MICRONUTRIENT STATUS AND ITS EFFECT ON INFANT NEUROCOGNITIVE DEVELOPMENT IN RURAL CAMBODIA

by

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A THESIS

Presented to the Department of Human Physiology
and the Robert D. Clark Honors College
in partial fulfillment of the requirements for the degree of
Bachelor of Science

May 2024

An Abstract of the Thesis of

Megan Oshiro for the degree of Bachelor of Science
in the Department of Human Physiology to be taken May 2024

Title: Micronutrient Status and Its Effect on Infant Neurocognitive Development in Rural Cambodia

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Micronutrient deficiencies can significantly impair cognitive development, especially during critical periods in early infant life. As well, micronutrient levels that are not explicitly deficient, but at sub-clinical levels, might confer developmental risk. The present thesis aims to assess severity of thiamine, iron, and vitamin A deficiency in a sample of infants participating in a randomized control trial (RCT) in rural Cambodia (Whitfield et al., 2019), and examine possible relationships of such deficiency to infants' neuro-cognitive development. Data were collected in Kampong Thom, Cambodia from exclusively breastfed infants (n=335) participating in a four-arm RCT; mothers were supplemented with thiamine (0, 1.2, 2.4, 10 mg/day dosage levels) across the first 6 months postpartum. Infant blood at 24 weeks was assayed for a) erythrocyte transketolase activity coefficient (ETKac) for thiamine, b) ferritin for iron, and c) retinol binding protein (RBP) for vitamin A. Neurocognition was assessed at 24 and 52 weeks via the Mullen Scales of Early Learning (MSEL). We found that infants were at higher risk for vitamin A deficiency compared to thiamine and iron (though the risk of thiamine deficiency was higher in the placebo group compared to the other supplementation groups). In addition, thiamine (ETKac) and MSEL baseline scores were significantly associated with MSEL motor and language scores at 24 and 52 weeks. However, the directionality of relationship between

ETKac and MSEL scores was opposite of our expectations, which is consistent with the hypothesis that early life lack of access to adequate thiamine reduces infant ETK production. In summary, these findings underscore that infants in rural Cambodia face the risk of multiple micronutrient deficiencies and these deficiencies – even at marginal levels – can be associated with negative consequences for their neuro-cognitive development. These findings will contribute in important ways to designing nutritional interventions to protect infants' neurocognitive outcomes.

Acknowledgements

I am extremely grateful to have had the best possible support and guidance throughout this entire process and cannot emphasize enough the roles of others in producing this thesis. Thank you to my advisor and mentor Dr. Dare Baldwin for welcoming me into the Acquiring Minds Lab and for guiding me through this process every step of the way. It has been such a privilege to work with someone who is as knowledgeable, understanding, and encouraging as her. I would also like to express my gratitude for Dr. Jeffrey Measelle who has provided meaningful insight to this thesis and to my own growth and learning throughout this endeavor. Dr. Baldwin and Measelle have both contributed in a multitude of ways to this research and I am deeply indebted to the patience, expertise, and time they have shared with me over the past year. I am sincerely thankful to Dr. Nicki Swann for being a wonderful professor and agreeing to be a part of this thesis committee in my process of acquiring honors in the Human Physiology Department. Thank you to my roommates and friends who have been by my side and cheering me on in every aspect. I am incredibly lucky to have had their thoughtful support. Lastly, I would like to thank my family for giving me the opportunity to come to the University of Oregon and for everything they do to always make me feel loved and supported.

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Introduction

Micronutrient deficiencies are strikingly common, affecting an estimated one third of people globally (Han et al., 2022). Infants in particular are at high risk of developing these deficiencies because they require more nutrients to support their rapid growth and development (Bailey et al., 2015). Inadequate nutrition during infancy has significant repercussions that threaten infant growth and neurocognitive development and can lead to long-term health impairments (Kirolos et al., 2024). Recent studies on effects of thiamine deficiency have shown some of the detrimental health impacts that micronutrient deficiencies can have on infant health. For example, in 2003 there was an outbreak of thiamine deficiency in Israeli children after thiamine was mistakenly left out of infant formula. A longitudinal study showed that the children who survived suffered long term developmental, gross motor, and medical impairments (Mimouni-Bloch et al., 2014).

