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IRON AND ZINC SUPPLEMENTATION AND THE EFFECTS ON MOTHER-INFANT INTERACTION

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ABSTRACT

Objective: The objective of this thesis was to investigate the effects of supplementing children ages 6-18 months with iron and zinc on mother-infant interaction.

Methods: This study is a double-blind, randomized controlled trial. Infants aged 6-18 months were recruited from a larger study investigating iron, zinc and diarrhea outcomes. Infants were randomized into the following supplementation groups: iron alone, zinc alone, iron and zinc in combination and iron and zinc separately. They were followed up at 3 and 6 months post-baseline and their mother-infant interaction was evaluated using the Emotional Availability Scales. An intent-to-treat analysis was conducted, comparing groups at each time-point (amount of change over time was not analyzed for this thesis). Blood data were taken at baseline and 6 months, but, as this was an intent-to-treat analysis, were not included in the present analysis. Socio-economic status, child age, and sex were controlled in all analyses.

Results: At 3 months, infants supplemented with iron and zinc in combination performed significantly better on the Child Involvement sub-scale than those supplemented with placebo ($p<0.05$), zinc-alone ($p<0.001$), and iron and zinc supplemented separately ($p<0.01$). Infants supplemented with iron-alone performed better than those supplemented with zinc-alone ($p<0.05$). At 3 months, child supplementation with iron and zinc in combination and with iron alone had an effect on Maternal Sensitivity such that both of these groups performed better than the zinc alone infants ($p<0.05$). At 6 months, effects of supplementation on mother-infant interaction were not evident.

Conclusions: At least transiently, when children were supplemented with iron and zinc in combination, child involvement with the parent improved. Interestingly, although mothers were not supplemented, maternal sensitivity improved as well.

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Chapter 1

Introduction

Nutrition plays an important role in child development. One hypothesis states that nutrition could influence the ability of the child to engage with the care-giver environment and thus affect child development. This thesis aims to investigate the effects of iron and zinc supplementation and their influence on the quality of mother-infant interaction. It is well understood that mother-infant interaction affects child development and, that child development in all countries, both developed and developing, is a crucial concern.

In general, child development is directly influenced by family socioeconomic status and follows a "social gradient." However, a low socio-economic status doesn't always predict poor child development outcomes if there are quality programs and interventions in place to act as moderators. For example, Cuba, although regarded as being a relatively impoverished country, has surprisingly good school outcomes. Upon closer look, it is not surprising as it provides remarkable early child development programs. Cuba's Minister of Education attributes the success to their early child development centers and the fact that almost every child attends one.[1]

Further, by investing in child development, not only do the individuals of the nations benefit but the nation as a whole can experience large economic returns. In the High/Scope Perry Preschool Program in the United States, high risk children were randomized into a high quality preschool program or a regular one. At 40 years of age, significantly more of those who attended the high quality preschool program were employed, had higher salaries, and had savings accounts. On a national level, this intervention yielded economic returns of $195,261 per participant to the general public, while the actual investment cost was $15,166 —that results in a return of $12.90 per dollar spent.[2]

Although programs supplying direct psychosocial stimulation have been shown to positively influence child development, effective interventions that improve long-term human development and functional outcomes need not be limited to these types of programs. Micronutrient deficiencies like iron and zinc are prevalent in lower income countries. Iron deficiency affects an estimated 2 billion people worldwide and results in health and economic costs.[3] While the lack of zinc biomarkers makes it more difficult to accurately measure, some regions of the world are predicted to have high rates of zinc deficiency: up to 71.2% of their nation.[4] By intervening nutritionally at an early age, long term effects on development have been observed. In her longitudinal study of Costa Rican infants, Lozoff et al. found that the infants whose iron status remained deficient after three months of iron therapy had significantly worse developmental scores growing up and ultimately demonstrates worse functional outcomes at 25 years compared to those who, as infants, were not iron deficient or whose iron status recovered after iron therapy.[5] In Walker et al.'s study, stunted infants were supplemented with a milk-based formula in addition to their mothers receiving a mother-interaction intervention for two years, supplemented alone or stimulated alone.[6] In late adolescence, infants that were stunted before two years of age exhibited poorer emotional and behavioral outcomes.[7] While supplementation was found to have no effect 20 years later, the mother-infant interaction intervention reduced violent behavior, and was related to less depression and social inhibition.[8] In an analysis of the overall contributions of the intervention to society, it was found that those who participated in the mother-infant psychosocial stimulation intervention were had 50% higher earnings than those who were not stunted and did not receive the stimulation. Because supplementation alone showed no long-term effects, all children who were psychosocially stimulated regardless of if they were supplemented as well were included in the analysis.[9] Even though no nutrition-specific effects were found, the effect of stimulation on behavioral outcomes could have been mediated by supplementation.

More research is needed to isolate the effect of child nutrition on mother-infant interaction. While iron and zinc status have been linked to brain and child development, the compounding variable of mother-infant interaction has not been isolated to further paint the picture of the many ways nutrition can affect child development. Figure 1 depicts a hypothesized pathway that iron and zinc deficiency could take to influence development mediated by mother-infant interaction. There is a need for placebo controlled studies to determine the effects of iron and zinc on mother-infant interaction and to develop successful interventions and policies. Specifically, intent to treat analyses are needed to guide policy makers. Assessing micronutrient status isn't always possible but providing a safe supplement might be more achievable.

Because iron and zinc are crucial for brain development, and because it has been observed that nutritional status changes a child's affect, the social interaction with their mother may also be affected and could change the quality of their interaction. However, zinc and iron may compete for absorption and less effectively improve biochemical and behavioral outcomes. Therefore, we hypothesize that when children are supplemented with iron and zinc in separation, the quality of mother-infant interaction will improve to a greater degree than when they are supplemented in combination or individually.

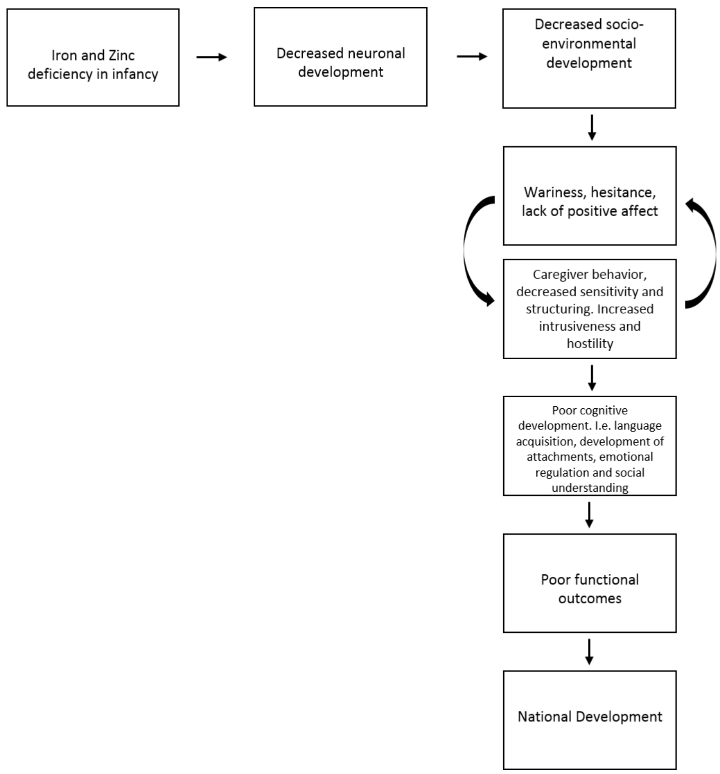

Figure 1: How iron deficiency, zinc deficiency, mother-infant interaction and cognitive development may affect national development

Chapter 2

Literature Review

I. Mother-Infant Interaction and Child Development

Mother-Infant interaction has many components that can have an effect on different domains of cognitive functioning. Each of these components could be influenced by nutritional status, and could be one way that nutritional status can dictate the course of a child's development. The Emotional Availability Scales used in this study outline the following components of mother-infant interaction: parental inputs: sensitivity, structuring, non-intrusiveness, non-hostility, and child inputs: responsiveness to parent and involvement with parent. [10] The emotional availability scales will be used as a framework to explain the various tangible effects of parent-infant interaction such as promotion of social understanding, development of attachments, acquisition of language and the maturity of emotional understanding.[11]

Child Responsiveness and Child Involvement with the Parent

It is important to conceptualize mother-infant interaction not as a mother molding her child, but as a reciprocal action. Several theories and models exist that explain the reciprocal action. One, the transactional principle, states that “infants both shape and are shaped by their experiences with parents in a reciprocal manner.”[12] Another, the Mutual Regulation Model, assumes that the infant is motivated to communicate with people and engage with objects. Engagement with the world will produce normal development while disengagement with the world will disrupt development. Two of the three processes required for successful engagement and thus development are involved with the integrity of the child's biological systems, behavior and communication system. Only the third process involves the capacity of the caretaker to respond to the infant.[13]

In a study measuring the behavior of iron deficient anemic children, even though the tester was blind to the child's nutrition status, the tester treated the iron-deficient anemic children differently than the iron sufficient children. Similarly, the care-givers showed less pleasure in the child, gave them less affection during a motor test, and provided less encouragement. [14] This kind of interaction with children could prevent children from developing to their full potential. Infant temperament is also know to affect how a caretaker responds to an infant.[15] In general, how healthy and developed the child is affects parental behavior.[15] Most certainly, there are nutritional factors involved in the health and socio-emotional affect of a child, thus dictating different parental affect in return. There is a need for additional studies involving both the mother and infant inputs to interaction in the context of nutrition.

