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Maternal Stress, Breastmilk IGF-1, and Offspring Growth among Breastfeeding Mothers-Infant Pairs in the Tampa Bay Area

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Maternal Stress, Breastmilk IGF-1, and Offspring Growth among Breastfeeding Mothers-Infant Pairs in the Tampa Bay Area

by

Lauren Gottfredson

A thesis submitted in partial fulfillment of the requirements for the degree of
Master of Public Health
Department of Global Health
College of Public Health

and

Master of Arts
Department of Anthropology
College of Arts and Sciences
University of South Florida

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Dedication

To those who supported me: Mom, Dad, Jeffrey, Michael, and Amy
Acknowledgments

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Abstract

**Background:** Maternal stress during utero has been shown to have negative health consequences on the offspring, including low birth weight and increased risk of adult disease. Variation in breastmilk may act as an environmental cue of maternal stress and continue to program the infant during early life. This research aimed to explore the role of breastmilk on developmental programming of the infant. Specifically, to examine how breastmilk may act as a medium for the exposure of stress between the mother and the offspring, and see if variation in insulin like growth factor-1 (IGF-1) a potential mechanism for the relationship.

**Methods:** Survey-interviews, anthropometrics of the mother and offspring (height and weight), and breastmilk samples were collected for 31 breastfeeding mother-offspring pairs in the Tampa Bay area. Breastmilk was analyzed for IGF-1 and fat content. Maternal stress was measured through the PSS-10 and two self-reported ten-point stress scales. Offspring length for age and weight for age Z-scores were calculated using LMS equation.

**Results:** PPS-10 score was negatively correlated with child length for age and weight for age Z-scores. Child length for age and weight for age Z-scores were also negatively correlated with the breastmilk fat variables (creamatocrit percent, fat g/dL, and kcal/dL). No relationships were found between breastmilk IGF-1 and offspring length for age, weight for age, or maternal stress.

**Conclusions:** Results indicate that maternal stress may negatively impact offspring growth. However, more research is necessary to better understand if or how breastmilk fat may act as a mechanism to mediate offspring growth due to maternal stress. This sample had low levels and prevalence of detectable IGF-1, which likely contributed to the lack of statistical relationships.
Further research using lower dilutions and larger samples sizes is necessary to better explore the potential role of breastmilk IGF-1 on offspring growth and/or its relationship to maternal stress.
Introduction: Statement of the Problem

With prevalence of non-communicable diseases rising globally, understanding the complex, developmental pathways that may increase disease risk is necessary for prevention. Maternal stressors before and during pregnancy have been shown to impact offspring health via increased risk of low birth weight and high risk of adult non-communicable disease (Adelman et al. 2008; Barker 1994; Bogin et al. 2007; Gluckman and Hanson 2006; Kuzawa and Sweet 2009). The Developmental Origins of Health and Disease (DoHAD) paradigm examines fetal and early life environment associated with adult disease risk. This thesis uses a DoHAD framework to understand how maternal environment via breastmilk may continue to program the infant after birth.

Research on human milk variation is gaining interest in the fields of anthropology and human biology (Quinn 2012). Breastfeeding, and therefore breastmilk, is an integral part of maternal and child health and well-being. The beneficial relationship of breastfeeding and infant health is well established. There is strong evidence to support the proposition that early induction of breastfeeding, as well as exclusive breastfeeding for the first six months, decreases infant risk of infection and increases immune function (Walker 2010). Moreover, breastfeeding is associated with healthy growth trajectories and decreased adult disease risk (Dewey et al. 1993; Gale et al. 2012; Hassiotou and Geddes 2014; Thompson 2012).

Breastmilk composition in regards to macronutrients is buffered from maternal condition. However, micronutrients, including vitamins, fatty acids, and hormones, are more likely to vary as a result of maternal factors (Thakkar et al. 2013), and thus reflect maternal environment. This
research explores the role of breastmilk as a programming mechanism on early life development. Specifically, how breastmilk may or may not be affected by maternal condition and thereby affect the infant. Understanding breastmilk composition and variation, and how it may affect early life development, has important implications on infant, maternal, and population health.
**Literature Review**

**Theoretical Background**

*Maternal Stress:*

Humans respond to stress with two main physiological systems; the hypothalamic-pituitary-adrenal axis (HPA) and the sympathetic adrenal medullary system (SAMS). The HPA axis produces cortisol, which is a commonly used marker to measure stress. Cortisol has been shown to increase during many stress events including lab studies, stressful jobs, stressful activities, and daily hassles (Pollard and Ice 2007 in Stinson et al. 2010B). The SAMS releases epinephrine and norepinephrine, which elude the “flight or fight” response to stress. This response is characterized by physiological changes that include a decrease in blood flow to the organs not needed for rapid movement to allow for an increase in heart rate, blood pressure, mental activity, and cellular metabolism (Brown 2006 in Stinson et al 2010; Stinson et al. 2010B). A benefit of using cortisol as a measure of stress is that it is commonly studied and thus the levels associated with stressful and non-stressful states are well established. However, it is also highly variable between individuals, populations, and throughout the course of the day.

Generally speaking, both systems work to release hormones that help to cope with stress. Acute stresses consist of an isolated stress incident, which induce an individual stress response. Chronic stress, however, can result in a continuous change in hormone levels, which can affect reproduction hormone secretions (Miller n.d.). Further, chronic elevation of cortisol has been shown to have negative physiological and psychological health consequences. These can include depression, immunosuppression, obesity, cardiovascular disease, diabetes, and osteoporosis.
(Stinson et al. 2010B: 474). These increased hormonal levels can inevitably impact
reproduction. Humans however, have the notable ability to adapt to such long-term stress. This
adaptability is known as allostasis and acts as a coping mechanism in which the individual can
“achieve stability through change” (Stinson et al. 2010B: 463). The level of chronic stress of the
individual throughout time can be measured through his or her allostatic load, which is
considered to be the body’s reaction and consequences of allostasis or adaption to stress (Stinson
et al. 2010B: 458). Additionally, there are cultural and individual variances in stress response.
An individual’s response to stress can vary depending on cultural or environmental influences.

Maternal stress has been shown to have negative reproductive consequences. Epigenetic
studies have shown that maternal stress not only affects the individual who was exposed to
stress, but can also be heritable when epigenetic changes occur in the gametes. Moreover, recent
research has shown that the mother’s fetal environment may have an even greater impact on
child’s health later in life, showing an effect of intergenerational inertia (Kuzawa 2005; Kuzawa
in press; n.d.). In particular, the severity of the effects of prenatal stress on birth outcome is
dependent on the timing of the exposure and the sensitivity of the developing system. If the
stress were to occur during fetal development of a more sensitive system the ramifications would
be more severe. Further, in research done on natural disasters, stress, and birth outcomes,
researchers found that objective hardship (what happened) had a greater impact than subjective
distress (how the women reacted) (Dancause et al. 2011: 817-818).

Research shows a multifaceted relationship between stress and lactation. Specifically,
increase in stress can result in a negative effect on the process of lactation, biologically and
found that sucking-induced oxytocin is pulsatile, or pulsating, and that psychological stress
reduces the pulsating. This relationship then suggests relaxation is necessary for an adequate let down response for the mother. Along with its role in the milk let down response, oxytocin also plays an additional role in social relationships and maternal bonding (Kumsta and Heinrichs 2013), and as a stress hormone (Lau 2001).

Originally, oxytocin was shown to have a negative relationship with stress in breastfeeding women, in which higher levels of oxytocin decreased the women’s stress response (oxytocin acted as a protective agent against stress). In more severe stress events, stress response inhibited the release of oxytocin and thereby the letdown response (Rudzik et al. 2014). However, further research has not supported this relationship between oxytocin and stress. Oxytocin may operate differently between breastfeeding and non-breastfeeding women (Rudzik et al. 2014), and/or differently between socially connective and stressful situations (APA 2014). In a study among first time mothers during the postpartum period in Sao Paulo, Brazil, Rudzik et al. (2014) found an increase in oxytocin was positively associated with an increase in perceived stress. This highlights the fact that further work on oxytocin’s role as a stress hormone is necessary to understand how oxytocin responds to, or affects the stress response, in both breastfeeding and non-breastfeeding women.

Nonetheless, it is clear that stress has an integrative relationship with lactation. In addition to oxytocin’s potential modulation of the stress response, opiate levels increase during breastfeeding and can provide a protective role to stress (Lau 2001: 399). The protective aspect of breastfeeding on stress has also been shown in lab studies on rats (Mezzacappa et al. 2003).

Stress during delivery can also have a direct impact on lactogenesis, or the onset of milk production following pregnancy. Labor can be associated with high levels of stress, which leads to hormone variation (Chen et al. 1998: 335). Thus, research has shown that high levels of stress
during delivery can impact mechanisms related to milk let down. Specifically, Chen et al. (1998) compared birth experience and lactogenesis (the onset of milk secretion or production) between multiparous and primiparous women. Lactogenesis was measured in three ways; by biomarkers including lactose concentration and casein (a breastmilk protein) in the milk, the women’s perception of breast fullness, and milk volume on day five. Stress was assessed through hormone levels in blood samples during pregnancy and lactation, and by direct observation of the deliveries by the researchers. The findings confirmed that stress during labor and delivery was associated with negative impacts on lactation including delayed fullness, delayed casein appearance, and lower milk volume. Additionally, it was found that primiparity and extended labor were risk factors for delayed lactogenesis (Chen et al. 1998). This helps to highlight the syndemic nature of health concerns such as these, in which stress impacts both birth and lactation, and birth itself can impact lactation.

**Maternal Stress as an Adaptive Mechanism:**

From an evolutionary perspective it is important to understand why we experience stress. If a stress response can ultimately lead to poor health outcomes, what was its value evolutionarily? Cortisol allows for energy mobilization and increased alertness. If an individual is placed into an acute stress event, he/she would benefit from having an increase awareness and energy in order to escape the situation. Additionally, epinephrine creates the sense of urgency and motivation to escape or deal with the stress event (Stinson et al. 2010B). Thus, individuals who release these hormones during stress events are more likely to survive and thus are likely to have higher fertility.

There has been research supporting maternally derived stress as an adaptive mechanism of the fetus and/or the mother as well. First proposed by Barker (1994), the Barker hypothesis,
also known as fetal origins hypothesis, proposes that stress in utero effectively programs the fetus to a stressful post-uterine environment. This programming is adaptive when the pre and post uterine environments are in synchrony but becomes maladaptive when they are mismatched, and can lead to cardiovascular disease and diabetes later in life. The role of maternal stress may have thus played an adaptive role for mother and offspring, as long as nutritional stress remain constant. According to this view, maternal stress and fetal programming reduces current investment on the part of the mother allowing her to put additional investment on future condition, benefiting her, but not the offspring (Nepomnaschy et al. 2006; Sheriff and Love 2013).

*Developmental Programming and Disease Susceptibility:*

There is strong evidence to support the relationship between low birth weight and increased risk of cardiovascular disease and diabetes later in life in nutrient rich environments. However, why this relationship exists is less clear. Fetal origins hypothesis argues that poor fetal environment caused by nutritional stress leads to underdeveloped organs. When the offspring is exposed to a mismatched environment, including a high caloric diet, the body is not adapted to cope with the high caloric intake, resulting in the onset of disease (Barker 1994). The predictive adaptive response hypothesis argues along the same lines; that the fetus receives signals from the mother on how the current environment is, effectively predicting its adult environment to be similar and acts to adapt to the expected adult environment while in utero (Gluckman et al. 2007). However, it is likely that humans evolved in environments that were rapidly changing, thus maternal cues during utero would have provided poor information on how the future environment would be. The idea of intergenerational inertia proposes that the information
received by the fetus in utero is actually an average of how the environment has been over a long maternal line, providing a more even keel of environmental indications (Kuzawa 2005).

The developmental programming hypothesis states that poor fetal environment leads to smaller adult body size, which is unhealthy in adulthood, regardless of environmental mismatch. This represents physiological disruption that is maladaptive rather than adaptive. Further, these effects of physiological constraint are permanent and put the adult at increased risk of disease later in life (Bogin et al. 2007). In sum, the developmental programming hypothesis states that low birth weight is not a form of adaptation to poor environment, but rather a negative consequence of energetic constraint.

