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A MICROANALYSIS OF IRON STATUS IN INFANTS AND MOTHER-INFANT INTERACTION

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ABSTRACT

Iron deficiency is the most prevalent single nutrient deficiency in the world, and mothers and children under the age of five are the most affected population. In children, non-optimal mother infant interactions have been linked to poor child development, which can be caused by iron deficiency. There is limited evidence from studies that looked directly at iron status and mother-infant interactions, and no studies that have observed the dyadic relationship at the microanalytical level. Thus, the objective of this thesis was to investigate how the iron status of infants impacts mother-infant interaction at a microanalytical level.

This study was part of a larger parent study exploring the effects of iron and zinc on diarrhea and anemia outcomes that used a longitudinal, randomized, double blind, factorial community trial design involving mother and infant dyads from Mirzapur, Bangladesh. Infants ranged from six to 18 months of age at baseline. The interaction between 19 of these mother-infant dyads was included in this substudy. All videos were coded using a microanalytical coding scheme using Mangold INTERACT. This coding scheme included child behaviors, maternal behaviors, and shared behaviors. The behaviors coded for the child included looking extra-dyadic, looking dyadic, non-distress vocalizations, and distress vocalizations. Behaviors coded for the mothers included looking extra-dyadic, looking dyadic, dyadic engagements, and latency to respond to the infant. Joint attention was coded as a shared behavior between the mother and her infant. Iron and inflammation status were assessed in infants at baseline via serum ferritin, transferrin receptor, calculated total body iron, hemoglobin, and C-reactive protein to classify iron deficient and iron sufficient groups. Other measurements that were collected and controlled for included socioeconomic status, mid-upper arm circumference, weight-for-age Z score, child's sex and age, exclusive days of breastfeeding, household size, number of children of particular

ages in the household, village of residence, and maternal depressive symptoms and reasoning ability.

There were no statistically significant differences seen between infant iron status groups when using ferritin, transferrin receptor, and body iron and Mangold INTERACT outcomes. These findings could be due to the small sample size of dyads and an even smaller sample size for each iron status group, and the inability to assess the outcome by maternal iron status and to control for it.

Treating iron biomarkers as continuous variables revealed positive contributors of hemoglobin and transferrin receptor on mother-child interactions. Other contributing factors included greater weight-for-age Z scores and mid-upper arm circumference. As mother-infant interaction is an important aspect of cognitive functioning and attachment in the infant, it is important that future studies and funders consider that our findings are limited by a small sample size and inability to control for maternal depressive symptoms and maternal iron status. As such, more studies are needed to understand the effects of iron status on mother-infant interactions at the microanalytical level.

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LIST OF ABBREVIATIONS

Abbreviation	*Definition*
DcytB	Duodenal Cytochrome B
DMT-1	Divalent Metal Transporter 1
FPN	Ferroportin
Ft	Ferritin
Hb	Hemoglobin
ID	Iron Deficiency
IDA	Iron Deficiency Anemia
MUAC	Mid-Upper Arm Circumference
PPD	Postpartum Depression
SES	Socioeconomic Status
Tf	Transferrin
TfR	Transferrin Receptor
WAZ	Weight-for-age z-score
WRA	Women of Reproductive Age

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Chapter 1: Literature Review

Iron Deficiency in Developing Countries

Prevalence, Progression and Symptoms of Iron Deficiency

Approximately two billion people, 33% of the world population, are affected by anemia in both developed and developing countries, about 50% of which is assumed to be due to iron deficiency (ID) (Stevens et al. 2013; Lopez et al. 2016; Camaschella 2015). The worldwide rates of anemia have decreased slightly from 33% to 29% in non-pregnant women of reproductive age (WRA), from 43% to 38% in pregnant women, and from 47% to 43% in children over the past 20 years (Stevens et al. 2013; Camaschella 2015). The populations of Central and West Africa and Central and South Asia have the highest rates of iron deficiency anemia (IDA) globally (Stevens et al. 2013; Camaschella 2015; Lopez et al. 2016).

Low physiological states of iron status can be categorized based on the level of severity as follows: depleted, deficient, and anemic. Depletion refers to a reduction in serum or plasma ferritin (Ft) concentrations, indicating depleted iron stores. Deficiency is defined by a decrease in tissue iron, which is measured by transferrin receptor (TfR). Deficiency precedes IDA and can lead to compromised functioning of iron related systems in the body (Camaschella 2015). As iron storage decreases, transferrin saturation and total iron binding capacity are diminished as well. Anemia, the most iron-deficient state, is evidenced by a drop in hemoglobin along with other biomarkers as seen in the states of iron depletion and iron deficiency without anemia, as well as microcytic hypochromic red blood cells (Gibson 2005).

In developing countries, poverty, malnutrition, and famine are common causes of IDA, especially in pregnant women and children (Camaschella 2015). IDA in early pregnancy has been associated with a higher risk of preterm delivery and putting the infant at risk for early

neonatal mortality (Scholl 2005). Common symptoms associated with IDA include weakness, headache, fatigue, and difficulty in concentrating, and these are caused by low delivery of oxygen to tissues and decreased activity in iron-containing enzymes (Camaschella 2015). Less common symptoms include vertigo, tachycardia, cardiac murmur, restless leg syndrome, atrophic glossitis, dry and damaged skin and hair, neurocognitive dysfunction, and angina pectoris (Lopez et al. 2016). In some patients anemia can be asymptomatic and diagnosis can only be made through the analysis of iron biomarkers in blood samples (Lopez et al. 2016).

Prevalence of Iron Deficiency in Bangladesh

The Demographic and Health Surveys Program found that the prevalence of anemia in Bangladesh of children 6-59 months was 51%. It was also found that 51% of women of reproductive age (15-49 years) were anemic ("Bangladesh: Nutrition Profile" 2018). In a rural area of Bangladesh, 27% of pregnant women had ID, while only 13.4% had IDA, although rates of IDA were much lower in rural communities with high groundwater iron (Ahmed et al. 2018). High rates of anemia have also been seen in urban areas of Bangladesh. The main reasons for anemia in educated, urban, university students were found to be poor dietary habits, menstrual blood loss, and lack of knowledge of ID consequences (Shill et al. 2014). In a study of 289 women aged 14-19 years from low socioeconomic status living in an urban area, 88% were anemic with Hb < 120 g/L and 79.5% were ID defined by Ft < 12 µg/L prior to the intervention. As it is common for women in Bangladesh to become pregnant during their teenage years, this is of particular concern for this population. The ID rate decreased by 90% in those taking iron with folic acid, and the anemia rate decreased by 92% in those taking iron with folic acid and vitamin A (Ahmed, Khan, and Jackson 2001). Alternatively, 68% of children who were anemic at baseline (Hb < 110 g/L) in a study conducted by Black et al. did not have improved rates of

anemia after a 6-month intervention with iron, zinc, iron and zinc in combination, and a micronutrient mix consisting of 16 vitamins and minerals (Black et al. 2004). This discrepancy in findings displays the need for more controlled iron intervention studies prior to making recommendations in Bangladesh.

Iron Bioavailability

The adult human body contains 3 to 5 g of iron, or approximately 55 mg/kg for males and approximately 44 mg/kg for females (Gkouvatsos, Papanikolaou, and Pantopoulos 2012; Ganz 2013). Iron in the human diet can be found in two forms, heme iron and non-heme iron. More than 70% of body iron is found in hemoglobin (Papanikolaou and Pantopoulos 2017). Heme is a stable porphyrin ring complex with an iron atom at its core and functions as an oxygen carrier in hemoglobin in erythrocytes and in myoglobin in the muscle tissue (Theil 2004). Heme iron in the diet comes from animal sources. Heme iron composes 40% of iron in these animal sources, with the other 60% being non-heme iron. Non-heme iron exists in two valence states, reduced ferrous iron (Fe^{2+}) and oxidized ferric iron (Fe^{3+}), with ferrous iron being more soluble than ferric iron (Teucher, Olivares, and Cori 2004). Reduced ferrous iron spontaneously converts to oxidized ferric iron via aerobic oxidation, which is insoluble at the physiological pH (Papanikolaou and Pantopoulos 2017). Non-heme iron absorption is less efficient than heme iron, with its sources being plant foods such as fruits, vegetables, grains, nuts, and dairy products. The source of the iron is not the only important consideration for bioavailability, as the chemical composition of food and the type and quantity of the iron determine its bioavailability within a meal (Theil 2004; Hurrell and Egli 2010).

The presence of enhancers and/or inhibitors of iron also serve to determine the bioavailability of iron. Enhancers are nutrients that aid in absorption of iron, with examples

being vitamin C (ascorbic acid), fructose, citric acid, animal tissues from meat, fish, and poultry, lysine, histidine, cysteine, and methionine. The lower pH produced by organic acids prevents the precipitation of ferric iron and reduces it to ferrous iron in the intestinal mucosal cells of the duodenum and upper jejunum (Hurrell and Egli 2010). Inhibitors impede iron absorption and include calcium, specific animal proteins such as casein, whey, and albumin, oxalic acid, tannins, phytates, polyphenols, carbonate, phosphate, fiber, and other metal ions (Teucher, Olivares, and Cori 2004; Hurrell and Egli 2010). Of these inhibitors, phytates are the main source of inhibition for non-heme iron, as they have six negatively charged phosphate groups at a pH between six and seven with a high affinity for binding to divalent metals such as iron. Other inhibitors that bind to divalent metals include fibers and polyphenols, although phytates have a higher affinity for binding (Mascitelli, Goldstein, and Zacharski 2015). In a low income country such as Bangladesh, phytate consumption is high due to the high consumption of a plant-based diet (Tetens et al. 2003; Gibson et al. 2010).

