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THE EFFECT OF MATERNAL SALIVARY CORTISOL LEVEL ON CHILD NUTRITION STATUS

ALLISON A. POHL
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Reviewed and approved by the following:

Katarzyna Kordas, PhD
Assistant Professor of Nutrition
Thesis Supervisor

Rebecca Corwin, PhD
Associate Professor of Nutritional Neuroscience
Honors Advisor

* Signatures are on file in the Schreyer Honors College

ABSTRACT

There is a unique connection between a mother and child that is unlike any other human relationship. The biological mother is the sole caregiver that supports the child from conception, through gestation, and throughout childhood and subsequent developmental periods. While the mother acts to support her child socially, emotionally, and financially, it is her biological influence that initially shapes her child's development. Specifically, the current study aims to address the role that maternal stress measured through salivary cortisol has on her child's nutritional state, measured through height, weight, and hemoglobin status. Overall, 56 mother and child pairs from Montevideo, Uruguay were included in the study. Maternal saliva and child blood samples were collected over a period of several weeks. The saliva samples were then analyzed for cortisol content using the ELISA method (Salimetrics, State College, PA) (HemoCue, Lake Forrest, CA) and child blood samples were analyzed for hemoglobin content using a portable instrument. Area under the curve (AUC) calculations were used to average maternal cortisol concentrations based on the two days of collection and simple linear and logistic regressions were run with AUC as the independent variable and child nutritional status as the dependent variables. Models were covariant-adjusted for child age, child sex, whether the child lived with one or both parents and the mother's age, years of schooling, maternal IQ score, depressive symptoms, stress, employment status, and finally, the parents' marital status, SES score, housing occupant density, and HOME Inventory score. The average maternal cortisol concentrations (expressed as AUC) for Day 1 and Day 2 of collection were 96.16 ±72.01µg/dL and 98.74 ± 84.05µg/dL, respectively. There was not a statistical association between maternal salivary cortisol levels and child height, weight, or hemoglobin concentration. Overall, this study concludes that maternal stress measured through salivary cortisol did not influence child

nutrition status. However, a large body of literature supports the effect that maternal stress plays in overall child development, thus, further studies with larger sample sizes are needed to clarify the results.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

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INTRODUCTION

The relationship between mother and child is a bond that is unlike any other human connection. Although several individuals may act as caregiver to a single child, it is the mother whose individual influence initially shapes the physical, cognitive, and emotional development of the child. Part of the exceptionality of the mother-child relationship is based upon the mother's unique role as sole nurturer, provider, and protector during the gestational period (Broad, Curley, & Keverne, 2006). Research supports the idea that the mother's health and quality of care is one of the largest determinants of the child's health and survival (Pavard, Sibert, & Heyer, 2007). Many maternal influences on the child begin in utero, before further exposure to and stimulation from external contacts. For example, due to the nine months of intimate bidirectional connection and influence between the mother and fetus, infants prefer their biological mother's voice, language, and dialect to that of a strangers (Kisilevsky et al., 2003). Similarly, due to the child's nutritional dependence on the mother, an infant can develop particular taste preferences for foods that the mother consumed during pregnancy (Brown et al., 2011). It has even been suggested that the level of maternal stress experienced during pregnancy may influence the rate of development of the fetal nervous system, though more research on the topic is necessary (DiPietro, 2004).

According to early child psychologists, Bowlby and Cassidy, the mother's influence continues throughout infancy because she is typically regarded as the infant's most significant means of emotional adaptation (Luijk et al., 2010). Also, such conditions observed in infants as fetal alcohol syndrome, low birth weight, decreased vascular functioning, growth restrictions, birth defects, and neonatal abstinence syndrome (withdrawal symptoms including physiological and behavioral problems in the infant such as blotchy skin coloring, fever, seizures, high-pitched

crying, tremors, irritability, vomiting, rapid breathing, and increased muscle tone that can occur when a newborn is exposed to addictive drugs while in utero) often develop as a result of maternal nutrient deficiencies as well as the ingestion and abuse of tobacco products, alcohol, and illicit drugs including, methamphetamines, opiates, and cocaine during pregnancy (Meyer-Leu, Lemola, Daeppen, Deriaz, & Gerber, 2011; Baily, McCook, Hodge, McGrady, 2011). While these examples demonstrate how maternal behaviors during gestation impact fetal as well as early infant development, the intimate and unique influence of the mother is expressed through all stages of development.

Due to various societal circumstances including maternity leave policies, financial concerns, and traditional preferences in marital and cohabitation relationships, mothers are still more likely than fathers to act as the primary caregiver to children (Amato, 1994). Even when fathers do spend time with their children, it is seldom without the presence and help of their wives and/or female partners (Amato, 1994). Also, because women, on average, still earn less than their male counterparts, many families choose to uphold traditional household roles and allow the father to act as the breadwinner while the mother raises the children and maintains a comfortable living environment. Due to socialization strategies, nurturance and compassion are characteristics that are instilled more strongly in females than males, thus creating a strong moral responsibility for maternal care-giving (Hyde, 2007). Even when mothers do split their time between parenting and work, they are often concerned about the damage their careers have on their relationships with their children. There is some evidence to suggest that excessive time that mothers spend away from their children may reduce their responsiveness to various infant cues, ultimately hindering infant development (Booth, Clarke-Stewart, Vandell, McCartney, & Owen, 2007). Thus, because mothers on average spend more time with their children than do fathers,

they may have a larger influence over their children's health and development than other caregivers even later in life.

Not only are there multiple social explanations supporting the unparalleled intensity of the mother-child relationship from gestation through adulthood, but also several biological reasons exist as to why mothers have such a powerful impact on their children. The hormone dopamine is a catecholamine neurotransmitter that is most well-known for its central role in motivation, reward, and reinforcement for such pleasurable experiences as eating and drug usage (Wise, 2006). Dopamine receptor sensitivity is enhanced by the presence of estrogen through the stimulation of oxytocin receptors (Parent et al., 2005). Animal studies have suggested that mothers may receive greater dopaminergic reinforcement for social interactions and responsiveness than do fathers due to the hormone fluctuations during and following pregnancy (Parent et al., 2005). Based solely on anatomical differences between men and women, mothers, unlike fathers, are able to provide nourishment to their children via breastfeeding. Women who breastfeed their children typically expose their offspring to an increased amount of exclusive bonding time with the mother. Mothers who choose to breastfeed their children further influence their child's health and development through the immunoglobulins, proteins, and overall nutrition present in the breast milk that they provide. For example, one study stated that exclusive breastfeeding can reduce an infant's risk of developing ear infections and diarrhea. Over a one year period, 86% of breastfed infants experienced no incidence of illness when compared to formula-fed babies who experienced illness more frequently (Cardenas & Major, 2005). Also, breastfeeding enhances and improves maternal influence, possibly explained by the unique bonding connection that mothers and infants experience (Wall, 2010). Clearly, mothers have both a unique and powerful impact on their children through all stages of development.

However, there remain many maternal factors and behaviors that are not well understood in terms of their effect on child development. One such factor is maternal stress and its effect on child growth and development.

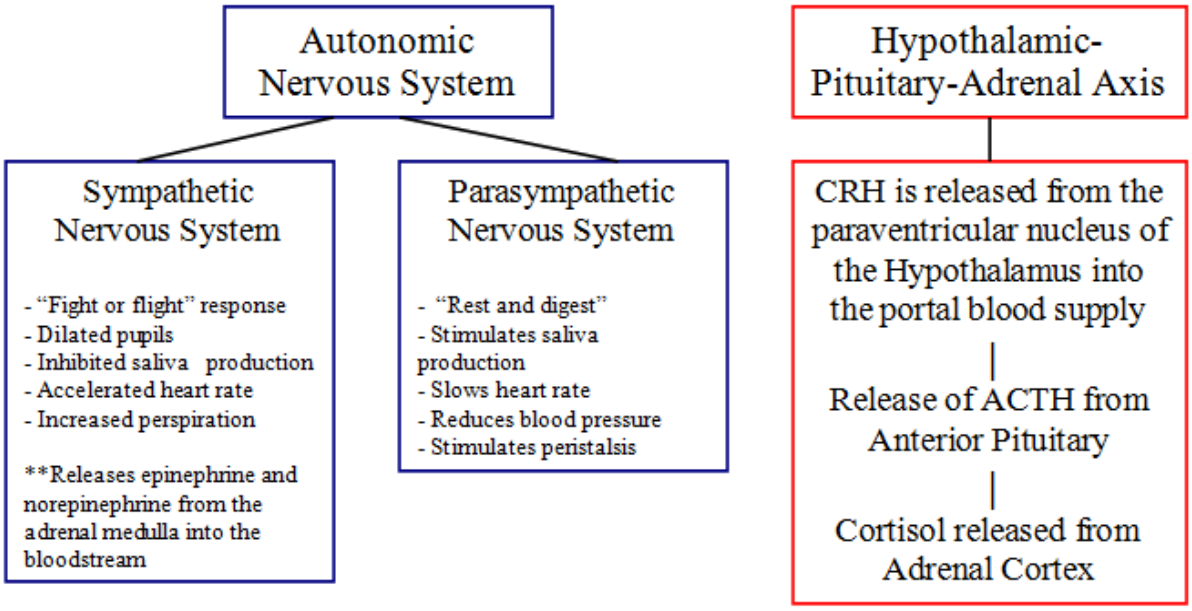
Stress is experienced by people of both genders, all age groups, occupations, ethnicities, and socioeconomic statuses. Stressors are defined as occurrences that could potentially impact an individual's physical, emotional, or psychological well-being (Kemeny, 2003). Thus, while some individuals may perceive certain situations or events as stressors, others may not value those same experiences as stressful. The actual stress response, on the other hand, is explained as physical or emotional responses that appear when exterior factors burden an individual's abilities (Charlton, 1992). From pressures at work, to the death of a family member, to physical tension, stress can manifest in different ways from person to person. Although stress has historically been viewed as a negative aspect of life, it does, in fact, have several necessary qualities. Our early ancestors used the biological stress response to their advantage as a means of survival and endurance during harsh or unfavorable environmental conditions. This sort of stress response is known as the "fight or flight" phenomenon and may actually benefit an organism throughout development (Badvaev, 2005). Not only does stress aid in survival during compromising situations, it can also aid in normal developmental processes such as skeletal growth and maturity (Badvaev, 2005). While these forms of stress may be beneficial and at times necessary for normal development, higher amounts of acute stress can have multiple negative effects including mood alterations, cognitive deficits, appetite changes, immune suppression, infectious disease development, increased risk for cardiovascular disease and mental illness, rheumatoid arthritis, and even memory loss (Kemeny, 2003; Robles, Glaser, & Kiecolt-Glaser, 2005; Hanson

& Chen, 2010). An individual who experiences these high levels of acute stress exposes his/her body to increased allosteric load and compromising health (Hanson & Chen, 2010).

