EARLY FEEDING PRACTICES AND BMI IN INFANTS

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Abstract: Obesity and childhood obesity are growing public health problems. Some studies have shown a link between early breastfeeding cessation and introduction of complementary foods may be a contributing factor to this problem. (Aims) The aims of this study were to determine any relation between breastfeeding and infant BMI, formula consumption and infant BMI, timing of complementary foods and infant BMI, and maternal vitamin D status and infant BMI. (Methods) 132 mother-infant pairs were seen at approximately 3, 6, and 9 months post-partum. Anthropometric measurements were taken at all time points. Mother gave 24-hour recall and infant weights before and after feeding at the three month visit, dietary questionnaires were filled out at 6 and 9 months visits. Blood samples were taken from mothers at 3 month visit to analyze for vitamin D status. (Results) No significant relations were found between breastfeeding, formula consumption, timing of complementary foods or maternal vitamin D status and infant BMI. (Justification of results) Our results are supported by what was found in the current literature.
# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Chapter</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>I. INTRODUCTION</td>
<td>1</td>
</tr>
<tr>
<td>Purpose of Research</td>
<td>1</td>
</tr>
<tr>
<td>Research Questions and Hypothesis</td>
<td>2</td>
</tr>
<tr>
<td>II. REVIEW OF LITERATURE</td>
<td>5</td>
</tr>
<tr>
<td>Infant Weight</td>
<td>6</td>
</tr>
<tr>
<td>Modes of Feeding</td>
<td>10</td>
</tr>
<tr>
<td>Nutrients of Concern</td>
<td>12</td>
</tr>
<tr>
<td>Appropriate Complementary Feeding Practices</td>
<td>14</td>
</tr>
<tr>
<td>III. METHODOLOGY</td>
<td>18</td>
</tr>
<tr>
<td>Sample</td>
<td>18</td>
</tr>
<tr>
<td>Procedures</td>
<td>19</td>
</tr>
<tr>
<td>Assessments/Questionnaires</td>
<td>19</td>
</tr>
<tr>
<td>Statistical Analysis</td>
<td>23</td>
</tr>
<tr>
<td>Chapter</td>
<td>Page</td>
</tr>
<tr>
<td>---------</td>
<td>------</td>
</tr>
<tr>
<td>IV. FINDINGS</td>
<td>25</td>
</tr>
<tr>
<td>Demographics</td>
<td>25</td>
</tr>
<tr>
<td>Research Questions</td>
<td>28</td>
</tr>
<tr>
<td>V. CONCLUSION</td>
<td>33</td>
</tr>
<tr>
<td>Specific Aims</td>
<td>34</td>
</tr>
<tr>
<td>Discussion</td>
<td>35</td>
</tr>
<tr>
<td>Limitations</td>
<td>38</td>
</tr>
<tr>
<td>Future Research</td>
<td>38</td>
</tr>
<tr>
<td>Implications for Practice</td>
<td>39</td>
</tr>
<tr>
<td>Summary</td>
<td>39</td>
</tr>
<tr>
<td>REFERENCES</td>
<td>40</td>
</tr>
<tr>
<td>APPENDICES</td>
<td>44</td>
</tr>
<tr>
<td>Table</td>
<td>Page</td>
</tr>
<tr>
<td>-------</td>
<td>------</td>
</tr>
<tr>
<td>Table 1. Mothers’ Demographic Information</td>
<td>26</td>
</tr>
<tr>
<td>Table 2. Infants’ Anthropometric Measurements</td>
<td>27</td>
</tr>
<tr>
<td>Table 3. Frequency of Formula Consumption</td>
<td>27</td>
</tr>
<tr>
<td>Table 4. Start of Complementary Foods</td>
<td>28</td>
</tr>
<tr>
<td>Table 5. Predominant Breastfeeding vs. Added Water or Formula</td>
<td>28</td>
</tr>
<tr>
<td>Table 6. Chi Square of BMI Groups by Breast vs Formula Feeding</td>
<td>29</td>
</tr>
<tr>
<td>Table 7. Mean and Standard Deviation of BMI z-score by Start of Solids</td>
<td>30</td>
</tr>
<tr>
<td>Table 8. BMI Groups Chi Square by Start of Solids at 6 and 9 Month Visits</td>
<td>31</td>
</tr>
<tr>
<td>Table 9. Vitamin D Supplementation of Infants</td>
<td>32</td>
</tr>
</tbody>
</table>
CHAPTER I

INTRODUCTION

Purpose of Research

The purpose of this research is to determine the relation between breastfeeding cessation and early introduction of solid foods and BMI in infants. There is a plethora of information supporting breastfeeding, yet breastfeeding statistics remain staggeringly low. According to the 2014 Breastfeeding Report Card (Centers for Disease Control and Prevention, [CDC] 2016b), 79% of babies in the United States are breastfed compared to only 71.2% of babies in Oklahoma. In the United States, 49% and 27% of babies are still breastfed at six months and twelve months of age, respectively. In Oklahoma, 38.4% and 22.6% of babies are breastfed at six and twelve months.

In the US, it is recommended that infants be exclusively breastfed for the first six months of life, and solid foods consisting of fruits, vegetables, cereals, and meats be introduced gradually after six months of age (Baird et al., 2008). One study found that close adherence to this recommendation resulted in slower growth and smaller size at six months of age. This study also
found many infants were eating solids before six months of age, but later introduction of solid foods was inversely correlated with weight at six months of age. The study also found longer breastfeeding duration resulted in slower increases in fat gain. Other studies found that exclusive breastfeeding for at least six months delayed the introduction of solid foods until the baby was six months of age and allows the baby more control over his own intake versus bottle feeding (Fisher, Birch, Smickilas-Wright & Picciano, 2000; Moss & Yeaton, 2014). Thus the question remains: Is the child at a healthy weight because of exclusive breastfeeding, or because of the delay in starting solids? Is it the breastmilk that is having the positive impact, or the baby’s control over his own intake?

In many developed countries, mixed-feeding (a combination of breastfeeding and formula feeding) has become commonplace (O’Sullivan, Farver & Smilowitz, 2015). The long-term effects of mixed-feeding on infants and children is still unclear, but it could be associated with inappropriate weight gain and childhood obesity. This is because formula feeding could “alter the microbiome of the infant gut, increasing pro-inflammatory taxa” (O’Sullivan et al., 2015, p. 2). These alterations can have an impact on immune health and food tolerance down the road.

Research Question/Hypothesis

The purpose of this research is to determine the relation between breastfeeding cessation and early introduction of solid foods and/or infant formula and BMI in infants. It has long been touted that exclusive breastfeeding is superior to formula feeding for many reasons including healthy infant weight. What may not be considered in this claim is that exclusive breastfeeding for at least six months delays the introduction of solid foods until
the baby is six months of age. The objective of this project is to determine to what degree formula feeding and solid food introduction predict infant weight at the 85th percentile or greater at three months, six months and 9 months of age. The hypothesis is that infant diet at three and six months of age is positively correlated with BMI status at subsequent visits.

Much of the published research has been conducted with low-income, low-education level, high risk populations (Dawson-McClure et al., 2014; Gaffney, Brito, Kitsantas & Kermer, 2016; Gross, Mendelsohn, Fierman, Racine, & Messito, 2012). The sample for this proposed research will include mothers from average to above average income levels; most with a college education.

The specific aims of this study include:

1. Determining the relation between breastfeeding and BMI at three, six and nine months of age.
   a. Use t test to determine if there is a significant difference in BMI z-score between infants exclusively breastfed (no formula) at each age and those receiving formula.
   b. Use chi square to determine if there is a relation between exclusive breastfeeding (yes/no) and BMI z-score greater than or less than 1.04 at each age. (z-score of 1.04 is used because it is the 85th percentile. Infants and children are considered overweight if they reach the 85th percentile on weight for age charts.)
2. Determining the correlation between amounts of formula consumed (oz.) at three months and BMI z-score.
   a. Determine the correlation between formula consumed at three months and BMI z-score at three, six, and nine months.

3. Determining relation between the introduction of solid foods (yes/no) and BMI z-score at three, six and nine months of age.
   a. Use t test to determine if there is a significant difference in BMI z-score between infants receiving solid foods or not at each age.
   b. Use chi square to determine if there is a relation between infants receiving solid food or not and BMI z-score greater than or less than 1.04 at each age.

4. Determining relation between a breastfeeding mother’s vitamin D status when the infant was three months of age and infant BMI at three, six and nine months.
   a. Conduct bivariate correlation analyses and two additional partial correlation analyses controlling: for vitamin D supplementation of breastfed infant and formula fed to infant that contained vitamin D.