Iron Metabolism

Absorption of Iron

Although heme iron is more readily absorbed in the body, most people consume a greater amount of non-heme iron than heme iron, making non-heme iron a greater contributor to overall iron status. After entering the digestive tract, non-heme iron gets reduced from the ferric form to ferrous iron by duodenal cytochrome B (DcytB), a ferrireductase located on the apical membrane of the enterocyte. Other reducing agents, such as ascorbate, can also reduce the ferric iron to its ferrous form. If iron is originally in the ferrous form in the dietary source, then it will not require reduction. The ferrous iron then enters the enterocyte via divalent metal transporter 1 (DMT-1). DcytB and DMT-1 are both regulated by iron status in the body, increasing their activity with ID

and decreasing it with normalized iron status (Lönnerdal, Georgieff, and Hernell 2015). The non-heme iron is then either delivered to ferritin (storage), apo-protein (metallation), or ferroportin (export) depending on where it is needed. This delegating process appears to be regulated by cytosolic iron chaperone proteins poly (rC)-binding protein-1 or -2 or PCBP1 or 2 (Bogdan et al. 2016; Muckenthaler et al. 2017). For the ferrous iron to be stored in the enterocyte, it must be oxidized by a ferroxidase enzyme and stored in a ferritin heavy chain. In the enterocyte the ferrous iron can also be used, as opposed to being stored, for metabolism in the mitochondria. If the iron is going to be used elsewhere in the body, it must be transported across to the basolateral membrane, although the mechanisms of transport are not well understood (Lönnerdal, Georgieff, and Hernell 2015; Papanikolaou and Pantopoulos 2017). Ferrous iron is exported by ferroportin (FPN) on the basolateral membrane of the enterocyte. Outside of the enterocyte, the soluble or membrane-bound multicopper ferroxidases hephaestin or ceruloplasmin oxidize the ferrous iron to ferric iron (Vashchenko and MacGillivray 2013). The ferric iron can then bind to transferrin (Tf) on one of its two iron-binding sites, which can then deliver it to tissues expressing transferrin receptor (TfR), such as bone marrow erythroblasts, via receptor-mediated endocytosis (Bogdan et al. 2016; Papanikolaou and Pantopoulos 2017).

Heme-iron must be hydrolyzed by proteases in order for it to be released from the globin portion of hemoglobin and myoglobin. The mechanism of absorption into the lumen is incompletely understood. Multiple theories to explain this process have been proposed including receptor-mediated endocytosis, passive diffusion, and active transport (Blanc, Garrick, and Arredondo 2012). However, it has been well established that heme iron absorption requires

catabolism of heme within enterocytes and release of $Fe^{2+,}$ which then follows the absorption of non-heme iron (Papanikolaou and Pantopoulos 2017).

Regulation of Iron Absorption

There are two mechanisms responsible for maintaining iron homeostasis, post-transcriptional modifications of TfR, Ft, and other metabolic factors at the intracellular level, and hepcidin binding to FPN at the systemic level (Bogdan et al. 2016). Iron regulatory proteins (IRP) and iron response elements (IRE) regulate the levels of Ft and TfR protein production intracellularly based on iron status. IRPs regulate the expression of genes involved in iron metabolism by binding to structures formed in untranslated regions of mRNA called IREs (Hentze et al. 2010; Zhang, Ghosh, and Rouault 2014; Bogdan et al. 2016; Papanikolaou and Pantopoulos 2017). IRE-containing transcripts are found in Ft, TfR, FPN, DMT-1, and the ALAS2 gene (Zhang, Ghosh, and Rouault 2014; Papanikolaou and Pantopoulos 2017). IRE-IRP binding occurs in conditions of low iron, causing decreased production of Ft but up-regulated production of TfR, leading to an increase in iron scavenging and limited sequestration or efflux and erythroid heme synthesis. IRE/IRP interactions do not occur in conditions of high iron, and non-binding results in up-regulated production of Ft but down-regulated production of TfR, promoting the storage of excess iron intracellularly and for utilization for heme synthesis in erythroid cells. TfR mRNA degradation and synthesis of ferritin, ferroportin, and ALAS2 also occur under conditions of non-binding (Zhang, Ghosh, and Rouault 2014; Bogdan et al. 2016; Papanikolaou and Pantopoulos 2017).

Iron absorption is regulated at a systemic level via hepcidin, a hepatic hormone that regulates how much iron exits enterocytes, macrophages, hepatocytes, and other cell types (Silva and Faustino 2015; Hentze et al. 2010). Hepcidin covalently binds to FPN, leading to down-

regulation of FPN and increased cellular retention of iron. Thus, iron accumulates in the intestinal cell, down-regulating DMT-1. Under conditions of low iron, hepcidin and FPN are regulated by the IRE-IRP system in order to repress hepcidin translation and stabilize FPN translation, thus allowing the release of iron from various cell types into the circulation (Bogdan et al. 2016).

Iron and Mother-Infant Interaction

Mother-Infant Interactions

The quality and quantity of mother and infant interactions impacts aspects of cognitive functioning throughout the lifetime, including social and emotional processes. From a young age, physical and psychological development in the infant is influenced by the parent-infant relationship, as both parents offer their children the support that they need to achieve milestones throughout their life (Bornstein 2002; Brinker, Seifer, and Sameroff 1994). The quality of caregiving is also essential in determining the level of children's socioemotional and cognitive development, especially in the form of sensitive and responsive parenting (Bornstein and Tamis-LeMonda 1989; Landry, Smith, and Swank 2006; Dexter et al. 2013; Wolff and van IJzendoorn, 1997). Maternal responsiveness and sensitivity refers to the quality with which the mother reacts to and interprets her child's cues in a timely and appropriate manner (Leerkes, Blankson, and O'Brien 2009; Dunst and Kassow 2014). Sensitive and responsive parenting helps to create a safe, loving environment where the infant can learn and play. How parents respond to negative emotions from their child teaches the infant about their own emotional states and how others respond (Leerkes, Blankson, and O'Brien 2009). Adequate physical, psychosocial, and economic conditions are able to positively aid in the child's psychological development, self-confidence, and happiness (Bornstein 2005).

The concept of attachment was first introduced by Bowlby (Bowlby 1969) and then expanded upon by Ainsworth and colleagues (Ainsworth 1979). The theory states that infants become attached to those who provide responses to the signals of the baby that are consistent, predictable, and appropriate (Bornstein and Lamb 2002). Secure attachment has been shown to positively influence child emotional as well as cognitive adaptation beyond infancy as a result of adequate maternal sensitivity (Ainsworth 1979; Dexter et al. 2013). A child that is more securely attached will be more willing to be independent and explore a new situation rather than being entirely fearful. Another positive impact of secure attachment between a mother and infant is the development of self-regulatory behaviors that involve the late-developing prefrontal cortex (in adolescence) and could potentially benefit the child later in life (Wachs et al. 2013). In contrast, studies in children who were exposed to violence indicated that they displayed higher levels of aggression, attention problems, and depression (Walker et al. 2011). Multiple psychosocial risk factors can affect the mother-infant relationship, including low family socioeconomic status, maternal depression, maltreatment, and family violence (De Falco et al. 2014; Lovejoy et al. 2000).

As stable mother-infant interactions at the age between six and 12 months have beneficial effects for later child development, early interventions could prevent long-term socioemotional dysregulation issues. This is especially important as the early years of life are considered an important time for intervention to reduce negative emotional and behavioral problems and to promote self-regulation or pro-social behaviors (Wachs et al. 2013).

This thesis analyzes mother-infant interaction from a microanalytic standpoint in order to understand which interactive events between the mother and infant are impacted by infant iron status. The microanalytic approach allows analysis of the interaction based on the behaviors of

both the mother and the infant that maintain, end, avoid, and begin social interaction and stimulation. Both dyadic and extra-dyadic behaviors are analyzed for the mother and the infant. An infant's visual system is important in regulating social behavior, and visual contact between mother and infant are cardinal features of attachment behaviors under mature voluntary control early in life (Stern 1971). Joint attention, or the "triadic relationship between self, other, and object" that develops before the age of three (Naber et al. 2007), is an important interactive behavior and is analyzed as a part of mother-child interaction in this study. Joint attention has been found to be the underlying mechanism of nonlinguistic interactions, and is important for the development of early language (Tomasello and Farrar 1986).

Risk Factors related to Poor Child Development

Risk factors have been identified that impede adequate brain development in children, which is rapidly occurring during the first two years of life. During this time, motor, cognitive, and social-emotional inter-domains largely shape development (Walker et al. 2007). These risk factors can be divided into psychological and biological risk factors, and can impact both the mother and infant.

Maternal depression is one of the main psychosocial risk factors related to child development. Maternal depression has been repeatedly reported as a predictor of negative outcomes in terms of maternal sensitivity and child attachment security (Wolff and van IJzendoorn, 1997; De Falco et al. 2014). Postpartum depression (PPD) impacts the mother's ability to function effectively and care for herself and the child (Lovejoy et al. 2000; Black et al. 2009; Walker et al. 2007; Black et al. 2011; O'Hara and McCabe 2013). PPD has the ability to decrease sensitivity and responsiveness of the mother towards the infant, and can diminish response to needs such as food, sleep, safety, and health (O'Hara and McCabe 2013).

Furthermore, PPD has been significantly associated with an insecure attachment style between mothers and their infants (Ikeda, Hayashi, and Kamibeppu 2014). Mothers with PPD have been shown to be less sensitive to and less involved with their infants in addition to having more negative interactions (Cooper et al. 1999). For the infant, PPD can be associated with long-term behavioral, cognitive, and health-related outcomes in the child (O'Hara and McCabe 2013).