Although the feelings associated with stress are universally recognized and experienced by all humans, the complex biological reactions that cause such familiar sensations are not as easily understood by the general public. Feelings of stress are primarily regulated by two systems within the body; the autonomic nervous system (ANS) and the hypothalamic-pituitary-adrenal axis (HPA axis) (Kivilighan, 2006). The autonomic nervous system consists of two divisions; the sympathetic nervous system (SNS) and parasympathetic nervous system (PNS) (Figure 1). The SNS is primarily responsible for initiating the "fight or flight" response that is experienced in stressful situations. This system will increase an individual's heart rate, level of perspiration, pupil size, and blood pressure while reducing saliva production (Kivilighan, 2006; Goeders, 2004). Norepinephrine, a common catecholamine, is released by the SNS primarily at the adrenal medulla as well as various other organs (Kemeny, 2003). This release of norepinephrine stimulates the release of epinephrine, also called adrenaline, from the adrenal medulla and into the bloodstream (Kemeny, 2003). Stimulation of the SNS and the subsequent increases in norepinephrine and epinephrine, result in an increased heart rate as well as the other aforementioned symptoms (Kemeny, 2003).

The PNS directly inhibits the reactions of the SNS through its control over regulatory biological functions such as digestion (Kivilighan, 2006). The PNS decreases heart rate and blood pressure while increasing saliva production. Thus, during a stressful event, the SNS is activated and the concentration of available glucose as an energy source is increased while the activities controlled by the PNS such as reproduction and growth are deactivated (Kemeny, 2006). Both processes of the ANS take place in a relatively quick time frame (Kivilighan, 2006).

Figure 1. ANS and HPA Axis Branches and Functions



While the ANS uses norepinephrine and epinephrine to control the "fight or flight" response as well as "rest and digest" regulatory activity, the HPA axis is activated by acute stressors and regulated via a negative feedback loop as well as a normal diurnal circadian rhythm pattern (Liddle & Island, 1970) (Figure 1). The HPA axis is triggered when the hypothalamus releases corticotropin-releasing hormone (CRH) which then binds to receptors in the anterior pituitary to produce adrenocorticotropin hormone (ACTH) (Goerders, 2004). ACTH travels to the adrenal glands which then stimulates the release of cortisol, an adrenocorticosteroid produced in humans from the adrenal cortex (Goerders, 2004). As an individual experiences an increased level of stress, the HPA feedback mechanism appropriately increases the level of corticotropin released and subsequently the amount of cortisol circulating through the bloodstream (Liddle &

Island, 1970). Cortisol, as a reflection of the level of stress experienced by an individual can be measured through blood, urine, and saliva (Kemeny, 2006).

Not only is the level of circulating cortisol regulated by external stressors, it is also controlled by an individual's natural nycthemeral rhythm (Liddle & Island, 1970). The nycthemeral pattern of cortisol secretion follows the typical circadian rhythm model. Normally, the diurnal pattern of cortisol secretion is higher upon awakening and then decreases throughout the day. Between 6:30 and 7:30 in the morning salivary cortisol levels are typically around 0.4 µg/dL. By 8:00 in the morning the concentration of cortisol measured through saliva rises to almost 0.5 µg/dL and then quickly declines to around 0.1 µg/dL between 11:30 to 12:30 in the afternoon. The level of salivary cortisol then continues to decrease, though less rapidly, throughout the remainder of the day, until the normal sleep pattern resumes around 11:00 at night. Cortisol level then begins to rise again until awakening (Aardal & Holm, 1995).

While cortisol secretion normally peaks and declines throughout a 24-hour period, the influence of daily stressors and the subsequent stress response can increase the level of cortisol in an individual's system. Upon experiencing a stressor, cortisol secretion begins to increase and peaks after approximately 15 to 20 minutes (Liddle & Island, 1970). Because cortisol has a longer half-life than the catecholamines norepinephrine and epinephrine, the HPA axis activation process is longer than that of the SNS (Kivilighan, 2006). Many studies use saliva samples to measure cortisol concentrations because it is usually the quickest, least invasive, and most accurate method. Cortisol in the blood cannot move through the bloodstream freely. That is, it must be bound to Cortisol Binding Protein (CBP) in order to travel through the bloodstream. Thus, a blood sample used to measure cortisol does not represent the total amount of free cortisol that is shown through a saliva sample (Kivilighan, 2006).

Although chronic peaks in cortisol secretion can disrupt the normal diurnal rhythm, cortisol can have several protective and beneficial qualities (Kivilighan, 2006). The main role of cortisol in the body is to increase the level of available glucose in the bloodstream. This increase in glucose provides a quick source of energy, which is needed to overcome a particular perceived stressor (Kivilighan, 2006). Similarly, a brief release in cortisol also helps a person mount a response to stress by aiding in amino acid breakdown while increasing memory function as well as tissue repair and also monitoring blood pressure. An increase in cortisol may be beneficial in the short term, but it can have various negative effects if elevated over long periods of time (Kivilighan, 2006). Such negative manifestations include immunodeficiencies, neural atrophy, and abdominal obesity (Kivilighan, 2006).

Clearly the negative effects of increased cortisol secretion brought on by chronic and reoccurring stress can have detrimental physical, psychological, and emotional manifestations on all affected persons. The aim of this thesis is to examine the possible nutritional repercussions that children may face due to increased levels of maternal stress measured through salivary cortisol.

Research has shown that the different ways in which mothers consider and perceive their roles as caregivers, nurturers, and providers for their children ultimately affect the types of attachment they will make with their offspring during the critical first year of life after birth (Pridham, Schroeder, & Brown, 2000). Factors that influence a mother's perception of her maternal role include her level of education, mental health status, the infant's temperament, and her personal evaluation of her ability to successfully parent (Pridham, Schroeder, & Brown, 2000). More specifically, the mother's working model of her own care-giving and her responsiveness to her child's evolving needs can be negatively affected by feelings of depression

and a lack of formal education (Pridham, Schroeder, & Brown, 2000). These two causative factors are interrelated and can, in fact, influence one another. For example, fewer years of formal education can result in limited career opportunities and lower-paying jobs, which can then result in subsequent financial burdens, work-family balance difficulties, as well as time pressures. These common negative outcomes that often result from a lack of education can also negatively affect an individual's emotional and cognitive well-being, resulting in depressive symptoms. Most studies regarding the relationship between cortisol levels and depression are conducted on severely and chronically depressed subjects. One such study found that individuals experiencing extreme feelings of depression and anxiety resulted in slightly higher levels of cortisol (Van Eck, Berkhof, Nicolson, & Sulon, 1996). In several instances high levels of maternal stress brought on by extreme situations during pregnancy have resulted in negative birth outcomes for the infant. For example, mothers who were impacted by the Belgrad bombing in 1999 typically delivered infants with low birth weights. (Bolton, Buske-Kirschbaum, Papousek, Pirke, & Hellhammer, 2010). Similarly, pregnant women during their first trimester who were living within a two-mile radius of the World Trade Centers on September 11, 2001 typically gave birth to below-average infants for both height and weight (Bolton, Buske-Kirschbaum, Papousek, Pirke, & Hellhammer, 2010).

In a low-income household, all family members are negatively affected both psychologically and socially. (Gundersen, Lohman, Garasky, Stewart, & Eisenmann, 2008). Often due to a lack of coping resources, these affected families have a difficult time appropriately managing the multiple stressors of daily living activities (Gundersen, Lohman, Garasky, Stewart, & Eisenmann, 2008). Parents are responsible for adequately providing food, shelter, nurturance, and an overall sense of security for their family members. When funds for

providing such necessities are consistently insufficient, several aspects of the family's home-life can suffer. For example, if parent must work multiple jobs or extended hours to make ends meet, the time they spend at home actually parenting their children as well as simply being present in the home around their children can be reduced (Gundersen, Lohman, Garasky, Stewart, & Eisenmann, 2008). Also, less free time and/or lack of education and money can result in the failure to purchase and prepare nutritional meals for their offspring (Gundersen, Lohman, Garasky, Stewart, & Eisenmann, 2008).

Previous studies show that adults experiencing excessive work stress, depression, fatigue, as well as other medical illnesses have abnormal diurnal cortisol patterns (Adam & Gunnar, 2001). In one study, researches found that women experiencing work stress have a flattened diurnal pattern, meaning that their levels of morning cortisol are lower than normal and the decline in cortisol throughout the day is much smaller than in normal individuals (Adam & Gunnar, 2001). The study reported that the number of children in the household, the age(s) of the child(ren), as well as the mother's job-status (full/part time employed or unemployed) strongly predicted a weaker diurnal pattern as shown by a flattening in cortisol throughout the day (Adam & Gunnar, 2001). Having older mothers and older children in the household predicted steeper and more predictable diurnal cortisol, while an increased number of children as well as an increased number of hours spent working outside of the home resulted in weaker cortisol patterns (Adam & Gunnar, 2001).