Micronutrient deficiencies are especially prevalent in developing countries due to food insecurity and a lack of dietary diversity. Infants in Southeast Asian countries, such as Cambodia, are at high risk for thiamine deficiency due to the reliance on polished white rice as a dietary staple. A randomized controlled trial (RCT) conducted recently showed the first experimental evidence for benefits of thiamine supplementation of breastfeeding mothers on infant neurocognitive development (Measelle et al., 2021). This raises questions about the potential developmental advantages to optimal micronutrient intake of other micronutrients that are known to play a role in brain development. Iron and vitamin A are both micronutrients known to be essential to brain development, but also are micronutrients infants tend to be deficient in (Allen & Haskell, 2001; Christensen et al., 2022). The purpose of this thesis was to

examine thiamine, iron, and vitamin A status in infants in rural Cambodia, and assess the collective impact of these micronutrient deficiencies on infant neurocognitive outcomes.

Literature Review

Malnutrition and Micronutrient Deficiency

Malnutrition is a threat to all ages, but it is particularly detrimental for children. Malnutrition interferes with physical growth and neurocognitive development, as well as increases the risk of infection and death. According to the World Health Organization (WHO), globally 148.1 million children under the age of 5 are stunted (low length for age) as a result of malnutrition, and 45% of deaths reported amongst children in that age range are linked to undernutrition (Clark et al., 2020). In addition, roughly 250,000 children who are stunted die annually, and over one million wasting children (low weight for length) also die annually (Mertens et al., 2023). In 2016 the World Health Assembly and Sustainable Development Goal set targets to address malnutrition by 2025. However, reports within the last few years have shown that insufficient progress has been made and the world is still far from achieving these goals (Govender et al., 2021).

While a lack of food due to food insecurity and distribution is one of the main factors contributing to the presence of malnutrition, there is another critical factor driving its continued prevalence. Despite having enough food to eat, people in many countries are lacking certain micronutrients in their diets, which fails to provide what the body needs to survive and is another form of malnutrition. Such micronutrient deficiency is also known as “hidden hunger” and is defined by the WHO as the “non-explicit need for one or more of the 26 micronutrients that are essential for adequate body function” (Weffort & Lamounier, 2023). Over two billion people

around the world are at risk of developing micronutrient deficiencies and these deficiencies are especially widespread in lower-middle-income countries (LMICs) (Dairo & Ige, 2009; Keats et al., 2019).

Many people living in LMICs rely on starch-based staples that comprise the majority of their meals, and therefore, they often have diets lacking diversity in nutrients. Cambodia is an LMIC that relies on polished rice as a dietary staple. However, nutrients such as thiamine are only found in the husk and bran of rice that is removed in the milling process to produce polished rice. As a result, the lack of thiamine consumption in Cambodia has led to the development of thiamine deficiencies, which are especially prevalent in lactating women and infants due to their increased metabolic needs (Whitfield et al., 2017).

Nutrition and Brain Development

Pregnant women and children below the age of 5 who live in LMICs are at highest risk for micronutrient deficiencies (Black, 2003). From conception to the first two years of life, brain development is particularly rapid which increases the demand for essential nutrients (Likhar & Patil, n.d.). Myelination and hippocampal growth both increase abruptly at 32 weeks gestation and is the most active until about 18-24 months (Cusick & Georgieff, 2016). In addition, the monoamine neurotransmitter system starts developing prenatally and continues till about 3 years after birth. All of these processes require several micronutrients such as thiamine, iron, and vitamin A and recent evidence has shown the significance of these micronutrients in regards to cognitive development (Likhar & Patil, n.d.).