Of the biological risk factors related to child development, nutrient deficiencies such as ID can have a negative effect on both the mother and the infant (Walker et al. 2007; Murray-Kolb and Beard 2009; Gunnar and Nelson 2013; Prado and Dewey 2014). IDA infants have been found to have altered mental and motor development (Lozoff et al. 2008; Shafir et al. 2008; Carter et al. 2010). Infants with ID exhibit less attentiveness, and are more fearful, hesitant, clingy, unhappy, inactive, and easily fatigued (Lozoff, Klein, and Prabucki 1986; Lozoff et al. 1998; Beard and Connor 2003).

Mixed findings have resulted from studies on how iron intervention in the first year of life impacts child development. These results are due to developmental delays that were caused by ID in the child. While some studies have shown that ID leads to long-lasting and irreversible impacts on cognition and developmental potential, others suggested that supplementation was able to reverse developmental delays (Grantham-McGregor and Ani 2003; Lozoff, Jimenez, and Smith 2006; Walker et al. 2007; Beard 2008). Other possible explanations for these results include not being blinded, not using appropriate psychological testing for cognitive and social-emotional outcomes, and differences in iron supplement dosage, duration of the study, severity of the ID in subjects, and ages of the children.

Mother-Infant Interaction and Iron Deficiency

There are few studies that have investigated the relationship between IDA and mother-child interaction. Despite the small body of evidence, results have shown improvement in both iron status and mother-infant interaction following iron supplementation. Murray-Kolb and Beard conducted a randomized, double-blind intervention trial in South Africa to examine the relation between maternal iron status and mother-child interaction (Murray-Kolb and Beard 2009). Baseline iron status was taken at six to 10 weeks postpartum and mother-child interaction was videotaped at 10 weeks and nine months postpartum. At ten weeks, maternal sensitivity and child responsiveness were significantly greater in the control, non-anemic mothers versus IDA mothers. At nine months, the control group and the iron supplemented IDA mothers no longer differed in terms of their interaction scores, but both groups interacted better with their children compared to IDA mothers given a placebo. This showed that IDA in mothers negatively impacts mother-child interaction, and iron supplementation of IDA mothers can improve these effects. One limitation of this study was that iron status was not assessed in the infants (Murray-Kolb and Beard 2009).

Armony-Sivan and colleagues compared mother-infant interactions during feeding in infants with and without IDA. Iron supplementation of the children began at nine to 10 months of age in an economically stressed, inner-city community in Detroit, and was continued for three months with infant assessment, interviews, and mother-infant interaction observed during feeding being completed at baseline and endline. The results showed that mothers of IDA infants responded with significantly less sensitivity to infant cues and that infants were rated lower on clarity of cues compared to the non-IDA group. This study was limited by its small sample size (n=77), inability to assess iron status in response to iron therapy in all infants as 46% of them did

not donate a blood sample at endpoint, and a lack of data on maternal iron status (Armony-Sivan et al. 2010).

Corapci, Radan, and Lozoff observed five-year-old Costa Rican children, who were either chronically ID or iron sufficient in infancy, with their mothers during a structured interaction task both in a laboratory setting and at home. The families were of working class living in an urban setting. Post intervention, children who were chronically ID in infancy displayed more negative behavior during mother-child interactions than iron-sufficient children, despite the 3 mg/kg iron supplement taken during infancy. Mothers of the children who were chronically ID children in infancy were less responsive in both of the settings in which mother-child interaction was observed. One limitation of this study was the lack of data on maternal iron status (Corapci, Radan, and Lozoff 2006).

In this thesis, mother-infant interactions were quantified via a coding scheme developed using established studies and executed using the Mangold INTERACT software (Bornstein 1985; Colombo and Horowitz 1985; Fogel, Toda, and Kawai 1988; Stern 1971, 1974; Tomasello and Farrar 1986; Bornstein et al. 1990). This coding scheme accounted for behaviors both by the mother (looking extra-dyadically and dyadically and dyadic behavior), the infant (looking, vocalizing), and in combination (joint attention). The objective of this thesis was to assess how the iron status of a sample of 19 infants in Bangladesh impacts mother-child interaction at a microanalytical level, cross-sectionally. It was hypothesized that microanalytically coded interactions would differ in an infant with impaired iron status compared to an infant with sufficient iron status such that the interaction would be less optimal in the iron deficient condition. This thesis adds to the body of literature investigating the relationship between IDA and mother-child interactions

Chapter 2: Methods

General Study Design

The data used for the current project are from a larger parent trial and its methods are briefly explained here; extensive details are reported elsewhere (Chang et al. 2009). This study was conducted in collaboration with the International Centre for Diarrhoeal Disease Research, Bangladesh (icddr,b) and used a longitudinal, randomized, double-blind, factorial community trial design involving mother and infant dyads (n=500). The participants were permanent residents of four villages located in Mirzapur, Bangladesh, ranging from 2.5 to 12 km walking distance from the Kumudini Hospital where all data were collected. Mirzapur provided a sample reflective of Bangladesh's national averages of birth, infant, and child mortality rates of 27/1000 population, 80/1000 live births, and 110/1000 live births, respectively (Chang et al. 2009). Children from this setting are generally breastfed for one to two years and then introduced to a plant based diet (Chang et al. 2009).

Recruitment and Screening

Mothers and their infants were eligible for screening if they were permanent residents of the study area and the infant was between six and 18 months old. Random selection of the participating child was used in families with multiple eligible children. Exclusion criteria included malnourishment requiring medical intervention (defined by weight for height z-score < -3 or hemoglobin < 70 g/L), chronic illness, inability to be fed, or a fever above 38℃ on the day of recruitment. If any of these symptoms were present, children were sent for evaluation at Kumudini Hospital. Dyads were also excluded if the family planned to relocate within six months.

Psychological Study Participants

Participants were eligible for randomization into the nested cognitive portion of the study if they had participated in the larger parent study and had been randomly assigned to have blood draws. 352 participants were included in the cognitive portion of the study, which assessed mother-infant interaction at baseline, three months, and six months after beginning the supplementation. For purpose of this thesis, mother-child interaction was only assessed microanalytically via Mangold INTERACT at baseline.

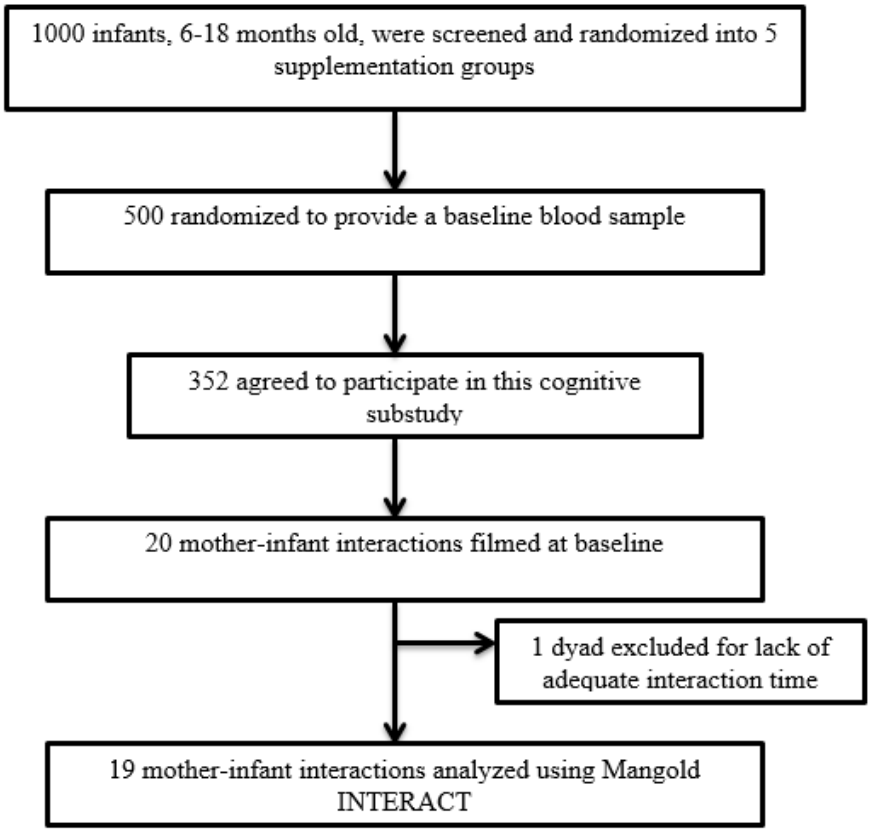

Figure 1. Flow Diagram of Dyads Included in the Study

Demographics, Descriptive Variables, and Anthropometrics

Home visits were conducted by village health workers to collect socioeconomic data, child sex (csex), age (cage), duration of breastfeeding (BF) and diarrhea, the number of children of particular ages in the household, the household size (hhsize), village of residence, and dietary intake data. Maternal depressive symptoms were assessed using the Center for Epidemiological Studies Depression Scale (CES-D) (Radloff 1977), and maternal reasoning ability was assessed via Raven's Standard Progressive Matrices (Raven and Court 1998). Both of these tests were administered at baseline, midpoint, and endpoint. An asset score was created for each of the households included in the study by evaluating access to washing water, drinking water, a latrine facility, durable foods, the number of animals owned by the household, homestead, land for cultivation, other land, and housing structure. The asset scores were then categorized into quintiles, which were then further divided into three groups, high, medium, and low to allow for easier data interpretation. The high group was composed of the highest quintile, medium of the second, third, and fourth quintiles, and low of the lowest quintile. Anthropometric variables collected included child mid-upper arm circumference (MUAC), and weight and length to calculate weight-for-age z-score (WAZ) using EPIinfo (Chang et al. 2009). All variables were collected at baseline using World Health Organization (WHO) Growth Standards.