If significant relations are determined in the previous analyses, a regression model will be tested to determine the impact of feeding practices on infant BMI. Regressions will be developed for all infants and for each gender.

The hypothesis of this study is that early breastfeeding cessation and/or early introduction of solid foods is associated with a higher BMI z-score for infants. Some topics of interest in the review of literature include infant weight as a measure of health, methods of infant feeding, nutrients of concern for breastfed infants, and complementary feeding practices.
Obesity is a public health problem. According to the Centers for Disease Control and Prevention, obesity among children aged 2-19 is 17% in the United States (CDC, 2016a). This can lead to high health care system costs as chronic obesity is associated with co-morbidities such as diabetes and heart disease. Childhood obesity is associated with weight-related conditions and complications previously only seen in adulthood such as type 2 diabetes, nonalcoholic steatohepatitis, and orthopedic complications (Shibli, Rubin, Akons & Shaoul, 2008). Data showing prevalence of overweight and obesity in infants (birth to one year) is scarce. This may be because there is no universally recognized definition of overweight and obesity in children under two years of age. It is apparent that the rate of childhood and adult obesity is increasing worldwide, but whether that obesity pattern starts in infancy is unclear.

A longitudinal retrospective review of well-child visits was conducted in 2013 that suggested obesity at five years of age would be predicted by infant weight starting around four months
(Gittner, Luddington-Hoe & Haller, 2014). This study asserted that children with excessive weight gain in the first five months of life had a higher risk of obesity at five years old. Excessive weight gain was determined by consistent and sequential increases in BMI percentiles on the growth chart.

For this study, exclusive breastfeeding was defined as an infant receiving no other food or drink besides human milk. Even giving an infant water would keep an infant out of the exclusive breastfeeding group. Not only does giving water temporarily satiate hunger, but water from a bottle can cause nipple confusion that interferes with breastfeeding (Zimmerman & Thompson, 2015). Predominant breastfeeding is defined as giving an infant mostly human milk, supplemented with formula or complementary foods. To be classified as predominantly breastfeeding in this study, infants could receive no more than 28 oz. of formula per week, or 4 oz. per day. It was preferred that mothers who were exclusively breastfeeding nurse, but were allowed to pump breast milk and give a bottle feed. There were no exclusion criteria associated with complementary feeding.

The remainder of this section will cover the topics of infant weight as a predictor of health and childhood obesity, modes of infant feeding, nutrients of concern for breastfed infants, and complementary feeding practices.

**Infant Weight as Predictor of Health/Childhood Obesity**

Weight is often used as a measure of infant health because an infant’s weight can more than triple in the first year of life (O’Sullivan et al., 2015). According to the Mayo Clinic, an infant should double his or her weight by five months of age, and triple it by one year. Early feeding practices largely determine the pattern of weight gain in the first year of
life, and therefore heavily impact infant and childhood weight status (Daniels et al., 2015; Fisher et al., 2000; Moss & Yeaton, 2014; Stang & Loth, 2011). When an infant is not gaining weight and body fat appropriately, it can have negative health effects in both the short-term and long-term.

This purpose of this research is to determine whether early introduction of solid foods causes larger BMI in infants, but smaller BMI can be detrimental as well. Failure to thrive (FTT) is a form of malnourishment that causes an infant to not gain enough weight. Although there is not a globally recognized definition of FTT, some of the traditional definitions include weight of less than 75% of median weight for age, weight less than 80% of median weight for length, BMI for age less than 5th percentile and weight deceleration across more than two major percentile lines since birth on the WHO growth chart (Larson-Nath & Biank, 2016). FTT and other forms of undernutrition can lead to negative health outcomes for infants. The short-term consequences of undernutrition for infants include stunting, underweight status, wasting, and iron deficiency anemia (Scherbaum & Srour, 2016). Long-term effects of undernutrition include higher susceptibility to infection and developmental delays and health problems that endure across generations. On the other side of the coin, over-nutrition and excessive weight gain can have deleterious effects as well. Infant overweight status is associated with many childhood outcomes like low self-esteem and diabetes, as well as adult outcomes like persistent obesity (Moss & Yeaton, 2014).

Excessive weight gain has been more difficult to define than FTT. There is currently no universally accepted definition of overweight and obesity in children under two years of age (Shibli et al., 2008). A search of Medline, Health Source, and Pubmed databases
yielded very few results for published works about infant weight gain, appropriate or
inappropriate. (CDC, 2016b; Ludington-Hoe, Gittner & Haller, 2013; Moss & Yeaton,
2014; Roy et al., 2016) Many of the publications referred to maternal weight gain or fetal
weight gain. Of the few sources that actually discussed infant weight gain after birth,
“excessive weight gain” was poorly defined at best. Many of the researchers fail to define
what is appropriate or inappropriate weight gain, what is excessive and what is not. If the
definition of excessive weight gain was included, it was often vague. There is evidence
that the rate at which an infant gains weight may be a predictor of obesity risk in older
childhood and even adulthood, but researchers have not answered the basic question of
“what is excessive?”

One research team described excessive weight gain as crossing two or more weight-for-
length percentiles on the CDC charts, but that is not exactly clear (Traveras et al., 2011).
Does that mean two percentiles, for example from the 10th percentile to the 12th
percentile? What is more likely is the CDC growth chart has weight-for-length curves for
the 5th, 10th, 25th, 50th, 75th, 90th, and 95th percentiles. This study probably showed that
infants who move up two or more percentile curves, for example from the 25th to the 75th
percentile curve, in the first six months of life have a higher prevalence of obesity in
adolescence. Another study described excessive weight gain as gaining 3 to 4 BMI
values, but never actually explained what a BMI value was (Ludington-Hoe et al., 2013).
Does that mean a tenth of a BMI measurement? Does it mean whole number change? The
definition of “BMI value” is never included in the paper. A study conducted in Japan
looked for a relationship between rapid weight gain patterns and anthropometric
measurements, but never described what “rapid weight gain” meant at all (Nanri et al.,
In all of the previously mentioned studies on “excessive weight gain,” none included any data or information about the infants’ diets.

It is easy to get confused about what it means for an infant to gain weight excessively. It is not defined in the literature. When a researcher or author uses the term, it is either used with the assumption that the reader is already an expert, with no description whatsoever, or it is described in a vague way that is different from other authors’ descriptions. Because of this, it is difficult for any clinician or student to draw any kind of conclusion about excessive infant weight gain.

The current research is mixed on whether early introduction of solid foods is causing over-nutrition in infants (Fewtrell et al., 2017). It may be the timing of complementary foods, it may be the types of foods introduced, or it may be a non-food risk factor that is causing a rise in obesity rates (Perez-Escamilla, Segura-Perez & Lott, 2017). There are studies that have shown certain minority groups are more likely to expose babies to obesity risk factors such as excessive TV time, insufficient sleep, and maternal depression, and these minority groups are also more likely to have larger infants.

The traditional method of measurement for children under 24 months of age is weight for length, or WFL set by WHO. However, a new study compared the use of BMI to WFL as a predictor of obesity later in life (Roy et al., 2016). This study found a significantly higher positive predictive value for obesity risk later in life using BMI rather than the WHO’s WFL chart. This study and others also asserted greater precision associated with using z-scores than percentiles. Z-scores for weight-for-length, weight-for-age, and other childhood measurements can define a child’s anthropometric measurements with much
more accuracy than the WHO growth chart, especially in the extreme cases (Larson-Nath & Biank, 2016). With the growth charts, the most accurate a description can get is >3rd percentile, but z-scores can allow for closer tracking of weight or height changes.

**Modes of Infant Feeding**

With the ever increasing prevalence of BMI greater than 30 in the United States, more research needs to be done to show effects of breastfeeding duration and delay of solid foods on healthy infant weight status. One study showed breastfeeding cessation before six months of age was linked to earlier introduction of solid foods, which is more commonly foods high in fat and sugar (Moss & Yeaton, 2014).

Mixed mode feeding, or alternating breastfeeding and bottle feeding, has been shown to lead to higher weight-for-age percentiles (Gaffney et al., 2016), while exclusive breastfeeding has well known protective effects in promoting healthy grown patterns (Daniels et al., 2015). Gaffney et al. compared the early feeding practices of low-income, Hispanic immigrant mothers to the results from the Infant Feeding Practices Study II (IFPS II) which is the largest longitudinal infant feeding practices study in the US (Gaffney et al., 2016). The results showed the low-income, Hispanic mothers were more likely than the average American mother to used mixed mode feeding, and the babies of the Hispanic mothers had higher weight status by 12 months of age. Another study with Hispanic mothers found breastfeeding knowledge may be lacking in low-income, minority mothers, and socioeconomic factors such as food insecurity were associated with obesogenic attitudes (Gross et al., 2012). One study in Canada found exclusive breastfeeding was protective against obesity when compared to exclusive formula
feeding, but a combination of breastmilk and formula seemed to neither protect against nor promote obesity (Rossiter et al., 2015).