Such programming effects can continue past utero and into early life. The developmental origin of health and disease paradigm (DoHAD) examines both early post-natal life and fetal environments in relation to adult health (Gluckman and Hanson 2006). The continued programming effects of mothering practices during infancy have been confirmed in animal studies (Hinde and Capitano 2010). Moreover, breastmilk may continue to use maternal cues to program the infant. Although few studies have examined programming effects of breastmilk, a couple have examined its role on infant behavior (Grey et al. 2013; Hinde and Capitano 2010). Additionally, it is already well known that the disease ecology of the mother is reflected in her breastmilk, in which breastmilk immunoglobulin A (IgA) contains an immunological memory of the mother’s pathogen exposure (Miller and McConnell 2012). This form of passive immunity is arguably an example of maternal programming via breastmilk, in which the breastmilk is programming the immune development of the infant via maternal pathogen exposure. Therefore, the use of maternal cues within breastmilk for other aspects of infant development can be expected.
All these hypotheses are based on concept of human phenotypic plasticity, and more specifically, the canalization of developmental plasticity. Many phenotypes are plastic, or are able to change and potentially adapt to the environment faster than genotypic change can occur. In fact, it is hypothesized that the large extent of human adaptability is due to plasticity, and that the plasticity of human phenotypes is an adaptation (Thomas 1998). Yet, it is developmental plasticity that is hypothesized to be causing the maladaptive adult phenotypes that are leading to the development of chronic disease, presenting a paradox. The maladaptive aspect may be due to developmental windows and canalization. Human plasticity is not always reversible and certain phenotypes develop within restricted windows. When environmental stressors are introduced during critical windows, development can be altered but cannot be reversed after the window closes. This is referred to as canalization. Further, there is variability in the length and timing of these windows between traits and between populations, which are potentially matched to maternal care (Wells 2014).

*Adaptation versus Accommodation:*

A part of adaptation implies increased fitness, which would need to be intergenerational. If a mother was extremely successful and had many offspring, but the offspring were all unsuccessful, the genetic line would still die out, meaning that the mother was ultimately unsuccessful as well. Therefore, while it may increase survivability of the mother to decrease resource allocation to the fetus during times of stress to reallocate to somatic maintenance, this would not be wholly adaptive since it would result in a poorly adapted offspring. During times of stress and low energy availability, decisions on allocation of energy between somatic and reproductive efforts must be made. There is good evidence to show that during such times, reproductive function ceases, not allowing for a pregnancy to occur (Ellison 2003). This could
very well be adaptive; in scarce times it would be detrimental for the mother to reproduce. However, once pregnancy is obtained, changes in energy allocation to the fetus are highly buffered, and spontaneous abortions due to lack of energy availability are infrequent. However, a decrease in energy does often lead to shortened gestation lengths since the mother is unable to support the energy requirements of the pregnancy (Ellison 2003). This is an example of how programming is not adaptive to the mother or the offspring, but a result of energetic constraint.

This distinction between adaptation and accommodation has led to a discrepancy with the concept of adaptability in the predictive adaptive response hypothesis (Gluckman et al. 2007). The hypotheses described in the previous section assume that programming is in fact adaptive rather than a result of constraint, pathology, or some form of accommodation. A working definition of adaptation implies that there is an increased benefit in survival, production (such as somatic maintenance and growth), and reproduction (Bogin et al. 2007). Accommodation on the other hand gives the organism the increased chance of survival in the short term, but does not imply long-term benefits. Both short and long term effects of developmental programming can have negative impacts on the individual in all three areas of adaptation. In this argument against predictive adaptive response, developmental programming is seen as pathology or as an accommodative effect (Bogin et al. 2007; Ellison and Jasienska 2007).

**Evolution of Breastmilk**

Milk is the evolved nutrient source for mammalian infants and provides the best and most complete nutrition. Milk also contains immunological and hormonal components, which facilitate in infant development (Hinde and Milligan 2011). There is little debate on the benefits of breastfeeding; it provides a critical part of maternal and child health and nutrition. Breastmilk decreases infant risk of infection and increases immune function (Walker 2010).
Research indicates that mechanisms for milk production evolved at least 160 million years ago and possibly up to 310 million years ago (Hinde and Milligan 2011; Oftedal 2011), before the evolution of mammals. Further, it is thought that the original function of milk production was for infant hydration, and that the increased nutritional aspects of milk were a secondary adaptation (Hinde and Milligan 2011). The production of milk provides multiple adaptive benefits and acts as a key aspect in the life history strategies of all mammals (Oftedal 2011). Pond (1977) proposed that the ability to lactate was the initial adaptation that allowed for the development of other mammalian characteristics including more rapid postnatal growth, ability to persist in poor and changing environments, and mutualistic social behaviors. This is due to three specific benefits of lactation; one, mothers do not have to forage or provision for their infant offspring, two, they can inhabit and reproduce in environments in would not be inhabitable for infants, and three, mothers can store fat, minerals, or other reserves to be transferred to offspring when the current environment is poor (Hinde and Milligan 2011).

The evolutionary origin of mammary glands in eutherian mammals is somewhat unclear, however it is thought that they developed from an apocrine-like gland (Oftedal 2011). In humans, milk is produced through a system of glands called the lobulo-alveolar duct system. The ability to lactate originates during puberty and is finalized during pregnancy. At both times, hormone signaling initiates physiological changes and development of the breast tissue including cell division within the lobulo-alveolar duct system and increased fat accumulation. During labor, oxytocin and prolactin are produced and aid the release, or let down response of milk. Nipple stimulation increases production of oxytocin and prolactin, and thus increases milk production, creating a feedback loop between supply of the mother and demand of the infant (Ellison 2001: 83-88). This relationship between the infant and the mother further emphasizes
the importance of early initiation of breastfeeding, as well as on demand feedings in order to maintain a healthy supply of milk.

Variation in milk composition reflects a species’ ecology, body size, growth rate, encephalization, and behavior (Hinde and Milligan 2011). For example, harp seals have rich milk with high fat content, while humans have watery milk with low fat content. This is due to the environmental differences and the demand of fat for seal pups, but also is due to the breastfeeding practices of the species. Species with more frequent feeds have more watery milk and those who have fattier milk have fewer feeds. This variation is also seen within primates, where primates who park their infants have fattier milk compared to those who carry them with them at all times, and are therefore likely to nurse frequently. This shows an inverse relationship between suckling frequency and energy density of milk. Further, within frequently suckling species, the energy density of the milk is negatively correlated with adult body size and positively correlated with growth rate. In other words, in species that feed often, energy rich milk is associated with smaller adult body size but more rapid growth (Hinde and Milligan 2011). Thus, behavioral, environment, and biology all interact to form the behavioral ecology of the species, which then affects the milk composition.

**Breastmilk Composition**

During the first week post-partum, mothers produce colostrum, which is a protein and antibody rich form of milk that is ideal for a newborn infant. After the first week, the mother starts to transition into the production of mature milk, which still contains antibodies, but is also higher in energy and fat. Mature breastmilk has an average fat content from 2.8-4.78 g/dl, average carbohydrate content of 6.5-8.0 g/dl and protein content 0.9–1.5 g/dl (Quinn 2012). However the composition and ratios of these macronutrients does vary throughout the lactation
from foremilk, which is more watery and lower in fat, to hind milk, which is higher in fat and promotes fullness (Ellison 2003; Quinn 2012).

Breastmilk also contains micronutrients including vitamins, minerals, hormones, and antibodies. These micronutrient components are more dependent on maternal diet and environmental exposures (Thakkar et al. 2013). In other words they are more susceptible to variation.

*Energy Content of Breastmilk:*

The energy content of breastmilk, or the amount of kilocalories per gram, comes from the fat and carbohydrate content. The fat component of breastmilk is the most variable (Thakkar et al. 2013), and provides much of the energy. There are multiple fatty acids found in breastmilk (Miller et al. 2013), and although overall fat content in generally buffered against maternal condition, maternal diet may influence the proportions of these fatty acids (discussed further below). There are two types of carbohydrates found in breastmilk. The primary carbohydrate is lactose, which is common in other mammalian milks, and acts as a nutrient source to the infant. The second is oligosaccharides, which are non-nutritive, and are present in a unique abundance and profile in breastmilk (Hinde and Milligan 2011; Miller et al. 2013).

*Proteins in Breastmilk:*

There are three main proteins in breastmilk, casein, whey, and milk fat globule membrane proteins. Proteins in breastmilk provide nutrition to the infant, help in digestion, protect against pathogens, and help promote healthy gut microbiota (Lopez Alvarez 2007). The proteins in milk also make up the immune factors and hormones.
Variations Found in Breastmilk

While some researchers note that milk is likely to vary based on maternal genetic makeup, environment, behavior, diet, or other factors (Thakkar et al. 2013), there is no evidence of macronutrient variance in breastmilk by diet, body mass index, or population (Quinn 2012). In fact, breastmilk composition is known to be buffered from maternal condition. In other words, the nutritional content of breastmilk is as equal in well-nourished mothers as it is in malnourished mothers (Ellison 2003). This is because lactating women can mobilize fat stores in order to create the necessary energy for milk production. In humans, female fat storage has been selected for as a way to increase energy status and protect against environmental instability in resources. An individual’s energy status is the amount of stored energy available that can be mobilized for reproduction. Females store fat during childhood and puberty, thus increasing their energy status and helping to buffer against poor environments (Ellison 2003; Lassek and Gaulin 2006). However, volume of breastmilk may be more susceptible to poor environment (Miller et al. 2013), causing a decrease in supply among poorly nourished women. On the other hand, breastmilk does exhibit some variation micronutrients, such as in levels of hormones and antibodies.

Within a population of Filipino mothers, Quinn et al. (2011) examined breastmilk macronutrient variation (fat, carbohydrates, and protein) in regard to maternal diet and maternal body mass index (BMI). They collected morning milk samples, anthropometrics, and dietary recall. They found no relationship between maternal diet and the content of fat, carbohydrates, or protein in breastmilk. They did find a slight inverse relationship between maternal adiposity and the sugar content of breastmilk. This, however, was the only significant association found in regard to maternal BMI. They did find variation in fat content of breastmilk, in which fat
increases with breastfeeding duration. This finding is confirmed in other studies as well, which show infant age as a predictor of breastmilk fat content. Variations in fat content may also be associated with nursing frequency (Quinn et al. 2011).

*Milk Variation by Psychological Distress:*

Rudzik et al. (2014) looked at levels of perceived stress among first time mothers during the postpartum period in Sao Paulo, Brazil. More specifically, the authors examine how perceived stress correlates with two known biomarkers of stress, oxytocin and Epstein-Barr virus (EBV-ab) in order to examine how closely linked variations in perceived stress are to biological responses to stress. They found no relationship between EBV-ab and any of the perceived stress scales used; however, they did find a significant positive relationship between oxytocin and all three perceived stress scales. This finding is somewhat incongruent with previous studies since their population is all breastfeeding women. Within a breastfeeding population the authors were expected to find an inverse relationship rather than a positive one, as discussed in the previous section on maternal stress. The authors discuss these differences in oxytocin’s response to stress within and outside of breastfeeding, but do not make a clear argument as to why they found a positive relationship in their sample (Rudzik et al. 2014).

Another study looked at psychosocial distress in relation to breastmilk transforming growth factor-beta (TGF-β) among a population of Japanese breastfeeding women (Kondo et al. 2011). TGF-β is a cytokine, which plays an important role in immune development and protection of asthma and allergies. The research found that high breastmilk TGF-β was strongly associated with poorer self reported health and postpartum depression. Further, there was seasonal variation in breastmilk TGF-β, in which levels were lower in August through October and during late afternoon. The authors note that this result may be due to climate or sunshine
hours, hinting at seasonal affective disorder. In sum, their findings indicate that depressive symptoms are associated with an increase in breastmilk TGF-β, however, they note that this does not imply that mothers with depression provide increased immune protection to their breastfed infants (Kondo et al. 2011). This research could have been strengthened by additional cross-cultural data, cultural context, and/or qualitative data.

Hormone variations in breastmilk have also been shown to affect infant temperament. Grey et al. (2013) examined breastmilk cortisol in relation to infant temperament, measured via the Rothbard Revised Infant Behavior Questionnaire (IBQ-R). According to the authors, this study was the first of its kind. Only two other human studies on the topic have been done, and they varied in regard to the methods and outcomes measured. The IBQ-R measures three dimensions of infant behavior: negative affectivity, surgency/extraversion, and orienting/regulation. The study found a positive relationship between breastmilk cortisol and the negative affectivity of the infant, indicating that breastmilk cortisol negatively effects infant temperament. This result remained significant after controlling for maternal psychological distress and socio-demographic factors. The relationship between breastmilk cortisol and orienting/regulation approached significance with a negative relationship, but there was no relationship between breastmilk cortisol and surgency/extraversion. Finally, the authors found stronger associations between breastmilk cortisol and behavior in female infants than in males. The authors note that this can be attributed to differences in environmental susceptibility between the sexes, although most evidence suggests that males are in fact more susceptible than females (Wells 2000). In sum, this study shows that maternal hormones can be transmitted to the infant via breastmilk and effectively alter their behavior and/or program the infant.
Effects of Breastmilk and Breastfeeding on Infant Health

Breastmilk contains the best macronutrient composition for the infant, as well as micronutrient components that cannot be found in other infant food sources. These micronutrients aid in proper growth and immune development of the child. Even in developed countries, bottle-fed infants have higher rates of morbidity and mortality than breastfed infants. The immune protection of the infant starts with antibodies passed through the placenta while in utero, and is then augmented by those in breastmilk. Antibodies in breastmilk protect against bacteria and viruses and strengthen the digestive and respiratory system (Walker 2010; Ellison 2001).