Blood Draw, Iron, and Zinc Assessment

Iron status was assessed at baseline and endpoint, six months after the initiation of supplementation, for both mother and infant at Kumudini Hospital. Blood was collected to measure serum zinc, Ft, TfR, and C-reactive protein (CRP). Hemoglobin (Hb) was assessed via Hemocue in the infant only using capillary blood from a finger prick.

Mothers were classified as iron depleted, but not yet iron deficient (ID), if serum Ft was less than 20 μg/L and as ID when serum Ft was below 15 μg/L (Brutsaert et al. 2003; Zhao et al. 2015). Infants were classified as ID if serum Ft was less than 23.7 μg/L (Siddappa et al. 2007; Zhao et al. 2015). The cut-off for adults for serum TfR for ID is greater than or equal to 8.3 mg/L for the assay used. This cut-off was used in the assessment of both mother and infant transferrin receptor measurements, as the cut-off for infants is not well defined (Skikne, Flowers, and Cook 1990; Olivares et al. 2000). Body iron was calculated using Cook's equation accounting for serum Ft and serum TfR (Cook, Flowers, and Skikne 2003).

$$body\ iron\ (mg/kg) = -\ [\log\ (TfR/Ft) - 2.8229]/0.1207$$

This equation has shown utility in population and intervention trials where individuals are more prone to ID, but has not been validated in children (Cook, Flowers, and Skikne 2003). Both mothers and infants were classified as ID if body iron was less than 0 mg/kg (Zhao et al. 2015).

Assessment of Mother-Infant Interaction

Ten minutes of naturalistic free play and a structured, stress-inducing interaction (changing the infant's clothes) were recorded via video camera in the Kumudini Hospital. The infant was alert and well fed prior to the time of filming. After the researcher set up the camera and provided instructions to the mother, the mother and infant were left alone in the room in order to capture naturalistic interactions. The mothers were instructed to play as they usually did at home using a basket of age-appropriate toys, until the changing of the infant's clothes was initiated by a knock on the door from the test administrator.

For the purpose of this thesis, the 20 videos recorded at baseline at the Kumidini hospital were assessed using a microanalytical approach. One dyad was excluded due to having significantly less recorded mother-infant interaction time than the other 19 dyads. A

microanalytical, or frame-by-frame, approach allows for the identification of specific behaviors of both mother and child that are significantly influenced by, but simultaneously influence, an interaction (Stern 1971).

The software Mangold INTERACT was used with an individual coding scheme that was adapted for both the mother and the infant using previously developed methodology (Bornstein 1985; Colombo and Horowitz 1985; Fogel, Toda, and Kawai 1988; Stern 1971, 1974; Tomasello and Farrar 1986; Bornstein et al. 1990). Child behaviors which were coded included: dyadic visual attention such as looking at the mother's face (CL_D_st), extra-dyadic visual attention including looking at an object or event in the environment (CL_ED), non-distress vocalization (C_NDV_st), distress vocalization (C_DV_st), and joint attention (JA_st). A vocalization was coded if it lasted a minimum of 0.3 seconds, and a second vocalization was credited if a change in the vocalization occurred or in the event of a silence of 2 seconds or more. A look, either dyadic or extra-dyadic, was coded if it lasted a minimum of 0.3 seconds. A new look was credited if another look occurred or the infant's eyes were closed for 1.5 seconds or more. Maternal behaviors coded included dyadic, indicating engaging in affective interpersonal attention (MD_st), nurturant, indicating engaging in a feeding, pacifying, or picking up the infant to comfort (M_N_st), imitative (M_I_st), dyadic visual attention (ML_D_st), and extra-dyadic visual attention (ML_ED_st). The "st" at the end of each code indicates that the proportion of time in that specific event in the video was used in the analyses. The events recorded were in reaction to a child-elicited behavior and, therefore, had to occur within 5 seconds of the child's behavior to be credited. Maternal latency to respond was also coded as M_LR_avg, indicating the time between the initiation of any child behavior expected to elicit a response and when the mother actually responded. These behaviors included distress vocalizations, non-distress

vocalizations, and looking dyadic. If the infant changed activities within the 5-second window and the mother responded differently to the new activity, each response was credited individually. The coding scheme as used in Mangold INTERACT can be seen below (Table 1).

Table 1. Microanalytic Coding Scheme from Mangold INTERACT

Variable	Abbreviation	Class
Looking Extra-Dyadic	ML_ED_st	Mother
Looking Dyadic	ML_D_st	Mother
Dyadic	MD_st	Mother
Latency to Respond	M_LR_avg	Mother
Nurturant	M_N_st	Mother
Imitative	M_I_st	Mother
Looking Extra-Dyadic	CL_ED_st	Child
Looking Dyadic	CL_D_st	Child
Distress Vocalization	DV_st	Child
Non-Distress Vocalization	NDV_st	Child
Joint Attention	JA_st	Both

Statistical Analyses

All statistical analyses were run in SAS 9.4 (SAS Institute, Cary NC). To characterize iron status and background characteristics, descriptive statistics were run using student's *t*-test for continuous variables and chi-square for categorical variables. Non-normally distributed variables were log-transformed prior to analysis. Statistical significance was considered at an alpha of $p < 0.05$. Microanalytic coding variables as used in Mangold INTERACT were compared via ANCOVA. Pearson correlations were used to identify variables that may covary with the outcomes of interest at $p < 0.1$. Potential covariates included SES, MUAC, WAZ, child sex and age, maternal depressive symptoms, maternal reasoning ability, and maternal and infant iron status. Maternal depressive symptoms data were missing for all 19 mothers, so it could not be controlled for. Stepwise regression models were conducted with microanalytic coding variables as outcomes of interest and the aforementioned covariates as predictors. Two sets of stepwise regression models were run, with the first including Ft and TfR as the only iron

predictors, and the second including BI as the only iron predictor. This is because BI is calculated based on Ft and TfR measurements. Four of the 19 mothers were missing iron biomarker data, so maternal iron variables was not used in the ANCOVA models or the stepwise regressions. The final sample size for the stepwise regression models was 16, as one mother was missing BF data, one outlier was removed from child TfR, and another outlier was removed from child CRP. The models were run with and without these outliers and the outcomes were altered such that different predictors were included in the stepwise regressions.

Chapter 3: Results

This chapter includes the overall characteristics for the entire sample analyzed in this thesis. Analyses done at baseline for ANOVA/ANCOVAs and stepwise regression models with relevant predictors are presented. As the significance level was set as alpha < 0.05, the p-values in each table below this cut-off are bolded.

Baseline Sample Characteristics

Sample characteristics of the included population can be found in Table 2. The household size for this sample was, on average, 4.9 people. As far as SES, 26.3% of participants were in the low SES group, 47.4% in the middle SES, and 26.3% in the high SES. The average age of the infants was 10 months, and 52.6% were male. The infant MUAC average was 132.5 mm, which falls within the range of 125 mm and 135 mm in which an infant would be considered at risk for acute malnutrition by WHO standards. The mean WAZ was -1.4. Infants were exclusively breastfed for an average of 125.9 days. The average maternal reasoning score was 21.3.

In this sample, infant Ft concentration average was 23.0 μg/L, with 55.6% of the infants being classified as ID by Ft. Average infant TfR concentration was 6.2 mg/L, with 16.7% of the infants being classified as ID by TfR. The mean for infant BI, which accounts for both serum Ft and TfR, was 3.1 mg/kg and showed that 17.7% of the infants were ID. The average infant Hb was 9.3 g/L, and the prevalence of anemia was 100%. None of the infants exhibited inflammation and the CRP average was 1.4 mg/L.

While maternal iron status was not included in statistical analyses, the average maternal Ft was 46.1 μg/L, with 13.3% being classified as iron depleted (Ft ≤ 20.0 μg/L) and 13.3% as ID (Ft ≤ 15.0 μg/L). This indicated that the mothers were either IS or ID by Ft, with no mothers falling into the iron depleted category only (Ft ≤ 20.0 μg/L > 15.0 μg/L). Average maternal TfR

was 3.4 mg/L, mean BI was 8.0 mg/kg, and mean CRP was 0.9 and none of the mothers had inflammation. The prevalence of ID in the mothers using TfR and BI was 0%.

Table 2. Overall Dyadic Characteristics, Demographics, and Blood Measures at Baseline; mean (SD or %)

	Variable Name	Unit	Total Sample
	n (range)		13-19
	Household Size (HHSIZE)	n	4.9 (1.6)
SES	Low (bottom 20%)	%	26.3
	Medium (middle 60%)	%	47.4
	High (top 20%)	%	26.3
Child	Child Age (CAGE)	months	10.0 (3.1)
	Child Sex (CSEX; male)	%	52.6
	Mean Upper Arm Circumference (MUAC)	mm	132.5 (7.6)
	Weight-for-age (WAZ)	Z-score	-1.4 (0.9)
Mother	Exclusive breastfeeding (BF)	days	125.9 (78.4)
	Maternal Reasoning (Raven's)	score	21.3 (9.0)
	Maternal Depressive Symptoms (CES-D)	score	N/A
Child Iron Status	Child Adjusted Ferritin (Ft)*	µg/L	23.0 (12.6)
	ID ≤ 23.7 µg/L	%	55.6
	Child Transferrin Receptor (TfR)	mg/L	6.2 (1.9)
	ID ≥ 8.3 mg/L	%	16.7
	Child Body Iron (BI)**	mg/kg	3.1 (3.1)
	ID < 0 mg/kg	%	17.7
	Child Hemoglobin (Hb)	g/L	93.0 (10.0)
	Anemia < 110 g/L	%	100.0
	Child C-Reactive Protein (CRP)	mg/L	1.4 (1.2)
	Inflammation > 10 mg/L	%	0.0
Maternal Iron Status	Maternal Adjusted Ferritin (Ft)*	µg/L	46.1 (23.4)
	Iron Depleted ≤ 20.0 µg/L	%	13.3
	ID ≤ 15 µg/L	%	13.3
	Maternal Transferrin Receptor (TfR)	mg/L	3.4 (1.5)
	ID ≥ 8.3 mg/L	%	0.0
	Maternal Body Iron (BI)**	mg/kg	8.0 (2.9)
	ID < 0 mg/kg	%	0.0
	Maternal C-Reactive Protein (CRP)	mg/L	0.9 (0.0)
	Inflammation > 10 mg/L	%	0.0

* Adjusted for inflammation via Thurnham's method (Thurnham et al. 2010);

** Calculated via Cook's equation (Cook, Flowers, and Skikne 2003).

Analysis of Covariance by Iron Status

Potential covariates were included in statistical models if they were related to the mother-infant interaction outcome as measured in Mangold INTERACT. The significance level for determining covariates was set at $p < 0.1$ in correlation analyses. Maternal iron biomarkers were not controlled for as four mothers were missing iron biomarker data. Correlations were run between Mangold INTERACT outcomes and iron biomarkers as well as demographic characteristics to identify covariates (Appendix A-1, A-2).