Thiamine

Thiamine, also known as vitamin B_1, is an important micronutrient (K. C. Whitfield et al., 2018). It acts as a coenzyme in the metabolism of carbohydrates and amino acids, and is essential

for cell development and growth (Fattal et al., 2011). Thiamine has a short half-life between 1-12 hours and is not produced by the human body endogenously. In addition, thiamine is a water-soluble vitamin, so it is only stored in small amounts and the excess is excreted in urine. Thus, regular consumption of thiamine is required to maintain adequate levels in the body.

The initial symptoms of thiamine deficiency include fatigue, poor memory, trouble sleeping, and abdominal discomfort (Fattal et al., 2011). Overtime, thiamine deficiency can lead to a reduction in enzymatic activity and clinical conditions such as Beriberi and Wernicke-Korsakoff syndrome. Beriberi is a potentially fatal form of severe thiamine deficiency that can affect the cardiovascular system as well as the peripheral nervous system. Wernicke-Korsakoff syndrome affects the nervous system and can cause nystagmus, ophthalmoplegia, psychomotor slowing, loss of sensation, and impaired consciousness. Both conditions when left untreated have the possibility of leading to death (Fattal et al., 2011).

Infants are especially at risk for developing thiamine deficiency due to the amount of growth and metabolic activity that happens within the first year of life (Fattal et al., 2011). Infants of thiamine deficient mothers who exclusively breastfeed are at the highest risk for thiamine deficiency. Before the 1990s, infants were brought into hospitals in Laos with an unidentifiable disease that was characterized by heart failure (Soukaloun et al., 2011). Later these cases were identified as “infantile beriberi”. This is the general term for a variety of health issues caused by thiamine deficiency, with a prevalence of cardiac and neurological symptoms. Infantile beriberi can be a very serious disease, and in regions known to have thiamine deficiency, infant mortality is associated with beriberi.

Beriberi can be easily misdiagnosed as other conditions such as a viral infection, pneumonia, or malaria. However, when identified quickly and treated immediately, recovery can

be seen within hours (K. C. Whitfield et al., 2018). Additionally, even sub-clinical levels of thiamine deficiency over long periods of time has been shown to have medical and developmental consequences (Harel et al., 2017). Many regions around the world are prone to thiamine deficiency, as a result of dietary reliance on carbohydrates, including polished white rice (Smith & Hess, 2021). Southeast Asia is one such region, including Cambodia. As of 2017, women and children in Cambodia have suboptimal thiamine status and 38-70% of infants, 6-12 months, were found to be thiamine deficient (K. C. Whitfield et al., 2017). This is an alarming statistic that this thesis aims to investigate.

Iron

Iron is a mineral that is absorbed through the gut and can be found circulating in blood, muscle tissue, or in its stored form (ferritin) within other various tissues. It is primarily used to create hemoglobin (an oxygen carrying protein in red blood cells) and many different enzymes that carry out various cellular functions (Abbaspour et al., 2014). A lack of iron can lead to anemia (low number of healthy red blood cells) causing symptoms such as fatigue, increased heart rate, and an enlarged spleen. Even mild iron deficiency anemia can impair bodily functions and affect cognitive development. Moderate iron deficiency anemia during the first month of life has been shown to produce poor neurodevelopment outcomes even if corrected at 5 years of age (González & Visentin, 2016), indicating that early deficiency has potentially life-long consequences.

On a cellular level, iron is an essential component of cytochrome C oxidase which is the last enzyme in a major metabolic pathway (McCann et al., 2020). Therefore, iron is essential to intracellular metabolism. During brain development there are several processes with high iron-related metabolic demands, meaning that iron deficiency at this time would limit an individual's

developmental potential. In addition, iron is found in enzymes that produces monoamines (ex. Adrenaline, dopamine, serotonin, etc.), each of which is important to key developmental achievements in socio-emotional development, executive functioning, and memory development. These functions thus are also all impacted by an early deficiency in iron. Furthermore, in animal trials, the hippocampus and myelination –both important for basic information processing—have been shown to be impacted by iron deficiency.