Compared to the milk of other mammals, human breastmilk, especially colostrum is particularly rich in immunoglobulin A (IgA). IgA is a part of mucosal immunity, and is not produced by the infant until after a few weeks of life, and even then it is produced at very low levels (Miller and McConnell 2012). Thus, breastmilk IgA provides an important immunological aspect to the infant. It is unknown whether high breastmilk IgA is a derived trait in humans or is primitive to apes, or even primates. Another unique aspect of breastmilk is the high proportion and profile of oligosaccharides. Oligosaccharides are non-nutritive simple sugars that promote the recruitment of Bifida bacteria, which prevent pathogens from attaching to the infants’ intestine (Hinde and Milligan 2011). Hinde and Milligan (2011) note that the unique profile of oligosaccharides in breastmilk may have evolved due to our disease ecology, in which high population density and large pathogen loads have lead to high rates of transmission and disease. Therefore, it is not such a stretch to think that increased levels of oligosaccharides were selected for.
**Breastmilk, Breastfeeding, and Infant Growth:**

Breastmilk has been shown to help promote healthy growth trajectories, while formula may program for more rapid, increased growth. Indeed breastfed infants have been shown to be longer and leaner than formula-fed infants of the same age (Dewey et al. 1993; Gale et al. 2012). One explanation for the increased weight gain among formula fed infants is the increased protein intake. Compared to formula, breastmilk is higher in fat and lower in carbohydrates and protein, which causes the breastfed infant to grow slower than formula fed infants. High levels of protein boost secretion of insulin like growth factor-1 (IGF-1), which than increases growth via the up-regulation of adipogenesis and adipocyte differentiation. This is in addition to the already high level of IGF-1 in formula. Although IGF-1 is present in breastmilk, it has higher concentrations in cows milk (a common supplementary food) and formula derived from cow milk (Hassiotou and Geddes 2014; Koletzko et al. 2009; Stolzer et al. 2011; Thompson 2012; Wiley 2012; Wiley 2005).

The high level of oligosaccharides in breastmilk may also affect infant growth. Recent evidence suggests that improper colonization of gut microflora may be linked to other health problems including growth, energy absorption, adipose development, cardiometabolic disease, and obesity risk. Formula does not have the same oligosaccharides and does not provide equivalent colonization of gut microflora. Therefore, absence of breastfeeding can lead to improper colonization, which may promote growth and increase risk of obesity (Thompson 2012).

Breastmilk contains a variety of bioactive hormones, including ghrelin, leptin, and adiponectin, which researchers propose may play critical roles in appetite promotion and regulation. In the short term they help regulate appetite control, but may also impact the
programming of long-term neuroendocrine pathways that regulate appetite regulation via hunger and satiety cues (Hassiotou and Geddes 2014; Thompson 2012).

Ghrelin is a hormone produced by the stomach that sends hunger signals and is positively associated with both weight and length in early life (Thompson 2012). It is possible that ghrelin influences energy balance by promoting hunger and thus food intake, as well as secretion of growth hormone (GH) (Castañeda et al. 2010). Indeed levels of ghrelin in breastmilk are increased during times of rapid growth. Additionally, there is a positive association between circulating ghrelin and between meal fasting times. In other words, longer fasting times between meals leads to an increase in circulating ghrelin, and therefore programs for increased appetite. Formula-fed infants have been shown to have higher levels of ghrelin, indicating a potential link between increased appetite and formula feeding (Thompson 2012). Although breastfeeding mother infant pairs may be more likely to have small feeds more often (Hassiotou and Geddes 2014), the causal link between bottle-feeding and ghrelin levels seems to be more likely due to feeding schedule than to formula use, and mothers who breastfeed on a schedule may be subjecting their infants to similar appetites. Indeed, many breastfeeding women feed on a set schedule rather than on demand.

Leptin, on the other hand, is a hormone produced by fat and helps to promote satiety and fullness, and is negatively associated with weight gain (Dundar et al. 2005 in Thompson 2012). Leptin may play a role in the slower weight gain of infants and programming of appetite control along with the fat content of breastmilk; leptin concentrations are higher in fattier milk, further promoting fullness towards the end of a feed (Daly et al 1993; Thompson 2012). In fact, lack of leptin release has been proposed as a potential “thrifty gene” associated with obesity and type-2 diabetes (Krebs 2009).
Finally, adiponectin is produced by adipose tissue although it is negatively associated with adipose mass (Thompson 2012). Further, it is associated with insulin resistance, with an inverse relationship, and is therefore important in regards to obesity and metabolic syndrome (Lihn et al. 2005). Evidence is unclear on the function and bioactivity of adiponectin in breastmilk, but it may avoid proteolysis, or the breakdown of proteins, in the stomach thereby promoting biological activity (Newburg et al. 2010 in Thompson 2012).

Insulin like growth factor-1 in milk: IGF-1 is a growth hormone that is present into adulthood, unlike IGF-2, which plays a vital growth role in utero (Genetics Home Reference 2014; Laron 2001). It is found in breastmilk, but is also produced by the individual, including infants. Thus, its role in breastmilk on growth not fully established (Wiley 2012).

Only two studies have examined the role of breastmilk IGF-1 on infant growth. Kon et al. (2014) found level of breastmilk IGF-1 to be positively associated with infant weight gain during the first three months of life. On the other hand, Khodabakhshi et al. (2014) found no difference in levels of breastmilk IGF-1 between their two groups of normal weight and obese infants. This area of research is recent, and the two studies are very different in design and thus difficult to compare. Further research is needed to better understand how IGF-1 functions to facilitate growth in breast and other milks.

However, because IGF-1 in formula and in cow’s milk may act to program the infant for increased growth (Thompson 2012; Wiley 2005; Wiley 2012), it likely has some influence on human growth within breastmilk as well. In work that looked at cow’s milk consumption in association with adult height, it was found that milk consumption was positively associated with adult height, although the amount of variance of adult height attributable to milk was minimal (Wiley 2005). IGF-1 is not the only aspect of cow’s milk that might contribute to increased
growth; cows milk also has protein, calcium, and calories, all which may be related to increases in growth (Wiley 2005). It may also be that the higher levels of protein stimulate IGF-1 production, therefore stimulating growth. Wiley (2012) reported that older infants who drank cows milk had increased growth compared to those who drank formula with lower protein levels. There were also differences in how cow’s milk consumption affected IGF-1 levels and/or growth rates at different times, suggesting that there may be critical windows in which the individual is more susceptible to changes in growth. On the other hand, there is some evidence to show that up-regulation of IGF-1 during childhood is associated with down-regulation of IGF-1 during adulthood, which may actually protect against some diseases (Wiley 2012).

**Feeding practices and infant growth:** Feeding practices and differential energy intake have been associated with growth differences between breastfed and formula fed infants. Breastfed infants self regulate volume and therefore intake less energy (Dewey et al. 1993) while formula or bottle-fed infants are often encouraged to empty the bottle, thus increasing energy intake (Dewey 1998 in Thompson 2012). Further, the fat content of breastmilk has been shown to increase with duration of a feed (Daly et al. 1993). Thus, infants who self-regulate to eat smaller volumes more frequently are consuming lower fat and energy feeds.

Li et al. (2010) found that infants who were exclusively breastfed during early infancy were less likely to finish a bottle during late infancy than those who had been fed by breast and bottle or than those fed exclusively by bottle. In fact, there was a dose response relationship where only 27 percent of exclusively breastfed infants finished the bottle, 54 percent of mix fed infants finished the bottle, and 68 percent of exclusively bottle-fed infants finished the bottle. The same relationship was found between feeding practice and finishing the bottle when breastmilk and formula were considered separately. Thus, type of feeding during early infancy
can impact the infants ability to self regulate intake during late infancy (Li et al. 2010). The importance of early feeding practices has implications for working mothers who often have to switch to bottle-feeding after maternity leave.

**Developmental Programming Effects of Breastfeeding on Later in Life Health**

Most research on the developmental programming effects of breastfeeding have been focused on how infant feeding practices affect later in life health. In other words, research thus far has aimed to examine if breastfeeding, duration of breastfeeding, and exclusivity of breastfeeding are related to a decreased risk in the development of obesity and/or metabolic syndrome, including type-2 diabetes.

*The Relationship Between Breastfeeding and Later in Life Disease Risk:*

The link between breastfeeding and the reduced risk of obesity was first proposed by McCance (1962), and supported empirically by Kramer (1981) with a case-control study on children aged 12 to 18 years. Data included anthropometric measurements to determine obese, overweight, or non-obese status, feeding history, family history, and demographics. Those who were not breastfed showed an increased relative risk of being overweight or obese compared to those who were breastfed. Further, the protective effect of being breastfed increased slightly with duration of breastfeeding. This result remained significant after controlling for confounders. Further epidemiological studies have since supported the role of breastfeeding on reduced obesity risk (Owen et al. 2005; Stolzer 2011).

Along with decreasing obesity risk, breastfeeding decreases the risk of developing metabolic diseases. Breastfeeding has been shown to impact cholesterol levels and decrease blood pressure, potentially acting as a protective measure. Additionally, exclusive breastfeeding has been associated with lower fasting insulin, lowered prandial blood glucose, and encouragement
of optimal lipoprotein metabolism and gastrointestinal adaptation, thereby decreasing risk of developing type-2 diabetes (Stolzer 2011). Indeed, studies have reported breastfeeding practices including duration and exclusivity to be protective against type-2 diabetes and high systolic blood pressure (Lawlor et al. 2005; Mayer-Davis et al. 2008).
Research Objectives and Hypotheses

The overall goal of this research project was to examine levels of breastmilk IGF-1 in relation to the mother’s self reported level of perceived stress. The specific research objectives are; 1) to examine the relationship between breastmilk IGF-1 and infant growth, 2) to examine the relationship between breastmilk IGF-1 and maternal perceived stress, 3) to examine the relationship between maternal perceived stress and infant growth, and 4) to determine if there is or is not a residual programming effects on the infant due to maternal stress via variation in breastmilk IGF-1.

Figure 2.1: Proposed Relationship between Maternal Stress, Breastmilk IGF-1, and Infant Growth
In order to assess these objectives I proposed five hypotheses: 1) there will be a positive correlation between infant length for age and level of breastmilk IGF-1, 2) there will be a positive correlation between infant weight for age and level of breastmilk IGF-1, 3) breastmilk IGF-1 will be negatively associated with levels of perceived stress, 4) infants born to mothers with higher levels of perceived stress will have smaller height for age, and 5) infants born to mothers with higher levels of perceived stress will have lower weight for age.
Methods

Data Collection

Population of Interest and Sampling:

The population of interest for this study is breastfeeding mother-infant pairs in the Tampa Bay Area. Multiple recruitment techniques were used. First was through La Leche League due to an established partnership and shared interest in breastfeeding research. This included posting a recruitment message onto the Brandon area La Leche League facebook page. In addition, I directly contacted individuals who had expressed previous interest in participating in this research project. These individuals had participated in a pilot study, which gave them the option to contact me via email if interested in further participation. Finally, I used snowball sampling through the mothers of La Leche League and other participants. Participants who enjoyed the experienced were asked to inform other breastfeeding mothers of the research and to give my contact information. This method drastically increased recruitment, as many of the women decided to repost my information into other forms of social media and breastfeeding websites including Tampa Bay Breast Friends and a Macdill Airforce Base mothers page. Participation of this research was fully voluntary and unpaid. Internal Review Board (IRB) approval was obtained from the IRB office at the University of South Florida (USF).

Types of Data:

This research combines qualitative and quantitative methods of both data collection and analysis. The data collection includes survey, anthropometrics, milk sample analysis, and informal or semi-structured interviews. Surveys were coded to correspond to the milk samples
as well as a contact sheet in case of follow up. However, no identifying information is included in analysis and/or write up.

*Semi-structured surveys:* The surveys include both quantitative and qualitative data and were administered by the interviewer in a semi-structured interview style. This allows for a biocultural approach in which biological information is collected, but also allows for the open-ended, more conversational type of ethnography to take place. First, demographics were collected. This included information such as participants’ age, age at first child, and age of their most recent child. Additionally the participants were asked brief questions on breastfeeding history and how actively involved they are in La Leche League. There were also more open-ended questions that asked the participants about what causes stress in their life and how they cope with stress. These questions were qualitative in nature and were used to help explain their stress levels. In order to quantify their stress I used the Cohen Perceived Stress Survey-10 (PSS-10) as well as a ten point self reported stress scale. The PSS-10 is a validated tool that examines perceived stress using ten questions. This tool is particularly valuable when making inter-population comparisons (Carnegie Mellon Psychology 2014). Because there is no baseline stress level determined for the population my sample comes from, inter-sample comparisons are more useful.