At baseline, ANOVA/ANCOVAs were conducted to compare each Mangold INTERACT outcome by infant iron status. Model 1 was a null model where each infant iron status variable was included. Model 2 included Model 1 plus the covariates indicated in Appendix A-1, A-2. Model 3 included Model 1 and controlled for all potential covariates considered in the methods section under the "Statistical Analyses" heading.

The ANOVA and ANCOVA models indicated in Table 3 showed that the infant iron status groups were not significantly different from each other and had no association with any of the Mangold INTERACT variables. However, Model 2 was significant overall for the proportion of the child looking extra-dyadic when characterized as iron deficient/sufficient based on Ft, TfR, and BI. Model 2 was also significant overall for the proportion of joint attention when characterized as iron deficient/sufficient based on Ft. Despite this overall significance, none of the individual covariates were significant for any of these models.

Table 3. Baseline Analysis of Covariance (ANCOVA) with Mangold INTERACT Outcomes by Iron Status; mean (SD)

		Model 1 (mean (SD))			Model 2 (mean (SD))			Model 3 (mean (SD))		
Mangold INTERACT Variable	**Iron Biomarkers**	**IS**	**ID**	**N**	**IS**	**ID**	**N**	**IS**	**ID**	**N**
Total Events	Ft	53.0 (15.1)	65.8 (24.5)	18	53.0 (15.1)	65.8 (24.5)	18	53 (15.1)	63.3 (24.7)	17
	TfR	62.5 (22.3)	62.7 (35.5)	18	62.5 (22.3)	62.7 (35.6)	18	60.7 (22.0)	62.7 (35.5)	17
	BI	62.6 (23.1)	48.7 (12.5)	17	62.6 (23.1)	48.7 (12.5)	17	60.7 (22.8)	48.7 (12.5)	16
Child Looking Extra-Dyadic Proportion	Ft	0.02 (0.02)	0.03 (0.02)	18	0.02 (0.02)	0.03 (0.02)	18	0.02 (0.02)	0.03 (0.02)	17
	TfR	0.03 (0.02)	0.02 (0.02)	18	0.03 (0.02)	0.02 (0.01)	17	0.03 (0.02)	0.02 (0.02)	17
	BI	0.03 (0.02)	0.03 (0.02)	17	0.03 (0.02)	0.03 (0.02)	17	0.03 (0.02)	0.03 (0.02)	16
Child Looking Dyadic Proportion	Ft	0.03 (0.04)	0.04 (0.06)	18	0.03 (0.04)	0.04 (0.06)	18	0.03 (0.04)	0.04 (0.06)	17
	TfR	0.04 (0.06)	0.02 (0.03)	18	0.04 (0.06)	0.02 (0.03)	18	0.04 (0.06)	0.02 (0.03)	17
	BI	0.04 (0.06)	0.01 (0.01)	17	0.04 (0.06)	0.01 (0.01)	17	0.04 (0.06)	0.01 (0.01)	16
Child Distress Vocalization Proportion	Ft	0.03 (0.07)	0.06 (0.12)	18	N/A	N/A	N/A	0.03 (0.07)	0.07 (0.13)	17
	TfR	0.06 (0.11)	0.00 (0.00)	18	N/A	N/A	N/A	0.06 (0.11)	0.00 (0.00)	17
	BI	0.06 (0.11)	0.00 (0.00)	17	N/A	N/A	N/A	0.06 (0.11)	0.00 (0.00)	16
Child Non-Distress Vocalization Proportion	Ft	0.08 (0.10)	0.11 (0.11)	18	0.08 (0.10)	0.11 (0.11)	18	0.08 (0.10)	0.08 (0.09)	17
	TfR	0.09 (0.10)	0.11 (0.13)	18	0.09 (0.10)	0.11 (0.13)	18	0.07 (0.08)	0.11 (0.13)	17
	BI	0.09 (0.10)	0.11 (0.13)	17	0.09 (0.10)	0.11 (0.13)	17	0.07 (0.09)	0.11 (0.13)	16
Joint Attention Proportion	Ft	0.20 (0.23)	0.31 (0.20)	18	0.20 (0.12)	0.31 (0.20)	18	0.20 (0.12)	0.28 (0.18)	17
	TfR	0.28 (0.19)	0.13 (0.13)	18	0.27 (0.19)	0.16 (0.16)	17	0.25 (0.16)	0.13 (0.13)	17
	BI	0.25 (0.17)	0.29 (0.25)	17	0.25 (0.17)	0.29 (0.25)	17	0.22 (0.14)	0.29 (0.25)	16
Mother Looking Extra-Dyadic Proportion	Ft	0.08 (0.07)	0.05 (0.04)	18	0.08 (0.07)	0.05 (0.04)	18	0.08 (0.07)	0.06 (0.04)	17
	TfR	0.06 (0.06)	0.10 (0.03)	18	0.06 (0.06)	0.09 (0.04)	17	0.06 (0.06)	0.10 (0.03)	17
	BI	0.06 (0.06)	0.06 (0.05)	17	0.06 (0.06)	0.06 (0.05)	17	0.07 (0.06)	0.06 (0.05)	16
Mother Looking Dyadic Proportion	Ft	0.03 (0.06)	0.04 (0.10)	18	0.03 (0.06)	0.04 (0.10)	18	0.03 (0.06)	0.05 (0.10)	17
	TfR	0.04 (0.09)	0.02 (0.02)	18	0.04 (0.09)	0.02 (0.02)	18	0.05 (0.09)	0.02 (0.02)	17
	BI	0.04 (0.09)	0.02 (0.03)	17	0.04 (0.09)	0.02 (0.03)	17	0.05 (0.09)	0.02 (0.03)	16
Maternal Dyadic Proportion	Ft	0.08 (0.10)	0.12 (0.12)	18	0.07 (0.10)	0.12 (0.12)	18	0.07 (0.10)	0.10 (0.11)	17
	TfR	0.11 (0.12)	0.07 (0.02)	18	0.11 (0.12)	0.07 (0.02)	18	0.10 (0.11)	0.07 (0.02)	17
	BI	0.12 (0.12)	0.09 (0.05)	17	0.12 (0.12)	0.09 (0.05)	17	0.09 (0.11)	0.09 (0.05)	16
Mother Latency to Respond Average	Ft	3.15 (1.46)	2.75 (1.35)	18	N/A	N/A	N/A	3.15 (1.46)	2.61 (1.34)	17
	TfR	3.10 (1.39)	2.92 (1.38)	18	N/A	N/A	N/A	3.03 (1.42)	2.92 (1.38)	17
	BI	3.06 (1.43)	2.79 (1.23)	17	N/A	N/A	N/A	2.98 (1.46)	2.79 (1.23)	16
Mother Nurturant Proportion	Ft	0.07 (0.08)	0.10 (0.11)	18	N/A	N/A	N/A	0.07 (0.08)	0.10 (0.12)	17
	TfR	0.08 (0.09)	0.01 (0.01)	18	N/A	N/A	N/A	0.09 (0.09)	0.01 (0.01)	17
	BI	0.09 (0.09)	0.00 (0.00)	17	N/A	N/A	N/A	0.09 (0.09)	0.00 (0.00)	16
Mother Imitative Proportion	Ft	0.00 (0.00)	0.00 (0.00)	18	N/A	N/A	N/A	0.00 (0.00)	0.00 (0.00)	17
	TfR	0.00 (0.00)	0.00 (0.00)	18	N/A	N/A	N/A	0.00 (0.00)	0.00 (0.00)	17
	BI	0.00 (0.00)	0.00 (0.00)	17	N/A	N/A	N/A	0.00 (0.00)	0.00 (0.00)	16

Model 1: null model; Model 2: controlled for covariates from Appendix A-1 and A-2; Model 3: controlled for all potential covariates

Stepwise Regressions

Multiple stepwise regression models were analyzed to predict the mother-infant interaction outcome by the variables listed in Table 1. There were six significant models when Ft and TfR were included in the model (excluding BI), with three being associated with maternal

outcomes and three with the infant. In the model including BI (excluding Ft and TfR), shown in Table 5, only the proportion of the mother looking extra-dyadic and the proportion of the mother looking dyadic were significant for the mother, with the same variables being significant for the child as in the model including Ft and TfR shown in Table 4.