Mixed feeding can also lead to changes in the infant gut microbiome (O’Sullivan et al., 2015). Babies who are exclusively breastfed have a higher concentration of the actinobacteria, a protective class of bacteria, in the gut whereas formula fed infants tend to have a higher concentration of the pro-inflammatory class γ Proteobacteria. This finding was consistent with the finding that formula-fed infants had a microbiome that more closely resembled that of an adult gut than did breastfed infants.

Breastfeeding can positively influence healthy eating behaviors, and subsequently, weight status by allowing the infant to control his own intake (Fisher et al., 2000). During breastfeeding, a mother has very little control over how much baby eats. The infant has all the control when it comes to choosing to latch on and suckle. In contrast, the caregiver has much more control over intakes when an infant is formula fed. The amount and concentration of formula offered is determined by the person mixing the bottle. This control has an impact on the infant’s ability to regulate his own intake. It also affects mothers’ attitudes about how much control her child should be allowed, both of which carry over into the toddler years. Fisher et al. (2000) determined that when infants were breastfed for the first year, they ate better as toddlers due to a sharing of the regulation between the toddler and mother. This assertion connects a positive association between prolonged breastfeeding and healthy growth patterns in young children. This connection is supported by another study conducted by Stang & Loth (2011) that found higher parental control over feeding results in a decreased ability to regulate caloric intake using internal cues like hunger and satiety (Stang & Loth, 2011).
Another way that breastfeeding can positively influence healthy eating behaviors is by delaying the introduction of solid foods until a baby is six months of age. In the 2002 Feeding Infants and Toddlers Study (FITS), it was discovered that nearly one third of infants were starting solid foods before four months of age, one quarter were given juice before six months of age, and one third of infants were introduced to cow’s milk before 12 months of age (Hendricks, Briefel, Novak & Ziegler, 2006). Other problematic feeding practices found in FITS included low fruit and vegetable consumption, and too many salty/sweet snacks.

Breastfeeding also influences healthy eating behaviors by helping to shape the infant’s taste preferences (Perez-Escamilla et al., 2017). Babies can actually taste what mothers have eaten during gestation when they swallow amniotic fluid. The flavors of food eaten by mothers also passes into the breast milk. If the mother is eating vegetables, which are typically bitter and not widely accepted by infants and toddlers, that bitter flavor will be in the breast milk. Babies who are fed breast milk tasting like vegetables are more likely to accept the bitter flavors of vegetables when they start taking complementary foods.

**Nutrients of Concern**

While it has been established that breastfeeding is the best source of nutrients for infants, it is not without its limitations. The nutrients available to the infants are determined by the mother’s nutrient status and diet. The American Academy of Pediatrics recommends that exclusively breastfed infants be supplemented with 400 IU of oral vitamin D daily as this is one nutrient that is insufficient in breast milk (Kang, Kim, Ahn, Yoo & Kim, 2015). This is to circumvent the effects of vitamin D deficiency such as growth and
developmental problems and rickets. A study in Korea found that maternal vitamin D status was closely, positively related to an infant’s vitamin D status when the infant was breastfed (Kang et al., 2015). In 2010, the IOM stated vitamin D deficiency as less than 20 ng/mL and in 2011 the Endocrine Society recommended that vitamin D levels be between 40-60 ng/ml for adults and children (Holick et al., 2011; IOM, 2010). To alleviate the vitamin D deficiency, the nursing mother can take an oral supplement of 25(OH) D daily (Hollis et al., 2015). According to the 2015 study, lactating mothers can safely and sufficiently transfer adequate amounts of vitamin D to the breast milk when taking an oral supplement of 6400 IU daily. The study also tested interventions with 400 IU and 2400 IU and found that the results from the 6400 IU group were superior to the other two groups when testing the vitamin D status of the breastfed infant. Vitamin D is included in our study because of the IOM recommendation and because a critical review done by Schwerz & Silveiro (2015) found that gestational vitamin D deficiency is linked to low birthweight and growth restriction in the neonate (Schwerz and Silveiro, 2015). Gestational vitamin D deficiency is also linked to poor mineralization of fetal bones, which can result in infants having trouble standing or walking. Infants with poor vitamin D status and poor bone mineralization are at high risk for developing rickets. It can be assumed that less bone mineralization would lead to lower body weight, and lower BMI.

Another nutrient of concern for exclusively breastfed infants is iron. Although it has rather high bioavailability, breast milk has a very low concentration of iron and cannot meet infant requirements (Ziegler, Nelson & Jeter, 2009). An infant’s iron stores are typically depleted by six months, and infants need iron supplementation. This can be
achieved through an oral supplement of ferrous sulfate or by feeding baby a fortified infant cereal. Either route has shown to be equally effective.

**Appropriate Complementary Feeding Practices**

Appropriate complementary feeding practices are an important factor in an infant’s nutrition and weight status. The World Health Organization recommends infants begin receiving complementary foods, or solid foods in addition to breast milk or formula, at six months of age (World Health Organization [WHO] 2017). The current recommendation takes into account that most infants have developed sufficient gastrointestinal tracts, kidneys and swallowing reflexes to take solid foods by four to six months of age (Fewtrell et al., 2017). The foods introduced should be offered two to three times daily from six to eight months of age, then increased to three to four times per day from nine to eleven months (WHO, 2017). When the infant is one to two years, one or two healthy snacks should be added to this feeding routine. Following these WHO guidelines can protect infants against undernutrition as well as over-nutrition.

In addition to the WHO guidelines, the Infant Feeding Practices Study II (IFPS II) outlined some common healthful complementary feeding practices (Fein, Labiner-Wolfe, Scanlon & Grummer-Strawn, 2008). The Fein study found an association between the adherence to the IFPS II guidelines and maternal education level. The guidelines include:

- Introduce solid foods between four and six months of age to ensure baby is developmentally ready.
- Introduce no more than three new foods per week to mitigate any allergic reaction.
- Do not feed juice before six months to prevent juice replacing milk and reducing protein and fat intake.
- Do not give cow’s milk before 12 months.
- Respond early and appropriately to hunger cues, children usually eat at least five times per day.
- Do not pre-masticate (chew) food for infants to prevent spread of illness.
- Limit added salt, naturally occurring sodium is sufficient.
- When adding salt, use iodized salt.
- Only give full-fat cow’s milk to infants between one and two years of age.
- Limit low-nutrient, high calorie foods.

Another study in 2016 supported the WHO and IFPS II guidelines as being protective against inappropriate weight in infants (Wang, et al., 2016). This study found introduction of complementary foods before 4 months of age increased an infant’s risk of becoming overweight and increased the risk of an infant becoming obese during childhood, but did not find any relation between delaying the introduction of solid foods beyond 6 months of age and overweight or obesity in childhood. However, a multi-country, prospective cohort study in the US, China and Mexico had findings that conflicted with the WHO and IFPS II guidelines (Woo et al., 2013). The findings from this study suggested that differences in infant weight status may be less associated with the age at which solid foods are introduced and more associated with the types of foods introduced. For example, higher protein foods, introduced earlier in China, may be associated with early adiposity. Mothers in Mexico were 38-40% more likely to introduce soft drinks before one year of age than mothers in the US or China, which can also lead
to overweight or obese status in infants. It is difficult to draw conclusions across populations, because the introduction of different food types had great variation in the three countries.

According to Perez-Escamilla et al. (2017), complementary feeding practices should also include responsiveness from the caregiver (Perez-Escamilla et al., 2017). They assert that parents and caregivers should be responsive to the infants’ cues for hunger and satiety. Much like how a breastfed infant can control his or her own intake, an infant with a responsive parent should also be able to control his or her own intake by signaling a need for more food or a desire to stop eating. The responsive parent will encourage and nurture these signals by responding appropriately for the child’s development and the situation. An unresponsive parent may maintain complete control over the child’s intake or relinquish all control to the infant, resulting in overindulgence.

Allergies

In addition to the effects of solid food introduction on BMI, the introduction of complementary foods may have some effect on food allergies. Increasing incidence of food allergies and auto-immune disease in the United States has led to a debate among experts on whether early introduction of solid foods prevents or boosts allergic response to food (Niinivirta, Isolauri, Nermes & Laitinen, 2014). The American Academy of Pediatrics recently released a consensus statement on the early introduction of peanuts and peanut containing foods (Fleisher et al., 2015). They now recommend introduction of peanut containing foods between four and eleven months in infants at high risk for peanut allergy. They also recommend infants who develop atopic conditions such as
eczema in the first four to six months of life see an allergist for an evaluation to diagnose food allergies.