*Anthropometrics and biological samples:* Because IGF-1 is a growth hormone, anthropometrics of height and weight of the mother and the infant were collected. Infant height and weight were compared to a reference population (the WHO) to get height and weight for age, as well as to calculate the ponderal index. The ponderal index is a value of the relationship between height and weight, which uses meters cubed, rather than meters squared as in body mass index (BMI). Due to complications in BMI patterns during infancy and childhood, the ponderal
index provides a better measure of adiposity in infants (Cooley et al. 2012). For the mother, both ponderal index and BMI were calculated.

Two-milliliter milk samples of foremilk from the left side were collected from all participants. Women were asked to refrain from feeding or pumping from the left side for an hour prior to collection. All participants hand expressed the samples into a small plastic cup, which was then poured by me into a flask and put immediately on ice. Milk can be stored at refrigeration temperatures for a couple of days, although this can lead to deterioration of hormones and cytokines (Miller et al. 2013). Samples were transported to USF where they were stored at negative 20 degrees Celsius. In circumstances where it was not possible to get the samples to USF the samples were stored in a home freezer until able to be brought to USF. Under these rare circumstances, they were always transported on ice to ensure they did not defrost during transportation. This will ensure minimal deterioration and bacterial growth (Miller et al. 2013).

Because milk changes over the course of a feeding, throughout the day, and over time, these variables need to be controlled for or used as a covariate. First, for the course of the feeding, mothers were asked to have refrained from feeding for one hour prior to sample collection to ensure all samples are foremilk. In some cases this was not possible, however all samples were collected with at least 30 minutes since last feed. Second, all samples were collected from the left side. Time of day cannot be controlled for due to the need to accommodate the mother’s schedules. Thus, time of day, as well as approximate last feeding time were collected and will be adjusted for in analysis. Finally, age of the infant was collected and used as a covariate to account for changes in breastmilk.
Data Analysis

Lab Analysis:

Milk samples were analyzed at the Health and Human Biology Lab at the University of South Florida. This lab is a part of the Department of Anthropology and is supervised by Elizabeth Miller, Assistant Professor in the Department. Level of breastmilk IGF-1 were analyzed using the Human IFG-1 Quantikine enzyme linked immunosorbent assay (ELISA) Kit from ALPCO. An ELISA, also known as a sandwich assay, uses a microtiterplate that is pre-coated with antibodies which bind with the target protein, in this case IGF-1. A second, biotinylated antibody is added to the plate to bind with the immobilized IGF-1. Finally, an enzyme conjugate is added to bind with the biotinylated antibody, and allowing for the levels of IGF-1 to be read by the plate reader as a measure of reflected light (ALPCO 2012). Although this kit was designed for serum or plasma, Khodabakhshi et al. (2014) reported using this kit along with the standard protocol in an analysis of breastmilk IGF-1 and found significant levels and results.

Figure 3.1: ELISA Sandwich Assay (Epitomics, n.d.)
Milk samples were first defrosted at room temperature and then thoroughly mixed using a vortex. Three hundred microliters (300 µl) were pipetted into test tubes and centrifuged at 30000 rpm for 15 minutes to separate the fat layer and the protein. The fat layer was removed and 20 µl of each sample was pipetted into test tubes containing 200 µl of Sample Buffer PP. The Sample Buffer PP is an acidic buffer used to disassociate IGF-1 from insulin like growth factor binding proteins (IGFBPs) (ALPCO 2012). Eighty (80) µl of Antibody Conjugate AK was pipetted into all wells of the assay plate followed by 20 µl of Sample Buffer PP, Standard A, Standard B, Standard C, Standard D, Standard E, Standard F, Control Sera KS1, Control Sera KS2, and all diluted samples. Buffer, standards, controls, and samples were pipetted in horizontal duplicate vertically and then across. After all samples were done, empty wells were assigned blank (Sample Buffer PP) and miscellaneous samples to test for consistency of technique. A sample map can be seen in figure 3.2.

The plate was sealed and then incubated for one hour at room temperature while on a plate shaker set to 350 rpm. After incubation, the plate was washed with 300 µl of Washing Buffer WP per well five times, using a plate washer. Then 100 µl of Enzyme Conjugate EP was pipetted into each well, the plate was again covered, and incubated at room temperature for 30 minutes on the plate shaker set to 350 rpm. The plate was then washed according to the same procedure. After the second washing, 100 µl of Substrate Solution S was pipetted into each well, and incubated for 15 minutes at room temperature in the dark. Finally, 100 µl of Stopping Solution SL was pipetted into each well, and the plate was read using a Bioteck plate reader.
In addition to the IGF-1 ELISA, fat component was analyzed using creamatocrit, or a fat centrifuge. Fat is a measure of energy, and therefore is an important factor in long term stored energy and growth. The creamatocrit method is fast and inexpensive, and has been found to correlate well with lipid and energy concentration of human milk (Lucas et al. 1978; Wang et al. 1999).

Samples were mixed using a vortex, and then placed into the creamatocrit tubes using capillary action. Tubes were then placed into the creatocrit centrifuge for 14 minutes in two sets, one set of 24 and one set of seven. Total column and fat layer was measured using a micrometer caliper placed to two decibels. Additionally, in samples that had two clearly separated lipid
layers (cream and free fat), the two lipid layers were also measured. Each measurement was taken twice.

*Statistical Analysis:*

Statistical analysis was done using Microsoft Excel and IBM SPSS. Data were manually input into Excel from the surveys. Z-scores were calculated using a lambda-mu-sigma (LMS) equation for infants (Table 3.1) and World Health Organization (WHO) data. The LMS equation provides a better representation of infant height and weight variation (Johnson 2015). WHO data were used instead of Center for Disease Control (CDC) because although the CDC data represent a U.S. population the WHO data better reflects breastfed growth patterns, as many U.S. infants are formula fed. Infant ages were rounded to the nearest month; children under three months were aged by week for a more accurate Z-score.

Mother and infant BMI and ponderal index were also calculated using Excel. Height/length measurements were converted into metric units and then put into the respective equations (Table 3.1).

Creamatocrit percent was calculated as the length of the total fat layer (cream plus free fat) over the length of the total column. Creamatocrit percent was converted into fat grams per deciliter to obtain fat percent. Finally, kilocalories from fat were calculated from the fat percent (Table 3.1).

Although Khodabakhshi et al. (2014) and Kon et al. (2014) reported significant levels of breastmilk IGF-1 levels in their results, this area of research is still very new and relatively untested. Based on the results of these studies, and that they did not report changing dilutions, our study samples were diluted in accordance to kit protocol. Kit protocol for sample dilutions are based on dilutions recommended for serum levels, thus levels of IGF-1 in the breastmilk
samples were very low and almost undetectable. Therefore IGF-1 level was converted into a
dichotomous variable, detectable or not detectable, rather than a continuous variable.

Table 3.1: *List of equations:*

<table>
<thead>
<tr>
<th>Use</th>
<th>Equation</th>
</tr>
</thead>
<tbody>
<tr>
<td>LMS equation for infant Z-scores</td>
<td>(((\text{measure}/M)^{L}-1)/(L/S))</td>
</tr>
<tr>
<td>BMI</td>
<td>(\text{Weight (kg)/Height (meters}^2))</td>
</tr>
<tr>
<td>Ponderal Index</td>
<td>(\text{Weight (kg)/Height (meters}^3))</td>
</tr>
<tr>
<td>Creamatocrit Percent</td>
<td>(\text{Fat layer (mm)/ total column (mm)})</td>
</tr>
<tr>
<td>Fat grams/dL</td>
<td>(((\text{Creamatocrit percent-0.59})/0.146)/10)</td>
</tr>
<tr>
<td>Kcal/dL</td>
<td>((6.2 \times \text{g/dL})+35.1)</td>
</tr>
</tbody>
</table>

Data were imported from Excel into SPSS. Continuous variables were tested for
normality. Because not all variables were found to be normal, Spearman’s Correlation was
performed to obtain a correlation matrix. The correlation matrix was inspected to determine
which variables were highly correlated to help in the multiple-regression model building process.
The best regression was chosen based on a maximized R2adj, minimized mean square error and
lack of multicollinearity among the independent variables.

Chi-square was used to compare detectable IGF-1 level between male and female infants.
Mann-Whitney t-tests was used to compare the stress level between IGF-1 groups (detectable,
non detectable). Independent samples t-tests were used to examine variations in breastmilk fat
content between males and females, as well as between samples with and without detectable
IGF-1. Finally, infant length and weight were regressed on breastmilk fat content.
Qualitative Analysis:

Themes, ideas, and major points were drawn out of the interviews to supplement the qualitative nature of the data. Data were coded by hand and analyzed by question. Surveys were printed into hard copies; I then went through each question and color coded for each time a participant mentioned a topic. In addition I noted the context of how the participant mentioned the topic in order to create subcategories later. The number of participants who mentioned a topic was counted and then categorized into themes. Interviews were not fully transcribed, but all interviews were recorded. Quotes were pulled from the audio recordings.
Results

Descriptive Statistics

The population consisted of 31 mother infant pairs \((n=31)\). Sixteen (16) of the infants were female and 15 were male. This was the first time breastfeeding for most \((n=21)\), of the mothers. Additionally, most of the mothers were working \((n=19)\), not in school \((n=28)\), and married \((n=28)\). Eleven of the women reported being members of La Leche League, seven reported being members “on Facebook”, and 12 identified as not members. A complete list of results from categorical variables can be seen in Table 4.1.

Table 4.1: Descriptive statistics of categorical variables:

<table>
<thead>
<tr>
<th>Variable</th>
<th>16 female</th>
<th>15 male</th>
</tr>
</thead>
<tbody>
<tr>
<td>Infant sex</td>
<td></td>
<td></td>
</tr>
<tr>
<td>First time Breastfeeding</td>
<td>21 yes</td>
<td>11 no</td>
</tr>
<tr>
<td>Mother working</td>
<td>19 working</td>
<td>12 not working</td>
</tr>
<tr>
<td></td>
<td>1 on maternity leave</td>
<td>2 work part time from home</td>
</tr>
<tr>
<td></td>
<td>5 work &gt;20 hours</td>
<td></td>
</tr>
<tr>
<td></td>
<td>4 work 20-39 hours</td>
<td></td>
</tr>
<tr>
<td></td>
<td>9 work 40 hours+</td>
<td></td>
</tr>
<tr>
<td>In school</td>
<td>6 in school</td>
<td>28 not in school</td>
</tr>
<tr>
<td>Married</td>
<td>28 married</td>
<td>2 live with boyfriend</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1 single</td>
</tr>
<tr>
<td>La Leche League Membership</td>
<td>11 not members</td>
<td>7 members “on Facebook”</td>
</tr>
<tr>
<td></td>
<td></td>
<td>12 not members</td>
</tr>
<tr>
<td>Detectable IGF-1</td>
<td>4 yes</td>
<td>27 no</td>
</tr>
</tbody>
</table>
Range, mean, median, and standard deviation were calculated for all the continuous variables. Age of the mother ranged from 24 to 37 years with a mean age of 28.45 years. A summary of all descriptive statistics for maternal characteristic variables including age, height in meters, weight in kilograms, BMI, and Ponderal index, can be found in table 4.2.

Table 4.2: Descriptive statistics for maternal characteristic variables:

<table>
<thead>
<tr>
<th>Variable</th>
<th>n</th>
<th>Min</th>
<th>Max</th>
<th>Mean</th>
<th>Median</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>n=31</td>
<td>24</td>
<td>37</td>
<td>28.45</td>
<td>27</td>
<td>3.44</td>
</tr>
<tr>
<td>Maternal Height (m)</td>
<td>n=31</td>
<td>1.51</td>
<td>1.78</td>
<td>1.65</td>
<td>1.65</td>
<td>0.06</td>
</tr>
<tr>
<td>Maternal Weight (kg)</td>
<td>n=31</td>
<td>45.6</td>
<td>114.4</td>
<td>72.18</td>
<td>68.9</td>
<td>15.49</td>
</tr>
<tr>
<td>Maternal BMI</td>
<td>n=31</td>
<td>17.95</td>
<td>39.5</td>
<td>25.91</td>
<td>25.08</td>
<td>2.62</td>
</tr>
<tr>
<td>Maternal Ponderal</td>
<td>n=31</td>
<td>11.26</td>
<td>23.21</td>
<td>15.67</td>
<td>15.66</td>
<td>2.62</td>
</tr>
</tbody>
</table>

Descriptive statistics were also obtained for infant age and anthropometrics. Infant age ranged from three weeks to 33 months. The mean age was 9.54 months and the median was 8.5 months. Infant age and anthropometrics of length in centimeters, weight in kilograms, BMI, Ponderal index, length for age Z-score, and weight for age Z-score, are summarized in table 4.3.