The significant stepwise regression models for the infant included the proportion of time of the child looking extra-dyadic, the proportion of time of the child looking dyadic, and the proportion of time the child expressed non-distress vocalizations (Table 4). The stepwise regression model was significant in the model including Ft and TfR and the model including BI for the proportion of the child looking extra-dyadic, where it was positively associated with MUAC and Hb (Table 4, 5). The proportion of the child looking dyadic was positively associated with WAZ in the model including Ft and TfR and the model including BI (Tables 4, 5). The proportion of time the child expressed non-distress vocalizations was positively associated with WAZ and BI in the model including Ft and TfR and the model including BI (Table 4, 5).

The significant stepwise regression models for the mother included the proportion of time the mother was looking extra-dyadic, the proportion of time the mother was looking dyadic, and the proportion of time joint attention between the mother and infant occurred (Table 4, 5). The stepwise regression model for the proportion of the mother looking extra-dyadic was associated with Hb only in the model including Ft and TfR and the model including BI (Tables 4, 5). The proportion of the mother looking dyadic was positively associated with WAZ in the model including Ft and TfR and the model including BI (Tables 4, 5). The average maternal latency to respond time was negatively associated with TfR only, and was only significant in the model including Ft and TfR (Table 4).

Table 4. Stepwise Regression Models of Covariates with Mangold INTERACT Outcomes at Baseline (Ft and TfR)

Dependent Variable	Step	Predictor Variable	Parameter Estimate	r^2	Predictor p-value	Overall p-value	Overall r^2	Sample size (n)
Child Looking Extra-Dyadic Proportion	1	MUAC	0.00	0.53	**0.00**	**0.00**	0.68	16
	2	Hb	0.01	0.15	**0.03**			
Child Looking Dyadic Proportion	1	WAZ	0.03	0.27	**0.04**	**0.04**	0.56	16
	2	CRP	-0.02	0.15	0.09			
	3	Hb	0.03	0.15	0.07			
Child Non-Distress Vocalization Proportion	1	WAZ	-0.06	0.35	**0.02**	**0.02**	0.47	16
	2	BF	0.00	0.11	0.12			
Mother Looking Extra-Dyadic Proportion	1	Hb	0.03	0.25	**0.05**	**0.05**	0.25	16
Mother Looking Dyadic Proportion	1	WAZ	0.05	0.27	**0.04**	**0.04**	0.53	16
	2	CRP	-0.03	0.16	0.08			
	3	Hb	0.04	0.11	0.11			
Mother Latency to Respond Average	1	TfR	-0.42	0.31	**0.02**	**0.02**	0.31	16

MUAC=mean upper arm circumference; Hb=child hemoglobin; WAZ=weight for age Z-score; CRP=child C-reactive protein; BF=continuous days of breastfeeding; BI=child body iron; TfR=child transferrin receptor. All models were significant at $p < 0.05$

Table 5. Stepwise Regression Models of Covariates with Mangold INTERACT Outcomes at Baseline (BI)

Dependent Variable	Step	Predictor Variable	Parameter Estimate	r^2	Predictor p-value	Overall p-value	Overall r^2	Sample size (n)
Child Looking Extra-Dyadic Proportion	1	MUAC	0.00	0.53	**0.00**	**0.00**	0.68	16
	2	Hb	0.01	0.15	**0.03**			
Child Looking Dyadic Proportion	1	WAZ	0.03	0.27	**0.04**	**0.04**	0.56	16
	2	CRP	-0.02	0.15	0.09			
	3	Hb	0.03	0.15	0.07			
Child Non-Distress Vocalization Proportion	1	WAZ	-0.06	0.35	**0.02**	**0.02**	0.57	16
	2	BF	0.00	0.11	0.12			
	3	BI	-0.01	0.10	0.11			
Mother Looking Extra-Dyadic Proportion	1	Hb	0.03	0.25	**0.05**	**0.05**	0.25	16
Mother Looking Dyadic Proportion	1	WAZ	0.05	0.27	**0.04**	**0.04**	0.53	16
	2	CRP	-0.03	0.16	0.08			
	3	Hb	0.04	0.11	0.11			

MUAC=mean upper arm circumference; Hb=child hemoglobin; WAZ=weight for age Z-score; CRP=child C-reactive protein; BF=continuous days of breastfeeding; BI=child body iron; TfR=child transferrin receptor. All models were significant at $p < 0.05$

Chapter 4: Discussion and Conclusion

Interpretation of Findings

This is the first study, to our knowledge, to focus on the relationship between infant iron status and microanalytical coding outcomes in rural Bangladesh. We hypothesized that microanalytically coded interactions would differ in an infant with impaired iron status compared to an infant with sufficient iron status. It was expected that in an infant with ID, the interaction would be less optimal. We did not see meaningful differences between infant iron status groups when using Ft, TfR, and BI and Mangold INTERACT outcomes at baseline. A potential explanation for these null findings is likely the small sample size of dyads overall and an even smaller sample size for each iron status group (ID/IS). When looking at maternal iron status, they were mainly iron sufficient at baseline with only 13% being ID, so we were not able to assess the outcome by maternal iron status nor to control for it. In contrast, infants were 56% ID and 44% IS at baseline as indicated by Ft, which allowed us to conduct group difference analyses. All infants in this sample were classified as anemic when assessed by Hb. As such, we were not able to look at group difference by anemia status.

Maternal Mangold INTERACT Outcomes

This study makes an important contribution by showing that infant iron status did not influence maternal or child Mangold INTERACT outcomes but the results should be interpreted with caution due to our small sample size. Factors associated with these outcomes were evaluated by stepwise regressions. The proportion of the mother looking extra-dyadic was positively related to infant hemoglobin such that the higher the infant hemoglobin, the more time the mother spent looking extra-dyadically. This accounted for 25% of the variance. The mean proportion of the mother looking extra-dyadic ranged from 0.05-0.08 seconds at baseline. This

accounted for a small proportion of the video, between 5 and 8%. This means that the mother was not looking away from the infant for large amounts of time, which is beneficial to the infant. Infant WAZ influenced the proportion of the mother looking dyadic, such that a higher WAZ was related to a larger proportion of time the mother spent looking dyadic. This accounted for 27% of the variance. The mean proportion of the mother looking dyadic ranged from 0.07-0.12 seconds, or 7-12% of the video. This represents the amount of time the mother was engaged in dyadic visual attention towards the infant. A higher proportion of the mother looking dyadic is indicative of a more engaged mother. The average maternal latency to respond time was influenced by child TfR, such that a lower TfR, indicating more sufficient iron status, was related to a longer average time period that it took for the mother to respond to the infant. This accounted for 31% of the variance. The average ranged from 2.61-3.15 seconds per video. A greater latency to respond time is associated with a mother who is less attentive to her infant's cues and behaviors. On average, the average maternal latency to respond time accounted for less than 1% of the total video which represents that the mothers were responsive to their infant. The rest of the maternal Mangold INTERACT outcomes were not associated with any of the included factors.

Child Mangold INTERACT Outcomes

The proportion of the child looking extra-dyadic was affected by MUAC and infant Hb, such that the higher the infant MUAC or the higher the infant Hb, the more time the child spent looking extra-dyadic. These accounted for 68% of the variance. The mean proportion of the child looking extra-dyadic ranged from 0.02-0.03, or 2-3% of the video. This is indicative of the child remaining focused on the interaction instead of gazing away from the mother. Infant WAZ influenced the proportion of the child looking dyadic, such that a higher WAZ was related to a

greater proportion of time the child looked dyadic. This accounted for 27% of the variance. The mean proportion of the child looking dyadic ranged from 0.01-0.04, or 1-4% of the video. This represents the amount of time the infant looked directly at the mother's face, which was not often. One possible explanation for this is that the mothers were instructed to keep the infant facing the camera, so any time the infant turned towards the mother, the response was to turn the infant back. Infant WAZ influenced the proportion of time the child expressed non-distress vocalizations, such that a child with a lower WAZ expressed more non-distress vocalizations throughout the interaction. This accounted for 35% of the variance. The mean proportion of non-distress vocalizations ranged from 0.50-0.80, or 5-8% of the video. Non-distress vocalizations were coded when the child babbled to himself or herself or the mother. On average, all of the infants expressed non-distressed vocalizations at some point of the mother-infant interaction.

Comparisons with Previous Research and Speculations

Overall, the mothers in this sample were engaging during the filmed interaction, and were distracted from the dyad for a small proportion of the total video, as indicated by looking extra-dyadic. Considering their biological iron status, the mothers were mainly IS, with only 13% ID as indicated by Ft. In the South African study conducted by Murray-Kolb and Beard, it was observed that control (iron sufficient) and iron supplemented mothers no longer differed in the way they interacted in terms of being more sensitive and exhibiting reduced intrusive and hostile behavior towards their infants post-supplementation (Murray-Kolb and Beard, 2009). These findings may explain why of the mothers in our sample had mostly positive interactions with their infants. However, a direct comparison cannot be made as mothers in the current study were not supplemented, and a microanalytical coding scale was used instead of a global coding scale, which was used in the Murray-Kolb and Beard study. In a study conducted by Armony-Sivan

and colleagues, it was found that mothers with IDA infants were less sensitive to their infant's cues and provided less cognitive and social-emotional fostering than non-IDA infants (Armony-Sivan et al. 2010). While these findings cannot be directly translated to the microanalytical coding scheme used in this study, they may suggest that mothers with IDA infants would have a greater average latency to respond time.

The infants in this sample were largely responsive to their mothers as expressed via looking dyadically and non-distress vocalizations such as babbling. They also involved their mothers in the interaction by playing with toys together. This was the case even though all of the infants were anemic as indicated by Hb, but only 56% were ID by Ft. Similarly, in the Murray-Kolb and Beard study, it was observed that control and iron supplemented mothers no longer differed in the responsiveness of their child throughout the interaction. We speculate that the sufficiency of the mothers in this study had a positive impact on the way the child responded to the mother, despite the 56% ID rate of the infants.