Much in the same way the school of thought on peanut allergy has changed, experts are looking at asthma development differently as well (Wyness, 2014). A British study found the effects of early introduction of common food allergens were not as detrimental as once thought. In the 1990’s it was recommended that common food allergens be avoided in infancy to mitigate allergy risk, but studies are debunking this view point. More research on this topic is needed to make a clinical conclusion.

**Summary**

Obesity is a growing problem, and although health professionals agree that it may start during infancy, they do not agree on what it means to have excessive or inappropriate weight gain in infancy (CDC, 2016a; Daniels et al., 2015; Fisher et al., 2000; Gittner et al., 2014; Moss & Yeaton, 2014; O’Sullivan et al., 2015; Shibli et al., 2008; Stang & Loth, 2011). The purpose of this research, which is to determine the relation between breastfeeding cessation and early introduction of solid foods and/or infant formula and BMI in infants, may help clarify this issue. Results may allow for additions to existing guidelines, like those from WHO and IFPS II, to help caregivers know what to feed infants (Fein et al., 2008; WHO, 2017).
CHAPTER III

METHODOLOGY

A study was conducted from 2008 to 2010, funded by the United States Department of Agriculture, to observe the relation between a mother’s micronutrient status and the cognitive development of the infant. This study is a part of that larger study, which was previously approved by the Oklahoma State University Review Board (AS0783) and the current project was given a “Non- Human Subjects designation on January, 27, 2017 (Appendix I).

Sample

The sample for this study included 132 mother-infant pairs, the infants being three months of age at the beginning of the study. All participants included in the study were full-term, single birth infants, who were predominantly breast fed. Those infants who were consuming more than 28 ounces of formula in a week were excluded, as well as those with current illness and those from mothers who had blood transfusions or illness. Each mother-infant pair was assessed when the infant was three, six, and nine months of
Procedures

Mothers filled out a demographic questionnaire and submitted to other assessments that were not analyzed in the current study prior to the initial testing of the infants at three, months of age. After the initial testing, the infants participated in visits at three, six and nine months at which anthropometric measurements were taken. When each infant was three months old, his/her mother completed a 24-hour food record for her infant by weighing the infant before and after nursing for a 24 hour period. Mothers filled out infant dietary questionnaires for the six and nine month visits.

Assessments/Questionnaires

Demographics

During the preliminary visit, the mothers filled out a demographic information questionnaire (see Appendix II). General characteristics were provided about the infant and related families for the sample population. Ethnicity, household income, mother’s education level, gender of the infant and state/federal financial assistance status were included as part of this questionnaire. Mothers also completed a slightly modified version of the Pregnancy Risk Assessment Questionnaire (PRAMS) which asked “How old was your baby the first time you fed him or her anything besides breast milk?” Mothers responded in weeks or months.

Anthropometry
Anthropometric measurements including weight and length of the infants, who were weighed wearing only a dry diaper and t-shirt, were assessed at each visit. Lengths were taken on a recumbent length board (Schorr Production, Olney MD, accuracy to 0.1 cm). Infants’ lengths were taken twice for accuracy, and were taken a third time if the first two measurements differed by more than one centimeter. If a third measurement was needed, the outlier was discarded and the two closer measurements were averaged. Two research assistants were used in taking the lengths measurements to ensure accuracy. One research assistant assured the infant was lying flat on the board, with his or her head against the base. The infant’s knees were then pressed toward the board to extend the legs. The second assistant stabilized the infant’s head at the top of the board for the most accurate length measurement. Infant weights were measured using a digital infant scale and an automatic average of multiple measurements was taken by the digital scale (Seca, Columbia, MD, accuracy to 0.002 kg). A non-stretchable, feed-through plastic measuring tape was used to measure head circumference above the eyebrows and ears, around the largest part of the head. An average of three head circumference measurements was used to calculate z-scores. The infants’ anthropometric measurements were entered into Health Organization (WHO) Anthropometry software v 3.2.2 (Geneva, CHE) and BMI and z-scores were calculated with this software.

*Dietary Assessment*

Before the first visit, predominant breastfeeding, or giving no more than four oz. formula per day, was confirmed for each infant in the study. Two methods of dietary assessment were used. At three months of age, infant weight was measured before and after
breastfeeding and mothers also reported foods consumed by the infant other than breast
milk. At six and nine months of age, a dietary questionnaire was utilized.

At the three-month visit, parents were provided with a digital scale (Seca, Columbia,
MD, accuracy to 0.002 kg) and were asked to use the scale provided to measure and
record infant weight before and after each feeding for a 24 hour period. They were also
asked to record the time of each feeding and describe any food consumed. Any liquid
given in a bottle, including breast milk and formula, was also to be recorded. For any
solid foods consumed by the infant, parents were asked to record the brand name and
save the labels for the next visit, if possible. The data collection form provided to the
parents included instructions for weighing infants on the scale, making sure the scale was
tared prior to weighing (see Appendix III). These instructions also specified to what
decimal place the weight was to be recorded, how the start time and end time of feeding
were to be recorded, and instructing parents to weigh their infant in a dry diaper and in
the same clothes each time weight was measured.

Eight of the 132 mother-infant pairs did not complete the 24-hour infant food record.
These eight pairs were excluded from the data set, leaving 124 mother-infant pairs
included in the data set. Four other 24-hour food records were discarded due to
incompletion or having only four or less feedings in the 24-hour period. Of the 120
remaining mother-infants pairs, 23 were missing one to two weight measurements. The
missing weights were explained in a note as feeding in the middle of the night or
“messiness requiring cleaning.” Other weight changes were averaged to the nearest
hundredth to replace the missing data. The weights were recorded in grams and the
weight change was converted to fluid ounces by dividing grams by 30.8. This number
comes from The Food Processor, Version 10.11.0, Salem Oregon. The derived volume of breast milk, in fluid ounces, was added to bottle fed breast milk or formula to determine a total feeding volume.

*Infant Dietary Questions*

Mothers completed a survey titled “Infant Dietary Questions” at the six and nine month visits. The specific questions are located in Appendix IV. General questions about breastfeeding duration, exclusivity and supplementation or medication received by the infants were included in the survey. The survey also included questions about the infants’ complementary feeding practices. Frequent foods were broken into categories including baby cereal, infant fruit, infant vegetables, baby meat, infant “dinners,” infant juice, infant desserts, other homemade puree or ground baby food, mashed table food, cereal (not infant), regular juice, cow’s milk, and other foods; a slightly more condensed list than that used in the FITS (Fox, Pak, Devaney & Jankowski, 2004). The survey also asked general complementary feeding questions such as frequency of infant meals.

*Vitamin D Methods*

Blood samples were taken from the mothers at the University Health Services Center on the Oklahoma State University Stillwater campus at the three month visit. Safety-multifly needles (Sarstedt Inc., Newton, NC) were used to fill 7.5 mL monovettes. The samples were frozen for 5-OH Vitamin D determination. Concentrations of 25-OH Vitamin D were determined using ELISA kits from ImmunoDiagnostic Systems. Each sample was analyzed in duplicate. Quality control samples were within expected ranges.
Statistical Analysis

Statistical Package for the Social Sciences software (SPSS) was used to analyze all data. Means and standard deviations (SD) were determined for continuous variables and frequencies for categorical ones. Using data obtained from the PRAMS at the three month visit, infants were grouped into those who were exclusively breastfed and those receiving formula or water. Using this grouping, a t-test was used to determine between group differences in BMI z-scores of infants three, six, and nine months of age. A chi square test using the same exclusive breastfeeding grouping and a BMI z-score greater or lesser than 1.04 was performed using three, six, and nine month data. Analysis was also be performed to determine the correlation between amounts of formula given as reported on the Infant Diet Questionnaire and BMI z-score at six, and nine months. Three correlations were performed: six month diet data was correlated with BMI at the six and nine month visits and nine month diet data was correlated with the nine month BMI.

Infants were grouped by responses on the nine months’ Infant Diet Questionnaire into those who received complementary foods before six months of age or not. A t-test was performed to determine any differences in BMI z-scores between groups using six and nine month BMI data. A chi-square test was done to determine any relation between infants receiving solid food or no and BMI z-scores higher than or less than 1.04 at six and nine months.