While it is clear that there is a link between daily maternal stressors and maternal salivary cortisol levels and patterns, it is much more difficult to draw connections between abnormal maternal cortisol measurements and child nutritional status. In animal studies researchers have found that pregnant rats that were exposed to stressful situations gave birth to offspring that

experienced impaired learning and coping behaviors along with weakened motor development (Welberg & Seckl, 2001). It has been seen in animal studies that mothers who are exposed to both chronic as well as single daily stressors transmit their own increase in cortisol secretion across the placenta. This increase in cortisol transmission stimulates the fetal HPA axis and can effect brain maturation, suggesting long-term consequences (Lupien, McEwen, Gunnar, & Heim, 2009). An increase in cortisol in utero can result in an increase in HPA activity throughout adulthood (Lupien, McEwen, Gunnar, & Heim, 2009). While it is difficult to directly depend on results from animal studies because of the higher complexity of human compared to animal fetal development, these studies suggest a possible connection between maternal stress and child well-being (DiPietro, 2004).

In the first few months of a child's life there is rapid brain maturation and development. Moreover, the first year of post birth life is the period when the brain's HPA axis is the most labile to external environmental influences (Lupien, McEwen, Gunnar, & Heim, 2009). Because the brain is responsible for receiving and transmitting stressors as well as the stress response, early exposure to high levels of gluccocorticoids can have irreparably damaging effects on infant and child development (Lupien, McEwen, Gunnar, & Heim, 2009). In a study conducted by Lupien et al. maternal stress during pregnancy led to increased HPA axis activity at 6 months, 5 years, and 10 years of age. While this increase in HPA activity has been shown to increase incidences in anxiety, depression, and poor cognitive functioning it is difficult to determine any nutritional implications (Lupien, McEwen, Gunnar, & Heim, 2009).

Some of the most supportive evidence regarding child nutrition status is that stress, depression, and anxiety as well as glucocorticoid therapy in pregnant mothers all result in lower birth weight and length for infants (Lupien, McEwen, Gunnar, & Heim, 2009). Less clinical

findings suggest that family dynamics play an important role in the stress level of mothers and the nutritional condition of their offspring. Due to financial strains, single parents often experience more stress both at the work-place as well as within the home. Of these single parent homes, those managed by females typically make less money, and thus endure greater amounts of stress (Gable & Lutz, 2000). Due to these increased home and work demands, parents often pay less attention to their children's nutritional as well as physical activity needs or are simply unable to adequately provide the proper diet for their family, and affected family members often experience unhealthy weight gain that can lead to obesity (Gable & Lutz, 2000). Similarly, parents who provide nutritious options to their children on a frequent basis, are more likely to promote a healthy weight for their family members (Gable & Lutz, 2000). Thus, parents who are unable to provide more healthy options may be more likely to have overweight or obese children

Clearly, poverty can lead to excess stress and can in turn affect the nutritional status of each family member including children. Not only can an infant's weight and height be affected by maternal stress, but also, a child's hemoglobin status. Early studies regarding family dynamics and health reported that income status was related to hemoglobin levels as well as other nutritional factors (Patterson & Albers, 2001). Specifically, one study reported that children living in impoverished or low-income households for 10 years or more, have more pronounced nutritional disparities, however, the research on this topic is inconclusive and requires more study (Patterson & Albers, 2001).

The specific question raised in this paper is whether or not there is any connection between maternal salivary cortisol levels and child nutritional status as measured by hemoglobin, height, and weight. Hemoglobin (Hb) accounts for about 65% of the protein component of red blood cells and is responsible for respiratory functions, specifically, carrying oxygen throughout

the body to organs while transferring carbon dioxide back to the lungs (World Health Organization, 2001). Dietary iron is necessary for Hb synthesis, thus a deficiency in dietary iron can result in inadequate Hb levels, which can then lead to anemia (Ramakrishnan, 2001). Not only can inadequate consumption of dietary iron lead to a decrease in Hb synthesis but also inadequate vitamin A intake can lead to a decrease in hemoglobin synthesis as well. Although more research may be needed on this topic, riboflavin (vitamin B2) has also shown to impact iron metabolism and in turn, Hb production (Ramakrishnan, 2001).

In adults, iron deficiency anemia can manifest in several symptoms such as fatigue, lack of energy, paleness, decreased performance in exercise and work activities, decreased immune functioning, irregular thermoregulation, pica, angular stomatitis, glossitis, and esophageal webbing (Ramakrishnan, 2001). In underdeveloped countries, approximately 50% of women capable of reproduction and 70% pregnant women experience iron deficiency anemia (Ramakrishnan, 2001). A deficiency in maternal iron status often results in a depletion of infant iron reserves, and mothers suffering from iron deficiency anemia are more likely to have infants with decreased red blood cell volume and total hemoglobin mass (World Health Organizations, 2001). Healthy infants typically have proper levels of iron stores that endure for roughly 6 months post-birth (Ramakrishnan, 2001). Infants must rely on these stores as well as their dietary intake to provide enough iron to meet their nutritional needs. While breast milk does not contain a large amount of iron, it has good bioavailability and, in conjunction with the infant's liver stores, is able to sustain an infant for around 6 months (Brown et al., 2011). Thus, the mother's iron status pre-pregnancy as well as during gestation clearly has an early impact on her child's iron levels.

Infancy and early childhood are critical times in which children most frequently experience iron deficiency and require a sufficient supply of dietary iron due to the rapid growth and low total iron content experienced during this developmental period (Filer, 1989). As infants grow, their blood volume as well as red blood cell mass increase and require an increased level of iron intake to maintain adequate hemoglobin levels (Filer, 1989). This increased need for iron consumption begins around 4-5 months post-birth and is greatly increased for premature infants (Filer, 1989). There have been suggestions from several research studies that infants experiencing iron deficiency may initially gain weight, due to a demand for increased dietary iron, but then lose weight as their iron deficiency anemia progresses (Filer, 1989). Many studies have been conducted regarding the connection, if any, between iron deficiency anemia in infants and young children and behavioral and mental abnormalities.

In one double-blind intervention trial conducted by Lozoff et al. children aged 12-23 months with iron deficiency anemia scored significantly lower on the Infant Behavior Record (IBR) test as well as the Mental Development Index (MDI) than non-iron deficient children. Similarly, in a long-term observational study conducted by Palti, Meijer, and Adler, children from birth to age 13 were more likely to experience iron deficiency later in life than infants and children who did not experience iron deficiency at a young age (Palti, Meijer, & Adler, 1985). Also, researchers found that though the mother's level of education as well as the child's sex were contributing factors, children with iron deficiency anemia had decreased educational achievement and lower scores for positive task orientation (Palti, Meijer, & Adler, 1985). Similarly, results from the Tennessee WIC from 1975-1984 showed that children categorized as living in low SES subpopulation households had a higher prevalence of anemia than children living in high SES environments (Filer, 1989).

While there clearly is an impact on both physical (birth weight and growth) as well as mental and behavioral development, the question still remains whether or not the maternal stress level affects child hemoglobin status and subsequently presence of iron deficiency anemia.

Because mothers are often responsible for providing meals for their children, their nutrition-related decisions as well as their financial ability to provide for their family greatly affect the health and nutritional status of their children. For example, if a mother is not educated about the importance of certain nutrients (i.e. iron for hemoglobin synthesis), she probably will not perceive their importance in the diet, and therefore not purchase and feed these particular foods to her children (Variyam, Blaylock, Lin, Ralston, & Smallwood, 1999). Similarly, mothers' food choices and cooking behaviors were influenced by their nutritional knowledge and overall level of education (Variyam, Blaylock, Lin, Ralston, & Smallwood, 1999). Also, the mother's physical financial ability to provide quality foods for her child/children can be threatened by low socioeconomic status and an impoverished environment (Chen 2004). Heme-iron is the form of iron that is most available for absorption by the body. However, foods that contain heme-iron are derived from animal sources such as liver, poultry, beef, oyster, and clams, and may be more expensive to purchase than non-heme iron sources. Financial insecurity may limit the ability for mothers to purchase these food sources of highly bioavailable iron. Children raised in impoverished homes were over three times more likely to suffer from iron deficiency, compared to children in financially stable homes (Seccombe, 2000).

Clearly there are many factors that influence the nutritional status of infants and young children. Maternal background, health, behavior, and diet remain some of the strongest determinants in the nutrition-related outcomes of the infant due to the uniquely intimate bond shared between mother and child from conception through postnatal development. While the

effect of these maternal factors on child nutrition has been the topic of many previous research studies, this paper focuses more specifically on the relationship between maternal stress and the nutritional status of her offspring, specifically the weight, height, and hemoglobin status. The main objective of this thesis is to determine the role that maternal stress, as measured through salivary cortisol concentrations, has of the child's overall nutrition status during infancy.

The potential implication of this work is that it may provide further evidence suggesting a direct association between maternal stress levels and child nutrition status. Overall, the central hypothesis of this study is that mothers with higher salivary cortisol levels are more likely to experience more stress and, as a result, their children are more likely to experience atypical height, weight, and hemoglobin statuses.

2. SUBJECTS AND METHODS

Setting

This study was conducted in Uruguay's capital city, Montevideo, located in the southern region of the country. Information was collected at the Our Lady of Fatima Parish in the Catholic University Faculty of Psychology Practicum Center located in the Cerro neighborhood in Montevideo, Uruguay. Previous studies report anemia, speculatively due to iron deficiency, among the population's children.

Participant Recruitment

The two-step recruitment phase began with a total of 244 children and mothers who previously took part in a blood screening of anemia and lead levels. Parents of the children were then re-contacted through letters requesting their return to the Practicum Center for the current study and phone calls were later made in an attempt to re-contact the mothers as well. Multiple mothers refrained from participation due to disinterest in the current study, transportation problems, relocation of the family, and disconnection of the family phone. In the second phase of recruitment, directors from six surrounding preschools were contacted via telephone, and meetings were arranged for those directors who were interested in the study. The details of the study were further explained, and with the directors' permission, educational letters explaining the study were delivered to parents of the preschool children. Additional meetings were conducted at the preschools to provide information to parents in a face-to-face setting. New participants were enrolled from five surrounding preschools. All five schools are located in the Cerro neighborhood and within close proximity of Our Lady of Fatima Parish. Children from the

age of 12-36 months were signed up for the current study. Appointments were later arranged at Our Lady of Fatima Parish for parents who agreed to participate.