A potential explanation for the optimal outcomes may be attributed to exclusive days of BF, with an average of 126 days being observed within the dyads. A study by Jonas and colleagues found that mothers who breastfed at three months postpartum were more sensitive to their infants in interactions six months postpartum, which predicted reduced levels of negative affectivity in infant temperament at 18 months postpartum (Jonas et al. 2015). Although the current study did not see any differences by iron status, Hb and TfR were significant predictors in the proportion of the child looking extra-dyadic, the proportion of the mother looking extra-dyadic and the average maternal latency to respond stepwise regression models. This suggests that anemia and iron status may be associated with some of the Mangold INTERACT outcomes used in this study.

Study Strengths and Limitations

This study has several strengths, the first one being the use of multiple iron biomarkers to assess infant and maternal iron status. Additionally, the use of the microanalytical coding approach allowed for the detection of subtle interaction details that occur within the mother-infant dyad. Another strength of this study is that it collected and accounted for relevant SES and caregiving characteristics that are known to influence mother-infant interactions, such as assets, the number of people in the household, maternal reasoning ability, days of exclusive breastfeeding, and infant anthropometry. This study contributes to the body of evidence surrounding iron status and mother-infant interactions. Specifically, it builds upon the Murray-Kolb and Beard study conducted in South Africa (Murray-Kolb and Beard, 2009).

Limitations of this study must also be considered. There was a small sample size of only 20 mother-infant dyads with mother-infant interaction footage, with one being excluded for inadequate interaction time at baseline. This may be the reason for no group differences being observed by iron status. All of the 19 mothers did not have CES-D data at baseline, so we were not able to control for maternal depressive symptoms, which are known to negatively impact the dyadic interaction. In addition, maternal iron status was not controlled for due to four of the 19 mothers included in the study having no iron biomarker data. However, the mothers in the sample were mainly iron sufficient at baseline, which prevented us from making ID and IS groups. Therefore, we could not assess Mangold INTERACT outcomes by maternal iron status.

Overall Conclusions and Future Directions

Although this study was limited by multiple factors, we were able to identify that this sample of mother-infant dyads had largely positive interactions when assessed microanalytically. While it was hypothesized that coded interactions would differ in an infant with impaired iron

status compared to an infant with sufficient iron status such that the interaction would be less optimal, few differences were seen between child iron status groups at baseline. The interactions were largely optimal, with the mother and infant exhibiting a relationship in which both are responding positively to the other. As all of the mothers in this sample were IS, it is thought that this may have positively influenced the interaction. Furthermore, significance was found among the Mangold INTERACT data when using child iron biomarker, child anthropometry, and demographic data. Significant Mangold INTERACT outcomes for the infants included the proportions of looking extra-dyadic, looking dyadic, and non-distress vocalizations. Significant Mangold INTERACT outcomes for the mothers included the proportions of looking extra-dyadic and exhibiting dyadic behavior, and the average maternal latency to respond time. The findings in this study should be replicated with a larger sample size to assess if the significance of the Mangold INTERACT outcomes for the mothers and their infants remain the same. Future studies should assess maternal depressive symptoms if possible for potential inclusion as a covariate and maternal iron biomarker data. Samples with greater variability in iron status should be considered in order to assess mother-infant interactions among IS, ID, and IDA groups. Assessing these videos with a global coding scale and the comparison of statistical outcomes may be an interesting way to evaluate the effectiveness of using a microanalytical coding scheme to assess mother-infant interaction.

The results observed in this study represent the first assessment of the relationship between microanalytical coding outcomes and infant iron status in a sample from rural Bangladesh. Policymakers and educators should consider that infant anthropometrics and anemia and iron status were important contributors for the dyadic relationship in this sample, such as the greater proportion of the dyadic interactions with higher MUAC, WAZ, Hb, and lower TfR.

Appendix A-1: Identification of Anthropometric and Demographic Covariates

Pearson Correlation Coefficients
Prob > |r| under H0: Rho=0
Number of Observations

	cage	csex	waz	muac	bf	hhsize	ucode	asset	ravtscore2	TotalEvents	CL_ED_st	CL_D_st	C_DV_st	C_NDV_st	JA_st	ML_ED_st	ML_D_st	MD_st	M_LR_avg	M_N_st	M_I_st
cage	1																				
	19																				
csex	0.13933	1																			
	0.5694																				
	19	19																			
waz	-0.39726	-0.32613	1																		
	0.0921	0.173																			
	19	19	19																		
muac	-0.22668	-0.583	0.78873	1																	
	0.3507	0.0088	<.0001																		
	19	19	19	19																	
bf	0.07741	0.37058	-0.05631	-0.33862	1																
	0.7601	0.1301	0.8244	0.1693																	
	18	18	18	18	18																
hhsize	0.69704	-0.03529	-0.06616	0.06604	0.11302	1															
	0.0009	0.8859	0.7879	0.7882	0.6552																
	19	19	19	19	18	19															
ucode	0	-0.32757	-0.00236	0.18448	-0.14699	0.02001	1														
	1	0.171	0.9924	0.4496	0.5605	0.9352															
	19	19	19	19	18	19	19														
asset	0.32869	0.15547	0.08548	0.27353	-0.40047	0.42607	0.0287	1													
	0.1694	0.5251	0.7279	0.2572	0.0996	0.0689	0.9072														
	19	19	19	19	18	19	19	19													
ravtscore2	0.11847	0.54041	0.17687	-0.12912	0.18809	0.03921	-0.06213	0.09674	1												
	0.6291	0.0169	0.4688	0.5983	0.4548	0.8734	0.8005	0.6936													
	19	19	19	19	18	19	19	19	19												
TotalEvents	0.23027	-0.07693	0.00049	0.00777	0.19217	0.39294	0.09173	0.23369	-0.05966	1											
	0.3429	0.7543	0.9984	0.9748	0.4449	0.0961	0.7088	0.3356	0.8083												
	19	19	19	19	18	19	19	19	19	19											
CL_ED_st	-0.05303	-0.21277	0.67208	0.56605	0.24631	-0.10296	0.06062	-0.1479	0.17676	-0.19905	1										
	0.8293	0.3818	0.0016	0.0115	0.3245	0.6749	0.8053	0.5457	0.4691	0.4139											
	19	19	19	19	18	19	19	19	19	19	19										
CL_D_st	-0.30968	-0.01798	0.53413	0.29055	0.17231	-0.08245	-0.27145	-0.15526	0.0821	0.28433	0.24364	1									
	0.197	0.9418	0.0185	0.2275	0.4941	0.7372	0.2609	0.5256	0.7383	0.2381	0.3148										
	19	19	19	19	18	19	19	19	19	19	19	19									
C_DV_st	0.2402	0.0118	-0.1116	-0.22187	0.15954	0.02142	-0.19208	-0.27687	0.13118	0.12636	0.12689	-0.06128	1								
	0.3219	0.9618	0.6363	0.3613	0.5271	0.9306	0.4308	0.2512	0.5924	0.6062	0.6047	0.8032									
	19	19	19	19	18	19	19	19	19	19	19	19	19								
C_NDV_st	0.57656	0.14854	-0.59747	-0.46079	0.31889	0.39347	0.25328	0.01694	-0.03839	0.46411	-0.32137	-0.28896	-0.04581	1							
	0.0098	0.5439	0.0069	0.0471	0.1971	0.0956	0.2955	0.9451	0.876	0.0453	0.1797	0.2302	0.8523								
	19	19	19	19	18	19	19	19	19	19	19	19	19	19							
JA_st	0.27928	0.06284	0.1865	0.08937	-0.18184	0.39621	0.10405	0.42833	0.33232	0.21715	-0.2393	0.14348	-0.40482	0.20013	1						
	0.2469	0.7983	0.4446	0.716	0.4702	0.0931	0.6716	0.0673	0.1645	0.3719	0.3238	0.5579	0.0856	0.4114							
	19	19	19	19	18	19	19	19	19	19	19	19	19	19	19						
ML_ED_st	-0.10027	-0.14776	0.07656	0.33086	0.22753	0.06161	0.17547	0.06572	-0.31919	-0.15979	0.45051	-0.11738	-0.06636	-0.1446	-0.59711	1					
	0.683	0.5461	0.7554	0.1665	0.3639	0.8021	0.4724	0.7892	0.1829	0.5135	0.0529	0.6322	0.7872	0.5548	0.0069						
	19	19	19	19	18	19	19	19	19	19	19	19	19	19	19	19					
ML_D_st	-0.25219	-0.12232	0.52649	0.32467	0.0864	-0.03259	-0.18025	-0.12983	0.05791	0.19619	0.27529	0.95293	-0.16225	-0.23044	0.25835	-0.11157	1				
	0.2976	0.6179	0.0206	0.175	0.7332	0.8946	0.4603	0.5963	0.8138	0.4208	0.254	<.0001	0.5069	0.3426	0.2855	0.6493					
	19	19	19	19	18	19	19	19	19	19	19	19	19	19	19	19	19				
MD_st	0.4284	-0.00288	-0.3151	-0.29384	0.14463	0.26584	0.20169	0.12685	0.04642	0.64302	-0.38962	-0.05472	-0.1413	0.72768	0.48944	-0.53437	-0.04953	1			
	0.0673	0.9907	0.1888	0.2221	0.5669	0.2713	0.4077	0.6048	0.8503	0.003	0.0992	0.8239	0.5639	0.0004	0.0334	0.0184	0.8404				
	19	19	19	19	18	19	19	19	19	19	19	19	19	19	19	19	19	19			
M_LR_avg	0.21607	0.24666	-0.13929	-0.09736	-0.01424	0.15261	-0.2453	0.16763	0.32914	0.13511	-0.22107	-0.12756	0.09679	0.15371	-0.00599	-0.24246	-0.29236	0.30673	1		
	0.3743	0.3087	0.5696	0.6917	0.9553	0.5328	0.3114	0.4927	0.1688	0.5813	0.3631	0.6028	0.6935	0.5298	0.9806	0.3172	0.2245	0.2015			
	19	19	19	19	18	19	19	19	19	19	19	19	19	19	19	19	19	19	19		
M_N_st	-0.10044	-0.15011	0.16341	0.00948	0.16212	-0.02766	0.04135	-0.2994	-0.17726	0.07098	0.02481	0.21131	0.51511	-0.17728	-0.09822	-0.05381	0.13516	-0.22464	-0.10404	1	
	0.6824	0.5396	0.5039	0.9693	0.5204	0.9105	0.8665	0.213	0.4678	0.7728	0.9197	0.3852	0.024	0.4678	0.6891	0.8268	0.5812	0.3552	0.6717		
	19	19	19	19	18	19	19	19	19	19	19	19	19	19	19	19	19	19	19	19	
M_I_st	0.23233	0.10201	-0.05838	0.13032	0.04167	0.03105	0.07258	-0.00979	0.22044	0.33901	0.12	-0.03027	0.28198	0.15529	-0.14297	0.06574	-0.14864	0.1213	0.37192	0.1579	1
	0.3385	0.6777	0.8123	0.5949	0.8696	0.8996	0.7678	0.9683	0.3645	0.1556	0.6246	0.9021	0.2422	0.5256	0.5593	0.7892	0.5436	0.6208	0.1169	0.5185	
	19	19	19	19	18	19	19	19	19	19	19	19	19	19	19	19	19	19	19	19	19