A Pearson correlation was done to determine any relations between the breastfeeding mother’s vitamin D status and BMI at three and six months, including two additional bivariate correlations controlling for vitamin D supplementation of the breastfed baby.
and any formula fed to an infant that contained vitamin D were completed. Pearson correlation coefficients are reported although Spearman correlation coefficients were also determined but did not change the results. *P*-value is set at < 0.05.
CHAPTER IV

FINDINGS

Demographics

The sample consisted of 132 mothers between the ages of 19 and 42 years and their infants. The mothers’ average age at child birth was 28 years. About 50% of the mothers classified themselves as not working outside the home, 16% said they had part-time employment and 26% were working full-time. Married mothers made up 85% of the study participants. See Table 1 for the full demographic profile of the mothers.

The birthweights of the babies were between 2.55 kg and 4.73 kg with the average birthweight being 3.47 kg. Seventy-one percent of the babies were born vaginally, and 22% were born by cesarean section. When asked about baby’s health, about 2% of mothers said their babies were less healthy than average and about 90% said their babies were generally healthy. Seventy-five percent of babies were reported as never having been seriously ill. At three months of age, 23 infants had a BMI z-score of more than 1.04. At six months of age, 35 infants had a BMI z-score of more than 1.04. At nine months of age, 41 infants had a BMI z-score of more than 1.04. See Table 2 for infant anthropometric measurements.
### Table 1: Mothers’ Demographic Information

<table>
<thead>
<tr>
<th>Category</th>
<th>n</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Infant Gender</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>76</td>
<td>57.6%</td>
</tr>
<tr>
<td>Male</td>
<td>56</td>
<td>42.4%</td>
</tr>
<tr>
<td><strong>Mothers’ Race/Ethnicity</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>White</td>
<td>115</td>
<td>87.1%</td>
</tr>
<tr>
<td>Hispanic</td>
<td>3</td>
<td>2.3%</td>
</tr>
<tr>
<td>Native American</td>
<td>9</td>
<td>6.8%</td>
</tr>
<tr>
<td>Asian</td>
<td>3</td>
<td>2.3%</td>
</tr>
<tr>
<td>Black</td>
<td>2</td>
<td>1.5%</td>
</tr>
<tr>
<td><strong>Mothers’ Education Level</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Less than HS diploma</td>
<td>1</td>
<td>0.7%</td>
</tr>
<tr>
<td>High school graduate</td>
<td>7</td>
<td>5.0%</td>
</tr>
<tr>
<td>Some college</td>
<td>39</td>
<td>27.9%</td>
</tr>
<tr>
<td>College graduate</td>
<td>35</td>
<td>25.0%</td>
</tr>
<tr>
<td>Post grad or above</td>
<td>50</td>
<td>35.7%</td>
</tr>
<tr>
<td><strong>Income Level (annually)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Under $15,000</td>
<td>15</td>
<td>11.5%</td>
</tr>
<tr>
<td>$15,001 to 25,000</td>
<td>21</td>
<td>16.2%</td>
</tr>
<tr>
<td>$25,001 to 40,000</td>
<td>30</td>
<td>23.1%</td>
</tr>
<tr>
<td>$40,001 to 60,000</td>
<td>31</td>
<td>23.8%</td>
</tr>
<tr>
<td>Over $60,000</td>
<td>33</td>
<td>25.4%</td>
</tr>
<tr>
<td><strong># Children born to each mother</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>63</td>
<td>48.1%</td>
</tr>
<tr>
<td>2</td>
<td>41</td>
<td>31.3%</td>
</tr>
<tr>
<td>3</td>
<td>17</td>
<td>13.0%</td>
</tr>
<tr>
<td>4</td>
<td>7</td>
<td>5.3%</td>
</tr>
<tr>
<td>5</td>
<td>3</td>
<td>2.3%</td>
</tr>
<tr>
<td><strong>Mother’s vitamin D level</strong></td>
<td>130</td>
<td>100%</td>
</tr>
<tr>
<td>Mean= 67.92 ± 17.56</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ranges:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>28-45</td>
<td>6</td>
<td>4.6%</td>
</tr>
<tr>
<td>46-65</td>
<td>61</td>
<td>46.9%</td>
</tr>
<tr>
<td>66-75</td>
<td>27</td>
<td>20.8%</td>
</tr>
<tr>
<td>76-85</td>
<td>19</td>
<td>14.6%</td>
</tr>
<tr>
<td>86-105</td>
<td>13</td>
<td>10.0%</td>
</tr>
<tr>
<td>106 and up</td>
<td>4</td>
<td>3.1%</td>
</tr>
</tbody>
</table>
Table 2: Infants’ Anthropometric Measurements

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>Mean Exact Age (in months)</th>
<th>Mean BMI for age z-score</th>
<th>Mean weight for age z-score</th>
<th>Mean Weight for length z-score</th>
<th>Length for age z-score</th>
</tr>
</thead>
<tbody>
<tr>
<td>3 month</td>
<td>132</td>
<td>3.06 ± 0.35</td>
<td>0.11 ± 1.05</td>
<td>0.01 ± 0.99</td>
<td>0.22 ± 1.08</td>
<td>-0.12 ± 1.08</td>
</tr>
<tr>
<td>6 month</td>
<td>127</td>
<td>5.96 ± 0.32</td>
<td>0.31 ± 1.12</td>
<td>0.07 ± 1.04</td>
<td>0.42 ± 1.10</td>
<td>-0.27 ± 1.09</td>
</tr>
<tr>
<td>9 month</td>
<td>119</td>
<td>8.92 ± 0.36</td>
<td>0.65 ± 1.02</td>
<td>0.20 ± 1.05</td>
<td>0.68 ± 1.00</td>
<td>-0.51 ± 1.15</td>
</tr>
</tbody>
</table>

Only three mothers reported giving their infant formula as part of the 24-hour food record at the three month visit. However, on the PRAMS questionnaire, 65.9% of mothers reported their babies had not been given anything but breastmilk; no formula, water, or complementary foods.

The data in Table 3 is from the 24-hour infant food record at the six and nine month visits.

Table 3.

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>Predominant breastfeeding (4 oz. formula or less per day)</th>
<th>Not predominantly breastfeeding (More than 4 oz. formula per day)</th>
<th>Formula consumption per day in oz. Mean and SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>6 month visit</td>
<td>121</td>
<td>99 (81.8%)</td>
<td>22 (18.2%)</td>
<td>4.21 ± 10.40 range: 0.00 – 32.50</td>
</tr>
<tr>
<td>9 month visit</td>
<td>119</td>
<td>85 (71.4%)</td>
<td>34 (28.6%)</td>
<td>5.58 ± 1.10 range: 0.00 – 49.00</td>
</tr>
</tbody>
</table>

Mothers were asked about the introduction of complementary foods at each visit. At the three month visit, no mothers reported giving any complementary foods. The responses differed between the six and nine month visits, with the nine month responses being more consistent with the 3 month reports. Table 4 shows the responses from each visit, but the nine month answers are subsequently used for the purposes of this study.
Table 4. Start of complementary foods

<table>
<thead>
<tr>
<th>6 month visit</th>
<th>n</th>
<th>Complementary foods before 3 months</th>
<th>Complementary foods before 6 months</th>
<th>Complementary foods after 6 months</th>
</tr>
</thead>
<tbody>
<tr>
<td>121</td>
<td>1 (0.8%)</td>
<td>82 (67.0%)</td>
<td>39 (32.2%)</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>9 month visit</th>
<th>n</th>
<th>Complementary foods before 3 months</th>
<th>Complementary foods before 6 months</th>
<th>Complementary foods after 6 months</th>
</tr>
</thead>
<tbody>
<tr>
<td>119</td>
<td>0 (0.0%)</td>
<td>85 (71.4%)</td>
<td>34 (28.6%)</td>
<td></td>
</tr>
</tbody>
</table>

Research Questions

Relation Between Breastfeeding and BMI z-score

Using a t-test, no significant difference in BMI z-score was found between infants (3 months old) who were exclusively breastfed and those receiving formula or water at three (p = 0.297), six (p = 0.940) and nine (p = 0.801) months visits. A chi square test with breastfeeding groups and infants grouped by a BMI z-score of less than or greater than or equal to 1.04 was also performed. There was no significant relation found at the three (p= 0.373), six (p= 0.826), or nine (p= 0.662) month visits.