Parental Sensitivity and Parental Structuring

Parental Structuring is the degree of attempts to set rules for play and make a point to follow the child's lead in play. Parental Sensitivity encompasses total affect of the parent, their responsiveness, and the accessibility and flexibility of their interaction.[10] These constructs go hand in hand. The caretaker who effectively structures play will be sensitive to their child's developmental progress in their attempts.[16]

Parent-infant interaction is undoubtedly related to attachment, and parental sensitivity is widely regarded as one mechanism for the development of attachments, as Bowlby and Ainsworth assert that infants become attached to those who provide "consistent, predictable, and appropriate responses to the baby's signals, as well as to their needs." [13] Infants who are more securely attached to their caregiver have more freedom to explore and interact with their environment and other people beyond their caretakers.[18] Stemming from Ainsworth's Strange Situation experiment, many people have investigated, in particular, which parental behaviors predict attachment. While studies have shown that sensitivity at an early age can predict the security of attachment later in infancy and toddlerhood,[19,20] a meta-analysis of parental inputs and attachments confirms that sensitivity is important for developing attachments yet it is not the only, or most important parental input, and point to parental synchrony and mutuality, and also infant temperament.[21] Reflecting this complexity, the Emotional Availability scales are rooted in the integration of emotional availability with attachment and attachment is woven throughout each construct of the scales beyond sensitivity.[10]

Social understanding and learning are processes that require joint problem solving, an interactive process between the caregiver and the infant, which is, essentially, Vygotsky's "zone of proximal development." Parental structuring is thus the attempt to teach infants and children tasks with guidance or "scaffolds" so that the "scaffolds" can be lessened or removed completely. [22]

Parental sensitivity has also been shown to have an effect on the infant's language acquisition as it is accelerated by maternal responsiveness to the infants' exploration of the environment[12,23]. A proposed mechanism is a structuring technique, where the bouts of joint attentiveness are characterized by interaction initiated either by the mother or child, where both are visually focusing on an object, and the infant is aware of the mother's interaction with her.[24] Tomasello et. al showed that when mothers responded to their infants attention to various objects instead of directing their infant's attention elsewhere within bouts of joint attentiveness at 15 months, it correlated with the infant's size of vocabulary at 21 months. Object references outside of joint attentiveness did not correlate with vocabulary size.[23]

Others have shown that mother's responsiveness can predict achievement of language milestones through attuned responses.[19,20] For example, Nicely et al. measured how mothers' attuned or non-attuned responses to infant expressivity at 9 months predicted language milestones at 13 months. Mothers' matching responses, a form of attuned responses, predicted age at 50 words of production and age of combinatorial speech.[19]

Parental Nonintrusiveness and Nonhostility

Parental intrusiveness is the overdirectedness and interference that could interfere with the child's autonomy. Intrusiveness could be thought of as over structuring and scaffolding, to the point where there is no space for the child to lead. A parent is considered to be hostile when the nature of the interaction with the infant is threatening and frightening, even sometimes in fantasy or pretend situations.[10] Depression may also be a consideration in maternal intrusiveness and hostility as those behaviors have been correlated with maternal depressive scores.[27]

The effect of taking over a child's autonomy can also manifest itself in parental intrusiveness influencing the development of emotional understanding and regulation.

Grolnick et al. demonstrated how mother-infant interaction can influence toddler distress. Food and gifts were presented and then withheld from the child. They found that when mothers over engaged with their child as they dealt with the delay before they could get their gift or food, it undermined the child's attempt at regulating their own emotions.[28]

Parental intrusiveness has also been associated with less secure attachments. In a study using the Emotional Availability scales, children who were more insecure had mothers who demonstrated higher levels of intrusiveness in the Strange Situation.[29] In the “Watch, Wait and Wonder” intervention program, mothers were instructed to allow their child to lead, and interact only at the child's initiative. Mothers who received Psychodynamic Psychotherapy were instructed to talk with the therapist as the mother and infant played. Mothers assigned to the “Watch, Wait and Wonder” program exhibited less intrusiveness at post-test and were more securely attached than the Psychodynamic Psychotherapy group.[30]

II. Direct Effects of Zinc and Iron on Memory and Learning

Zinc and iron influence the functioning of many brain regions and metabolic processes. In particular, zinc and iron both play a role in memory and learning, likely as a result of their roles in the hippocampus, one brain region known to be particularly important for learning and memory.[31]

Zinc

Impairments in memory, learning and attention may be due to zinc deficiency causing the hippocampus to immaturely develop, have lower amounts of DNA, RNA and protein.[32] Halas et al. examined the effects of zinc deficiency on memory and learning by doing a series of three experiments evaluating the performance of rats on a radial maze. Zinc deficient rats demonstrated a severe learning deficit and some working memory deficit when compared to adequately nourished and undernourished rats. Their abilities to learn a new task in the second experiment after performing a similar task in the first experiment was significantly decreased, showing that their performance was mostly attributable to their ability to learn versus their forgetfulness.[33]

There is conflicting research about zinc's effect on attention. In a matched-pair double-blind zinc supplementation trial in boys aged 5-7 years, no effects on attention were found, but increases in growth and taste acuity were observed.[34] Conversely, Golub et al. found differences in attention when prepubertal monkeys were induced with a short-term zinc deficiency,[31] and Sandstead et al. found that Chinese children aged 6-9 years that were repleted with zinc in addition to other micronutrients improved more on attention and reasoning than children repleted with a mixture of other micronutrients not including zinc.[35] Aside from one study that linked pregnant mothers' consumption of animal sources of food (presumably being higher in zinc) to increased infant attention to maternal stimuli,[36] there is limited research about zinc and attention in infants. Clearly, more research is needed before definitive conclusions can be made.

Iron

Iron deficiency in utero and postnatally can cause significant deficits in brain functioning. Iron is required for normal oligodendrocyte function because of its essentiality to lipid production and its role in oxidative metabolism and thus myelination.[37 62] Lack of iron has been associated with reduced amounts of myelin, and abnormal looking immature oligodendrocytes, which leads to an inability to accumulate iron in the brain.[40] Iron is also involved in neurotransmitter metabolism. In addition to dopaminergic insults, iron deficiency causes GABA, an important inhibitory neurotransmitter, to be elevated in the hippocampus, striatum and globus pallidus.[39]

Aside from involvement in general brain development and functioning, iron's involvement with a multitude of processes in the hippocampus may account for much of the learning and memory issues observed in iron deficient humans and animals. In iron deficient rat neonates, it was found that there was significantly decreased CytOx activity, a terminal enzyme that contains iron that is required for oxidative phosphorylation in most regions of the hippocampus while other brain regions not involved in higher functioning were maintained.[31] Additionally, since the hippocampus rapidly develops in the late fetal to postnatal periods, concurrent iron sufficiency is particularly crucial. Iron deficiency truncates the development of apical dendrites in the CA1 region of the hippocampus in particular, as iron is critical for the signaling pathways involved.[41]

Rats who were iron deficient during gestation, lactation, and after weaning, had a greater latency to reach the platform in a spatial water maze, an overall longer path length, and spent less time in the correct quadrant of the platform at P25 compared to iron-supplemented rats. At P100, the effects continued as the iron deficient group still had a greater latency and increased thigmotaxis (staying close to walls). Rats supplemented with iron had better iron concentrations in the cerebellum, frontal cortex, striatum and hippocampus than the iron deficient rats. Longer term, the iron-supplemented rats continued to have higher concentrations of iron in the hippocampus, cerebellum and midbrain at P100. Additionally, supplemented rats had higher levels of dopamine in all of the previously listed brain regions.[50]