Many studies have demonstrated that iron deficiency during infancy is associated with delayed development (Saloojee & Pettifor, 2001). Infants have an increased demand for iron due to their rapid growth, with the need for iron increasing specifically between 4-6 months. Though there is some information on the prevalence of anemia in Cambodia there is not much information on the rates of iron deficiency specifically, and even less data on iron deficiency in infants. One aim of this thesis was to provide some much-needed data that describes the presence of iron deficiency in Cambodian infants.

Vitamin A

Vitamin A is a fat-soluble micronutrient that regulates vision, reproduction, the immune system, and growth and development. The biologically active form of vitamin A is retinoic acid which regulates gene expression and is a key signaling molecule during embryogenesis (Olson & Mello, 2010). It also plays a role in the continued creation, differentiation, and maintenance of neuronal phenotypes and neural tube development. Therefore, retinoids have been found to be involved with learning, memory, synaptic plasticity, and sleep (Olson & Mello, 2010). In a study with mice lacking receptors for retinoic acid in their hippocampus, they found that the mice experienced cognitive deficits for learning, motor control, and balance compared to wild type mice (Chiang et al., 1998).

Approximately one third of children under 5 in LMICs are deficient for vitamin A (Weffort & Lamounier, 2023). Vitamin A deficiency (VAD) can lead to night blindness and if untreated could eventually lead to true blindness. Xeropthalmia is the clinical presentation of VAD which includes night blindness, Bitot's spots, and corneal scarring. According to the WHO, 500 million children are blind due to VAD and 50% die within a year of blindness (Song et al., n.d.). In addition, vitamin A is involved with the maintenance of mucosal membranes. VAD can cause a breakdown of mucosal membranes in the lungs and intestines as well as immune dysfunctions that increase the frequency of infection and chronic inflammation leading to anemia (Hodge & Taylor, 2024). In developing countries where infectious diseases are highly prevalent and deadly, a compromised immune system due to VAD can be an underlying factor of mortality.

Additionally, mothers postpartum have lower retinol levels within the first year which decreases the amount of vitamin A they have in their breast milk. Usually, the concentration of vitamin A in the breastmilk only meets infants' daily needs, so vitamin A is not readily stored leading to vitamin A deficiency after weaning from breastmilk. Therefore, it is suggested that vitamin A supplementation is important during lactation. Recent studies have shown that only 6.4% of women in Cambodia are vitamin A deficient. However, in 2000, 22% of rural children in Cambodia aged 5-59 months had severe vitamin A deficiency with no change as of 2005 (Johnston & Conkle, n.d.). Although severe vitamin A deficiency is well-known to undercut healthy neurocognitive development, little research has explored negative consequences that sub-clinically low levels of vitamin A might have. This thesis sought to assess the current state of vitamin A deficiency in infants in Cambodia and the effect it might have on their neurocognitive development.

Randomized Control Trial: Benefits of Maternal Thiamine Supplementation

In response to new findings on the effects of thiamine deficiency on infant health and development, a randomized controlled trial (RCT) was conducted to assess the impact of daily thiamine supplementation of exclusively breastfeeding mothers on mother and infant thiamine status between 2 and 24 weeks postnatal. A follow up was done at 52 weeks to assess long-term impacts of supplementation. Secondary goals of the RCT were to examine the extent to which thiamine supplementation protected infant neurocognitive development, with a particular interest in motor, language, and cognitive development.

The findings of the RCT revealed that daily thiamine supplementation increased maternal milk thiamine concentration (Gallant et al., 2021). In terms of infant neurocognitive development, maternal thiamine supplementation produced significant benefits for infant language development, as measured at 24 weeks post-partum. In particular, maternal supplementation level dosage was positively associated with Mullen Scales for Early Learning (MSEL) receptive language and expressive language scores (Measelle et al., 2021). Furthermore, thiamine supplementation was shown to protect infants' language processing at 24 weeks (Baldwin et al., 2021). These outcomes highlight the importance of adequate thiamine status for infant neurocognitive development. However, the existing studies have only assessed the associations between thiamine and neurocognitive outcomes without consideration for the status of other micronutrients such as iron and vitamin A. This thesis is a secondary data analysis of the RCT that examines the prevalence of iron, and vitamin A deficiency in infants in rural Cambodia, as well as their thiamine status, and assesses the individual and combined predictive capabilities of these micronutrient deficiencies on infant neurocognitive outcomes at 24 and 52 weeks. The specific research questions were as follows:

1. In addition to thiamine levels, what does iron and vitamin A status look like in infants in Cambodia at 24 weeks? How prevalent and severe are these micronutrient deficiencies?