Sample

Originally, 124 children and mother pairs were enrolled in the study. A total of 15 sibling pairs were evaluated as part of the original sample, however only the youngest of each pair was ultimately included in the current study. Thus, the study consisted of a total of 109 children (age 20-43 months) and their mothers.

Ethical Concerns

Both the Office of Research Protections at the Pennsylvania State University and the Ethics Committee at the Catholic University of Uruguay approved the study. During the first visit to the Center the consent form was clearly and thoroughly read to each of the mothers prior to the beginning of the study. The leading psychologist and/or pediatrician were responsible for reading the consent form and answering any questions asked during the session. At the conclusion of the meeting parents signed the form and maintained a copy for themselves while returning the original to the coordinator.

Procedure

The procedure consisted of two different assessment meetings both of which were conducted at the Practicum Center. During the first visit anthropometric measurements were taken, biological samples were collected, mother-child play interactions were recorded, and demographic questionnaires were administered. Both visits required from 1.5 to 2 hours and an

additional assessment period was arranged if the original sessions were not finished. Reasons for the scheduling of an additional session included limited availability of the Practicum Center and/or child restlessness. Due to the 2-day saliva collection procedure, the two sessions were arranged about one week apart. Prior to the saliva collection, mothers were informed and shown the correct way to collect saliva samples. Parents were compensated for their time and participation through the payment of both their child and their own transportation expense, while children were given a toy.

Assessments

Anthropometry:

Standard anthropometric procedures were used for weight and length measurements of the children. A single nurse was responsible for performing all weight measurements in triplicate, and children wore just their underwear or clean, unsoiled diapers. Two separate scales were used for weighing the children. For older participants a children/adults scale was used while younger children used a Seca Infant Scale (Seca 872, Shorr Productions, Olney, MD). Weight was documented in kilograms to 1 decimal place.

With regards to length/height measurements, children who were less than 24 months old were assessed based upon their horizontal length and children older than 24 months of age were assessed while standing. The height board used was custom-made for this particular study and was constructed with a firm base with side and backboards. Height was assessed through the use of a tape measure as well as a movable head piece that were connected to the backboard. The board's structure followed the World Health Organization's arrangement of standard height

boards, and height was documented in centimeters to 1 decimal place. The average for heights and weights was calculated.

Biochemical

Non-fasting blood samples from both the mothers and the children were taken between the hours of 8am-11am. A 25-guage blood collection set with a butterfly needle was used to collect blood from child participants (Vactainer, Becton Dickinson, Franklin Lakes, NJ). A lithium herapin tube was used to collect whole blood for lead level testing (Vacutest Plast, Italy). Hemoglobin in blood was assessed directly after sampling with a HemoCue 201+ portable hemoglobinometer (HemoCue, Lake Forest, CA). The blood tube remained on ice until it could be stored at -20 degrees Celsius at the Department of Toxicology, University of the Republic.

Mothers were given small bags of eight 2ml tubes and eight small, white straws to collect their own saliva over a 2 day period (Salicaps, IBL-Transatlantic Corp., Toronto, ON). Instructions were also provided with the tubes and straws to aid the mothers in their saliva collections. Women were advised to think of their favorite foods and/or move their jaws in a manner similar to chewing. Women were instructed to fill the tube at least half way and store the saliva in a refrigerator or freezer until they could take the samples back to the Practicum Center. Four saliva samples were to be collected on each of the 2 days, however, women who were ill were instructed not to provide samples. The first sample was collected immediately after waking, but before getting out of bed. The women were instructed to collect the second sample 30 minutes after the initial sample was collected. The next saliva collection was just before lunch time, from 11am-12pm, and the fourth and final sample was to be collected before eating dinner from 4pm-6pm.

Collection logs were included in the bags issued for saliva collection for women to keep track of their collection times, when they awoke and went to bed, how many times they awoke throughout the night, how many hours they slept, and when they ate their meals. During their second visit to the Practicum Center mothers were instructed to turn in their saliva samples. Maternal samples were kept on ice and then transported to the laboratory.

Cognitive/Psychological

Psychologists and advanced psychology students were in charge of conducting the cognitive assessment portion of the study. Training sessions were administered to researchers and a pilot study was conducted with a similar population prior to the commencement of the current study. Symptoms of depression in the mothers were evaluated using the Beck Depression Inventory II (BDI II). Twenty-one items were used to assess sadness, pessimism, feelings of failure and guilt, self-criticism, and suicidal thoughts. The Parenting Stress Index, 3rd Edition (PSI) was used to assess stress levels in the mothers. The test consisted of thirty-six categories where the mothers were asked to select responses ranging from “strongly agree” to “strongly disagree.” Maternal IQ was assessed using the Wechsler Adult Intelligence Scale III (WAIS III) where subcategories including similarities, arithmetic, vocabulary, block design, and object assembly were evaluated.

Laboratory Analysis of Saliva Samples

The laboratory analysis took place at the Pennsylvania State University. Salimetrics High Sensitivity Salivary Cortisol Enzyme Immunoassay Kit was used for the assessment of cortisol levels in each sample of maternal saliva. The level of cortisol measured indicates the amount of

serum cortisol in circulation. A microtitre plate coated with monoclonal antibodies to cortisol was used during the assessment. The cortisol that is present in the provided standards and unknowns competes for antibody binding sites with the cortisol that is coupled to horseradish peroxidase (HRP). Following incubation, unbound cortisol is washed away and the bound cortisol can be measured through the reaction between the peroxidase enzyme and the substrate tetramethylbenzidine (TMB). The reaction between these two compounds produces a blue color and a yellow color will be produced after sulfuric acid is added to stop the reaction. The intensity of the blue color observed is a measurement of the amount of cortisol peroxidase present and is inversely proportional to the amount of cortisol present.

Thirteen kits total were used to complete the analysis of all saliva samples from the 60 mothers. All kits were kept at 2-8 degrees Celsius in the laboratory's refrigerator until use.

Materials supplied in each kit included: one 96-well microtitre plate coated with monoclonal anti-cortisol antibodies, six vials of cortisol samples (3.0, 1.0, 0.333, 0.111, 0.037, 0.012 µg/dL) 500µL each, two vials of cortisol controls (high and low, 500µL each) and both containing cortisol, buffer, and preservative, one 100mL bottle of wash buffer concentrate (10x) containing phosphate buffer, detergent, and preservative, one 60mL bottle of assay diluent containing phosphate buffer, pH indicator, and preservative, one 50µL vial of cortisol enzyme conjugate containing cortisol conjugated to HRP, and preservative, one 25mL bottle of TMB substrate solution, one 12.5mL 3 M stop solution containing sulfuric acid, and one strip of non-specific binding (NSB) wells which do not contain anti-cortisol antibody.

Materials that were needed but were not supplied in the Salimetrics kits were precision pipette to deliver 15 and 25 µL, and precision multichannel pipette to deliver 50µL and 200µL, a vortex, a plate reader with 450nm filter, computer software for data reduction, deionized water,

reagent reservoirs, one disposable tube per kit for holding 24mL for the assay diluent, pipette tips, and a serological pipette to deliver 24mL.

Saliva samples were kept in the laboratory's freezer and were frozen at -20 degrees Celsius until their use. Each saliva sample was run in duplicate thus, each 96-well plate provided could be used to analyze 38 samples taking into account wells for controls, standards, and conjugate. On the day of the assay, 38 samples were removed from the freezer and thawed in the laboratory's refrigerator for approximately one hour. After an hour of thawing the samples were placed on the laboratory bench and brought to room temperature. At the same time one kit containing a microtitre plate, cortisol standards, cortisol controls, wash buffer concentrate, assay diluent, cortisol enzyme conjugate, TMB substrate solution, stop solution, and NSB was removed from the refrigerator and allowed to thaw to room temperature on the laboratory bench. The 38 samples were centrifuged at 1500 x g (@ 3000 rpm) for 15 minutes to evenly distribute the precipitated mucins.

Prior to beginning the assay, the 10x wash buffer was diluted by 10-fold with room-temperature deionized water (100mL wash buffer to 900mL of deionized water). The microtitre plate was prepared prior to the beginning of the assay as the regular wells in H-1,2 were replaced with 2 NSB wells. The extra NSB wells were placed back into their foil packaging and stored in the refrigerator for future assays.

25 µL of each standard were pipetted from the highest to the lowest cortisol concentration in wells A-1 through F-1. The standards were then duplicated into wells A-2 through F-2. The high control was pipetted in wells A-3 and A-4 and the high control was pipetted into wells B-3 and B-4. The 38 unknown saliva samples were pipetted in duplicate from wells C-3 and C-4 through wells H-11 through H-12. 25µL of the assay diluent were pipetted

into well G-1 and G-2 to serve as zero wells. 25µL of the assay diluent were also pipetted into the NSB wells (H-1 and H-2). Controls, standards, and saliva samples were all pipetted within 20 minutes or less to ensure the highest quality assay results. After all samples had been pipetted, 24mL of the assay diluent was pipetted into a disposable tube. A 1:1600 dilution of the conjugate was prepared by adding 15µL of the conjugate to the 24mL disposable tube containing the assay diluent. The diluent and conjugate were immediately mixed in the disposable tube and 200µL of the diluted conjugate solution was dispensed into each of the 96 wells using a multichannel pipette.

The plate was placed onto a rotator for 5 minutes at 500rpm and then incubated at room temperature for an additional 55 minutes.

After an hour of incubation the plate was washed four times with 1X wash buffer: 300 µL of wash buffer was pipetted into each well and the liquid was then discarded by inverting the plate over the sink. After each wash, the plate was blotted on paper towels before being turned upright.

200 µL of the TMB solution was added to each well using a multichannel pipette. The solution was then mixed on a plate rotator for 5 minutes at 500 rpm and then incubated in a dark section of the laboratory for an additional 25 minutes. 50 µL of the stop solution was added after incubation using a multichannel pipette. The plate was then gently tapped for about 3 minutes to mix the solution. A plate cloth was used to wipe the bottom of the plate dry and the plate was then inserted into reader and read for optical density at 450nm.