In piglets induced with a severe or mild iron deficiency, spatial learning and memory was evaluated using a hippocampal-dependent, spatial T-maze task. Because the development of a neonatal piglet parallels that of humans, and they are born with lower iron stores like humans are, they are particularly good subjects for investigating the effects of nutritional deficiencies early in life. The severely iron deficient piglets had fewer correct choices in the maze. Tested again six days later, the severely iron deficient piglets had not improved while the mildly deficient and control piglets had. The severely iron deficient piglets also had lower levels of iron in the hippocampus. This demonstrates the effect that iron deficiency has on the hippocampus and the implications for learning tasks.[43]

Studies examining the effect of iron on infant attention are limited. However, there are some studies that discuss attention in school-aged children. Berglund et al. supplemented low birth weight infants with varying doses of iron or with placebo at 6 weeks. His findings demonstrate a protective effect of iron supplementation as a neonate on attention 3 years later.[44] In adolescent girls and boys, iron supplementation resulted in better scores on the Digit Span, mazes and clerical task test than controls, demonstrating improved attention, auditory memory, recent memory, and visual perception.[45]

III. Direct Effects of Socio-Emotional Development

For this study, socio-emotional development is of interest because of the changes in affect and temperament that influence the mother-infant dyad. In general, however, few human studies that have used a placebo control exist due to the ethical difficulty in executing longitudinal placebo-controlled trials with infants identified to have a nutritional deficiency.

Zinc

Most of the research about socio-emotional development is centered around reduced activity of zinc deficient infants and little research about the direct effects of zinc deficiency or supplementation on socio-emotional behavior has been done. Some animal models demonstrate conclusive evidence but results may not translate to humans. In one rat model, pups that were prenatally zinc deficient showed more aggression than those who were undernourished or those that were normally fed at 75 and 105 days.[46] Golub et al. demonstrated that when zinc deprivation was induced at a time of rapid development, rhesus monkeys were seen to be emotionally less mature and had difficulty with separation.[47] Black et al. found that in Bangladeshi infants, no effect of supplementation could be be found on emotional regulation, but it did improve other developmental outcomes.[48] More studies investigating the role of zinc on emotionality in humans are needed.

Iron

Research on iron and socio-emotional development has less to do with motor development and activity and more to do with direct effects on positive or negative affect. For example, Lozoff and De Andraca found meaningful results when they supplemented non-anemic iron deficient children with iron at 4 months and followed up at 12 months. While there were no significant differences on the motor tests or the Behavior Rating Scales, those infants that were not supplemented showed less positive affect and no attempt to interact socially.[49] Also, a Costa Rica sample of children at ten years of age who were chronically iron deficient had more parent and teacher reports of anxiety, depression, attention and social problems.[50]

One study found that, in newborns of Peruvian women, lower levels of hemoglobin and serum iron in cord blood were associated with higher levels of negative emotionality and distress in the hospital, and negative emotionality and reduced overall ability to be soothed at home. [37] Not all studies have found iron to be effective for socio-emotional development. Mofatt et al. in their prevention trial that was a double-blind randomized control trial, found conflicting results. Infants were given either regular infant or iron-fortified formula. At 15 months, there were some transient effects of regular formula on the Psychomotor Development Index from the Bayley Scales of Infant Development, but no difference in socio-emotional development or behavior was found.[52]

IV. Iron and Zinc Indirectly Effecting Development through their Effect on the Child's Ability to Engage with the Environment

Functional isolation is the theory that nutritional deficiencies can impact development through the care-giver environment.[53] When a poor care-giver environment is combined with malnutrition, the effects on exploratory behavior are exaggerated,[54] resulting in less involvement with the parent or responsiveness to the them, and thus impairment of socio-emotional and cognitive development.

Zinc

Most of the research about zinc deficiency and engagement with the environment revolves around activity and motor development. Animals and humans with zinc deficiency are less active than zinc sufficient counterparts and exhibit motor development deficits.[31,55,47] In their studies on rhesus monkey behavior and zinc deficiency, Golub et al. found that both short term and continuous zinc deficiency lead to behavioral deficits in a cross-over design. The monkeys' performance after 15 weeks of zinc deprivation was compared with a period of zinc adequacy. Results showed that short term zinc deprivation reduces spontaneous motor activity, in addition to other behavioral deficits such as short term memory and attention. These effects also appeared before a noticeable biochemical change in plasma zinc, illustrating the subclinical effects of dietary zinc deprivation and also the insensitivity of zinc biomarkers.[31] Specific findings included a 50% reduction in spontaneous movement compared to controls at 1 month, and a 7-10% reduction at 3 months. Further, lower levels of climbing and exploration were observed at 12 months in the zinc-deprived monkeys.[47]

Reduced activity has been shown to have negative effects on cognitive development as it may prevent children from developing more complex skills and engaging with their environment.[56] Golub and Black's findings illustrate one avenue by which zinc deficiency may alter cognitive and socio-emotional development. If the infant is less active, they are less likely to explore and thus seek opportunities for development in their environment. The care-giver is also included in this environment. In a prospective double-blind randomized controlled trial, low birth weight term infants were found to be more responsive to the tester if supplemented with zinc at 12 months.[57] Further illustrating the combined effects of environmental isolation and malnutrition, one study investigating children aged 9-30 months, had four treatment groups: zinc supplementation, an intervention aimed at improving mother-child interaction by teaching the mother how to psychosocially stimulate her child, both treatments, and no treatment. Zinc supplementation only improved the mental developmental quotient of the children when they were also psychosocially stimulated by their mother. The group receiving both treatments had the highest scores, above zinc supplementation alone and psychosocial intervention alone. [58]

Iron

Iron's role in engagement with the environment may be related to its involvement with dopaminergic tracts in the brain. In neurotransmitter production, iron is a cofactor that regulates the activity of tryptophan hydroxylase, producing serotonin, and tyrosine hydroxylase, producing norepinephrine and dopamine.[38] Therefore, during iron deficiency, marked effects can be observed on dopaminergic tracts.[55] The ability to process the environment is dependent upon the integrity of these systems.[60] For example, it has been shown that rats that have suffered a dopaminergic insult early in life are more likely to be distracted, and hesitant to engage in new tasks. Not only are they directly cognitively impaired, the social affect of the iron deficient rat is different than the iron-sufficient rat.[27] This may translate to humans as a proposed mechanism for indirectly delaying their development and producing long term behavioral effects.[61]

Lozoff et al. found that infants who were not supplemented with iron, and preschoolers who were iron deficient anemic were both slower to touch toys for the first time and engage with the mother in “social looking”.[62,49] Social looking is the equivalent of joint-attentiveness, previously discussed as a mechanism for language acquisition.[23,24] Additionally, infants who were not supplemented with iron crawled later than infants that were, similar to the effects that zinc might have on motor development and activity.

Iron and Zinc in Combination

Zinc and iron could work in tandem to improve engagement with the environment. Black et. al found, in a sample of Bangladeshi children, that iron and zinc supplementation in combination had a protective effect at 6 and 12 months on infant motor development and exploration, perhaps encouraging future cognitive development.[48]

V. Double Blind Randomized Controlled Supplementation Trials in Infants

Zinc

Six notable double-blind randomized controlled trials of zinc supplementation have been done on human infants and they reveal mixed results. Three of these studies evaluated development using the Bayley Scales of Infant Development, one of which found negative effects of supplementation. Ashworth et al. found no effects of supplementation on the Psychomotor Development Index (PDI) or Mental Development Index (MDI) but observed that infants were more responsive to the tester at 12 months.[57] Castillo-Durán et al. found no differences on the PDI but a significantly greater percentage of the placebo group did worse on the MDI.[63] Hamadani et al. actually found that providing zinc supplementation to 1 month olds resulted in decreased MDI scales at 7 and 13 months. They cautioned against single nutrient supplementation.[64]

Two studies found that zinc supplementation increased activity. Bentley et al. observed that infants who were supplemented with zinc were more active and spent more time sitting up instead of lying down. Although it wasn't significant, a trending inverse relationship between zinc supplementation and time crying was observed. However, language milestones were unaffected by supplementation. [65] Sazawal et al. also found that after at least one month of supplementation, infants spent more time performing activities that required high movement and in general had a higher energy expenditure.[55]

Finally, as described previously, one study uniquely tested zinc supplementation with and without psychosocial intervention. Zinc supplementation coupled with a psychosocial intervention resulted in infants with a significantly higher developmental quotient than those supplemented with zinc alone and those that didn't receive any treatment . Zinc alone improved hand-eye coordination.[58]

In general, while it is clear supplementation of zinc alone has some effect on development, the results of these studies do not show consistent effects of supplementation.