2. Do any associations exist between thiamine, iron, and vitamin A status and one another?

3. How does thiamine, iron, and vitamin A deficiencies relate to infant neurocognitive development (individually and on a combined level) as measured by MSEL scores at both 24 and 52 weeks?

Hypothesis

To investigate these questions, exploratory secondary analysis of the existing RCT data was conducted using descriptive statistics and multiple regression models. Due to thiamine supplementation as the intervention for this RCT, we expected the majority of the sample to be sufficient in thiamine. However, it was predicted that the placebo group might have higher proportions of marginal deficiency/deficiency for thiamine than the other treatment groups. Furthermore, the government in Cambodia maintains a standard of care that pregnant women receive prenatal vitamins that have 30-60 mg of iron (Um et al., 2023). Therefore, it was predicted that most of the infants would not be deficient for iron. Vitamin A status of Cambodian infants was predicted to trend towards deficiency.

Limited information exists on the relationship between thiamine status and iron or vitamin A status. However, evidence suggests an association between iron and vitamin A deficiency (Saraiva et al., 2014). As a result, we expected a positive association between iron and vitamin A status in our sample. Further, we predicted that micronutrient status would be associated with neurocognitive outcomes; in particular, micronutrient deficiency was expected to predict reduced MSEL scores over time.

Methods

The data from this double blind, four parallel arm RCT were collected between September 2018 and December 2019 in participants' homes. The National Ethics Committee for Health Research (Cambodia), Mount Saint Vincent University Research Ethics Board (Canada), and the University of Oregon Institutional Review Board (United States) all provided ethical approval of this RCT. The full protocol for this RCT has been published (K. C. Whitfield et al., 2019).

Participants

Participants were healthy mother-infant pairs (n=335) recruited during antenatal care visits at one of eight health centers in Kampong Thom, Cambodia. Eligibility criteria for this study included mother's (18-45 years old) most recent pregnancy being "normal" (i.e. no preeclampsia, no gestational diabetes, no chronic conditions, etc.) and intention to exclusively breastfeed for six months. Infants were singletons born without any complications. The participants resided in Kampong Thom, Cambodia and were not planning to move in the next six months. In addition, the participants' entire household had to be willing to only consume salt provided by the study. Lastly, the participants were not enrolled in any other nutritional programs at the time and did not take any thiamine supplements up to 4 months before the start of their participation. Throughout the pregnancy and lactation all mothers who participated received an average of 84 iron tablets (SD 25) and 32 folic acid tablets (SD 15), which represents the standard-of-care in Cambodia.

Sample Characteristics

Characteristics for infants, mothers, and households of the sample appear in Table 1. 335 infants participated in the study at baseline and usable cognitive data was available for 295 infants at 24 weeks and 309 infants at 52 weeks. Loss to follow up was mainly due to migration of participants. Infant length-for-age at birth was below WHO Z-score means, and the majority of women and their husbands had achieved only a primary-level education. Though the baseline MSEL scores were lower than the US T-score norms, there were no significant differences in baseline scores between treatment groups (Measelle et al., 2021).