Excel was used to enter the cortisol concentrations for each mother. Each of the two optical densities were entered and used to calculate the average optical densities of each sample as well as the average optical density of the NSB wells. The log of the known standard

concentrations was also calculated. The calculated concentration of cortisol in each collection was derived from dividing the corrected average optical density by the average optical density of the NSB well. The coefficient of variance was also calculated to compare differences between the replicated samples and to ensure precision.

Statistical Sample

57 women provided saliva samples. Of those, one was excluded from analysis because her wake-up and collection times for both days were reported at evening or nighttime hours, which differed from the instructions and the rest of the sample (Figure 2).

Figure 2. Model for the calculation of area under the curve.

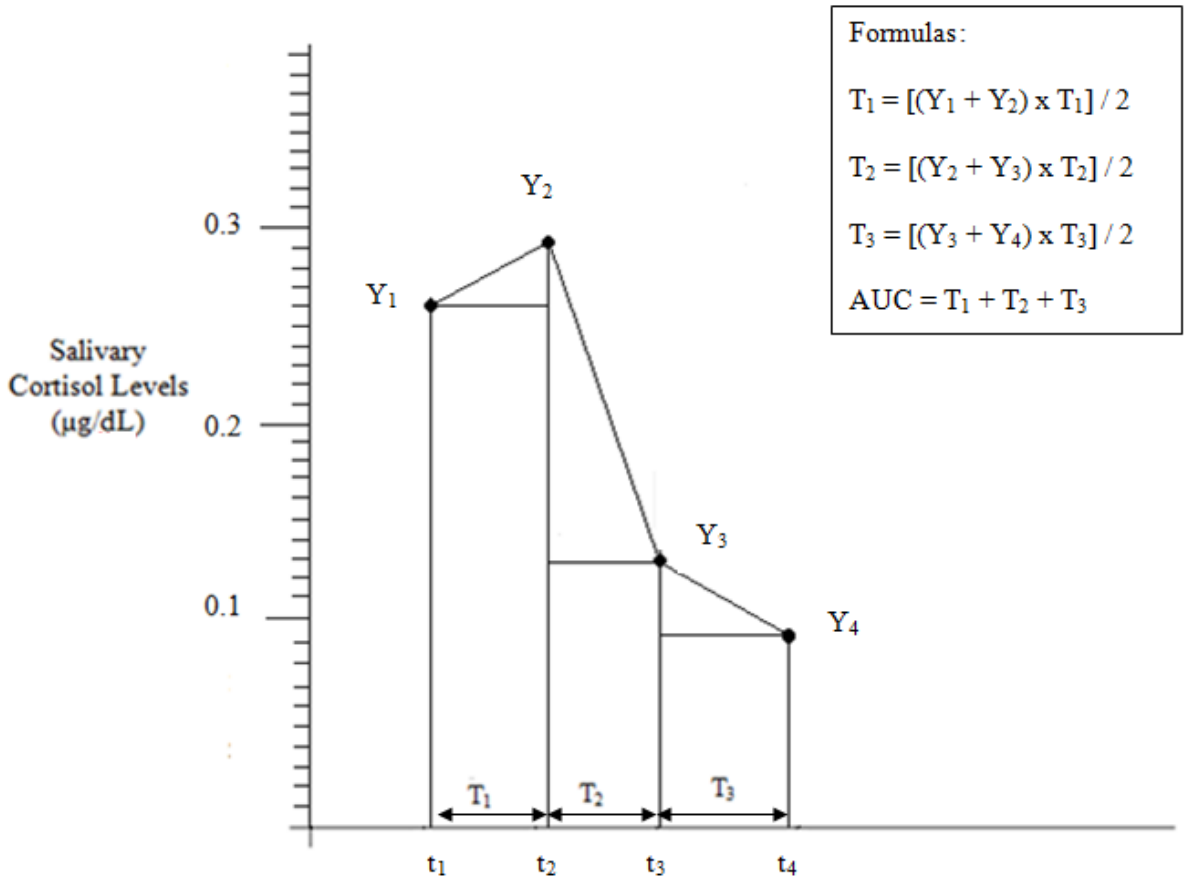


Figure 2. This figure represents the AUC calculations that were used to determine the cortisol values for each of the individual collections provided by the 57 mothers. In the figure, Y_1-Y_4 represent the cortisol values for each mother, $t_1 – t_4$ represent each collection time, and T_1-T_3 represent the intervals between each collection time. The area of each of the three trapezoids was calculated and then summed to find the amount of cortisol each mother secreted throughout day. This formula was used again to calculate maternal cortisol levels for day two of collection.

Statistical Analysis

The objective of this study was to investigate the relationship between salivary cortisol levels in the mother and the nutritional status in their children. Three measures of nutritional status were considered: 1) hemoglobin concentration (g/dL), 2) height (cm), and 3) weight. Maternal cortisol levels, or more specifically, area under the curve (AUC) of the four daily measures averaged over the two days of collection, was the main predictor variable.

Area under the curve (AUC) was calculated for each day based on the four cortisol values obtained for each woman and the time interval between successive saliva collection (Figure 3). In a questionnaire accompanying the saliva collection kit the woman was asked to note down the time (t_i) at which she collected her sample. These times were used to calculate the interval between collections (T_1 for the interval between 1st and 2nd collection; T_2 for the interval between the 2nd and 3rd collection; T_3 for the interval between 3rd and 4th collection). Mean interval, $T_{1\text{-}3}$ was calculated based on existing non-missing observations. When a time t_i was missing or when the time provided was implausible (for example, t_2= 11:15am whereas t_1= 11:45am) and T_i could not be calculated, the mean sample T_i was used to substitute for the missing values.

Successive cortisol values, (Y_i) for each woman were added following the formula, (Figure 2):

$$T_1 = [(Y_1 + Y_2) \times T_1] / 2$$

$$T_2 = [(Y_2 + Y_3) \times T_2] / 2$$

$$T_3 = [(Y_3 + Y_4) \times T_3] / 2$$

In cases where a successive cortisol value was missing, the next value was used to calculate the area of the trapezoid, along with the summed timed intervals between the first and the

subsequent collection (for example, if Y_2 was missing, Y_1 and Y_3 were added and T_1 and T_2 were added). Finally, area under the curve was calculated by summing the three trapezoids,

$$AUC = T_1 + T_2 + T_3$$

to attain the total amount of cortisol produced by the women during the time between the first and last saliva collection (approximately 11 hours).

This process was carried out for each of the two saliva collection days, yielding AUC1 (n= 53) and AUC2 (n= 52). Because the two values were correlated [correlation value (0.3087) and p-value (0.0309)], they were averaged (AUC_G). When only one value was available, an average could not be calculated and the existing value was used for AUC_G.

In a preliminary analysis, women who provided saliva samples were compared to women who did not provide samples on demographic, cognitive/emotional, and household characteristics using two-sided t-tests and chi^2 tests.

Regression models were constructed to investigate the association of maternal cortisol values (as AUC_G) and child nutrition status. First, (Model 1) simple linear and logistic regressions were run with AUC_G as the independent variable and child nutritional status as the dependent variables (each nutritional status measure in a separate model). Subsequently, models were covariate-adjusted (Model 2).

Variables were chosen as potential covariates based on previous studies of child nutritional status or maternal stress. These potential covariates included variables on the child: age (months), sex, whether the child lived with one or both parents; the mother: age (years), years of schooling, maternal IQ score, BDI score, PSI TS score, employment status (mother works vs. does not work); and the family: parents' marital status, SES score, housing occupant density (number of occupants/total rooms), and HOME Inventory score. To select a covariate for

inclusion in multivariate models, each potential covariate was entered into a simple regression with AUC_G or each outcome variable, and was included in the multivariate model if the achieved p-value was <0.20. The final covariates were child age, occupant density, SES score, moderate/severe depressive symptoms in the mother, maternal employment status, and maternal age.

3. RESULTS

Of the 109 mothers included in the study sample, 57 provided saliva samples. One woman was excluded due to inaccurate self-reporting of saliva collection times (Figure 3).

Differences between the mothers who provided saliva samples and mothers who did not provide saliva samples were compared to assess any population biases between the two groups (Table 1). Overall, five characteristics significantly differed between the two groups of women. The highest statistical significance in difference between the two groups was observed in PSI, dysfunctional child interaction and PSI, total score where scores were higher for both measures in the group of mothers who provided samples. A statistical difference was also observed in PSI, parenting distress, where mothers who provided samples scored higher than mothers who did not provide samples. Child hemoglobin levels as well as child blood lead levels were also significantly higher in the group of women who provided saliva samples.

The reported times that the mothers recorded for their 8 total saliva collection times were evaluated for accuracy and averaged for further statistical analysis (Table 2). According to the results presented in Table 2, the mothers correctly followed the directions that were outlined in the packets given to each study participant.