Iron

Only two longer-term (2-6 months) double blind randomized controlled trials have been done with human infants.[66,67] Other studies have been done, but have used repleted, formerly iron-deficient infants as the control group. Only one demonstrates clear effects of iron supplementation on development. Idjiradinata and Pollitt's study in 1993 supplemented iron-deficient anemic (ID+A), iron deficient (ID), and iron sufficient 12-18 month children with either an iron supplement or placebo for four months. The ID+A children that received an iron supplement reversed their developmental delays while their counterparts who took the placebo did not. Iron supplementation or placebo had no effect on the ID or iron sufficient children, but ID and iron sufficient children were not significantly different on the Bayley Scales at baseline, while ID+A was significantly different than both.[67]

Aukett et al.'s study which supplemented ID+A children ages 17-19 months with iron and Vitamin C or just Vitamin C, they found that there was no significant difference in psychomotor development on the Denver Screening test, but more children who received iron achieved the appropriate rate of achievement. Despite being supplemented, their hemoglobin levels did not improve, and a portion of the ID+A children did not catch up developmentally as expected.[66].

Although not randomized control trials, some longitudinal studies have shown the relationship between iron deficiency, development, and the effect of supplementation. In research conducted by Lozoff and colleagues, children in Costa Rica were followed for 25 years and, despite restored hematological markers, suffered long term consequences of once being severely iron deficient. Five and ten years later, those that had been chronically iron deficient were scoring significantly lower on mental and motor scales.[,50, 61] At 25 years, the long-term consequences for development ultimately manifested in poor functional outcomes and continued socio-emotional problems.[5]

Iron and Zinc in Combination

The research described in this thesis investigates iron and zinc supplemented in combination, given separately, and alone, versus placebo. Iron and zinc have some overlapping and complimentary effects on brain functioning, socio-emotional development and cognitive development. However, zinc and iron have antagonistic effects on each other during absorption and could limit the success of supplementation. In a community control trial of Indonesian infants, supplementation in combination was found to be less effective in altering biochemical outcomes than supplementing with zinc and iron alone.[68] Two notable randomized control supplementation trials that have included a supplement of iron and zinc in combination are discussed.

Surkan et al. supplemented a large sample, 544 Nepali children, ages 4 months to 17 months with placebo, iron alone, zinc alone, and iron and zinc in combination. They were followed up at 3 month intervals for one year when language and motor milestones were measured. Truly an intent-to-treat analysis, no blood was drawn and nutritional status of the population was unknown. Additionally, the analyses were not stratified by age despite the large range, and only language and motor components of development were measured. The findings were that despite zinc and iron supplementation, infants still failed to meet language milestones.[69]

Black et al. supplemented children with iron and zinc in combination, iron alone, zinc alone, a mix of micronutrients, or riboflavin. No differences were found on the MDI. Significantly higher scores were found in infants who were supplemented with iron and zinc or a mix of micronutrients on the PDI compared to infants who were supplemented with riboflavin alone, although all groups' scores decreased from 6-12 months. Additionally, more exploratory behavior (orientation-engagement) was observed in infants supplemented with iron alone, zinc alone, and iron and zinc in combination than riboflavin alone, although again, all scores did decrease from 6-12 months.[48]

VI. Conclusion

In conclusion, the maternal components of mother-infant interaction such as sensitivity, structuring, non-intrusiveness and non-hostility have a significant effect on secure attachments, language acquisition, and emotional and social understanding. In turn, the child contributes to mother-infant interaction and influences their own development as well. One way that they do this is by altering the way their mother responds to them, creating a reciprocal cycle of feedback and development.

Iron and zinc both have independent direct effects on infant development of memory, learning and attention, as well as socio-emotional development. Iron and zinc both have independent indirect effects on the infant's development by limiting or enhancing their ability to engage with the environment. Although iron and zinc supplementation in combination is less effective in improving biochemical outcomes than iron and zinc supplementation alone, iron and zinc supplementation in combination can result in improved behavioral and developmental outcomes. However, limited double-blind randomized controlled trials in infants have been done with either micronutrient. More data isolating the direct effects of these micronutrients on mother-infant interaction and child development is needed.

Chapter 3

Methods

The particular details of the study from which these data were obtained are discussed in a previous article.[23] Briefly, the methods are explained here. This study is part of the cognitive portion of a larger study carried out by staff at the International Centre for Diarrhoeal Disease Research, Bangladesh (ICDDR,B). This was a longitudinal, double-blind, randomized, placebo-controlled community trial that took place in Mirzapur, Bangladesh. Participants were mothers and their children aged 6-18 months at baseline. Participants were recruited from several villages surrounding Kumudini Hospital, the hospital that serves all of Mirzapur, ranging from 2.5km to 12 km walking distance. Exclusion criteria included being severely malnourished, severely anemic, having chronic illness or an active fever. Mirzapur's birth, infant, and child mortality rates of 27/1000 population, 80/1000 live births, and 110/1000 live births, respectively, are reflective of Bangladesh's national averages. Most children are breastfed for 12-24 months, exclusively for six-months, and are introduced to a plant-based diet around six months of age.

I. Supplementation Intervention

Children were randomized into 5 different six-month supplementation regimens. Fieldworkers, mothers and children were blind to their assignment. Supplementation analyses were done in an intent-to-treat manner, meaning that only the original assignment was considered, not the eventual treatment received. The supplementation groups were daily placebo, daily alternating iron/folic acid and placebo (iron alone), daily alternating zinc and placebo (zinc alone), daily alternating iron/folic acid/zinc and placebo (zinc and iron in combination), daily alternating zinc and iron/folic acid (zinc and iron separately). The zinc supplement was a dose of 5mg per day dissolvable tablet, the iron supplement was a dissolvable tablet with a dose of 6.25mg/d for children 12 months and older and half tablets of 3.125mg/d for children less than 12 months, and 50 IU folic acid. The placebo was identical in shape, taste and color to the other supplements. The tablets were manufactured by Nutriset S.A.S (Malaunay, France). Supplements were administered to children by reconstituting them in 5 mL of water on an empty stomach. When doses were missed, children were given one dose in the morning and another that night. Village health workers (VHW) demonstrated this process, instructed women on how to give them to their children, and supervised the administration.

VHWs had all completed, at the minimum, 8 years of school and were preferred to be a permanent resident of the village they were working. Training for the VHWs included 7 days on recruitment protocols, supplementation training, data collection methods, and after three months, they received a 7-day refresher. Groups of 6 VHWs were overseen by one of four trained supervisors, all of which had 12 years of school and research experience. Once a week, the supervisor checked the VHWs' assessments and personally accompanied them on their home visits. Follow-up forms were double-checked for missing data and standardized forms were used to assess accuracy in adherence reporting.

II. The Cognitive Study Participants

Only those participants in the larger parent study that had been randomly assigned to have blood draws were eligible for randomization into the nested cognitive portion of the study.

352 participants were included in the cognitive study that examined mother-infant interaction which was assessed at baseline, three months, and six months. However, because funding for the cognitive study was received after the parent study began, some of the participants had already begun supplementation and were too far along to include a 'baseline' measure of outcomes for the cognitive sub-study. Therefore, for the purposes of this analysis, 117 children whose baseline mother-infant interaction was assessed within one month of beginning supplementation were included in these analyses (Figure 2). Three participants were excluded because they were not followed-up at three month or six months, resulting in an n of 114. The baseline demographic data of the smaller sample was compared to the larger sample using an Analysis of Variance.