Table 1: Infant, Mother, and Household characteristics

	TOTAL (N=335)
Infant	
Sex, *female*	161 (48%)
Length for age at 2 weeks	-0.62 (1.02)
MSEL[1] at 2 weeks	
Gross Motor	36.91 (7.18)
Fine Motor	33.89 (5.96)
Expressive Language	38.10 (1.36)
Mother	
Age, *years*	28.1 (6.2)
Parity, *multiparous*	57 (69%)
Ethnicity, *Khmer*	335 (100%)
Marital Status	
Married	330 (98%)
Divorced/Widowed/Separated	5 (<1%)
Education	
None	40 (12%)
Primary	161 (48%)
Lower Secondary	83 (25%)
Upper Secondary	43 (13%)
Higher Education	8 (2%)
Treatment Group	
Placebo (0 mg)	83 (25%)
1.2 mg	86 (26%)
2.4 mg	81 (24%)
10 mg	85 (25%)
Household	
Father Education	
None	38 (11%)
Primary	151 (45%)
Lower Secondary	97 (29%)
Upper Secondary	34 (10%)
Higher Education	15 (4%)
Wealth Index Score*	
Poorest	81 (24%)
Second	69 (21%)
Third	108 (32%)

Fourth	54 (16%)
Wealthiest	23 (7%)

Data reported as means and (SD) or n (%). Percentages may not add up to 100% due to rounding. [1]MSEL = Mullen Scales for Early Learning. *Wealth equity index (WEI) based on Demographic Health Survey (DHS) Program guidelines (USAID); developed using 2014 DHS data.

Data Sample

Thiamine Supplementation Treatment Group

Mothers were randomly assigned to one of four thiamine supplementation treatment groups: placebo (0mg), 1.2 mg (Estimated Average Requirement, EAR), 2.4 mg (2 X EAR), and 10 mg (positive control). Each mother consumed 1 capsule with respective thiamine dosage daily from 2-24 weeks postpartum. The capsules were made of gelatin with identical sizes and shapes. They contained various amounts of thiamine hydrochloride (respective to the corresponding supplementation group) as well as cellulose which served as a filler. The content of the capsules was randomly tested before and during the trial with only slight deviations from desired content (97-106% expected thiamine content). Double blinding was achieved by labeling the capsules with alpha-numeric codes that correspond to a treatment group. These codes were created by statisticians at the South Australian Health and Medical Research Institute (SAHMRI). The capsules were formulated, compounded, and packaged at the Quinpool Wellness Center in Halifax, Canada.

Compliance

Compliance was assessed every 2 weeks by field staff who collected the old capsule packet, counted the number of remaining capsules, and gave them a new capsule packet. At this time, the field staff also asked if the participant had shared or sold the salt, about infant feeding

habits, and about eating from the common household pot. To build rapport and encourage high compliance, participants interacted with the same field staff throughout the study. In addition to the fortnightly in-person check-ins, participants would receive a daily text and weekly call to remind them to consume the capsules daily. A participant was considered compliant if they consumed more than 80% of capsules within the 22-week intervention.

Measures

Biological Samples

Blood samples from mothers were taken at 2 and 24 weeks, and infant blood samples were collected at 24 weeks. Phlebotomists from the National Institute of Public Health Laboratories (NIPHL) met with the mothers and infants in their homes or a central village location to collect the blood samples in EDTA-coated tubes. 9 mL of blood were collected from the mothers and 5 mL from infants. The samples were placed on ice and transferred within 5 hours of collection to a field lab in Kampong Thom. Before blood sample processing, the sample tube was inverted several times. Then one drop of the sample blood was used to record hemoglobin and one capillary tube was filled to record hematocrit. The remaining sample was then centrifuged for 15 minutes at 3000 rpm and 4° C. Next, the buffy coat and plasma were removed, and the erythrocytes washed 3 times using PBS. Samples were stored for up to 10 days at -20° C before being moved to the NIPHL in Phnom Penh for storage at -80° C. The samples were batch shipped on top of dry ice to participating laboratories for analysis.

Anthropometrics

The infant anthropometrics collected included length, weight, and head circumference. Infant anthropometrics were collected at 2, 12, and 24 weeks using calibrated instruments and anthropometric indicator measurement guide (Cogil, 2003).