Three stress scales were used; 1) a ten-point self reported breastfeeding stress scale, 2) a ten-point self reported general stress scale, and 3) the PSS-10 stress scale. The PSS-10 is a validated scale with a theoretical range of zero to 40. The breastfeeding stress scale had a wide range from one to nine, with a mean of 2.71 and a median of 2.5. The general stress scale had a range of two to ten with a mean of 5.1 and a median of 5. Finally the PSS-10 had a range of four to 31, also representing a wide range. The mean score was 13.97 and the median was 13. The descriptive statistics of the stress scales and shown in Table 4.4.
Breastmilk fat content was analyzed using creamatocrit. Creamatocrit percent, or the proportion of the fat layer compared to total column, ranged from 0.97 to 13.71 with a mean percent of 7.18 and almost equal median of 7.17 percent. Fat grams/deciliters (g/dL) was calculated from creamatocrit percent. Fat g/dL ranged from 0.26 to 8.99, the mean was 4.51 and the median was 4.49. The kilocalories per deciliter (kcal/dL) were then calculated from the fat g/dL. Kcal/dL ranged from 41.09 to 120.12, the mean was 79.63 and the median was 79.4. This information is summarized in table 4.5.
Table 4.5: *Descriptive statistics for breastmilk fat:*

<table>
<thead>
<tr>
<th>Variable</th>
<th>n</th>
<th>Min</th>
<th>Max</th>
<th>Mean</th>
<th>Median</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Creamatcrit%</td>
<td>n=31</td>
<td>0.97</td>
<td>13.71</td>
<td>7.18</td>
<td>7.17</td>
<td>3.11</td>
</tr>
<tr>
<td>Fat g/dL</td>
<td>n=31</td>
<td>0.26</td>
<td>8.99</td>
<td>4.51</td>
<td>4.49</td>
<td>2.13</td>
</tr>
<tr>
<td>kcal/dL</td>
<td>n=31</td>
<td>41.09</td>
<td>120.12</td>
<td>79.63</td>
<td>79.4</td>
<td>19.29</td>
</tr>
</tbody>
</table>

**Analytic Statistics**

Parells’s test for correlation was performed on all of the continuous variables to create a correlation matrix. A full correlation matrix of positive correlations can be found in table 3.6. Correlations were determined statistically significant when the p-value was less than 0.05 ($\alpha=0.05$). A moderate positive correlation was found between mother’s age and breastfeeding stress scale, indicating that as maternal age increases, so does stress associated with breastfeeding. Maternal height was positively correlated with infant length for age $Z$-score. Interestingly, maternal height, weight, and BMI were negatively correlated with other infant anthropometrics including infant BMI and infant Ponderal index. Maternal PPS-10 score was negatively correlated with infant length for age $Z$-score. In other words, higher levels of stress were associated with lower length for age $Z$-scores. Figure 4.1 shows a scatter chart of infant length for age $Z$-scores plotted against maternal PSS-10 score.

Infant length for age $Z$-score and weight for age $Z$-score were also negatively correlated with the breastmilk fat variables (creamatoctic percent, fat g/dL, and kcal/dL). A summary of all significant correlations can be found in Table 4.6.
Table 4.6: *Summary of significant correlations* (at $\alpha=0.05$)

<table>
<thead>
<tr>
<th>Correlated variables</th>
<th>Correlation coefficient</th>
<th>$p$-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mother’s age and breastfeeding stress scale</td>
<td>.419</td>
<td>0.019</td>
</tr>
<tr>
<td>PSS-10 and ten point stress scale</td>
<td>.808</td>
<td>0.000</td>
</tr>
<tr>
<td>PSS-10 and infant length for age Z-score</td>
<td>-.495</td>
<td>0.005</td>
</tr>
<tr>
<td>Maternal height and infant length for age z-score</td>
<td>.506</td>
<td>0.004</td>
</tr>
<tr>
<td>Maternal height and infant BMI</td>
<td>-.373</td>
<td>0.039</td>
</tr>
<tr>
<td>Maternal height and infant Ponderal index</td>
<td>-.366</td>
<td>0.043</td>
</tr>
<tr>
<td>Maternal weight and infant BMI</td>
<td>-.415</td>
<td>0.020</td>
</tr>
<tr>
<td>Maternal weight and infant Ponderal index</td>
<td>-.410</td>
<td>0.022</td>
</tr>
<tr>
<td>Maternal BMI and infant Ponderal index</td>
<td>-.316</td>
<td>0.045</td>
</tr>
<tr>
<td>Infant length for age Z-score and infant weight for age Z-score</td>
<td>.683</td>
<td>0.000</td>
</tr>
<tr>
<td>Infant length for age z-score and creamatocrit percent</td>
<td>-.420</td>
<td>0.019</td>
</tr>
<tr>
<td>Infant length for age z-score and fat g/dL</td>
<td>-.420</td>
<td>0.019</td>
</tr>
<tr>
<td>Infant length for age z-score and kcal/dL</td>
<td>-.420</td>
<td>0.019</td>
</tr>
<tr>
<td>Infant weight for age z-score and creamatocrit percent</td>
<td>-.406</td>
<td>0.024</td>
</tr>
<tr>
<td>Infant weight for age z-score and fat g/dL</td>
<td>-.406</td>
<td>0.024</td>
</tr>
<tr>
<td>Infant weight for age z-score and kcal/dL</td>
<td>-.406</td>
<td>0.024</td>
</tr>
</tbody>
</table>
Figure 4.1: *Infant length for age Z-score against maternal PSS-10.* Graph showing the inverse relationship between infant length for age Z-score and maternal PSS-10 score. The correlation coefficient was -.495 with a p-value of 0.005.

*IGF-1:*

Only four of the breastmilk samples had detectable levels of IGF-1, leaving 27 sample without detectable IGF-1 levels. Chi square was used to examine the relationship between detectable IGF-1 and sex. No significant relationship was found. A non-parametric test of two independent samples (t-test), Mann-Whitney, was performed to test if there were differences in level of stress between the two IGF-1 results (detectable, non-detectable). There was no significant relationship between detectable breastmilk IGF-1 and the ten-point self reported stress scale, nor the PSS-10. Mann-Whitney was also used to test relationships with IGF-1 and infant age length for age, and weight for age, with no significant relationship found.
Creamatocrit:

Independent samples t-tests was performed to compare the mean differences in breastmilk fat variables of creamatocrit percent, fat g/dL, and kcal/dL, between males and females. There were no statistically significant differences between the two means for any of the three variables. In other words, fat content was not significant related to infant sex. I also performed simple regression of infant length in cm on the three fat content variables, with none of them being significant.

Finally, independent samples t-test was use to examine if there was a significant difference in fat content between the IGF-1 groups. No significant difference was found in creamatocrit percent, fat g/dL, or kcal/dL between samples with detectable levels of IGF-1 and those without detectable levels of IGF-1.

Hypothesis Testing:

Hypotheses are shown in table 4.7 below. Based on the low levels of IGF-1 in breastmilk, the variable had to be treated as dichotomous, and correlations could not be examined. I did use Mann-Whitney tests to examine the relationship between IGF-1 and infant length and weight and maternal stress, with no significant relationships found. Thus, hypotheses one, two, and three were rejected. Hypotheses four was accepted: there were moderate inverse relationships between the PSS-10 stress scale and infant length for age Z-score showing that as PSS-10 score increased, Z-scores decreased. The relationship between PSS-10 score and infant weight for age Z-score was not significant therefore hypothesis five was also rejected.
Table 4.7: Summary of hypotheses

<table>
<thead>
<tr>
<th>Hypothesis</th>
<th>Accept/Reject</th>
</tr>
</thead>
<tbody>
<tr>
<td>1) There will be a positive correlation between infant length for age and</td>
<td>Rejected</td>
</tr>
<tr>
<td>level of breastmilk IGF-1.</td>
<td></td>
</tr>
<tr>
<td>2) There will be a positive correlation between infant weight for age and</td>
<td>Rejected</td>
</tr>
<tr>
<td>level of breastmilk IGF-1.</td>
<td></td>
</tr>
<tr>
<td>3) Breastmilk IGF-1 will be negatively associated with levels of perceived</td>
<td>Rejected</td>
</tr>
<tr>
<td>stress.</td>
<td></td>
</tr>
<tr>
<td>4) Infants born to mothers with higher levels of perceived stress will</td>
<td>Accept</td>
</tr>
<tr>
<td>have smaller height for age.</td>
<td></td>
</tr>
<tr>
<td>5) Infants born to mothers with higher levels of perceived stress will</td>
<td>Reject</td>
</tr>
<tr>
<td>have lower weight for age.</td>
<td></td>
</tr>
</tbody>
</table>

**Qualitative Results**

*Why Breastfeed:*

When asked, “what led you to choose to breastfeed” three major themes emerged. The first theme was perception of breastfeeding (n=30). This theme included general responses that breastfeeding is better or best (n=22), such as “breast is best” [interview 14], or “it’s better for babies” [interview 9]. Of those 22 mothers, nine of the women specifically mentioned researching or looking up the many benefits of breastfeeding over formula. Perception of breastfeeding also include responses that breastfeeding is natural, or just what you do (n=10). One woman stated, “I am conscious about what I eat, and what I buy, and I can’t imagine feeding my baby milk that, um, you know, comes from a can” [interview 29]. Other responses included things such as, “it was an automatic expectation” [interview 17], or “for me it was like… never really a question that I was gonna use formula” [interview 5]. Finally, this theme
included women who mentioned that they “wanted the experience” [interview 7], or simply wanted to breastfeed (n=3).

A second major theme identified was specific benefits to the infant (n=13). Most women mentioned health and nutrition benefits of breastfeeding (n=13), stating things such as its “it’s healthier than formula” [interview 31], or even more specifically “antibody protection, I just wanted to make sure he was as healthy as he could be and grew at a proper pace, and has as much immunity as I could give him, particularly in the first months of life” [interview 24]. A few (n=4) specifically mentioned future or developmental benefits of breastfeeding for infant; “it sets them up for the best future”[interview 1], or “give him the best start on life possible” [interview 7].

The third theme was benefits to the mother (n=20). This included general responses, “all the benefits to her, but me also” [interview 26], but also more specific health benefits to the mother, like it “lowers the risk of PPD (post partum depression)” [interview 2]. Convenience (n=8) was also mentioned as a benefit of breastfeeding, “it’s just a whole lot easier, than having to worry about preparing bottles, and, you know, heating them, storing them correctly” [interview 8]. The cost of formula, or the fact that breastfeeding is free, was also mentioned often by the mothers (n=14). One woman responded that, “we would have a lot more expenses if we were doing formula, so I didn’t wanna risk that” [interview 17] associated with formula feeding. Finally, bonding (n=7) was included as a benefit to the mother.

Causes and Types of Stress:

Mothers were asked, “What causes stress in your life?” Two major response themes emerged, family and maintenance. Family (n=25) included stress related to their children (n=12), their husband or partner (n=6), and other family (n=11). For children, answers related to
kids being difficult, “getting into stuff” [interview 4], or when they do not listen [interviews 1, 13], as well as concern for them, “wanting the best for him” [interview 3]. Stress related to husbands and partners related to fighting with them, “when he (boyfriend) gets home it’s usually a loud bickering conversation, argument” [interview 17], not having enough time for their relationship, “husband gets put on the back burner” [interview 5], or their partner’s work, “my husband’s work schedule” [interview 13]. Other family was stress mostly caused by in-laws or parents; “family sometimes drives us nuts” [interview 20], but was also sometimes due to distance from family.

Maintenance (n=23) included day-to-day issues that were not related to the family or the individual. Topics within maintenance included having to balance too much to do and household maintenance (n=9); “balancing work and home with two small children” [interview 2], “managing a home” [interview 5]. Another topic included in maintenance was money or finances. Women stated that “planning for the future” [interview 24] or “unexpected bills” [interview 11] were sources of stress. Work and school related stress was also included in this theme. Responses included general stress related to work and school, but also “not wanting to go back to work” [interview 9], and coworker stress.

Women were also asked to categorize their type of stress as being due to daily hassles, specific events, or chronic problems. Most women (n=17) termed their stress as being due to daily hassles. Five termed their stress as being chronic problems, and four as being caused by specific events.

Coping Mechanisms:

Women were asked how they coped with stress. The most common ways in which women reported coping with stress was talking to their husbands, family, or friends (n=14). One
woman stated, “I talk a lot, even if just to her (baby). Talk it out” [interview 28]. Another common coping mechanism was getting out of the house (n=8); “make a point of getting out, take a walk” [interview 20]. Prayer or meditation (n=8) was another common coping mechanism. A few women also reported exercising (n=5), although some of them also stated that they do not exercise as much as they did before pregnancy; “before he was born I did a lot of yoga and ballet and exercise to manage stress, but I can’t do that as much now, with him” [interview 3]. Having a glass of wine (n=5), reading or watching television (n=5), or crying (n=4) were other coping techniques mentioned, “at the end of the day… he sits with her (baby) and I have glass of wine or something and watch Modern Family” [interview 26]. Less common were eating (n=3), shopping (n=2), preparing or planning (n=2), and hobbies (n=2).
Discussion

The purpose of this research project is to examine the relationship between maternal perceived stress, variation in breastmilk IGF-1, and infant growth. Specifically, it seeks to explore if there is a relationship between maternal perceived stress and variation in breastmilk IGF-1 or infant growth, and if variations in breastmilk IGF-1 impact infant growth (see figure 1.1).