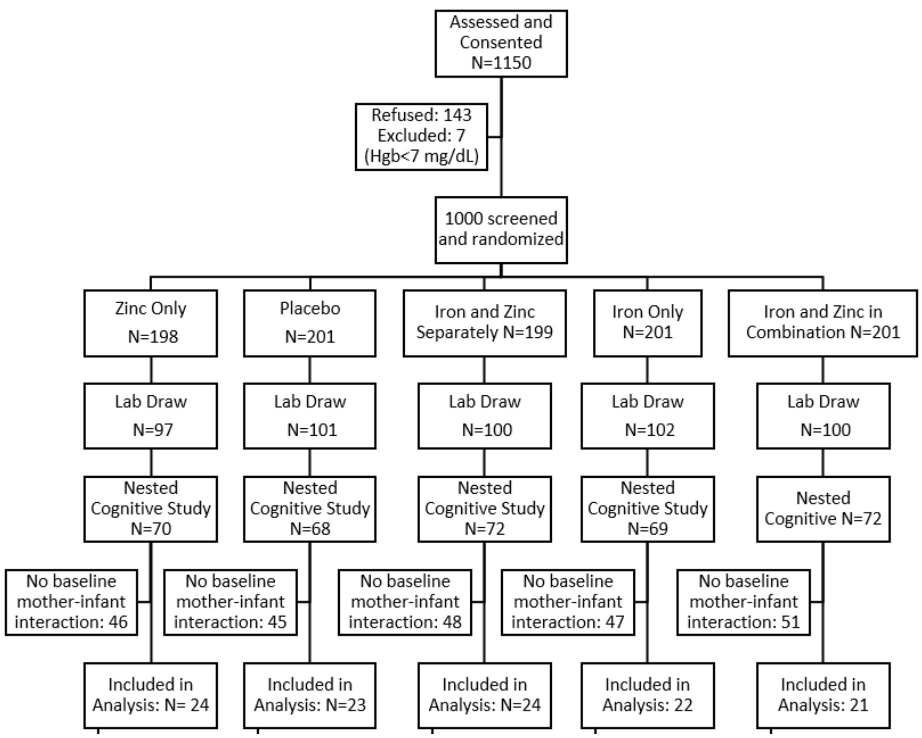

Figure 2: Participant Flow

While all participants included in this analysis provided an endpoint blood sample and follow up survey, mother-infant interaction was not tested at endpoint in 3% of the participants. The reasons for being unable to obtain endpoint mother-interaction data are not known.

III. Assessment of Anthropometrics and Demographics

Village health workers conducted home visits and collected socioeconomic data. Information about duration of breastfeeding and diarrhea, the number of children of particular ages in the house, the household size and dietary intake was collected. An Asset Quintile was created 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. If the family confirmed that they would not be moving in the next six months, they were sent to Kumudini Hospital to be assessed further by a study nurse. Child anthropometrics were taken at the hospital as well as a child blood sample. Inclusion criteria included a weight for height z-score equal to or more than -3SD, and a hemoglobin value greater than or equal to 7 mg/dL.

IV. Assessment of Mother-Infant Interaction

In the Kumudini Hospital, 10 minutes of naturalistic free play as well as a structured interaction of changing the infant's clothes were recorded via video footage. The mother was given a basket of age-appropriate toys to use in play with her child. The researcher instructed them to play "as you usually play at home," and after setting up the camera, exited the room so as not to influence the interaction. This took place at a time when the baby was alert and well-fed.

The videos were coded using the Emotional Availability scales.[12] These scales (4 parental scales: sensitivity, structuring, nonintrusiveness and non-hostility; 2 child scales: responsiveness and involvement with the parent) integrate emotional availability perspectives and attachment and can assess both the child and maternal inputs to interaction. These scales have been used before and have been sensitive to maternal iron status.[24] A team of researchers who had experience in the assessment of child development were chosen to collect this data. This cognitive team was trained to code videos as per the Emotional Availability Scales protocol. They reviewed sample videos, coded them with the scales, and sent them back to Dr. Murray-Kolb to be approved. On-site supervision occurred by the head of Child Development at ICDDR,B.

The subscales are scored on a Likert scale. Maternal sensitivity, child responsiveness to the parent and child involvement with the parent are all scored on a 7-point scale. Maternal structuring, non-intrusiveness and non-hostility are scored on a 5-point scale. The higher number indicates more of that trait.

V. Assessment of Nutritional Status

Blood draws to assess nutritional status of the child were done during enrollment and follow-up six months later. Venous blood was spun, centrifuged, and stored at -20 C before transfer to the ICDDR,B laboratory every 2 weeks on dry ice. Zinc was measured using atomic absorption spectrometry (model AA-6501S, manufacturer Shimadzu Co, Japan, Zinc stock standard 1000 mg/l, BDH VWR UK, Quality control from UTAK Lab Inc). For hemoglobin, a trained laboratory technician used capillary blood from a finger prick by Hemocue. Serum transferrin receptor was measured by particle-enhanced immunoturbidimetric assay (Hitachi- 902, manufacturer Roche, calibration kits from Roch, Mannheim, Germany). Each lot of 35 samples used both standard and commercial machine kits to calibrate. Samples were also pooled in 10 and any lot with a value greater or less than 2 s.d. was re-analyzed.

Ferritin was assessed via a Ramco kit. Because ferritin is an acute-phase protein, it is elevated in the presence of inflammation. Therefore, C-reactive protein was measured and high CRP was classified as ≥5 mg/L. For those participants with indications of inflammation, plasma ferritin was adjusted using the Thurnham method.[70] Participants, their mothers, and all researchers were blinded to the child's nutritional status.

VI. Statistical Analyses

The data were analyzed using SAS software version 9.3 (SAS Institute Inc, Cary, North Carolina) and the analysis was performed in a blinded manner. Height and weight z-scores were calculated with EPIinfo. To determine differences between groups at baseline, categorical variables were compared using a x^2 test, and continuous variables were compared using ANOVA. Mother-infant interaction outcomes were analyzed separately at each time-point (baseline, three, and six months) using ANOVA, controlling for baseline scores, child sex and age, and percent lowest quintile of socioeconomic status. Comparisons were considered significant at the $p<0.05$ level.

Covariates were selected by the following process: First, potential confounding variables were selected based on previously published literature, such as maternal depressive symptoms, breastfeeding, measures of socioeconomic status and child characteristics (age, sex). These were correlated against each other in a matrix. When 2 or more variables were highly correlated, only one variable was retained to eliminate redundancy. For socio-demographic variables, number of children under 5, household size and the asset quintile were redundant. Asset was retained because of the larger variation throughout the sample. The resulting variables were correlated with each of the outcome variables (all six Emotional Availability scales). Breastfeeding and depressive data were excluded as potential covariates here because of the lack of variation in the sample, and the lack of significant correlations with the sub scales. The variables that correlated with a p value of 0.1 or lower with one or more of the scales were included in the statistical model for analyzing all of the outcome variables. Thus, child sex, age, and asset quintile were used as covariates in the statistical model.

My role in the project was data cleaning, analysis, and writing the report under my advisers' guidance. Specifically, while I was not involved in the experimental design or data collection, I developed my own research question and hypothesis, conducted the literature review, and independently considered the implications and relevancy of my findings.

Chapter 4

Results

I. Baseline Demographics and Nutritional Status

At baseline, the intervention groups were not significantly different in sociodemographic variables or in nutritional status (Table 1). Overall, 53.6% of the participants were male. While, on average at enrollment, the children were 8 months of age, there was a wide range of ages (5 to 17 months). All but 10% of the children were breastfed, reflective of Bangladesh's culture of breastfeeding. Additionally, maternal depressive symptoms were not significantly different among groups (data not shown).

Nearly all children were anemic. The average hemoglobin for the entire sample was 8.8 mg/dL. Iron deficiency did not appear to be a primary cause of anemia as there was no correlation between hemoglobin levels and ferritin and transferrin receptor values (r= -0.06, p=0.5) and (r=0.09, p=0.3) respectively. Mean ferritin value for the sample was 25.4 mg/mL, within the normal range.[71] Mean serum transferrin receptor, 6.3 µg/mL, was also within the normal range and was not up-regulated. In general, a slight zinc deficiency was observed across the sample with a serum zinc level of 0.66 mg/L (less than 0.70 mg/L is considered to be deficient) coupled with mild stunting as the mean height-for-age z-score was -1.21.[72] However, plasma zinc is not a sensitive measure of status, and the deficiency is mild.[73] Although these lab values were measured, because this analysis is designed as intent-to-treat, lab values were not included in the statistical model. Finally, at baseline, there were no significant differences among groups for mother-interaction video scores (Tables 2-7). The smaller sample analyzed here differed from the larger parent study in the following ways: the enrollment age was younger, 8.0 vs. 9.6 months (p=0.01), and subjects in the present analyses had a lower mean hemoglobin of 8.77 mg/dL vs 9.1 mg/dL (p=0.03).