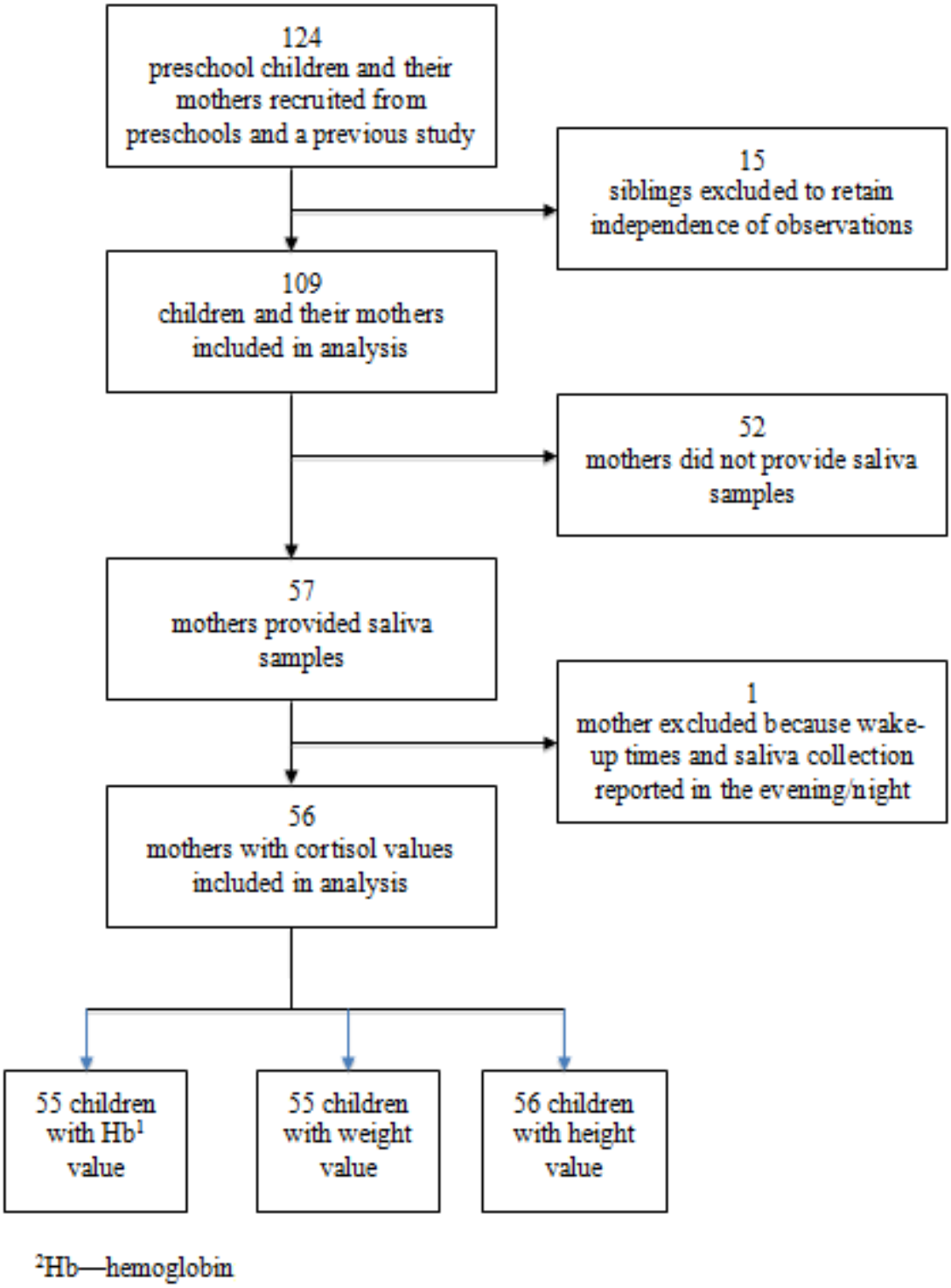
The correlation between individual cortisol values within and between saliva collection days 1 and 2 are shown in Table 3. The strongest correlation in cortisol values was seen between consecutive saliva collections taken within the same day. This is shown by the relatively high correlation coefficients between collection 1 and collection 2 of day 1 (0.5068), collection 2 and collection 3 on day 1 (0.2620), and collection 3 and collection 4 on day 1 (0.3993). Correlation coefficients between consecutive saliva collections on day 2 maintain a similar pattern of moderate-to-high correlation. High correlations between the collections were also observed in

the same collection times between days 1 and 2. For example, the correlation between collection 1, day 1 and collection 1, day 2 is 0.4951, the correlation between collection 2, day 1 and collection 2, day 2 is 0.2653, the correlation between collection 3, day 1 and collection 3, day 2 is 0.4835, and the correlation between collection 4, day 1 and collection 4, day 2 is 0.5459.

The collection times that the mothers reported as well as the levels of cortisol calculated for each maternal saliva sample were used to determine the mean AUC in $(\mu g/dL)^2$ for day 1 and day 2 of the collection period. Table 4 shows the maternal cortisol measures in µg/dL with the mean and standard deviations, median, and range for each of the four collection times for both day 1 and 2. The four reported collection times for day 1 and day 2, respectively, were used to calculate the average time lapse between the first collection and each consecutive collection. This calculated time lapse, along with the mean cortisol calculations were used to determine the average AUC for day 1 $(96.16 \pm 72.01\ \mu g/dL)^2$ and day 2 $(98.74 \pm 84.05\ \mu g/dL)^2$. The table shows that the mean, standard deviations, and median cortisol values for the same collection times during day 1 and day 2 were similar. Also, the total AUC for day 1 and day 2 were comparable.

Associations between maternal salivary cortisol values and child nutrition status were assessed to measure the effect of maternal stress on child anthropometric and hemoglobin values (Table 5). A statistical association was seen between child age and hemoglobin, child age and weight, and child age and height. There was no statistical association between maternal salivary cortisol levels and child hemoglobin, height, or weight status.

Figure 3. Selection of study participants.



[2]Hb—hemoglobin

Table 1. Characteristics of women who did and did not provide a saliva sample.

Characteristic	Did woman provide saliva sample?			
	N	No	N	Yes
Maternal Age (y)	50	28 ± 7.2	56	29.1 ± 7.5
Maternal IQ (points)	33	80.9 ± 17.3[1]	53	80.6 ± 11.8
Maternal schooling (y)	50	8.7 ± 3.2	56	8.8 ± 3.0
Number of siblings	38	2.1 ± 1.9	41	2.3 ± 2.2
Child's birth order	40	2.4 ± 1.8	45	2.7 ± 1.9
Child's age (mo)	53	30.0 ± 9.1	56	32.5 ± 10.2
Beck Depression Inventory score	45	16.2 ± 11.8	54	15.8 ± 10.8
Spousal support score (house)	43	16.9 ± 5.2	50	17.8 ± 3.5
Spousal support (money)	40	5.8 ± 2.7	47	5.8 ± 2.5
PSI, parenting distress	45	70.0 ± 24.7	53	81.1 ± 19.6*
PSI, difficult child	45	79.4 ± 15.6	53	80.8 ±14.8
PSI, dysfunctional child interaction	45	58.2 ± 28.5	53	75.0 ± 19.5**
PSI, total score	45	76.7 ± 17.7	53	86.6 ± 11.7**
HOME Inventory score	45	9.1 ± 2.3	53	8.5 ± 2.5
Occupant density (persons/room)	49	2.0 ± 1.6	56	1.7 ± 0.8
SES score	51	5.8 ± 2.5	56	6.5 ± 2.3
Maternal hemoglobin (g/dL)	43	13.7 ± 1.5	51	13.7 ± 1.6
Child hemoglobin (g/dL)	45	11.9 ± 1.7	55	12.7 ± 1.6*
Maternal blood lead level (µg/dL)	44	5.9 ± 2.4	53	5.6 ± 2.7
Child blood lead level (µg/dL)	40	5.3 ± 2.9	51	6.7 ± 3.3*
Parents' marital status	52			
Married/living together		75.0%		76.7%
Separated/divorced		25.0%		23.2%

[1] Value given as M ± SD or %; *p<0.05, **p<0.01

Table 2. Mean saliva collection times for study women.

Day	Wake up	1st collection	2nd collection	Breakfast	3rd collection	Lunch	4th collection	Dinner
1	7:30 ± 1.07	7:44 ± 1:11	8:16 ± 1:17	8:52 ± 1:08	11:49 ± 0:32	13:10 ± 0:52	17:36 ± 1:36	20:47 ± 2:54
2	7:25 ± 1:17	7:38 ± 1:18	8:28 ± 1:48	8:55 ± 1:17	11:42 ± 0:30	13:16 ± 0:51	17:18 ± 2:08	21:16 ± 1:58

Table 3. Correlations among individual cortisol values within and between days of saliva collection.

		Day 1				Day 2			
		Col. 1	**Col. 2**	**Col. 3**	**Col. 4**	**Col. 1**	**Col. 2**	**Col. 3**	**Col. 4**
Day 1	**Col. 1**	1.0000							
	Col. 2	0.5068	1.0000						
	Col. 3	0.0597	0.2620	1.0000					
	Col. 4	0.0320	0.2323	0.3993	1.0000				
Day 2	**Col. 1**	0.4951	0.2785	-0.0208	-0.0001	1.0000			
	Col. 2	0.0980	0.2653	0.0584	-0.2129	0.1815	1.0000		
	Col. 3	0.1200	0.1862	0.4835	0.1057	-0.0179	0.1871	1.0000	
	Col. 4	-0.0496	0.1027	0.4830	0.5459	-0.1578	-0.0183	0.4109	1.0000

Table 4. Time lapse between successive collections, cortisol levels, and area under the curve (AUC).

Day	1st cortisol M ± SD (µg/dL) Median (Range)	ΔTime2-1 (min)[1]	2nd cortisol M ± SD (µg/dL) Median (Range)	ΔTime3-2 (min)	3rd cortisol M ± SD (µg/dL) Median (Range)	ΔTime4-3 (min)	4th cortisol M ± SD (µg/dL) Median (Range)	AUC (µg/dL)[2]
1	0.26 ± 0.27 0.17 (0.02 – 1.42)	37 ± 28	0.29 ± 0.25 0.25 (0.02 – 0.97)	216 ± 57	0.13 ± 0.13 0.09 (0.01 – 0.72)	346 ± 60	0.09 ± 0.14 0.04 (0.01 – 0.69)	96.16 ± 72.01 75.85 (9.59 – 352.39)
2	0.25 ± 0.19 0.19 (0.01 – 0.98)	35 ± 13	0.30 ± 0.23 0.27 (0.02 – 0.96)	210 ± 49	0.14 ± 0.22 0.06 (0.01 – 1.08)	347 ± 47	0.09 ± 0.11 0.04 (0.01 – 0.56)	98.74 ± 84.05 70.66 (8.40 – 469.29)

[1]ΔTime—Lapse of time between successive saliva collections. For example Time2-1 refers to number of minutes between first and second collection; [2]AUC—Area under the curve, represents the total amount of cortisol secreted by the woman between the first and the last saliva collection time (approximately 10 hours).