This area of research has important implications in regards to the developmental origins of health and disease paradigm (DoHAD). There is empirical evidence to support the proposition that maternal nutritional and social stressors are associated with increased risk of low birth weight offspring (Adelman et al. 2008; Kuzawa 2005; Kuzawa and Sweet 2009). Further, research supports long term negative health impacts of being born low birth weight, including increased risk of obesity, type-2 diabetes, cardiovascular disease, and hypertension (Barker 1994; Bogin et al. 2007; Gluckman and Hanson 2006). DoHAD research utilizes this knowledge to extend the critical window of disease susceptibility into early life. In other words, DoHAD examines uterine and early life environments as early determinants of health in order to understand relationships to later in life health outcomes (Gluckman and Hanson 2006).

Breastmilk may also play a role in developmental programming of early life environment and could have important longitudinal health implications related to disease risk. Indeed, there is evidence for variation in milk composition due to offspring sex (Fujita et al. 2012; Hinde 2009; Powe et al. 2010) and maternal stress (Grey et al. 2013; Kondo et al. 2011; Rudzik et al. 2014). Further, increased levels of IGF-1 in formula, along with high levels of protein, which increase
the secretion of IGF-1 in the infant, may be linked to rapid growth and increased risk of obesity later in life (Hassiotou and Geddes 2014; Koletzko et al. 2009; Stolzer et al. 2011; Thompson 2012; Wiley 2012; Wiley 2005). A recent study found breastmilk IGF-1 to be positively linked to infant weight gain within the first few months of life (Kon et al. 2014). Conversely, Khodabakhsi et al. (2014) found no relationship between breastmilk IGF-1 and infant obesity. The literature lacks an understanding on how variation within breastmilk, including IGF-1 and fat levels, relate to or impact infant growth, or moreover, how it may be affected by maternal stress. Understanding disease risk associated with early life can have important public health and policy implications (Hanson and Gluckman 2011).

**Variation in Breastmilk Fat Content**

Data analyzed in this project indicated the presence of a moderate yet statistically significant correlation between maternal stress, measured via the PSS-10, and infant length for age Z-score. The correlation was inversely related, indicating that as maternal stress increases, infant length for age decreases. Thus, hypothesis four was accepted (table 2.7); infants born to mothers with higher perceived stress were shorter length for age. There is no research that examines how variation in breastmilk fat relates to infant growth. However, when compared to formula, breastmilk fat is associated with longer length (Dewey et al. 1993). Thus, the inverse relationship between breastmilk fat and infant length for age is an interesting one and highlight the need for more research.

Breastmilk fat content was inversely associated with infant length for age (-0.420) and weight for age (-.406), indication that as breastmilk fat content increased, infant length for age and weight for age decreased. This result seems to be opposite of what would be expected, since breastmilk fat has been reported to be associated with increased child growth (Dewey 1993). It
would be expected that fat content would be positively associated with infant length and/or weight for age. However, there could be other potential confounders to this finding. Number of feeds per day is inversely associated with fat content; the more often an infant feeds the less fatty the breastmilk (Hinde and Milligan 2011; Quinn et al. 2011). Thus, it is possible that longer infants are feeding more often, and therefore their mother’s are producing breastmilk with lower fat. Other confounders could be other nutrient sources for the child and maternal height. Indeed, maternal height and infant length for age Z-score were positively correlated, indicating that maternal height could have confounded the relationship between breastmilk fat and infant length.

The levels of fat for this sample fell outside the average range of 2.8-4.78 g/dL (Quinn 2012) with a sample range of 0.26 to 8.99 g/dL. The average fat content for this sample was, however, on the upper limit of the standard range with a mean of 4.51 g/dL (see table 2.7). This is particularly interesting since all samples were foremilk, which tends to be lower in fat and higher in water (Ellison 2003; Quinn 2012). In other words, milk tends to become higher in fat during the duration of the feed, thus since all of the samples were foremilk, it would have been expected for the average fat content to fall on the lower end of the spectrum rather than the higher.

There could be multiple explanations for the higher fat content of this sample. First is the number of feeds through the day. While number of feeds was not collected within this data, U.S. mothers tend to feed less often compared to a global breastfeeding population in which the average range is based upon. Therefore a higher fat content of milk may be attributed to decreased feeding frequency (Hinde and Milligan 2011; Quinn et al. 2011).

Another explanation could be maternal diet. Although generally speaking it is true that breastmilk nutrient content is buffered from maternal condition, fat is the most variable of the
macronutrients in breast milk. This is specifically true in regards to different fatty acids found within milk (Miller et al. 2013; Thakkar et al. 2013). Unfortunately, I did not collect data on maternal diet or fatty acid composition of the breastmilk analyzed. Thus, it cannot be concluded that this is the cause of the variation in breastmilk fat.

Infant age has also been shown to be a predictor of breastmilk fat content (Quinn et al. 2011). Generally as an infant is introduced complementary foods, breastfeeding frequency decreases and fat increases (Quinn et al. 2011). However, in contrast with other studies, no correlation was found between breastmilk fat content and infant age within this sample.

Sex was also examined as a potential predictor of breastmilk fat. According to the Trivers Willard Hypothesis there could be variations in breastmilk fat related to offspring sex and maternal condition. The Trivers Willard hypothesis states that male reproductive success is more conditional to parental investment than is female reproductive success. Therefore, in resource poor environments it is more advantageous to have and sustain girls than boys, but that in resource rich environments it is more advantageous to have and sustain boys than girls (Trivers and Willard 1973). Thus, according to the hypothesis, there would be evolutionary mechanisms that select between the sexes based on maternal environment.

In a study on well-nourished mothers, it was found that the energy content of milk was 25 percent higher in mothers of males than females (Powe et al. 2010). Similarly, Fujita et al. (2012) found that offspring gender and socioeconomic status interacted to affect the energy content of milk. Specifically, they hypothesized that in women of lower socioeconomic status, the energy content of their milk would be richer in mothers of daughters, but for women of higher socioeconomic status, the energy content of the milk would be higher in mothers of sons. They were able to support this hypothesis with their data, and created a model that estimated that
economically sufficient mothers produced richer milk for sons than daughters by 2.8 vs. 0.6 g/dL, while poor mothers produced richer milk for daughters than sons with by a difference of 2.6 vs. 2.3 g/dL. On the other hand, Quinn (2013) did not find evidence to support gender-biased variation in fat, carbohydrate or protein content of milk in regard to socioeconomic status within her sample of Filipino mothers.

Unfortunately, I did not examine socioeconomic condition of the mothers. Therefore I am not able to fully test if fat content in breastmilk varies by infant sex in relation to mothers’ socioeconomic condition. However, this thesis reinforces the need for more research on breastmilk variation in order to gain a better understanding of it affects on infant health.

**Difficulties Measuring IGF-1**

The ELISA assay used in this project was designed to analyze IGF-1 levels in serum and plasma (ALPCO 2012). Because analysis of IGF-1 is breastmilk is new in the field, a proper protocol for dilution had not been established. Thus, samples were diluted to kit protocol based on results given by Khodabakhshi et al. (2014). These authors did not report changing the dilutions to their samples and reported significant levels of (55.35-117.41 ng/ml) of breastmilk IGF-1. However, within this sample, dilutions were too high and levels of IGF-1 were very low and almost undetectable. Breastmilk IGF-1 was therefore treated as a dichotomous variable, with only four samples having detectable levels of IGF-1.

Because breastmilk IGF-1 had to be treated as a dichotomous variable, correlations between it and the other variables could not be determined. Further, because of the disproportionate comparison groups, these data had reduced statistical power. This made it difficult to effectively use IGF-1 in statistical analyses.
When IGF-1 was analyzed as a dichotomous variable, this study showed no relationship between breastmilk IGF-1 and infant length for age, weight for age, or maternal stress. Breastmilk IGF-1 was not found to be a significant predictor of infant length for age, or weight for age, nor was it associated with infant sex, infant age, or level of maternal perceived stress. Due to the limited number of samples with detectable IGF-1, these conclusions are only tentative.

**Perceptions of Breastfeeding**

Within this sample, perception of breastfeeding was a common reason for why women chose to breastfeed. It stands to reason that within a population of breastfeeding women, that the perception of breastfeeding would be positive. A large majority of the women referenced breastfeeding as being better or best in a general way and multiple women reported having a desire to breastfeed, or simply wanting to have the experience.

Although most, if not all, physicians and researchers would agree that breastfeeding is better for the infant than formula, it would be inaccurate to state that this sample is representative of all mothers, or even of all mother who breastfed for any duration their children. Because recruitment for this sample was done in part through La Leche League, the sample represents a population with strong positive sentiments toward breastfeeding, and moreover, for exclusive breastfeeding for an extended duration. Snowball sampling would encourage like-minded individuals within the sample, regardless of being members of La Leche League. Further, snowball sampling often occurred through participants posting my information to online mother support groups such as “Tampa Bay Breast Friends” and a MacDill Moms group. There would be bias as to who is more likely to be members of such online groups that would skew the results of this question.
Conclusions

In sum, this was novel research, which turned out interesting results, albeit not as expected. Significant results were found between infant growth and maternal stress as well as breastmilk fat. More specifically, there was a negative correlation between maternal perceived stress and infant length for age and weight for age Z-scores, as well as a negative correlation between breastmilk fat and infant length for Z-score. This indicates that maternal stress may negatively impact infant growth. However, more research is necessary to better understand if or how breastmilk fat may act as a mechanism to mediate infant growth due to maternal stress.

IGF-1 could not be determined to be a potential mechanism for variations in infant growth. Unfortunately IGF-1 could not be detected in a majority of samples, so statistical results involving IGF-1 are based on a reduced sample size, and need to be taken as preliminary. Further research using lower dilutions and larger samples sizes is necessary to better explore the potential role of breastmilk IGF-1 on infant growth and/or its relationship to maternal stress.

Applications to Applied Anthropology and Public Health

This research contributes to knowledge on stress related to health, human milk variation, how stress impacts breastmilk, the relationship between maternal stress and infant growth, and fetal programming. There is a growing pool of literature that examines how prenatal maternal stress may impact infant health, introducing an intergenerational aspect to health and stress research (Bogin 2007; Kuzawa 2005). Breastfeeding, and specifically breastmilk research is also gaining popularity (Quinn 2012), due to its positive relationship with health. This research contributes to knowledge on human milk variation, how stress impacts breastmilk in regards to
variation in IGF-1, the relationship between maternal stress and infant growth, and fetal programming.

Early life environment has been linked to adult disease risk, specifically in regard to non-communicable diseases. Breastfeeding has been shown to reduce adult disease risk (Kramer 1981; Lawlor et al. 2005; Mayer-Davis et al. 2008; McCance 1962; Owen et al. 2005; Stolzer 2011). The DoHAD paradigm allows researchers to examine the longitudinal outcomes associated with early life, and the integrated relationship between environment and stressors, genetics, and development (Hanson and Gluckman 2011). Research based on the DoHAD paradigm suggests that early life is a critical window for disease susceptibility. Thus, prevention strategies should also target early life, and programs should shift from secondary or tertiary care to primary prevention (Barouki et al. 2012). An emphasis on maternal and child health programs, to promote healthy pregnancies, improve birth outcomes, and promote breastfeeding are good starting points for such interventions (Mania 2011; Hanson and Gluckman 2011). In order to reduce the intergenerational impact of stress, the aim of interventions should be to identify the negative health impacts of stress, and then provide positive and useful ways to deal with stress as a health issue. Moreover, these programs should be implemented during pregnancy as well as postpartum.

Within the context of this research, the aim would be to encourage healthy breastfeeding practices while also targeting maternal stress and providing coping mechanisms to decrease levels of perceived stress. One promising method for such an intervention is increasing social support networks. Studies integrating social support theory into public health have shown that social support and social networks are a significant predictor of health outcomes, and act as a protective effect against disease (Uchino 2005). Moreover, social support has been shown to be
particularly beneficial in protecting against stress and adverse life events as well as aid in successful breastfeeding practices (Bonita et al. 2013)

Although tackling such a complex issue seems daunting, health researchers, applied anthropologists, and public health practitioners should not be discouraged. Addressing developmental aspects of health is feasible and has the potential of having a major public health impacts (Hanson and Gluckman 2011). Further, intergenerational influences of development and health imply improved health for future generations as well.

**Strengths, Limitations, and Recommendations for Future Research**

The strength of this research is its novelty; research that examines variation in breastmilk hormones in relation to infant growth and health is new, but gaining interest. Specifically looking at IFG-1 as an indicator for infant growth is a new idea. Indeed, at the onset of this study no other research examining breastmilk IGF-1 and infant growth had been published, yet two have been published during the course of this study (Khodabakhshi et al. 2014; Kon et al. 2014). Additionally, this research incorporates the DoHAD paradigm with fetal programming to form a new perspective of breastfeeding as a reflection of maternal environment and its role on developmental programming.