Table 1: Baseline characteristics of children included in the present analysis (mean ± SD)

	Zinc Alone	Placebo	Separate Iron and Zinc	Iron Alone	Combined Iron and Zinc
n	24	23	24	22	21
Child Age (months)	8.22 (3.41)	8.13 (2.60)	9.00 (3.45)	8.36 (3.05)	9.00 (4.00)
Children Under 5 (n)	0.52 (0.66)	0.48 (0.59)	0.63 (0.77)	0.59(0.67)	0.86(0.91)
Household Size (n)	5.61 (2.33)	5.35 (1.87)	6.13 (2.49)	6.32 (2.64)	5.14 (1.88)
Weight-for-Age (Z-score)	-1.34 (.94)	-1.39 (1.11)	-1.13 (1.13)	-1.15 (1.03)	-1.32(0.85)
Height-for-Age (Z-score)	-1.13 (0.81)	-1.31 (0.75)	-0.99 (0.63)	-1.06 (0.95)	-1.10 (1.01)
Weight-for-height (z-score)	-0.70 (0.79)	-0.49 (1.13)	-0.55 (1.31)	-0.46 (1.09)	-0.61 (0.92)
Mean Upper Arm Circumference	132.87 (10.98)	131.04 (10.49)	135.00 (12.09)	133.82 (10.56)	131.14 (7.50)
Number of Days BF	90.91 (70.89)	88.00 (71.97)	105.48 (79.15)	74.25 (62.98)	103.57 (76.76)
Serum Zinc (mg/l)	0.63 (0.12)	0.61 (0.09)	0.65 (0.08)	0.60 (0.07)	0.6 (0.11)
Hb (mg/dL)	9.03 1.07	8.731.07	8.80 1.34	8.79 1.24	8.50 0.98
Serum Transferrin Receptor (μg/ml)	6.16 (1.97)	6.56 (2.3)	6.54 (2.20)	6.28 (2.41)	5.58 (1.72)
Adjusted Serum Ferritin (mg/ml)	24.75 (16.67)	24.52 (18.45)	20.19 (21.56)	25.17 (16.53)	31.22 (18.72)
C-reactive Protein (mg/l)	2.99 (3.50)	4.02 (4.89)	7.29 (15. 87)	2.10 (2.30)	2.37 (1.78)
% Male	65.22	47.83	54.17	63.64	28.57
% Anemic	91.30	95.45	91.67	100.00	100.00
% Inflamed (>5%)	18.18	27.27	25.00	9.09	9.52
% Lowest Quintile	21.74	4.35	8.33	27.27	0.00

II. Mother-Infant Interaction Scores

Analyses run with and without covariates did not differ. Therefore, analyses adjusting for covariates are presented here. Significant differences among groups were seen at 3 months on some of the subscales. No significant differences were seen at endpoint.

Maternal Sensitivity (Table 2): At midpoint, the combined iron and zinc group scored significantly higher than the zinc group ($p<0.001$). The iron alone group also scored significantly higher than the zinc alone group ($p<0.05$). The combined iron and zinc group was not significantly different from the iron-alone group, however. The combined supplementation group was higher than the zinc alone group by 1 point. This difference is around one standard deviation. At endpoint, there were no significant differences.

Maternal Structuring, Non-Intrusiveness and Non-hostility (Tables 3, 4, and 5): No significant differences among groups were detected at any time point.

Child Responsiveness to the Parent (Table 6): There were no significant differences at midpoint or end point. However, at midpoint there were insignificant but trending differences among groups ($p=0.088$ with controls), but no post-hoc analyses were run.

*Child Involvement with the Parent (Table 7):*At midpoint, the combined iron and zinc group scored significantly higher than the zinc alone group ($p<0.001$), the placebo group, ($p<0.04$) and the iron and zinc separately group ($p<0.01$). The iron alone group scored significantly higher than the zinc alone group ($p<0.05$). There were no significant differences at endpoint.

Table 2: Maternal Sensitivity

Supplementation Group	Baseline Mean (s.d.)	Midpoint Mean (s.d..)	Endpoint Mean (s.d.)
Zinc Alone	4.20 (1.17)	3.90 (0.92)	3.45 (0.95)
Placebo	4.35 (1.55)	4.14 (1.00)	3.46 (.94)
Iron and Zinc in Separation	4.04 (1.36)	4.32 (1.19)	3.61 1.01)
Iron Alone	4.59 (1.17)	4.72 (1.15)[a]	3.73 (1.23)
Iron and Zinc in Combination	4.60 (1.37)	4.78 (1.35)[a]	3.99 (1.09)

[a]greater than zinc alone ($p<0.05$)

Table 3: Maternal Structuring

Supplementation Group	Baseline Mean(s.d.)	Midpoint Mean (s.d.)	Endpoint Mean (s.d.)
Zinc Alone	2.52 (0.65)	2.74 (.0.59)	2.34 (0.51)
Placebo	2.70 (0.85)	2.87 (0.60)	2.32 (0.48)
Iron and Zinc in Separation	2.50 (0.74)	2.85 (0.56)	2.47 (0.57)
Iron Alone	2.82 (0.72)	3.12 (0.49)	2.50 (0.49)
Iron and Zinc in Combination	2.69 (0.84)	2.91 (0.66)	2.61 (0.57)

Table 4: Maternal Non-Intrusiveness

Supplementation Group	Baseline Mean (s.d.)	Midpoint Mean (s.d.)	Endpoint Mean (s.d.)
Zinc Alone	3.52 (.92)	3.12 (0.93)	3.04 (0.72)
Placebo	3.37 (1.02)	2.96 (0.82)	2.85 (0.72)
Iron and Zinc in Separation	3.06 (1.17)	2.97 (0.79)	3.11 (0.64)
Iron Alone	3.30 (0.92)	2.96 (.0.81)	2.80 (0.54)
Iron and Zinc in Combination	3.31 (0.98)	2.89 (0.86)	2.86 (0.54)

Table 5: Maternal Non-Hostility

Supplementation Group	Baseline Mean (s.d.)	Midpoint Mean (s.d.)	Endpoint Mean (s.d.)
Zinc Alone	3.46 (0.72)	3.65 (0.44)	3.35 (0.46)
Placebo	3.70 (0.82)	3.68 (0.50)	3.48 (0.39)
Iron and Zinc in Separation	3.38 (0.89)	3.77 (0.70)	3.44 (0.47)
Iron Alone	3.89 (0.71)	3.92 (0.58)	3.42 (0.42)
Iron and Zinc in Combination	3.71 (0.64)	3.88 (0.72)	3.53 (0.37)

Table 6: Child Responsiveness to the Parent

Supplementation Group	Baseline Mean (s.d.)	Midpoint Mean (s.d.)	Endpoint Mean (s.d.)
Zinc Alone	4.02 (0.79)	4.13 (0.52))	4.10 (0.70)
Placebo	4.26 0.98)	4.30 (0.57)	4.03 (0.46)
Iron and Zinc in Separation	3.90 (0.75)	4.13 (1.00)	4.10 (0.81)
Iron Alone	4.45 (0.75)	4.40 (0.90)	4.10 (0.71)
Iron and Zinc in Combination	4.17 (1.06)	4.68 (0.67)	4.35 (0.64)

Table 7: Child Involvement with the Parent

Supplementation Group	Baseline Mean (s.d.)	Midpoint Mean (s.d.)	Endpoint Mean (s.d.)
Zinc Alone	3.87 (1.05)	3.60 (0.77)	3.66 (0.88)
Placebo	3.83 (1.11)	3.95 (0.79)	3.51 (0.58)
Iron and Zinc in Separation	3.65 (0.87)	3.81 (1.08)	3.68 (0.89)
Iron Alone	4.09 (1.13)	4.17 (0.90) [a]	3.81 (0.90)
Iron and Zinc in Combination	3.90 (1.23)	4.57 (0.69) [a*bc]	4.00 (0.82)

[a] greater than zinc alone ($p<0.05$)
[a*] greater than zinc alone ($p<0.001$)
[b] greater than iron and zinc in separation ($p<0.01$)
[c] greater than placebo ($p<0.05$)

Chapter 5

Discussion and Conclusions

I. Interpretation of Findings

In this study, 5-17 month old children were enrolled, supplemented with either iron and zinc in combination or separately, iron alone, zinc alone, or placebo, and assessed for mother-infant interaction using the Emotional Availability Scales at baseline, three, and six months. We hypothesized that at end-point, those supplemented with separate iron and zinc would perform better on the Emotional Availability Scales. Two sub-scales at midpoint, maternal sensitivity and child involvement with the parent, showed significant differences among groups. One additional sub-scale was trending, child responsiveness to the parent. The reasons why any group effects were not apparent at endpoint are puzzling, and certainly is an area for more research. Regardless, it is important to note that the findings are likely clinically significant as the combined iron and zinc group scored more than a standard deviation higher than those in the zinc alone or placebo groups. Despite the sample being relatively iron sufficient and only mildly zinc deficient, iron and zinc supplementation has, at least transiently, increased the child's involvement with the parent, in turn increasing maternal sensitivity, thus increasing the opportunity for further child development.