Table 5. Predominant Breastfeeding vs. Added water or formula

<table>
<thead>
<tr>
<th></th>
<th>Predominantly Breastfeeding</th>
<th>Added water or formula</th>
<th>T value, P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>3 months</td>
<td>n= 87</td>
<td>n= 45</td>
<td>t= 1.046, p= 0.297</td>
</tr>
<tr>
<td></td>
<td>mean= 0.18 ± 1.05</td>
<td>mean= -0.02 ± 1.05</td>
<td></td>
</tr>
<tr>
<td>6 months</td>
<td>n= 99</td>
<td>n= 22</td>
<td>t= -0.075, p= 0.940</td>
</tr>
<tr>
<td></td>
<td>mean= 0.34 ± 1.13</td>
<td>mean= 0.33 ± 1.05</td>
<td></td>
</tr>
<tr>
<td>9 months</td>
<td>n= 85</td>
<td>n= 33</td>
<td>t= 0.252, p= 0.801</td>
</tr>
<tr>
<td></td>
<td>mean= 0.64 ± 1.09</td>
<td>mean= 0.86</td>
<td></td>
</tr>
</tbody>
</table>
Table 6. Chi Square of BMI groups by breast vs formula feeding

<table>
<thead>
<tr>
<th>Predominant Breastfeeding (Y/N)</th>
<th>Underweight/Normal BMI z-score</th>
<th>Overweight/Obese BMI z-score</th>
<th>Total</th>
<th>Chi square</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yes, 3 months</td>
<td>80.5% (70)</td>
<td>19.5% (17)</td>
<td>87</td>
<td>$X^2 = 0.794$</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>$p = 0.373$</td>
</tr>
<tr>
<td>No, 3 months</td>
<td>86.7% (39)</td>
<td>13.3% (6)</td>
<td>45</td>
<td></td>
</tr>
<tr>
<td>Yes, 6 months</td>
<td>71.7% (71)</td>
<td>28.3% (28)</td>
<td>99</td>
<td>$X^2 = 0.383$</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>$p = 0.826$</td>
</tr>
<tr>
<td>No, 6 months</td>
<td>72.7% (16)</td>
<td>27.3% (6)</td>
<td>22</td>
<td></td>
</tr>
<tr>
<td>Yes, 9 months</td>
<td>67.1% (57)</td>
<td>32.9% (28)</td>
<td>85</td>
<td>$X^2 = 0.826$</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>$p = 0.662$</td>
</tr>
<tr>
<td>No, 9 months</td>
<td>61.8% (21)</td>
<td>38.2% (13)</td>
<td>34</td>
<td></td>
</tr>
</tbody>
</table>

Correlation Between Formula Consumed (oz) and BMI z-score

Only three mothers reported giving their infants formula at the three month visit, a correlation using this variable at that time point was not performed.

Using a Pearson correlation, no significant relation was found between the amount of formula consumed at six months and BMI z-score at the six ($r = 0.038$, $p = 0.681$) and nine ($r = 0.043$, $p = 0.648$) month visits. At the nine month visit, there was no significant relation between the amount of formula consumed and BMI z-score. ($r = 0.059$, $p = 0.529$)

Relation Between Introduction of Solid Foods and BMI z-score

Using a t-test, no significant difference in BMI z-scores was found between all of those children reported to be receiving solid food before six months of age (yes/no) at the six ($t = -1.722$; $p = 0.088$) and nine ($t = -0.455$; $p = 0.650$) month visits.
Because of the different growth rates in boys and girls and the near significance of the findings at the six month visit, additional gender specific analyses were performed. Female infants who started eating solid food before six months of age had a higher BMI z-score than those who were not started on solids before six months (t= -1.993; p= 0.050.) This difference disappeared at the nine month visit (t= 0.276; p= 0.784) and was not seen in male infants at the six (t= -0.571; p= 0.571) or nine month (t= -1.328; p= 0.191) visits. Using a chi square test to examine the relation between solid food introduction before 6 months (yes/no) and infant overweight (BMI z score >1.04), there was no significant relation at the 6 month visit (chi square = .110, p = 0.740) nor at the nine month visit (chi square = .071, p = 0.790).

Table 7. Mean and standard deviation of BMI z-score by start of solids

<table>
<thead>
<tr>
<th></th>
<th>Started solids before 6 months</th>
<th>Did not start solids before 6 months</th>
<th>t value, p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>All children at 6 months</td>
<td>n= 82</td>
<td>n= 39</td>
<td>t=-1.722, p=0.08</td>
</tr>
<tr>
<td></td>
<td>mean= 0.09 ± 1.11</td>
<td>mean= 0.46 ± 1.11</td>
<td></td>
</tr>
<tr>
<td>Females at 6 months</td>
<td>n= 49</td>
<td>n= 22</td>
<td>t=-1.993, p=0.05</td>
</tr>
<tr>
<td></td>
<td>mean= 0.39 ± 0.97</td>
<td>mean= -0.11 ± 1.00</td>
<td></td>
</tr>
<tr>
<td>Males at 6 months</td>
<td>n= 33</td>
<td>n= 17</td>
<td>t=-0.571, p=0.57</td>
</tr>
<tr>
<td></td>
<td>mean= 0.57 ± 1.29</td>
<td>mean= 0.35 ± 1.22</td>
<td></td>
</tr>
<tr>
<td>All children at 9 months</td>
<td>n= 76</td>
<td>n= 38</td>
<td>t=-0.455, p=0.85</td>
</tr>
<tr>
<td></td>
<td>mean= 0.69 ± 1.11</td>
<td>mean= 0.60 ± 0.83</td>
<td></td>
</tr>
<tr>
<td>Females at 9 months</td>
<td>n= 45</td>
<td>n= 21</td>
<td>t=0.276, p=0.78</td>
</tr>
<tr>
<td></td>
<td>mean= 0.43 ± 1.17</td>
<td>mean= 0.51 ± 0.82</td>
<td></td>
</tr>
<tr>
<td>Males at 9 months</td>
<td>n= 31</td>
<td>n= 17</td>
<td>t=-1.328, p=0.19</td>
</tr>
<tr>
<td></td>
<td>mean= 1.06 ± 0.91</td>
<td>mean= 0.70 ± 0.87</td>
<td></td>
</tr>
</tbody>
</table>
Table 8. BMI groups chi-square by start of solids at 6 and 9 month visit

<table>
<thead>
<tr>
<th></th>
<th>Underweight/Normal BMI z-score</th>
<th>Overweight/Obese BMI z-score</th>
<th>Total</th>
<th>Chi square</th>
</tr>
</thead>
<tbody>
<tr>
<td>6 mo- started solids before 6 months</td>
<td>25.6% (31)</td>
<td>6.6% (8)</td>
<td>39</td>
<td>n = 121</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>X²=1.639</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>p= 0.200</td>
</tr>
<tr>
<td>6 mo- did not start solids before 6 months</td>
<td>46.3% (56)</td>
<td>21.5% (26)</td>
<td>82</td>
<td></td>
</tr>
<tr>
<td>9 mo- started solids before 6 months</td>
<td>20.9% (24)</td>
<td>12.2% (14)</td>
<td>38</td>
<td>n = 115</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>X²=0.106</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>p= 0.745</td>
</tr>
<tr>
<td>9 mo- did not start solids before 6 months</td>
<td>44.3% (51)</td>
<td>22.6% (26)</td>
<td>77</td>
<td></td>
</tr>
</tbody>
</table>

Relation Between Maternal vitamin D Status and Infant BMI z-score

All mothers in this study had a normal or higher than normal vitamin D status, which can be found in Table 1. Using a Pearson correlation, no significant relation was found between maternal vitamin D status and BMI z-score at the three (r= -0.035, p= 0.692), six (r= -0.020, p= 0.824), or nine (r= 0.042, p= 0.657) month visits. When controlling for vitamin D contained in formula received at six months of age, no significant correlation was found at the six (r= 0.007, p= 0.941) or nine (r= 0.064, p= 0.503) months visits. When controlling for formula received at nine months of age, no significant correlation was found between maternal vitamin D status at infant BMI z-score (r= 0.037, p= 0.697). Infant formula consumption was reported by the mothers in the diet questionnaires at the six and nine month visits.

Using a Pearson correlation, controlling for vitamin D containing formula consumption and vitamin D supplements given to the infants at six months of age, no significant correlation was
found between maternal vitamin D and infant BMI z-score at the six (r= 0.003, p= 0.973) or nine (r= 0.048, p= 0.618) month visits. When controlling for formula consumption and vitamin D supplementation at nine months, no significant correlation was found between maternal vitamin status and infant BMI z-score (r= 0.029, p= 0.761). The vitamin D supplementation was reported on the diet questionnaire, completed by the mothers at the six and nine month visits and can be found in Table 9.

Table 9. Vitamin D Supplementation of Infants

<table>
<thead>
<tr>
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<th>6 months, n= 132</th>
<th>9 months, n=132</th>
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<tbody>
<tr>
<td>Receiving vitamin D supplement</td>
<td>11 (8.3%)</td>
<td>9 (6.8%)</td>
</tr>
<tr>
<td>Not receiving vitamin D supplement</td>
<td>121 (91.7%)</td>
<td>123 (93.2%)</td>
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</table>
CHAPTER V

CONCLUSION

This study sought to determine any relation between breastfeeding cessation and early introduction of solid foods and BMI in infants. The study included 132 mother-infant pairs with the mothers being between the ages of 19 and 42 years, and the average age at first childbirth being 28 years. All infants were within the normal range for birthweight. The infants were reported by the mothers to be more healthy than unhealthy.