Table 5. Associations of maternal salivary cortisol levels with children's nutritional status.

Statistical model	**Hemoglobin (g/dL)**	**Weight (kg)**	**Height (m)**
Model 1			
Salivary cortisol AUC	-0.001 ± 0.004	0.003 ± 0.006	0.0001 ± 0.0002
	48	48	49
Model 2			
Child age (mo)	0.06 ± 0.02*	0.12 ± 0.03**	0.006 ± 0.001**
Occupant density	-0.16 ± 0.33	0.40 ± 0.43	0.01 ± 0.01
SES score	-0.06 ± 0.11	0.14 ± 0.14	0.002 ± 0.003
BDI score > 19	-0.51 ± 0.55	-0.88 ± 0.70	-0.01 ± 0.02
Mother works	-0.84 ± 0.50	1.18 ± 0.64	0.02 ± 0.01
Maternal age (y)	0.002 ± 0.03	0.05 ± 0.04	0.001 ± 0.001
Salivary cortisol AUC	-0.003 ± 0.004	-0.01 ± 0.005	-0.0002 ± 0.0001

AUC—Area under the curve; Values given as β ± SE from a linear regression model*$p<0.05$, **$p<0.01$.

4. DISCUSSION

Overall, the results for this study do not support an association between maternal stress levels and child nutrition status. No positive association was observed between maternal salivary cortisol levels and either child height, child weight, or child hemoglobin status. The literature to date shows that normal ranges for adult salivary cortisol level for women aged 21-30 years is 0.112-0.743 µg/dL and 0.094-1.515 µg/dL for women between 31-50 years of age (Aardal & Holm, 1995). The averages presented in the current study fall within these established limits, suggesting that the subjects overall had normal cortisol levels. Although this current study does not show any association between maternal stress and child nutrition status, several other studies indicate that maternal stress influences child development, suggesting that maternal cortisol levels do have an impact on normal infant and child overall health and possibly nutrition.

Though there is a great deal of published research that examines the relationship between mothers and their children, the complex mechanisms of stress and the stress response, as well as the consequences of chronic stress on overall human health, the literature lacks studies that specifically analyze the effect of elevated maternal cortisol on child nutritional status. A great deal of the studies presented examine the effect that maternal dietary and behavioral factors on infant and child cortisol levels. Thus, infant cortisol levels are often presented as the outcome measure instead of the various nutritional outcomes that were measured in the current study. Additionally, the existing literature is mainly based upon studies that examine the relationship between maternal factors and fetal growth outcomes in utero rather than the infant or child nutritional status that the current study analyzed. Due a scarcity of directly comparable studies in the literature, studies that analyzed the relationship between maternal and fetal/child cortisol levels and developmental outcomes were reviewed and included in this discussion.

The period of fetal development is a vulnerable stage subject to a great level of change in fetal tissues and organs (Davis, Glynn, Waffarn, & Sandman, 2011). Major programming factors influencing fetal development include maternal health and functioning (Davis, Glynn, Waffarn, & Sandman, 2011). The HPA axis remains the main mechanism explaining the influence of maternal stress on fetal and infant development. Cortisol is able to travel through the placenta and, thus, the level of circulating maternal cortisol is directly correlated to fetal cortisol concentrations (Davis, Glynn, Waffarn, & Sandman, 2011). The fetal nervous system is then subject to the effects of excessive maternal HPA stimulation and cortisol secretion, resulting in poor stress response regulation (Davis, Glynn, Waffarn, & Sandman, 2011).

Research regarding the relationship between child cortisol levels and poor nutritional outcomes have been conducted. Several studies have demonstrated that increased amounts of maternal cortisol and reported stress have resulted in behaviors such as increased fear and reactivity in children, due to increased fetal HPA axis activity. Also, additional behavioral, physiological, psychological, and nutritional impacts that maternal stress may have on child health and development have been reported (Davis, Glynn, Waffarn, & Sandman, 2011). One such physiological impact of increased fetal HPA axis stimulation in children is increased fat mass (Dimitriou, Maser-Gluth, & Remer, 2003). In one cross-sectional study, urinary cortisol samples were examined in three different groups of children (aged 4-5 years, 8-9 years, and 13-14 years) and analyzed for a possible association with BMI, fat mass, and body fat percentage of the children. Results from the study showed that increased presence of glucocorticoid metabolites (a reflection of overall daily cortisol secretion) were positively associated with increased values for all three measures of body fat (Dimitriou, Maser-Gluth, & Remer, 2003). Similarly, higher salivary cortisol concentrations in children 8-10 years of age were associated

with higher incidence of growth stunting and cardiovascular response to stressors in Jamaican children (Fernald & Grantham-McGregor, 1998). Based upon the results brought forth by previous studies, an association between maternal stress and child development, and possibly nutritional status remains possible. Unfortunately, most of the literature focuses on how the mother's health and nutrient intake affects fetal, infant, and child stress levels, thus the connection between the literature and the current study is distant and indirect.

The unique connection between the mother and infant in relation to stress and fetal development is strongly supported by a study conducted by Stenius, Theorell, Lilja, Scheynius, Alm, and Lindbald. The study assessed the correlation between cortisol levels in the mother and child compared to the correlation between cortisol levels in the father and child. The results showed that the association between maternal and infant cortisol levels were far greater than the association between father and infant cortisol levels, thus supporting the greater biological connection that infants experience with their biological mothers (Stenius, Theorell, Lilja, Scheynius, Alm, & Lindbald, 2008).

The connection between low socioeconomic status and maternal stress has already been introduced and discussed extensively in this paper. More evidence supporting this connection between income, financial level, and career status is supported by the direct relationship between economic stress and vitamin A deficiency (Keith, West, & Mehra, 2009). Keith, West, and Mehra, observed an association between strained economic conditions brought on by acute financial crisis as well as chronic poverty and vitamin A deficiency. The paper reinforces the importance of the mother as the primary caretaker, and how a low education level and financial privation can lead to a lack of knowledge and/or monetary means to purchase high quality food sources of this specific micronutrient (Keith, West, & Mehra, 2009).

Further evidence supporting the unique biological relationship between mother and child is presented in a study conducted by Davis, Glynn, Waffarn, and Sandman where the potential role of maternal stress in fetal programming was examined in a population of 116 mother-infant pairs. Maternal cortisol levels and psychological stress were assessed via serum samples and questionnaires, respectively. Infant salivary cortisol level as well as infant responses to stressful situations were also assessed. Overall, the results of this study show that maternal cortisol levels can influence fetal programming by influencing the fetal stress regulatory system through cortisol's action on the CNS. Also, the study showed that increased maternal psychosocial stress increases the probability of a slower recovery from stress in the child (Davis, Glynn, Waffarn, & Sandman, 2011).

It has been shown that gestational maternal malnutrition as well as excessive maternal psychosocial stress often contribute to suboptimal birth outcomes (Van Dijk, Van Eijsden, Stronks, Gemke, & Vrijkotte, 2011). And though there is limited evidence supporting lifelong neurological deficits, some research suggests that maternal cortisol levels during pregnancy may mediate fetal brain growth and development (Li et al., 2012). One study conducted by Li, Wang, Chen, Dong, Shuai, Xiao, Reichetzeder, and Hocher measured the effect of maternal blood serum salivary cortisol measures on fetal brain development and infant blood cortisol concentrations in 423 mother-infant pairs (Li et al., 2012). Maternal blood serum cortisol samples were taken in the hospital just before delivery, child blood samples were collected approximately ten minutes after birth, and child brain development was assessed through ultrasound technology during early, middle, and late pregnancy. The results for this study showed that while there was no significant association between maternal cortisol levels and birth weight there was a significant negative association between maternal cortisol and child

abdominal and head circumference, femur length, and abdominal and pectoral diameter in early, middle, and late stages of pregnancy (Li, et al., 2012). Though this study similarly supports the null association between maternal cortisol levels and child weight status presented in the current study, the authors present a significant association that maternal cortisol may have with other parameters of child growth and overall health.

In another study, differences between male and female children in relation to adiposity levels and maternal cortisol levels were analyzed in 6,735 mother and child pairs. The mothers' blood samples were taken during pregnancy and the children were followed five years post birth (Van Dijk, Van Eijsden, Stronks, Gemke, & Vrijkotte, 2011). The study assessed the influence that maternal stress, measured through job strain questionnaires and serum cortisol samples, influenced child anthropometric outcomes (height, weight, BMI, waist circumference, waist-to-height-ratio (WHtR), and fat mass index (FMI)) five years after birth. While the results showed no association between maternal job strain and child BMI, FMI, or WHtR, there was a significant association between higher maternal cortisol levels and increased FMI in female children only (Van Dijk, Van Eijsden, Stronks, Gemke, & Vrijkotte, 2011). The results of this study suggest that a more longitudinal analysis of the current study may be necessary to observe positive associations between maternal stress and child nutritional status.

An interesting role that maternal nutrition may play in the development of the stress response in children has been proposed. Mead (2007) explains how the maternal diet during pregnancy can influence the infant's response to stress in the future. The basis for this assertion lies in the discovery that a higher maternal consumption of fish and meat results in higher fetal fasting plasma cortisol concentrations (Mead, 2007). According to the article, adult children whose salivary cortisol concentrations were measured after completing stressful tasks (ie: public

speaking and mental math problems) increased in proportion to the amount of meat and fish that their mothers consumed during the final trimester of pregnancy (Mead, 2007). While the mechanism of this association is not fully understood, animal studies have proposed that a balance that is disproportionately high in protein, may act as a maternal stressor due to ketoacidosis, and increase cortisol within her system and also within her fetus (Mead, 2007).