This was a cross sectional study, in which all data were collected during a single interview. Cross sectional studies allow for research to be done quickly and inexpensively, with minimal invasion of the participants’ time and without loss to follow up. However, the results of this study would have benefitted from a longitudinal design in which the milk samples could be collected from mothers with children of approximately the same age, ideally at birth, and then be followed to examine both changes and variations in IFG-1 levels, stress, and infant growth. This
would strengthen any associations found and allow for a better examination of causation between the three variables.

Small sample size was another limitation of this study. Although a sample of 31 is large enough to perform multiple regression analysis, it is still a small sample size with limited statistical power. Because of recruitment through La Leche League, and the use of snowball sampling, there is the issue of some homogeneity within this sample. Having a larger sample size would have made finding statistical correlations more likely, as well as diversified my population. Future research of this kind should use a larger sample size.

Ideally this research would look at both IFG-1 and IFG-2. While IGF-1 is an important factor in post-natal growth IFG-2 is associated with growth in utero. Since this research is attempting to look at hormone variation in relationship to stress and fetal programming, examining IFG-2 could have been valuable. Cortisol is another hormone that would be interesting to study within this research due to its relationship with stress and growth. Recent work has focused on the relationship between cortisol and growth due to energetic constraints (Nyberg et al. 2012).

Future research exploring the relationship between maternal perceived stress, breastmilk IGF-1, and infant growth is needed within the literature. A replication of this study should lower the dilutions of the breastmilk samples in order to obtain readable, quantitative levels of breastmilk IGF-1.

There are many potential confounders to infant growth, not all of which were accounted for within this study. Future research should include more in depth collection of complementary infant foods and analysis of maternal condition as potential confounders to infant growth. Because not all participants of this study were the same age, or even all infants, the amount of
complementary foods consumed varied widely and may have impacted growth. Using length for age and weight for age partially adjusts for this, but the lack of uniformity in time and amount of complementary foods consumed needs to be controlled for.

Since my data indicated that there is a moderate inverse relationship between maternal stress and infant growth (as stress increased length and weight for age decreased) it is important not to patient blame, or imply that they are independently at fault or in charge of their stress and therefore poor health. It is not the mother who is at fault for the manifestations of her stress. Rather, patients or participants should be given information on stress, how to cope with stress in a healthy manner, and ways in which to reduce stress. Further, it is important that the participants do not feel judged or decimated against based on their level of stress, ways they perceive stress, or ways they cope with stress. Future research should consider the sensitivity of how relationships between maternal stress, IGF-1, and infant growth could be interpreted, and exercise caution on how data are collected, written up, disseminated, and applied to programming. Yet, because this data does indicate a relationship between infant growth and maternal stress, public health measures should be taken to reduce stress among pregnant and breastfeeding women.

**Ethical Considerations**

As with all human subjects research, ethical considerations are of great importance. This is true within this research as it pertains to the sensitive nature of stress. Moreover, because of the implications on infant health, it was important to make sure the participants do not feel judged, discriminated against, or biased based on their survey answers and/or lifestyle choices. Finally, because collecting milk samples takes away a nutrient source for the infant, it comes with its own ethical considerations (Miller et al. 2013). That said, this study put participants at
minimal risk. Participants were not asked any identifying information such as name or social security number. Participation of this study did not increase any risk of physical or mental health, or injury. Finally, because this is not a nutritionally stressed population, the contribution of milk samples was not significantly detrimental to the mother or the infant. All participation of this study was completely voluntary and informed consent was collected from all participants. Approval from the internal review board (IRB) was completed for this study from the IRB at the University of South Florida.
References


6. Barker, David


7. Blyth, Rosemary, Debra K. Creedy, Cindy-Lee Dennis, Wendy Moyle, Jan Pratt, and Susan M. De Vries


8. Bogin, Barry, Silva, Maris Ines Varela and Luis Rios.


9. Bonia, Kimberly, Laurie Twells, Beth Halfyard, Valerie Ludlow, Leigh Anne Newhook, and Janet Murphy-Goodridge


10. Brown, Daniel E.


12. Carnegie Mellon Psychology


14. Center for Disease Control


15. Chen, Dorothy C., Laurie Nommsen-Rivers, Kathryn G. Dewey, and Bo Lönnerdal.


18. Daly, SE, A. Di Rosso, RA Owens, and PE Hartmann

19. Dancause, Kelsey N., David P. Laplante, Carolina Oremus, Sarah Fraser, Alain Brunet, and Suzanne King


21. Dewey, Kathryn G.


21. Dewey, Kathryn G.


22. Dundar, Nihal Olgac, Ozden Anal, Bumin Dundar, Hasan Ozkan, Sezer Caliskan, and Atilla Büyükgebiz


23. Ellison, Peter T.


24. Ellison, Peter T.

25. Ellison, Peter T., and Grazyna Jasienska

26. Epitomics
   2014 Main steps of the ZAP-70 Total and Phospho Sandwich ELISA assay.

27. Ertem, Ilgi Ozturk, Nancy Votto, and John M. Leventhal

   2012 Relationship of Insulin, Glucose, Leptin, IL-6 and TNF-α in Human Breast Milk with Infant Growth and Body Composition. Pediatric Obesity 7(4):304-312.

29. Fujita, Masako, Eric Roth, Yun-Jia Lo, Carolyn Hurst, Jennifer Vollner, and Ashley Kendell

30. Gale, Chris, Karen M. Logan, Shalini Santhakumaran, James RC Parkinson, Matthew J. Hyde, and Neena Modi
   Gilman et al. 2006
31. Gluckman, Peter D., and Mark A. Hanson

32. Gluckman, Peter D., Hanson, Mark A., and Alan S. Beedle

33. Grey, Katherine R., Davis, Elysia Poggi, Curt A. Sandman, and Laura M. Glynn

34. Hanson, Mark A. and Peter D. Gluckman

35. Hassiotou, Foteini, and Donna T. Geddes

36. Hill Kim, and Kaplan, Hillard

37. Hinde, Katie, and Lauren A. Milligan
38. Hinde, Katie, and John P. Capitanio
   

39. Insaf, Tabassum Z., Renée Turzanski Fortner, Penelope Pekow, Nancy Dole, Glenn Markenson, and Lisa Chasan-Taber
   

   

41. Koletzko, Berthold, Rüdiger von Kries, Ricardo Closa Monasterolo, Joaquín Escribano Subías, Silvia Scaglioni, Marcello Giovannini, Jeannette Beyer, Hans Demmelmaier, Brigitte Anton, Dariusz Gruszfeld, Anna Dobrzanska, Anne Sengier, Jean-Paul Langhendries, Marie-Francoise Rolland Cachera, and Veit Grote
   
42. Kon, Igor Ya, Natalia M. Shilina, Maria V. Gmoshinskaya, and Tatiana A. Ivanushkina

43. Kondo, Naoki, Yuki Suda, Atsuhito Nakao, Kyoko Oh-Oka, Kohta Suzuki, Kayoko Ishimaru, Miri Sato, Taichiro Tanaka, Akiko Nagai, and Zentaro Yamagata

44. Kramer, Michael S.

45. Krebs, John R.

46. Kumsta, Robert, and Markus Heinrichs

47. Kuzawa, Christopher

48. Kuzawa, Christopher and Elizabeth Sweet
49. Kuzawa, Christopher, Thyer, Zaneta Marie, and Ruby Fried
   N.d Biological Memories of Past Deprivation: Lifecourse and Intergenerational Transmission of Embodied Health Inequality. Unpublished, Department of Anthropology, Northwestern University.

50. La Leche League International

51. Lau, C.

52. Lau, C., N. M. Hurst, E. O. Smith, and R. J. Schanler

53. Laron, Z.

54. Lassek, William D., and Steven JC Gaulin

   2005 Infant feeding and components of the metabolic syndrome: findings from the European Youth Heart Study. Archives of Disease in Childhood, 90(6), 582–588. doi:10.1136/adc.2004.055335
56. Li, Ruowei, Sara B. Fein, and Laurence M. Grummer-Strawn


57. Lihn, AS, Steen Bønløkke Pedersen, and Bjørn Richelsen


58. López Alvarez, MJ


59. Lucas, A., J. A. Gibbs, R. L. Lyster, and J. D. Baum


60. Maina, William K.


2008 Breast-Feeding and Type 2 Diabetes in the Youth of Three Ethnic Groups: The Search for Diabetes in Youth Case-Control Study. Diabetes Care 31(3):470-475.

62. McCance, Robert A.

63. Mezzacappa, Elizabeth Sibolboro, Andrea Y. Tu, and Michael M. Myers.  

64. Miller, Elizabeth M., Marco O. Aiello, Masako Fujita, Katie Hinde, Lauren Milligan, and E. A. Quinn  

65. Miller, Elizabeth M., and Daniel S. McConnell  

66. Miller, Elizabeth  

67. National Institutes of Health  
2014 Genetics home Reference. Electronic Document,  

68. Nepomnaschy PA, Welch KB, McConnell DS, Low BS, Strassmann BI, and England BG.  

69. Newburg, David S., Jessica G. Woo, and Ardythe L. Morrow  
70. Northeast Florida Healthy Start Coalition Inc.

2013 Breastfeeding rates in Florida drop significantly,


71. Nyberg, Colleen H., William R. Leonard, Susan Tanner, Thomas Mcda, Tomas Huanca, and Ricardo A. Godoy

2012 Diurnal Cortisol Rhythms and Child Growth: Exploring the Life History
Consequences of HPA Activation among the Tsimane'. American Journal of Human

72. O’Brien, Maxine, Elizabeth Buikstra, and Desley Hegney

2008 The Influence of Psychological Factors on Breastfeeding Duration. Journal of
Advanced Nursing 63(4):397-408.

73. O’Campo, Patricia, Ruth R. Faden, Andrea C. Gielen, and Mei Cheng Wang

1992 Prenatal Factors Associated with Breastfeeding Duration: Recommendations for

74. Oftedal, Olav T.


75. Owen, Christopher G., Richard M. Martin, Peter H. Whincup, George Davey Smith, and Dereck G. Cook

2005 Effect of Infant Feeding on the Risk of Obesity Across the Life Course: A
76. Pollard, Tessa M., and Gillian H. Ice


77. Pond, Caroline M.


78. Powe, Camille E., Cheryl D. Knott, and Nancy Conklin-Brittain


80. Quinn, Elizabeth A.


81. Rudzick, Alanna, Breakey, Alicia, and Ricahrd Bribiescas


82. Sheriff, Michael J., and Oliver P. Love

83. Sperlings Best Places

2012 Stressful Cities 2012 List. Electronic Document,

84. Stinson, Sara. Barry Bogin, and Dennis O’Rourke (eds.).


85. Stinson, Sara. Barry Bogin, and Dennis O’Rourke (eds.).


86. Stolzer, Jeanne M.


87. Thakkar, Sagar K., Francesca Giuffrida, Cruz-Hernandez Cristina, Carlos Antonio De Castro, Rajat Mukherjee, Liên-Anh Tran, Philippe Steenhout, Le Ye Lee, and Frédéric Destaillats

88. Thomas, R. Brook
   1998 The Evolution of Human Adaptability Paradigms: Toward a Biology of Poverty.”
   Building a New Biocultural Synthesis: Political-Economic Perspectives on Human
   Biology. Alan H. Goodman and Thomas L. Leatherman. Ann Arbor: The University of

89. Thompson, Amanda L.
   2012 Developmental Origins of Obesity: Early Feeding Environments, Infant Growth,

90. Trivers, Robert L., and Dan E. Willard
   1973 Natural Selection of Parental Ability to Vary the Sex Ratio of Offspring. Science
   179:90-92.

91. Uchino, Bert N.
   2004 Social Support and Physical Health: Understanding the Health Consequences of
   Relationships. Yale University Press.

92. Ueda, Toshihiro, Yuji Yokoyama, Minoru Irahara, and Toshihiro Aono
   1994 Influence of Psychological Stress on Suckling-Induced Pulsatile Oxytocin

93. U.S. Census Bureau
   2013 State & County QuickFacts. Electronic Document,

94. Walker, Allan
   2010 Breastmilk as the Gold Standard for Protective Nutrients. The Journal of
   Pediatrics 156(2, Supplement):S3-S7.
96. Wang, Christine D., Patricia S. Chu, Beverly G. Mellen, and Jayant P. Shenai. 

1998 Creamatocrit and the nutrient composition of human milk. Journal of 

95. Wells, Jonathan C. K. 

2003 The thrifty phenotype hypothesis: Thrifty offspring or thrifty mother? Journal of 
Theoretical Biology 221(1):143-161.