Maternal sensitivity according to the Emotional Availability scales, is rated on a 7-point scale. The average scores observed across group ranged between 3.9 and 4.8. A “5” describes inconsistent sensitivity. A mother may fluctuate between being joyfully involved with her child to being preoccupied with other tasks. A “3” describes the mother as being somewhat insensitive. The mother may be active and harsh or passive, flat and non-interactive. The zinc-only group mothers, therefore, were exhibiting behaviors that were between somewhat insensitive and inconsistently sensitive. The iron only and iron and zinc in combination group mothers were exhibiting behaviors that were less insensitive and fell in the category of inconsistently sensitive.

Child involvement with the parent according to the Emotional Availability scales, is rated on a 7-point scale. The average scores observed across groups ranged between 3.6 and 4.6. A “3” is the rating for somewhat non-optimal in involving behaviors. In general, the child does not draw the parent into play very often, is avoidant and exhibits more autonomous pursuits than involving behaviors. If the child is over involving of the parent, unable to play by themselves, they would also be ranked as a 3. A “5” is described as moderately optimal in involving behaviors. Still, at a 5, involving behavior with the mother is still being overshadowed by the infant leaning towards autonomous play. There is a periodic request of the parent's attention. Therefore, in our sample, those children that were supplemented with zinc and iron in combination were closer to being moderately involved with the mother, while the zinc-alone group and placebo group were somewhat non-optimal in their involving behaviors.

II. Relation to previous research and speculation

The findings are consistent with the research surrounding nutrition and socio-economic development, affect and the subsequent effects on interactions with the care-giver. However, little research has been done analyzing the effects of supplementation without taking nutritional status into consideration. Studies usually identify iron deficient children and choose to supplement them while using iron sufficient children as the control group. An exception is Surkan et. al who also did not randomize supplementation exclusively to iron deficient children, but to a larger sample of Nepali children. The children who were supplemented with iron, zinc, or both did not improve the attainment of motor or language milestones one year later.[69]

Other trials have stratified their data by nutritional status. Murray-Kolb and Beard used the Emotional Availability scales to assess mother-infant interaction in South Africa. At baseline, the iron deficient anemic group scored significantly lower on the Maternal Sensitivity and Child Responsiveness scales than controls, but at 9 months (after the iron deficient anemic group had been supplemented with iron), there were no differences between groups.[74] This points to iron deficiency being the cause of differentiating scores, and the ability of supplementation to ameliorate the effects. Lozoff and colleagues also stratified infants by nutritional status and assigned the placebo supplement to iron sufficient children. Despite being iron sufficient at enrollment, the unsupplemented group had more negative socio-emotional affect at follow up.[49]

In our study, without regard to baseline nutritional status, the group that took zinc and iron in combination had significantly better biochemical outcomes than the group that just took zinc alone or the placebo. Those supplemented with the zinc and iron in combination had significantly lower transferrin receptor levels (5.4 µg/L) than placebo (8.91 µg/L) and zinc alone (7.4 µg/L) at end-point. And finally, ferritin levels were also higher for supplements taken in combination (35.1 mg/mL) than zinc (21.4 mg/mL), or placebo (21.62) and trended towards being higher than iron and zinc in separation (26.2 mg/mL, p=0.088). It is possible that supplementation prevented iron deficiency from occurring, or corrected an iron depletion not quite considered iron deficiency. While the combination group was not significantly different than the iron alone group on any of the biochemical outcomes, the iron alone group was not significantly different than any other group in ferritin levels, and only placebo for transferrin receptor. It is also possible that the competition between iron and zinc, in terms of absorption, did not play a significant role in this population since they were not iron deficient and only mildly zinc deficient at baseline.

Combined iron and zinc effects on increasing child involvement with the mother is consistent with the observation of Lozoff et al. described above, that iron deficient children do not attempt to socially interact, while their supplemented counter-parts do.[49] The present findings are also consistent with research studying the behavior of iron deficient anemic infants at play.[14,75]

At the crux of child involvement is the ability of children to attend to their parents instead of non-relevant stimuli. It has been demonstrated iron deficient and zinc deficient children may have shorter attention spans than iron sufficient children.[47,50,76] Perhaps the involvement of iron with the dopaminergic tracts in the striatum[59] and hippocampus are playing a role.[57] Although there are limited data supporting direct effects,[77] interruptions in the action of dopamine have been associated with disrupted cognitive function, including attention. Zinc's influence on the hippocampus and cerebellum may also be responsible for the differences among groups. The hippocampus is known to be involved with learning, memory and attention.[78]

Interestingly, even though only the child received supplementation, the mother's sensitivity was affected as well. This is an example of the transactional principle at work. The transactional principle states that infants influence the quality of the interaction with their caretakers just as caretakers shape the interaction with their infant.[12] Lozoff et al. observed similar effects in a study evaluating the behavior of iron-deficient anemic infants when testers blind to the child's nutritional status, treated iron deficient anemic children differently than iron sufficient children.[14] The Mutual Regulation Model also credits infants not only with the ability to shape the quality of the interaction with their caretaker, but also with the ability to shape their development. It is demonstrated in this study, as it is with the Mutual Regulation Model, that interaction is bidirectional, and has consequential effects on the behavior of both the mother and child.

Further illustrating this, Murray-Kolb & Beard used the Emotional Availability scales to assess mother-infant interaction in South Africa. At baseline, the iron deficient anemic group of mothers scored significantly lower on the Maternal Sensitivity subscale and their children scored significantly lower on the Child Responsiveness subscale compared to control (iron sufficient) mothers and their children, but at 9 months, there were no differences between groups.[74] This suggested that iron deficiency was the cause of differentiating scores and that supplementation ameliorated the effects. Because their study only supplemented the mothers, iron supplementation is thought to have directly affected maternal sensitivity and, in turn, indirectly affected child responsiveness to the mother. Our study found the reciprocal. Through supplementing the child, the childrens' behavior was directly affected and the mothers' sensitivity was in turn indirectly affected. It is demonstrated in this study, as it is with the Mutual Regulation Model, that interaction is bidirectional.

It is notable that, on both significant subscales (maternal sensitivity and child involvement with the parent), iron alone performed better than zinc, and iron and zinc in combination scored better than zinc. However, iron alone and iron and zinc in combination were never significantly different from each other. Logically, it may be that the iron was driving the improvement, and our group of children was not very iron deficient. Further, zinc and placebo were never significantly different.

We hypothesized that iron and zinc separate supplementation would have higher mother-infant interaction scores than combined iron and zinc supplementation. However, on the child involvement with the parent sub-scale the combined zinc and iron group scored significantly higher than the separate iron and zinc. In a trial investigating the effects of single vs. combined supplementation of zinc and iron, six months later, zinc alone improved growth outcomes while iron alone improved mental scores on the Bayley Scales of Infant Development. No effect was found in infants who were supplemented in combination.[68] A review by Walker et. al concluded that while there are not enough studies to truly understand the effects of iron and zinc co-supplementation, the biochemical outcomes will not change if supplementation is given in combination or separately.[79] Indeed, our results show that zinc status was not different between groups after supplementation (data not shown).

It is curious that the combination group had better iron outcomes than the iron-alone group, however, it could be explained by diarrhea outcomes. In the ICDDR,B sample our groups were taken from, groups that were supplemented in combination had better diarrhea outcomes. Iron alone increased poor diarrhea outcomes, but zinc attenuated the effects, resulting in the iron and zinc in combination group obtaining optimal hospitalization outcomes. [80] Another sample in Bangladesh from 2003 also showed improved diarrhea outcomes with zinc and iron supplementation given in combination.[81] Perhaps if diarrhea was being controlled, gut absorption in general would be increased for all nutrients.

Not only could diarrhea be influencing biochemical outcomes but cognitive outcomes as well. As mentioned before, diarrhea prevents the absorption of micronutrients critical for child development early in life. High amounts of childhood diarrhea in the first two years of life are associated with long-term cognitive deficits later in life.[42] Guerrant et al. proposed updating the DALYs for diarrheal disease partially because of the negative effects diarrhea has on mental development.[44] Thus, the success of the zinc and iron combination group with mother-infant interaction could be attributed to diarrheal outcomes and gut integrity.