Neurocognitive Data & Questionnaire Data

Infant cognitive development was assessed at 2, 12, 24, and 52 weeks postpartum using the MSEL (in participants' homes). The MSEL is a performance-based standardized developmental test that assesses child development in the domains of gross motor, fine motor, visual reception, receptive language, and expressive language. Since this was the first known time that the MSEL had been used with Cambodian infants, a translated version of the MSEL was tested with 10 Cambodian infants to establish test validity before use in this study. No adjustments were made to the MSEL stimuli or procedures except slight modifications of the toys used. For each subscale infants were rated based on their success completing the performance-based task (0 for failed and 1 for passed). The scores were calculated by adding the child's points for each MSEL scale. Raw scores were transformed into age-normal T-scores (based on a US norming sample). In this thesis the MSEL scores at 24 and 52 weeks were used for data analysis. Demographic (ex. ethnicity, parental education) and socioeconomic (ex. household income) information were also taken from each participant at 2, 12, 24, and 52 weeks.

Analytic Approach

Micronutrient Biomarkers and Cut-Offs

Infant blood samples at 24 weeks were used for analysis of biomarkers. Participants were separated into groups based on micronutrient cut-offs for sufficiency, marginal deficiency, and deficiency. Thiamine deficiency was assessed by the ETKac ratio. ThDP (a phosphorylated form of thiamine in humans) is a cofactor for many metabolic enzymes including erythrocyte transketolase (ETK). The ETKac ratio is the amount of stimulated ETK activity (by addition of exogenous ThDP) in relation to the basal ETK activity (Jones et al., 2021). In the case of thiamine sufficiency, the ETKac ratio is thought to be close to 1 since the addition of thiamine would have little effect on adequately saturated ETK. In the case of thiamine deficiency, the ETKac ratio is thought to be high due to a lack of endogenous thiamine to be metabolized. The cut-offs used for thiamine-related analysis were <1.15 for thiamine sufficiency, 1.15-1.25 for marginal deficiency and >1.25 for deficiency (Whitfield, 2024).

Iron deficiency was assessed using ferritin levels. Ferritin is a measure of the amount of stored iron and is commonly used to assess iron deficiency (Al-Naseem et al., 2021). The cut-offs used for iron-related analysis were <30 μg/L for sufficiency, 9-30 μg/L for marginal deficiency, and <9 μg/L for deficiency (personal communication, Wieringa, 2023).

Lastly, vitamin A deficiency was assessed using retinol binding protein (RBP) levels. RBP is a protein used to transfer vitamin A in the tissues (Mahmood et al., 2008). The RBP cut-offs in data analysis were >1.05 μmol/L for sufficiency, 0.7-1.05 μmol/L for marginal deficiency, and <0.7 μmol/L for deficiency (personal communication, Wieringa, 2023).

Inflammation has been shown to impact micronutrient status, particularly for ferritin and vitamin A (Tomkins, 2003). LMICs have a higher prevalence of inflammation which has been

shown to impair cognitive development (Page et al., 2018; Adelantado-Renau et al., 2020). Therefore, ferritin and RBP were corrected for inflammation before assigning deficiency status using previously calculated correction factors (Thurman et al., 2010; Thurman et al., 2003). See appendices A and B for ferritin and RBP correction factors.

Statistical Analysis

After participant data were trichotomized (into sufficient, marginally deficient, and deficient categories) using the deficiency cut-offs of each micronutrient, SPSS was used to determine the frequency of the trichotomized groups for each micronutrient (Table 2). Gallant et al. examined the same RCT and calculated the proportions of trichotomized groups (for thiamine) by treatment group. These data were used to create Figure 1, which visualizes thiamine deficiency at 24 weeks by treatment group. Next, correlations models were used to assess associations among thiamine, iron, and vitamin A (using both continuous and trichotomized variables). Another set of correlations were calculated to assess thiamine, iron, and vitamin A (continuous and trichotomized variables) in relation to MSEL scores at 24 and 52 weeks. Then, multiple regression models were run to assess the combined effects of thiamine, iron, and vitamin A (continuous and trichotomized variables) on MSEL scores at 24 and 52 weeks. The models were first partially adjusted using thiamine treatment group as a control to account for maternal thiamine supplementation in the RCT. Then the models were fully adjusted using thiamine treatment group, infant sex, infant birthweight, and MSEL 2 week (baseline) scores in 24 week models or MSEL 24 week (endline) scores in 52 week models.