Appendix A-2: Identification of Iron Status Covariates

Pearson Correlation Coefficients
Prob > |r| under H0: Rho=0
Number of Observations

	ctfr_1	ccrp_1	cAdjFt_1	CBIC_1	hb_1	TotalEvents	CL_ED_st	CL_D_st	C_DV_st	C_NDV_st	JA_st	ML_ED_st	ML_D_st	MD_st	M_LR_avg	M_N_st	M_I_st
ctfr_1	1																
	18																
ccrp_1	0.15912	1															
	0.5418																
	17	18															
cAdjFt_1	-0.66854	0.14231	1														
	0.0033	0.5732															
	17	18	18														
CBIC_1	-0.81196	0.06345	0.94971	1													
	<.0001	0.8088	<.0001														
	17	17	17	17													
hb_1	0.04758	0.34559	0.21235	0.21645	1												
	0.8513	0.1601	0.3976	0.404													
	18	18	18	17	19												
TotalEvents	-0.02376	-0.37507	-0.00709	0.08691	0.31088	1											
	0.9254	0.1251	0.9777	0.7402	0.1952												
	18	18	18	17	19	19											
CL_ED_st	0.39281	0.46592	-0.23575	-0.40285	0.22405	-0.19905	1										
	0.1068	0.0513	0.3463	0.1089	0.3565	0.4139											
	18	18	18	17	19	19	19										
CL_D_st	0.0482	-0.16846	0.03486	-0.02422	0.26662	0.28433	0.24364	1									
	0.8494	0.504	0.8908	0.9265	0.2699	0.2381	0.3148										
	18	18	18	17	19	19	19	19									
C_DV_st	-0.28071	-0.01643	0.02968	0.15749	0.02016	0.12636	0.12689	-0.06128	1								
	0.2592	0.9484	0.9069	0.5461	0.9347	0.6062	0.6047	0.8032									
	18	18	18	17	19	19	19	19	19								
C_NDV_st	-0.20281	-0.22219	0.10875	0.18888	0.10825	0.46411	-0.32137	-0.28896	-0.04581	1							
	0.4196	0.3755	0.6675	0.4678	0.6591	0.0453	0.1797	0.2302	0.8523								
	18	18	18	17	19	19	19	19	19	19							
JA_st	-0.18924	-0.40293	-0.2608	-0.2177	-0.17915	0.21715	-0.2393	0.14348	-0.40482	0.20013	1						
	0.452	0.0973	0.2959	0.4013	0.463	0.3719	0.3238	0.5579	0.0856	0.4114							
	18	18	18	17	19	19	19	19	19	19	19						
ML_ED_st	0.41671	0.41183	-0.00115	-0.01604	0.31283	-0.15979	0.45051	-0.11738	-0.06636	-0.1446	-0.59711	1					
	0.0854	0.0895	0.9964	0.9513	0.1922	0.5135	0.0529	0.6322	0.7872	0.5548	0.0069						
	18	18	18	17	19	19	19	19	19	19	19	19					
ML_D_st	0.12022	-0.19429	-0.04243	-0.11887	0.19913	0.19619	0.27529	0.95293	-0.16225	-0.23044	0.25835	-0.11157	1				
	0.6347	0.4398	0.8672	0.6495	0.4138	0.4208	0.254	<.0001	0.5069	0.3426	0.2855	0.6493					
	18	18	18	17	19	19	19	19	19	19	19	19	19				
MD_st	-0.29846	-0.2911	0.1194	0.11715	-0.00208	0.64302	-0.38962	-0.05472	-0.1413	0.72768	0.48944	-0.53437	-0.04953	1			
	0.229	0.2412	0.637	0.6543	0.9933	0.003	0.0992	0.8239	0.5639	0.0004	0.0334	0.0184	0.8404				
	18	18	18	17	19	19	19	19	19	19	19	19	19	19			
M_LR_avg	-0.45847	0.18024	0.29439	0.3312	0.13016	0.13511	-0.22107	-0.12756	0.09679	0.15371	-0.00599	-0.24246	-0.29236	0.30673	1		
	0.0557	0.4742	0.2357	0.1941	0.5953	0.5813	0.3631	0.6028	0.6935	0.5298	0.9806	0.3172	0.2245	0.2015			
	18	18	18	17	19	19	19	19	19	19	19	19	19	19	19		
M_N_st	-0.38013	0.11681	-0.00422	0.30665	-0.12703	0.07098	0.02481	0.21131	0.51511	-0.17728	-0.09822	-0.05381	0.13516	-0.22464	-0.10404	1	
	0.1197	0.6444	0.9867	0.2312	0.6043	0.7728	0.9197	0.3852	0.024	0.4678	0.6891	0.8268	0.5812	0.3552	0.6717		
	18	18	18	17	19	19	19	19	19	19	19	19	19	19	19	19	
M_I_st	-0.16776	-0.18127	-0.17586	0.05764	-0.01448	0.33901	0.12	-0.03027	0.28198	0.15529	-0.14297	0.06574	-0.14864	0.1213	0.37192	0.1579	1
	0.5058	0.4716	0.4852	0.8261	0.9531	0.1556	0.6246	0.9021	0.2422	0.5256	0.5593	0.7892	0.5436	0.6208	0.1169	0.5185	
	18	18	18	17	19	19	19	19	19	19	19	19	19	19	19	19	19

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Academic Vita

Marin Nagelberg

EDUCATION

The Pennsylvania State University, Schreyer Honors College — **University Park, PA**
Nutritional Science Major (Basic Sciences Option), Neuroscience Minor — *May 2019*
Dean's List Fall 2015 - Fall 2018

WORK EXPERIENCE

Pearle Vision — **Commack, NY**
Receptionist, Salesperson, Technician — *August 2014 – August 2018*

- Assist customers in selecting frames and lenses based upon prescription, price total frame and lens packages, sell and distribute contacts, answer phone calls, schedule appointments
- Pre-test patients for their appointment utilizing the auto-refractor and taking fundus photography
- Execute training of first time contact lens wearers

Sole Provisions — **Ronkonkoma, NY**
Assistant to Comptroller — *June 2012 – August 2016*

- Arranged invoices from vendors and processed payments from customers using QuickBooks

LEADERSHIP EXPERIENCE

Peers Helping Reaffirm, Educate, and Empower (PHREE) — **University Park, PA**
President — *August 2017 – Present*

- Develop programs to present to students about topics pertaining to sexual assault and resources
- Collaborate with student government to host Sexual Violence Awareness and Prevention Week and Red Zone Action Week to raise awareness about sexual assault statistics at Penn State

Greeks Care — **University Park, PA**
Facilitator — *September 2016 – November 2018*

- Delivered six-week program each semester to members of Greek Life discussing topics including sexual assault, gender roles, barriers to reporting, and resources on campus

HealthWorks Peer Educators — **University Park, PA**
Health Disparities Initiative Co-Lead — *January 2016 – December 2018*

- Presented workshops encouraging healthy habits including good nutrition, stress reduction, and getting enough sleep
- Worked to plan events to increase awareness of health disparities present at Penn State

Dancer Relations Committee — **University Park, PA**
Member, THON 2017, 2018, 2019 — *October 2016 – February 2019*

- Supported individual dancer during 46-hour annual dance marathon using proper health and safety procedures learned during weekly meetings

Sigma Delta Tau — **University Park, PA**
Standards Chairwoman — *November 2016 – November 2017*

- Enforced sorority bylaws by working with executive board members to ensure members followed expectations
- Formed a standards board and conducted monthly meetings for assistance in monitoring the chapter

RESEARCH EXPERIENCE

Dr. Laura Murray-Kolb Laboratory — **University Park, PA**
Research Assistant — *August 2016-Present*

- Study consequences of iron deficiency on mothers and children in developing countries via analysis of biochemical data, emotional response and cognitive ability

Dr. Gregory Shearer Laboratory — **University Park, PA**
Research Assistant — *January 2016 – April 2016*

- Analyzed the impact of diet on the production of lipid mediators based on polyunsaturated fatty acid consumption by preparing assays and quantifying fatty acids using GC-MS