The infants were seen at approximately three months, six months, and nine months of age. At each visit, mothers were asked to fill out questionnaires regarding breastfeeding, formula, and complementary foods (Appendix IV). Three mothers reported giving infant formula before three months of age. No mothers reported giving complementary foods before three months of age. At the six month visit, 99 mothers (81.8%) were predominantly breastfeeding or giving no more than four oz. of formula per day. According to the diet questionnaire completed by the mothers at the nine month visit, 76 (61.8%) of infants received complementary foods before six months of age.

The study found some transient, gender-specific effects of early introduction of complementary
foods on the BMI z-score of infants. At the six month visit, female infants who had been introduced to complementary foods before six months of age had a higher BMI z-score than female infants who had not been introduced to complementary foods before six months of age. This difference disappeared by the nine month visit and was not seen in male infants at any time point.

**Specific Aims**

*Determining the relation between breastfeeding and BMI at three, six and nine months of age.*

No significant difference was found between BMI z-scores in infants predominantly breastfed and those predominantly formula fed.

*Relation between amounts of formula consumed and BMI z-score*

No significant difference was found between BMI z-score in infants and the amount of formula consumed.

*Relation between introduction of solid foods and BMI z-score*

When looking at all infants, no significant relation was found between timing of solid food introduction and BMI z-score. When looking at male infants only, no significant difference was found in BMI z-scores between males who started solids before six months and those who did not. When looking at female infants, a significant difference was found between girls who started solid foods before six months of age at the six month visit, but not at the nine month visit.
Relation between maternal vitamin D status and BMI z-score

No significant relation was found between maternal vitamin D status and infant BMI z-score, even when controlling for vitamin D supplements and formula containing vitamin D given to the infants.

The hypothesis of this study was that early breastfeeding cessation and/or early introduction of solid foods is associated with a higher BMI z-score for infants. Our findings did not support accepting this contention.

Discussion

Moss and Yeaton (2014) found a positive impact on healthy weight status associated with exclusive breastfeeding and the delay of introducing complementary foods until six months of age. Moss and Yeaton were looking for “healthy weight status” or “obese weight status” in children at nine months, two years, and four years of age, and obtained diet information from a retrospective interview with the mother. Their study found babies who were breastfed, at any time, had lower odds of obesity than infants who were never breastfed. They also found that infants who did not receive solid food until four or five months of age had lower odds of obesity than infants who received solid foods before four months of age. This study did not find significant difference in weight status between the infants were breastfed or formula fed nor between infants who started complementary foods before six months or after six months, except for the transient difference found in girls at six months of age. However all infants in the present study would have been in Moss and Yeaton’s low risk group.
Gaffney et al. (2016) claimed that mixed mode feeding, or alternating between breastfeeding and bottle feeding has been shown to lead to higher weight-for-age percentiles. The findings of the present study do not support this claim. In the present study several mothers used mixed mode feeding, but their infants were not significantly heavier than those who were breastfed only. However, the Gaffney study included Medicaid and WIC eligible, Hispanic mother-infant pairs, where the present study included mostly middle-income class, Caucasian mother-infant pairs which might contribute to the differential findings.

A study by Hendricks et al. (2006) found exclusive breastfeeding delayed the introduction of complementary foods until a baby was six months of age. The findings of the present study do not support this claim. At six months of age, 82% of the infants in the present study were still predominantly breastfed, but 68% had already been introduced to solid foods. The present study population does not match that of the Hendricks study. The Hendricks study had a larger, more random sample whereas the present study was small and homogenous.

A study by Wang et al. (2016) found a higher risk of infants being overweight or obese when introduced to solid foods before four months of age. None of the mothers in the present study reported introducing solids before four months of age; no significant relation was found between timing of complementary foods (between four and six months) and infant BMI z-scores.

Studies by Fisher et al. (2008) and Wang et al. (2016) suggested that breastfeeding exclusively lowers the likelihood of maternal control over infant feeding. Both of these
studies asserted breastfeeding allows the infant to remain in control of his or her own intake during infancy and this behavior follows the infant into childhood. The infant’s control over his or her own intake has beneficial effects on obesity risk. The present study did not follow the infants into childhood, or examine parental vs. infant control. Although a difference was not found in BMI z-scores between infants who were breastfed and those who were formula fed, all infants in the present study were breastfed for the first three months of life.

The current literature is confusing. There is currently no universally accepted definition for overweight/obesity in children under two years of age (Shibli et al., 2008). The present study had its own definition of overweight as well. While the CDC growth charts were used, the present study used BMI z-scores rather than BMI. The current research is also confusing whether or not early introduction of solid foods causes over-nutrition in infants (Fewtrell et al., 2017). Fewtrell suggested the problem could be timing of complementary foods, types of food introduced, or some other factor. The present study showed that starting solids between four and six months of age did not cause a significant difference in BMI z-scores in infants who were predominately breast fed. Introducing solid foods at four months of age would be considered early by the WHO recommendation, which was used by Fewtrell.

Some researchers have stated that early feeding practices largely determine the pattern of weight gain in the first year of life, impacting infant and childhood weight status (Daniels et al., 2015; Fisher et al., 2000; Moss & Yeaton, 2014; Stang & Loth, 2011). However, overweight and obesity are not well defined for infants under the age of two years (Shibli et al., 2008). Current research is mixed on whether early introduction of solid foods is
causing over-nutrition in infants (Fewtrell et al., 2017). As previously mentioned, the present study showed only a transient difference in female infants mentioned before. When all infants were included, or just the male infants, starting solids before six months of age did not significantly increase the infants’ BMI z-scores. The present study did not help to clarify this topic.

In late 2016, a systematic Cochrane review of 11 clinical trials was conducted by Smith and Becker to evaluate the benefits and harms of early complementary foods. They found no risk of negative weight status outcomes associated with early introduction of solid foods, defined as introduction between four and six months of age. This review supports the findings of the present study.

Limitations
The limitations of the present study are mostly related to the sample population. The mothers included were homogenous. The majority of the mothers included were white, college educated, and had one or two children. All mothers had a normal or above normal vitamin D status. Most mothers reported their infant as being more healthy than not healthy and all mothers were breastfeeding at the start of the study. The results of this study may not apply to infants who received more formula early in life. This study included infant diet records that were reported by the mothers, increasing the risk of bias or error. The questionnaires given to the mothers were short and not in-depth, not quantitative and did not start before three months of age. All of these things are limitations to this study. Another limitation is the fact that infant overweight, infant obesity, and excessive infant weight gain are still poorly defined, making it difficult to compare the results of this study to those of previous studies.
Future Research

Much more research is needed in this area. While the present study mostly included breastfed infants, it would be useful to see studies conducted with mostly formula fed infants. Another useful topic of research would be breastfed, nursed compared to bottle fed with breast milk. This would be of interest due to the research showing maternal control over intake increases obesity risk, but infant control lowers obesity risk (Fisher et al., 2008; Wang et al. 2016). Although breast milk is beneficial, the shift of control from infant to mother might play a role in infant weight status. It is also important to clarify the definition of overweight/obesity in infants and the risk for overweight/obesity in infants.

Implications for Practice

The World Health Organization recommends children begin receiving complementary foods at six months of age. Cooperative Extension Educators or WIC Dietitians should also recommend introducing complementary foods at six months of age; however, in working with women in the Stillwater, OK area (where this study was conducted) it is easy to see that pediatricians often recommend starting cereal at four months of age. It can be frustrating when mothers are receiving different information from different health care providers. For practicing dietitians, the results of this study are good news. This study supports the recommendations already being made, but also provides pardon for mothers who might have started their infants on complementary foods between four and six months of age.