Although child height, weight, and hemoglobin status were the outcome measures that were used to assess the health status of the children in the current study, other measures of nutritional status such as the child's blood pressure have also been studied in relation to maternal glucocorticoids including cortisol and cortisone. Specifically, maternal glucocorticoids were measured as a marker of the placental concentration of the enzyme, 11-beta-hydrosteroid-dehydrogenase type 2, which allows entry of maternal glucocorticoids across the placental barrier into the fetal nervous system (Huh, Andrew, Rich-Edwards, Kleinman, Seckl, & Gillman, 2008). Not only does the 11-beta-hydrosteroid-dehydrogenase type 2 control the transport of cortisol from the mother across the placental barrier, it also controls fetal programming of hypertension. In this study, venous cord blood was assessed from 286 infants at birth and was used to determine an association between fetal cortisol levels as a marker of 11-beta-hydrosteroid-dehydrogenase type 2 activity and incidence of hypertension three years after birth. The results showed a positive association between higher levels of infant glucocorticoids and hypertension, suggesting that outcomes other than height, weight, and hemoglobin status may be valuable in assessing the relationship between maternal cortisol and child health status (Huh, Andrew, Rich-Edwards, Kleinman, Seckl, & Gillman, 2008).

While there are numerous studies that address the negative effect that increased chronic maternal stress can have on fetal growth and development, few studies to date have analyzed the

direct connection between elevated maternal cortisol levels and child nutritional status. Though the connection has been infrequently addressed in the literature, there have been a small number of human and animal studies that briefly address the possible role that cortisol has on nutritional status. The hormone, insulin-like growth factor (IGF) plays a major role in normal fetal growth. Specifically, IGF-1 plays the most prominent role in regulating fetal growth. The hormone is released in response to fetal glucose levels and is then responsible for controlling the movement of glucose into peripheral fetal tissues as well as its storage as glycogen and fat in liver and extra-hepatic tissues (Allen, 2001). In times of maternal starvation, fetal IGF-1 is decreased, stunting fetal growth (Allen, 2001). And while maternal IGF-1 does not cross the placenta to the fetus, it can, along with IGF-2, negatively affect the placenta's role and, thus, indirectly affect fetal growth (Allen, 2001).

Though IGF-1 and IGF-2 play the main role in regulating fetal growth in-utero, cortisol also has also been shown to affect fetal growth. In animal studies cortisol has shown to arrest longitudinal growth during the late gestational period in sheep fetuses (Allen, 2001). One proposed mechanism by which cortisol affects fetal grow is its suppression of IGF-2 as well as its tendency to promote cell differentiation over proliferation (Allen, 2001). Because maternal cortisol can pass through the placenta to the fetus, an increase in maternal cortisol level may promote an increase in circulating fetal cortisol, thus blocking normal fetal growth mechanisms.

Though the results from the current paper presented no association between maternal cortisol levels and child hemoglobin status, the mechanism of interaction between cortisol and hemoglobin has been studied and suggests that there is a relationship between these two factors. During times of iron deficiency norepinephrine concentrations increase which triggers an increase in corticotrophin releasing hormone (CRH) and subsequently cortisol as well (Allen,

2001). Little work has been conducted to study the consequence of iron deficiency in relation to cortisol secretion, but one animal study with rats showed that an iron-free diet resulted in an increased serum cortisol concentration (Allen, 2001). Further implications of this study suggest that mothers who experience iron deficiency and/or anemia due to diet choices, economic influences, or cultural preferences while pregnant are more likely to have increased circulating cortisol levels, which may be passed on to their children leading to growth inhibition.

One maternal factor that is probably more predictive of child hemoglobin status than cortisol level is maternal feeding practices during infancy. In one cohort study conducted in Montreal, Canada 299 disadvantaged infants aged 10 to 14 months and their mothers were assessed to determine the prevalence of iron deficiency anemia (Lehmann, Gray-Donald, Mongeon, & Di Tommaso, 1992). Infant blood samples were collected to determine hemoglobin and serum ferritin levels, as well as mean corpuscular volume and mothers completed a questionnaire regarding infant feeding practices (Lehmann, Gray-Donald, Mongeon, & Di Tommaso, 1992). Results from the study showed that 25% of the infants suffered from iron deficiency anemia, and serum ferritin levels and mean corpuscular volume were both predicative of iron deficiency anemia. High levels of iron deficiency anemia can be attributed to the introduction of whole cow's milk before 6 months of age and iron-fortified infant cereal for 6 months or less (Lehmann, Gray-Donald, Mongeon, & Di Tommaso, 1992). Similarly, high levels of iron deficiency anemia were observed in infants who had a low birth weight and were also fed iron-fortified cereal before 6 months of age (Lehmann, Gray-Donald, Mongeon, & Di Tommaso, 1992).

Another factor that may also be more predictive of infant nutritional status than maternal cortisol levels is the presence of maternal depression and other psychiatric disorders. In a

prospective cohort study conducted in Rawalpindi, Pakistan, 160 healthy mothers and 160 depressed mothers were assessed during their final trimester. Depressed mothers were reassessed at 2, 6, and 12 months following birth and infant weights and height measurements were taken at birth and again at 2, 6, and 12 months of age (Rahman, Iqbal, Bunn, Lovel, & Harrington, 2004). The study showed that more growth deficits were observed in infants of clinically depressed mothers and relative risk of being stunted and underweight were significantly increased in infants of depressed mothers. In addition, infants who were born to depressed mothers were more likely to suffer from more diarrhea (Rahman, Iqbal, Bunn, Lovel, & Harrington, 2004). Though the literature presents a compelling connection between the presence of maternal depression and compromised child nutritional status, the current study did not find and association. Thus, further studies must be conducted to draw conclusive results.

Other moderating factors presented in the literature include maternal age and marital status. In a large longitudinal cohort study that followed 8,958 children from birth through 10 years of age, maternal and environmental factors were assessed to determine a relationship with child psychosocial stress (Montgomery, Ehlin, & Sacker, 2006). Children who were formula-fed and who came from single family homes were more likely to experience higher levels of stress and negative developmental consequences than children who were formula-fed and from non-divorced/separated homes (Montgomery, Ehlin, & Sacker, 2006). Similarly, the prevalence of child malnutrition is higher in developing countries, where an estimated 20% of children less than or equal to 5 years of age are underweight (Imdad, Yakoob & Bhutta, 2011). Lastly, the mother's age must be taken into consideration when examining infant and child developmental outcomes. One study presented by Finlay, Ozaltin, and Canning showed that teen mothers along

with mothers up to age 27 who were giving birth for the first time had the highest risk for negative child birth and subsequent growth outcomes (Finlay, Ozaltin, & Canning, 2011).

The results of these studies show that while elevated or abnormal maternal cortisol may have an effect on child development, infant feeding practices, maternal presence of depression, maternal age at birth, industrialization of the country of origin, parity, and marital status may be more predictive of child nutrition outcomes.

The current study presents a possible connection between mothers and their children that has yet to be studied in the existing literature. Much of the review evidence supports the strength of the mother-infant relationship through the biological, emotional, and social connection that spans from conception through adulthood. Infant dietary and language preferences along with body weight, fat percentage, intellectual stimulation, and even stress responses can be influenced by the mother throughout the gestational period.

While the intensity of the mother-child relationship in utero is widely understood and accepted, fewer studies have examined the direct maternal effects that remain after birth. Several maternal factors that continue to influence child growth and development include maternal depression and anxiety, socioeconomic status, parity, job status, educational level, time spent at home, marital status, and age. Because there has been little literature published that directly relates maternal stress levels and child nutrition status specifically, the current study uses these biological and environmental maternal factors for the basis of examining a more direct association between maternal salivary cortisol levels and child nutrition status.

Although no association was observed between maternal cortisol levels and child height, weight, and hemoglobin status, further research is needed regarding this relationship in order to draw more conclusive associations. As presented in the existing literature, other child nutritional

status markers such as marginal iron deficiency, hypertension, and other micronutrients should also be analyzed. Also, several studies show that factors such as maternal depression and infant feeding practices may provide a stronger association between child nutrition status than maternal cortisol level. Also, more conclusive results may be drawn with a larger study sample. Other limitations of the current study are that other factors that significantly influence child nutritional status were not taken into consideration. Such factors include the mother's attitude and knowledge towards food and nutrition, the meals that she cooks for her child(ren), her own eating patterns, as well as the child's diet and eating behaviors. Also, because the current study was a cross-sectional design it cannot be determined from the results if increased maternal cortisol concentrations lead to poor child nutritional outcomes or vice versa. Ultimately, the existing research supports an indirect relationship between maternal stress levels and child nutrition status and thus, further studies are needed to ascertain a conclusive direct association.

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Academic Vita

Local
119 Harley Alley Apt. 2
State College, PA, 16801
(610) 999-3758

Allison A. Pohl

Home
510 Dayspring Lane
West Chester, PA, 19382
(610) 455-1969

Education:

Bachelor of Science Degree in Applied Nutritional Sciences, Penn State University — Spring, 2012
Minor in Health and Human Development
Honors in Nutrition
Thesis Title: The effect of Maternal Salivary Cortisol Levels on Child Nutrition Status
Thesis Supervisor: Katarzyna Kordas

Nutrition Research:

Stress and Nutrition Honors Thesis- Dr. Katarzyna Kordas — 2010-Present

- Determined maternal salivary cortisol levels
- Entered and analyzed data using Excel
- Collaborated with thesis advisor to complete senior thesis paper

Binge Eating Undergraduate Research Assistant- Dr. Rebecca Corwin — Spring, 2010

- Fed and recorded fat intake of research animals
- Prepared brain tissues for microscope slides
- Analyzed and recorded hormonal synapses in brain nuclei

Related Experience:

The Village at Penn State — 2011-Present

- Served lunch and dinner to over 100 residents in the facility's dining room
- Shadowed registered dietitian in assisted living and skilled nursing departments
- Assisted with patient care plans and assessments, attended resident life meetings, and observed patient dietary patterns.

State College Food Bank — Summer 2011

- Inventory control and stockroom organization
- Prepared food and basic necessity bags for clients
- Assisted with customer service and donation deliveries

Food Preparation Course Teaching Assistant- Chef Anne Quinn Corr — Spring, 2011