native American Nation: __________________
1 African American
2 Hispanic
2 Asian
96 White
1 Multiethnic Describe: __________________
__ Other Describe: __________________

Please place a check mark next to the highest grade you completed in school.

____ 6th grade
____ 7th grade
____ 8th grade
____ 9th grade
____ 10th grade
____ 11th grade
____ 12th grade
____ some vo-tech
____ some college courses
____ vo-tech graduate
____ college graduate

Please place a check mark next to the highest grade your spouse/partner completed in school.

____ 6th grade
____ 7th grade
____ 8th grade
____ 9th grade
____ 10th grade
____ 11th grade
____ 12th grade
____ some vo-tech
____ some college courses
____ vo-tech graduate
____ college graduate

Your current household income per month before taxes (please check one)

___ $ 0 – 100
___ $ 100 – 499
___ $ 500 – 999
___ $ 1000 – 1499
___ $ 1500 – 1999
___ $ 2000 – 2499
___ $ 2500 – 2999
___ $ 3000 – 3499
___ $ 3500 – 3999
___ $ 4000 plus

Is your current spouse/partner the father of the baby (check one)

___ yes ___ no

Ethnic group of the biological father of the baby. (please check)

___ Native American Nation: ___________________
___ African American
___ Hispanic
___ Asian
___ White
___ Multiethnic Describe: ___________________
___ Other Describe: ___________________

Do you currently receive state or federal financial assistance? (check as many as apply)

26 WIC
0 TANF
0 School lunch/breakfast
8 Food Stamps
3 Indian Health Services
2 Unemployment benefits
1 Energy assistance
2 Social Security/SSI
12 Medicaid

For how many years have you received such assistance? (check one)

20 five or more years
7 four years
4 three years
1 two years
4 one year
3 less than one year

My child seems to be less healthy than other children I know.

1 strongly agree

1 ___ agree
1 ___ do not agree or disagree
11 __ disagree
95 _ strongly disagree

My child has never been seriously ill.
__ Agree
__ disagree

Where did you hear about our study? Were you referred by a friend or did you see a poster, newspaper ad, poster, etc? Friend, flyer, doctor, poster, breastfeeding class, OSU professor or intern

APPENDIX II

SIX AND NINE MONTH INFANT DIETARY QUESTIONS

6 and 9 Month Infant Dietary Questions

Are you exclusively breastfeeding? Yes No

What kind of formula (or milk) do you use? _______________________
Note to interviewer: make sure to check if the formula is iron fortified or not

How often do you generally give formula? _____________________

How much formula does *(name)* generally take at a feeding? _______________________

When did *(name)* start taking solid food like cereal? _______________________months

What kinds of foods does *(name)* take now?
Note to interviewer: check all that apply

___ baby cereal
___ infant fruit
___ infant vegetables
___ baby meat
___ infant "dinners"
___ infant juice
___ infant deserts
___ other homemade puree/ground baby food
___ other foods: _____________________________

___ mashed table food
___ cereal: example cheerios/oatmeal (not infant)
___ regular juice/ juice drinks
___ cow's milk
___ whole ___ 1 or 2%

When did you start giving *(name)* pureed meat, infant dinners or other meat products? __________ months of age.

How many times a day does *(name)* eat these foods? _________________

Do you give *(name)* any supplements or medications routinely? ________

Note to interviewer: if yes please list all.

Appendix II

6-Month Reported Supplement Frequency

Supplement or Medication	N	%
None	87	78%
Acid Reflux Medicine	1	0.9%
Zyrtec	1	0.9%
Amoxicillin	1	0.9%
Allergy Medications (Claritin, Zyrtec)	2	1.8%
Homeopathic Teething	1	0.9%
Gas Drops	1	0.9%
Multivitamin (Infant) Polyvisol	3	2.7%
Teething Tablets	1	0.9%
Vitamin D	7	6.3%
Tylenol	2	1.8%

9-Month Reported Supplement Frequency

Supplement or Medication	N	%
None	87	78%
Acid Reflux Medicine	1	0.9%
Zyrtec	2	1.8%
Benadryl	1	0.9%
Claritin	4	3.6%
Eldeberry Juice	1	0.9%
Eye Drops	1	0.9%
Gas Drops	1	0.9%
Fluoride Drops	2	1.8%
Multivitamin (Infant) Polyvisol	3	2.7%
Teething Tablets/oil	3	2.7%
Vitamin D	5	4.5%
Albuterol	1	0.9%
Tylenol	1	0.9%
Motrin	1	0.9%
Omega 3 and DHA	1	0.9%

VITA

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Master of Science

Thesis: COGNITIVE AND GROWTH COMPARISON BETWEEN BREASTFED AND FORMULA FED INFANTS THROUGH NINE MONTHS OF AGE

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This study examined the relation between duration of breastfeeding, cognitive development, and growth of infants between three and nine months of age. The design of the study was longitudinal and observational. Participants were from a rural community in Oklahoma. Data was collected using a demographic questionnaire, infant anthropometric and visual habituation variables. The final analysis was on 111 infants who were predominantly breastfed at three months of age. Of the 111 infants, 73% were still breastfed at nine months, 12% stopped breastfeeding between six and nine months and 15% stopped breastfeeding between three and six months. General linear modeling was used to assess the interaction between growth and visual habituation with breastfeeding duration groups over the nine-month study period. Analyses found no significant interaction between breastfeeding duration groups and visual habituation over time. Changes in weight-for-age z score were significantly less for those infants who were breastfed for the entire nine months than for those that stopped before six months of age ($p = 0.012$). When length-for-age at three months was used as a covariate there was a significant interaction between change in length z score and breastfeeding group over time ($p = 0.026$). However, there was no relationship between breastfeeding duration groups and changes in BMI z score over time ($p = 0.537$). In conclusion, over the short time period (three to nine months) duration of breastfeeding did not have any impact on cognitive development as evidenced by visual habituation, but small differences were seen in changes in weight and length z scores. The effect of breastfeeding in all of these variables may become more evident in additional longitudinal studies. This project was supported by National Research Initiative Grant 2008-35200-18779 from the USDA National Institute for Food and Agriculture.

ADVISER'S APPROVAL: Dr. Tay Kennedy