Another consideration is that across all of the intervention groups, there were high rates of anemia. Only five children out of the entire sample were not anemic. Because their lab values indicate they were iron sufficient, the anemia may be due to another cause such as other micronutrient deficiencies or parasitic infections. [84]

The wide-spread anemia in our sample could have an effect on mother infant interaction. Because the groups' hemoglobin levels did not significantly differ, anemia, although not specifically tested, did not seem to have an effect on mother-infant interaction or mitigate any differences among treatment groups. However, anemia could certainly be what is most limiting mother-infant interaction and affecting the ability of infants in all groups, regardless of treatment in this study, to effectively engage with their mother. Perhaps if the children in our sample were relieved of anemia, they could more effectively respond to treatment and reflect more noticeable differences. However, Black et al. found that hemoglobin levels did not contribute to psychomotor and mental development scores in a sample of Bangladeshi infants. Therefore, the effect of anemia is inconclusive.[48]

III. Strengths and Weaknesses

Our strong study design, a randomized controlled trial, allows us to likely attribute our findings to the effect of supplements on the child and mother's behavior, given the differences found among groups. However, a narrow focus was taken to truly isolate the question of nutrient supplementation on mother-infant interaction. While this objective was achieved through the study design, a disadvantage is that other information that could answer other research questions was not included. For example, nutritional status, child development scores and mother's nutritional status were not included in this analysis. An advantage of doing a true, intent-to-treat analysis is that our findings are particularly useful to policy-makers. The study demonstrates that nutritional supplementation interventions can, at least transiently, improve mother-infant interaction even if nutritional status is not taken into account. Testing for nutritional status can be expensive, time-consuming, and simply not feasible in some populations.

Other weaknesses include the small sample size allowing for around 20-24 participants per group. Additionally, there were some differences between this smaller sample and the larger parent sample as this sample was more anemic and younger at enrollment. While the mean age at enrollment was 8.0 months, the age range was large, 5 to 17 months. Age was controlled in the analyses, but our data cannot answer age-specific research questions.

Despite weaknesses, our study addresses a need in the research for placebo-controlled trials investigating nutrition, mother-infant interaction and child development. Our results indicate that iron and zinc supplementation could be incorporated into future interventions to improve mother-infant interaction, and thus long-term developmental outcomes.

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ACADEMIC VITA

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Education

THE PENNSYLVANIA STATE UNIVERSITY |
UNIVERSITY PARK, PA *Graduation Expected*
May 2014
Schreyer Honors College

Major: Nutritional Sciences, Bachelor of Science
Minor: Global Health: Fieldwork in South Africa

Research

RESEARCH ASSISTANT TO DR. LAURA MURRAY-KOLB
January 2013-October 2013
Penn State University

≅ Statistically models and analyzes blood iron and zinc data and the emotional availability of Bangladeshi mothers and children via SAS Statistical Programming
≅ Assisting Dr. Murray-Kolb in her study with ICDDR,B collaborators

SCHREYER HONORS COLLEGE UNDGRADUATE THESIS
January 2013-February 2013
Penn State University

≅ Topic: Mother-Infant Interaction and Zinc and Iron Supplementation
≅ Currently analyzing data collected in Dr. Murray-Kolb's lab in an intent-to-treat fashion to guide further policy on supplementation and nutrition

RESEARCH ASSISTANT TO DR. KASIA KORDAS
August 2012-December 2012
Penn State University

≅ Conducted a literature review to be incorporated into a grant proposal for a psychosocial intervention for children with high blood lead levels in Uruguay

Professional Experience

GLOBAL HEALTH FIELDWORK
May 2013- July 2013
Polokwane, Limpopo, South Africa

≅ Analyzed the determinants of health in South Africa by traveling to and observing various public clinics and hospitals
≅ Focused on international nutrition and the epidemiological transition by interviewing individuals of various socioeconomic statuses
≅ Shadowed dietetic students during their practicum and sat in on discussions of the pathophysiology of medical nutrition therapy of unique diseases that were led by their professor
≅ Evaluated various intervention techniques and identified holes with South Africans and brainstormed new approaches to solving problems with many cultural and political factors involved

NUTRITON CURRICULUM DESIGNER
November 2010- July 2013
State College, Pennsylvania

≅ Developed a nutrition curriculum and materials for grade-school teachers that is available at the State College local library
≅ Mentored by Star Campbell, nutrition education professional and consultant
≅ Taught and piloted self-designed lesson plans in a local third grade classroom, resulting in a new nutrition unit including more interactive, effective lesson plans for Radio Park Elementary.

INTERN AT THE FOOD MARKETING INSTITUE
June 2012 – August 2012
Arlington, Virginia
- ≅ Analyzed and compiled the FMI "Retailer Contributions to Health and Wellness" survey and wrote the final research report, published on their company website
- ≅ Overhauled the health and wellness center on the FMI website. Created an effective and interactive resource for food retailers developing their health and wellness initiatives
- ≅ Designed the Contributor Package for the "Let's Put Our Plates Together" family meals campaign for food retailers

DIETARY AIDE AND PREP COOK AT FOXDALE RETIREMENT HOME
May 2008– August 2011
State College, Pennsylvania
- ≅ Served elderly residents in their rooms and in the dining room and worked with nurses to provide the most comfortable experience
- ≅ When working as a prep cook, prepared and cooked food for special diets particular to the elderly population.
- ≅ Learned how to best care for the elderly population through personal interaction with the residents and observing their reservations and concerns about diet

Honors and Awards

ALUMNI RECOGNITION FOR STUDENT EXCELLENCE AWARD
- ≅ One student from each department of the Health and Human Development College at Penn State were selected to receive this award for outstanding academics and contributions to the college and department.

SCHREYER HONORS COLLEGE ACADEMIC SCHOLARSHIP
- ≅ Chosen scholarship recipient and student in the honors college upon admission to Penn State

MARY BOYLE WEAVER AND REBECCA BOYLE SUTHERLAND SCHOLARSHIP
- ≅ Scholarship recipient chosen for academic excellence by the Health and Human Development College at Penn State

MARIE RADOMSKY AND VERNON W. ELLZEY HONORS SCHOLARSHIP
- ≅ Scholarship recipient chosen for academic excellence by the Health and Human Development College at Penn State

DEAN'S LIST
- ≅ Listed as a Dean's List scholar for academic achievement for all semesters

HEALTH AND HUMAN DEVELOPMENT GRANT WINNER
- ≅ Awarded the Small Project Grant by the Health and Human Development College Dean as well as the Awards Committee and Alumni Society Board to create nutrition curriculum kits for teachers via the local library.

SERV SAFE CERTIFIED
- ≅ Passed the certification exam in food safety and preparation with over 95%

Leadership Activities/Association Memberships

PRODUCTION MANAGER OF IN THE HEIGHTS PRESENTED BY THE PENN STATE THESPIANS
November 2013– April 2014
Penn State University
- ≅ Overhauled the hierarchal and communication structure of the Penn State Thespians Production Staff to be streamlined and consistent
- ≅ Oversees the Technical Director, the technical staff, and the Director and the Rehearsal staff
- ≅ Instituted weekly reports and documentations of all tasks and jobs of a production head to aid the organization in the future
- ≅ Manages a ten-thousand dollar budget by organizing receipts, verifying need and reporting purchases to multiple funding sources

DIRECTOR OF CHICAGO PRESENTED BY THE PENN STATE THESPIANS

September 2013–October 2013

Penn State University

- ≅ Designed the artistic vision for the show, and led a production staff of 25 members to realize that vision
- ≅ Led a rehearsal staff of 10 in managing cast rehearsals
- ≅ Coordinated a cast of 28 people and led rehearsals to develop the best possible acting technique and production value

PUBLIC RELATIONS CAPTAIN FOR PENN STATE DANCE MARATHON (THON)

October2013–February 2013

Penn State University

- ≅ Selected to be a leader of the largest student run philanthropy in the world, raising over 12 million dollars in 2013 alone for the fight against pediatric cancer
- ≅ Marketed, promoted, rehearsed and organized a large entertainment fundraiser for the entire THON community
- ≅ Enhanced THON's promotional efforts by participating in marketing and promotion focus groups

MORALE CAPTAIN FOR PENN STATE DANCE MARATHON (THON)

September 2012–February 2013

Penn State University

- ≅ Managed a committee of 32 volunteers throughout the THON season, held weekly meetings and planned committee events
- ≅ Trained volunteers in dancer wellness to provide support to those dancing/standing in the 46 hour no-sleeping no sitting Dance Marathon
- ≅ Mediated discussions and coordinated the creation of the Line Dance, a hallmark tradition of every THON weekend intended to motivate and stretch those standing on their feet for 46 hours