Summary

Obesity and childhood obesity are public health problems. However, obesity/overweight status in infancy, based on the results of the present study, it does not appear to be related to early feeding practices. Much more work needs to be done before adding to mothers’ stress. Dietitians need to focus on what they know works: breastfeed, add complementary food as developmentally
appropriate, and encourage mothers/parents be responsive to hunger cues. The proper timing for introducing solid foods is largely dependent on the infant’s development. All babies are different, and mothers should be reassured by that fact and encouraged to act based on their knowledge of their child’s individuality.
REFERENCES


APPENDICES
Appendix I: Application for Review of Human Subjects Research

Oklahoma State University
University Research Compliance
Institutional Review Board

Application for Review of Human Subjects Research
Pursuant to 45 CFR 46

<table>
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<tr>
<th>Name of Main PI</th>
<th>Jessica Riggin</th>
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Application for Review of Human Subjects Research
Pursuant to 45 CFR 46

Name of Main PI: Jessica Riggin

Co-PIs and other research team members information:

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Is this research being funded by an external funding agency? ☐ Yes  ☑ No

Part 2: Determination of Research

Will the data be obtained in a systematic manner?  Yes

Will the intent of the data collection be for the purpose of contributing to generalizable knowledge (the results of the activity are intended to be extended beyond a single individual or an internal program to be widely applicable)?  Yes

Part 3: Determination of Human Subject

Does the research involve obtaining information about LIVING individuals?  Yes

Does the study involve intervention or interaction with a "human subject"?  No

Does the study involve access to identifiable private information?  No
Application for Review of Human Subjects Research

Pursuant to 45 CFR 46

Name of Main PI: Jessica Riggin

Are data received by the investigator with identifiable private information? No
Are the data coded such that a link exists that could allow the data to be reidentified? No

Your research project does not qualify as human subjects research per 45 CFR 46.
Please proceed to the next section to complete the Determination of Non-research or Non-human Subjects Form.

Please describe the subject population you have chosen for this project including inclusion or exclusion criteria for subject selection.

Subjects are 132 mother-infant pairs recruited as part of a completed study Maternal Dietary Nutrients and Neurotoxicants in Infant Cogitative Development (IRB application AS0783). The sample for this study includes 132 mother-infant pairs, the infants being three months of age at the beginning of the study. All participants included in the study are full-term, single birth infants, who were predominantly breast fed. Those infants who were consuming more than 28 ounces of formula in a week were excluded, as well as those with current illness and those from mothers who had has blood transfusions or illness. Each mother-infant pair was assessed when the infant was three, six, and nine months of age. Mothers provided written informed consent for themselves and assent for their infants.

Please provide a brief summary of this project. Include the research question and a brief description of the methodology, including recruitment and how data will be collected. Please attach a copy of your survey.

The purpose of this research is to determine the relation between breastfeeding cessation and early introduction of solid foods and/or infant formula and BMI in infants. It has long been touted that exclusive breastfeeding is superior to formula feeding for many reasons including healthy infant weight. What may not be considered in this claim is that exclusive breastfeeding for at least six months delays the introduction of solid foods until the baby is six months of age. The objective of this project is to determine to what degree formula feeding and solid food introduction predict infant weight greater than the 75th percentile at three, six months and 9 months of age. The hypothesis is that the diet of infants at three and six months of age is positively correlated with BMI status at subsequent visits.

How will the results of this project be used? (e.g., Presentation? Publication? Report to funding agency?)

Thesis and perhaps publication
Application for Review of Human Subjects Research
Pursuant to 45 CFR 46

Name of Main PI: Jessica Riggin

IRB Number: [Blank]

Part 9: Signatures

Principal Investigator's Assurances

1. I certify that all information provided in this application is complete and correct.

2. I understand that, as Principal Investigator, I have the ultimate responsibility for the conduct of this study, the ethical performance of this project, the protection of the rights and welfare of human subjects, and strict adherence to any stipulations imposed by the OSU IRB.

3. I agree to comply with all OSU policies and procedures, as well as with all applicable federal, state, and local laws regarding the protection of human subjects, including, but not limited to the following:
   a. Conducting the project by qualified personnel according to the approved protocol.
   b. Implementing no changes in the approved protocol without prior modification approval from the IRB.
   c. Obtaining informed consent from each participant or their legally responsible representative prior to their participation in this project employing only the currently approved means of consent (e.g., form).
   d. Promptly reporting unanticipated problems and/or adverse events to the IRB in writing within 5 working days after learning of the occurrence.
   e. Conduct this study only during the time period approved by the OSU IRB.

4. I will prepare and submit a continuation request and supply all supporting documents to the IRB office at least two weeks before the approval period has expired if it is necessary for me to continue the research project beyond the time period approved by the OSU IRB.

My signature indicates that I have read, understand and agree to conduct this research project in accordance with the assurances listed above.

Signature: Jessica Riggin

Date: Dec 13, 2016
Application for Review of Human Subjects Research
Pursuant to 45 CFR 46

Name of Main PI  Jessica Riggs  IRB Number
Office Use Only
Office Use Only

Faculty Advisor Assurances

1. I have read the protocol submitted for this project for content, clarity, and methodology.

2. By my signature as faculty advisor on this research application, I certify that the student or grant investigator (i.e. visiting scholar) is knowledgeable about the regulations and policies governing research with human subjects and has sufficient training and experience to conduct this particular study in accord with the approved protocol.

3. I agree to meet with the investigator on a regular basis to monitor study progress. Should problems arise during the course of the study, I agree to be available, personally, to supervise the investigator in solving them.

4. I assure that the investigator will promptly report unanticipated problems and/or adverse events to the IRB.

5. If I will be unavailable, I will arrange for an alternate faculty sponsor to assume responsibility during my absence.

6. If the investigator is unable to fulfill requirements for submission of continuations or modifications, I will assume that responsibility.

Signature  Tay Kennedy  Date

Part 10: Training & Reminder Checklist

Human Subjects Training Needed:
Please complete training at the Collaborative Institutional Training Initiative (CITI) website. Specific enrollment instructions can be found at the OSU Training & Education Web Page. Please submit your training completion report with your application.

The training requirements listed below were identified as necessary due to responses made in this application. All PIs and research staff must complete and pass these modules.

Documents Needed:
Please submit the following documents, if applicable, as an attachment to your email when submitting the application. Include all attachments in either MS Word or Adobe PDF document format only. All pages for each appendix should be combined into one file labeled with the appendix letter and title. Applications and appendices are only accepted electronically via the main PI’s OSU email account. Select the Submit button below to email the IRB. If you use a webmail client (e.g. Outlook or Orange Mail) you will need to submit your form manually not using the submit button to irb@okstate.edu.

Forms and templates are available on the OSU IRB website.
Application for Review of Human Subjects Research
Pursuant to 45 CFR 46

Name of Main PI: Jessica Riggin

☐ Appendix G: Surveys/Measures/Demographics/Interview Questions
Appendix II: Demographic Questionnaire

Demographic Information Questionnaire

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

Gender of baby: __ Male __ Female

Birthdate of baby:
Month ___ Day ___ Year ___

Birth weight of baby: ___ lbs ___ oz

Date of expected birth (due date):
Month ___ Day ___ Year ___

Was the baby born by C-section? YES NO

Maternal Information
Birthdate:
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):
___ Native American Nation: ____________________
___ African American
___ Hispanic
___ Asian
___ White
___ Multiracial:
___ 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
Appendix III: Infant Diet Record with Weighing Instructions

Dietary Intake Record

<table>
<thead>
<tr>
<th>Time</th>
<th>Food</th>
<th>Description</th>
<th>Weight before meal</th>
<th>Weight after meal</th>
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For breast-fed and/or formula-fed infants also receiving solid food.
Dietary Intake Instructions

To find out how much your child is eating, we would like to weight your child before and after each feeding over a 24-hour period.

Please remember to:

♦ Record the time each feeding begins and ends.
♦ Weigh the child in the same clothes or blankets each time.
♦ DO NOT change diapers.
♦ Make sure the scale is zeroed before weighing.
♦ Write down all 4 numbers in the weight.

If you give your child any additional food or liquids besides breast milk, please WRITE THESE ITEMS DOWN AS WELL.

For bottle feeding: Include all liquids and or solids given in a bottle.

For solid food: write down the type of food including the brand name. Saving labels for us is a good idea.

Call **405-744-5965** with any questions!
VITA

Jessica Riggin

Candidate for the Degree of

Master of Science

Thesis: EARLY FEEDING PRACTICES AND BMI IN INFANTS

Major Field: Nutritional Sciences

Biographical:

Education:

Completed the requirements for the Master of Science in Nutrition at Oklahoma State University, Stillwater, Oklahoma in May 2018.

Completed the requirements for the Bachelor of Science in Nutrition at Oklahoma State University, Stillwater, OK in 2015.

Completed the requirements for the Bachelor of Science in Hotel/Restaurant Administration at Oklahoma State University, Stillwater, OK in 2006.

Experience:

Family Consumer Sciences Educator for Oklahoma Cooperative Extension Service, June 2008 to present

Professional Memberships:

American Academy of Nutrition and Dietetics, student member

Oklahoma Academy of Nutrition and Dietetics, student member

National Extension Association of Family Consumer Sciences

Oklahoma Extension Association of Family Consumer Sciences