96. Wells, Jonathan C. K. 

2014 Adaptive Variability in the Duration of Critical Windows of Plasticity: 
Implications for the Programming of Obesity. Evolution, Medicine, and Public Health 

97. World Health Organization 

2013 World Health Organization Ten Facts About Breastfeeding. Electronic Document, 
http://www.who.int/features/factfiles/breastfeeding/facts/en/index9.html, accessed July 1, 
2013.

98. Wiley, Andrea S. 

2005 Does Milk make Children Grow? Relationships between Milk Consumption and 

99. Wiley, Andrea S. 

2012 Cow Milk Consumption, Insulin-Like Growth Factor-I, and Human Biology: A 
Appendices

Appendix A: University of South Florida IRB Approval Letter

September 15, 2014

Lauren Gottfredson
Anthropology
Tampa, 33612

RE: Expedited Approval for Initial Review
IRB#: Pro00017508
Title: Maternal Stress, Breastmilk IGF-1, and Infant Growth among Breastfeeding Mothers-Infant Pairs in the Tampa Bay Area

Study Approval Period: 9/15/2014 to 9/15/2015

Dear Ms. Gottfredson:

On 9/15/2014, the Institutional Review Board (IRB) reviewed and APPROVED the above application and all documents outlined below.

Approved Item(s):
Protocol Document(s):
Gottfredson Thesis Proposal V1.docx
Study involves children and falls under 45 CFR 46.404: Research not involving more than minimal risk.

Consent/Assent Document(s)*:
Informed consent audit ver#1.docx.pdf

*Please use only the official IRB stamped informed consent/assent document(s) found under the "Attachments" tab. Please note, these consent/assent document(s) are only valid during the approval period indicated at the top of the form(s).

It was the determination of the IRB that your study qualified for expedited review which includes activities that (1) present no more than minimal risk to human subjects, and (2) involve only procedures listed in one or more of the categories outlined below. The IRB may review research through the expedited review procedure authorized by 45CFR46.110 and 21 CFR 56.110. The research proposed in this study is categorized under the following expedited review
category:

(3) Prospective collection of biological specimens for research purposes by noninvasive means.

(4) Collection of data through noninvasive procedures (not involving general anesthesia or sedation) routinely employed in clinical practice, excluding procedures involving x-rays or microwaves. Where medical devices are employed, they must be cleared/approved for marketing.

(6) Collection of data from voice, video, digital, or image recordings made for research purposes.

(7) Research on individual or group characteristics or behavior (including, but not limited to, research on perception, cognition, motivation, identity, language, communication, cultural beliefs or practices, and social behavior) or research employing survey, interview, oral history, focus group, program evaluation, human factors evaluation, or quality assurance methodologies.

As the principal investigator of this study, it is your responsibility to conduct this study in accordance with IRB policies and procedures and as approved by the IRB. Any changes to the approved research must be submitted to the IRB for review and approval by an amendment.

We appreciate your dedication to the ethical conduct of human subject research at the University of South Florida and your continued commitment to human research protections. If you have any questions regarding this matter, please call 813-974-5638.

Sincerely,

Kristen Salomon, Ph.D., Vice Chairperson
USF Institutional Review Board
Appendix B: University of South Florida IRB Amendment Approval Letter

10/9/2014

Lauren Gottfredson
Anthropology
4202 E. Fowler Avenue
Tampa, FL 33620

RE: Expedited Approval for Amendment
IRB#: Amel_Pro00017508
Title: Maternal Stress, Breastmilk IGF-1, and Infant Growth among Breastfeeding Mothers-Infant Pairs in the Tampa Bay Area

Dear Ms. Gottfredson:

On 10/8/2014, the Institutional Review Board (IRB) reviewed and APPROVED your Amendment. The submitted request has been approved for the following:

Approved Item(s):
Protocol Document(s):
protocol v2.10.8

Recruitment Document(s):
Participant Recruitment Language v2.docx

We appreciate your dedication to the ethical conduct of human subject research at the University of South Florida and your continued commitment to human research protections. If you have any questions regarding this matter, please call 813-974-5638.

Sincerely,

[Signature]
John Schinka, Ph.D., Chairperson
USF Institutional Review Board
Appendix C: Recruitment Message

Email recruitment/Facebook:

Subject: Participation opportunity for breastfeeding women

Body: Hello, if you are a breastfeeding mother who is interested in breastfeeding research, then you might be interested in participating in this study. I am a graduate student at the University of South Florida (USF). I am conducting a research study (USF IRB #pro00017508) for my thesis on the relationship between maternal stress, breastmilk variation, and infant growth. Participation in this research will include a one on one interview in which I will ask you questions about stress and breastfeeding. I will also take height and weight measurements of you and your child. Finally, I will collect a small sample of breastmilk, 1 to 2ml. Participation should take about 30 to 45 minutes your time. If you are interested, please contact me by email at lgottfire@mail.usf.edu. I am happy to answer any of your questions and set up a time and place that is convenient for you. Thank you for your interest!
Appendix D: Adult Informed Consent

Informed Consent to Participate in Research Involving Minimal Risk
Information to Consider Before Taking Part in this Research Study

IRB Study # Pro00017508

You are being asked to take part in a research study. Research studies include only people who choose to take part. This document is called an informed consent form. Please read this information carefully and take your time making your decision. Ask the researcher or study staff to discuss this consent form with you, please ask him/her to explain any words or information you do not clearly understand.

We are asking you to take part in a research study called:

Maternal Stress, Breastmilk IGF-1, and Infant Growth among Breastfeeding Mothers-Infant Pairs in the Tampa Bay Area

The person who is in charge of this research study is Lauren Gottfredson. This person is called the Principal Investigator. However, other research staff may be involved and can act on behalf of the person in charge. She is being guided in this research by Lorena Madrigal, Elizabeth Miller, and Ricardo Izurieta.

Purpose of the study

The purpose of this study is to examine the relationship between maternal stress, levels of breastmilk IGF-1, and infant growth. More specifically, it aims to see if maternal stress impacts infant growth via variation in breastmilk IGF-1, a naturally occurring growth hormone.

Why are you being asked to take part?

We are asking you to take part in this research study because you are a breastfeeding mother in the Tampa Bay area.

Study Procedures: What will happen during this study?

If you take part in this study, you will be asked to:

- Participate in Semi-structured Surveys. The surveys will include both quantitative and qualitative data and be administered by the interviewer in a semi-structured interview style. Information collected will include demographic information and questions on stress and breastfeeding history.
• Anthropometrics of height and weight of the mother and the infant will be collected.
• Milk samples will be collected from all participants. Women will be asked to hand express the samples. If they do not know how, I will be able to instruct them on the process. If they do not feel comfortable hand expressing, they will be allowed to use their own pump as long as they are the only individual to have used the pump. People with shared pumps will not be allowed to use their pumps for the sample expression.
• Total participation should take a single visit of approximately one hour. In some cases a follow up visit may be asked for by the researcher, but can be turned down.
• With permission of the participant, interview sessions will be audio recorded for transcription and researchers reference.

Total Number of Participants
About 30 individuals will take part in this study at USF.

Alternatives / Voluntary Participation / Withdrawal
You do not have to participate in this research study.
You should only take part in this study if you want to volunteer. You should not feel that there is any pressure to take part in the study. You are free to participate in this research or withdraw at any time. There will be no penalty or loss of benefits you are entitled to receive if you stop taking part in this study.

Benefits
You will receive no direct benefit(s) by participating in this research study.

Risks or Discomfort
This research is considered to be minimal risk. That means that the risks associated with this study are the same as what you face every day. There are no known additional risks to those who take part in this study.

Compensation
You will receive no payment or other compensation for taking part in this study.

Privacy and Confidentiality
We will keep your study records private and confidential. Certain people may need to see your study records. By law, anyone who looks at your records must keep them completely confidential. The only people who will be allowed to see these records are:

• The research team, including the Principal Investigator, study coordinator, and all other research staff.
• Certain government and university people who need to know more about the study. For example, individuals who provide oversight on this study may need to look at your
records. This is done to make sure that we are doing the study in the right way. They also need to make sure that we are protecting your rights and your safety.

- Any agency of the federal, state, or local government that regulates this research. This includes the Department of Health and Human Services (DHHS) and the Office for Human Research Protection (OHRP).
- The USF Institutional Review Board (IRB) and its related staff who have oversight responsibilities for this study, staff in the USF Office of Research and Innovation, USF Division of Research Integrity and Compliance, and other USF offices who oversee this research.

We may publish what we learn from this study. If we do, we will not include your name. We will not publish anything that would let people know who you are.

You can get the answers to your questions, concerns, or complaints

If you have any questions, concerns or complaints about this study, or experience an unanticipated problem, call Lauren Gottfredson at (650) 799-6103 or email her at lgottfre@mail.usf.edu.

If you have questions about your rights as a participant in this study, general questions, or have complaints, concerns or issues you want to discuss with someone outside the research, call the USF IRB at (813) 974-5638.

Consent to Take Part in this Research Study

It is up to you to decide whether you want to take part in this study. If you want to take part, please sign the form, if the following statements are true.

I freely give my consent to take part in this study. I understand that by signing this form I am agreeing to take part in research. I have received a copy of this form to take with me.

Signature of Person Taking Part in Study

Date

Printed Name of Person Taking Part in Study

Statement of Person Obtaining Informed Consent

I have carefully explained to the person taking part in the study what he or she can expect from their participation. I hereby certify that when this person signs this form, to the best of my knowledge, he/ she understands:

- What the study is about;
- What procedures will be used;
- What the potential benefits might be; and
• What the known risks might be.

I can confirm that this research subject speaks the language that was used to explain this research and is receiving an informed consent form in the appropriate language. Additionally, this subject reads well enough to understand this document or, if not, this person is able to hear and understand when the form is read to him or her. This subject does not have a medical/psychological problem that would compromise comprehension and therefore make it hard to understand what is being explained and can, therefore, give legally effective informed consent.

___________________________________________________
Signature of Person obtaining Informed Consent

_______________________________________________________________
Printed Name of Person Obtaining Informed Consent
Appendix E: Survey-Interview

Survey ID#:

**Anthropometrics:**

Mother:
- Height
- Weight
- BMI
- Ponderal Index

Child:
- Height
- Weight
- BMI
- Ponderal Index
Demographics/Background Questions:

1. How old are you?_________
2. How old were you when you had your first child?_______
3. How old is the child you are currently breastfeeding in months? _______
4. Child's gender________
5. Do you have other children? If so, how many and what are their ages?

6. Are you currently employed?
   __Yes  ___No
   a. How many hours do you work each week?_______

7. Are you currently in school?
   __Yes  ___No
   a. How many hours do spend on school each week?_______

8. Are you married, in a relationship, or single?__________

9. Are you a member of La Leche League?
   __Yes  ___No
   a. What brought you to join La Leche League?

Stress Scales:

10. On a scale of 1 to 10, with 1 being not stressful at all and 10 being very stressful, how would you rate your experience with breastfeeding in the last month?
    ____1  ____2  ____3  ____4  ____5  ____6  ____7  ____8  ____9  ____10

11. On a scale of 1 to 10 where 1 is not stressed at all and 10 is always very stressed, how would you rate your general stress level of the last month?
    ____1  ____2  ____3  ____4  ____5  ____6  ____7  ____8  ____9  ____10

12. The questions in this scale ask you about your feelings and thoughts during THE LAST MONTH. In each case, please mark the box representing HOW OFTEN you felt or thought a certain way.

83
<table>
<thead>
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<th></th>
<th>0- Never</th>
<th>1- Almost never</th>
<th>2- Sometimes</th>
<th>3- Fairly often</th>
<th>4- Very often</th>
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<tbody>
<tr>
<td>1. In the last month, how often have you been upset because of something that happened unexpectedly?</td>
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<td>2. In the last month, how often have you felt that you were unable to control the important things in your life?</td>
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<td>3. In the last month, how often have you felt nervous and &quot;stressed&quot;?</td>
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<td>4. In the last month, how often have you felt confident about your ability to handle your personal problems?</td>
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<td>5. In the last month, how often have you felt that things were going your way?</td>
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<td>6. In the last month, how often have you found that you could not cope with all the things that you had to do?</td>
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<td>7. In the last month, how often have you been able to control irritations in your life?</td>
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<td>8. In the last month, how often have you felt that you were on top of things?</td>
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<td>9. In the last month, how often have you been angered because of things that were outside of your control?</td>
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<td>10. In the last month, how often have you felt difficulties were piling up so high that you could not overcome them?</td>
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**Qualitative Questions:**

13. Have you breastfeed all of your children/is this your first time breastfeeding?

a. What led you to choose to breastfeed?
b. What other foods does your child currently eat?

14. Describe your breastfeeding experience(s):
   
a. Did you have any obstacles or difficulties?

15. Do you consider yourself to be a stressed person?
   
a. What causes stress in your life?
      
i. Is it more daily hassles, specific events, or chronic problems

16. How do you cope with daily stressors?

17. What about more severe stressors?