Results

Micronutrient Status of Sample

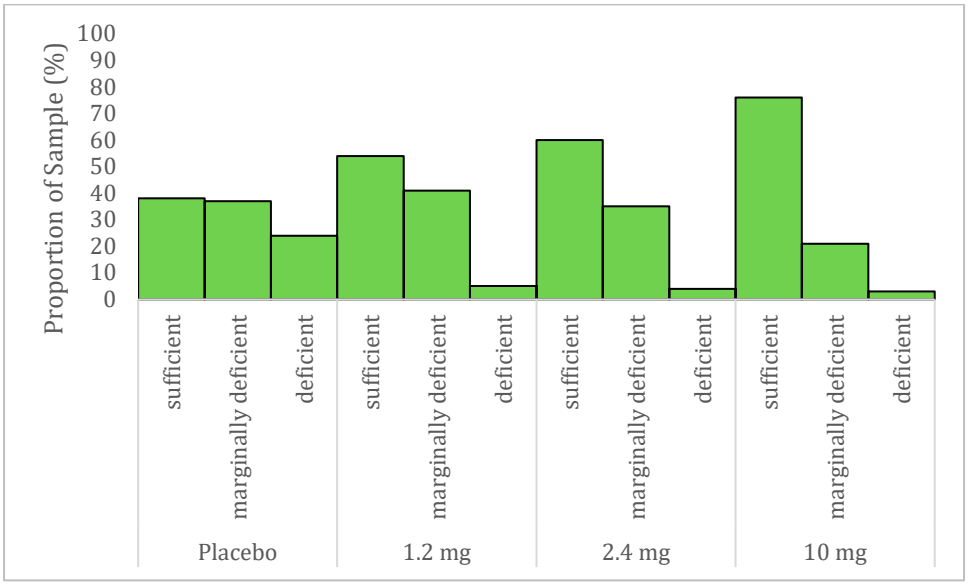
First, we provide descriptive information about the proportion of infants who met criteria for sufficiency, marginal deficiency, and deficiency for each micronutrient of interest (see Table 2). The status cut-offs are as follows: thiamine (sufficient: ETKac <1.15, marginally deficient: 1.15-1.25, deficient: >1.25), iron (sufficient: ferritin >30 μg/L, marginally deficient: 9-30 μg/L, deficient: < 9 μg/L), and vitamin A (sufficient: RBP >1.05 μmol/L, marginally deficient: 0.7-1.05 μmol/L, deficient: <0.7 μmol/L). Then we examined the percentage of infants who met criteria for sufficiency, marginal deficiency, and deficiency for thiamine with respect to each of the maternal thiamine supplementation treatment groups (see Figure 1).

Table 2: Proportion of Micronutrient Sufficiency, Marginal Deficiency, and Deficiency

	Sufficient	Marginally Deficient	Deficient
Thiamine	56.3 (%)	37.9 (%)	5.9 (%)
Iron	63.3 (%)	32.2 (%)	4.5 (%)
Vitamin A	26.5 (%)	67.1 (%)	6.4 (%)

Though technical deficiency of micronutrients was rare, marginal deficiency was common. Less than 7% of the sample was deficient for each micronutrient according to the cut-offs used. However, over 30% of the sample were marginally deficient for thiamine and iron, and the majority (67.1%) of the sample was marginally deficient in vitamin A (Table 2). The results match our predictions that iron deficiency would be less prevalent compared to vitamin A deficiency, with the possible explanation of pregnant women receiving prenatal iron as a standard of care in Cambodia.

Figure 1: Proportions of thiamine sufficiency, marginal deficiency, and deficiency for each treatment group



Note: There were 83 (placebo), 86 (1.2 mg), 81 (2.4 mg), and 85 (10 mg) participants in the treatment groups.

The placebo group (as shown in Figure 1) had the highest proportion of thiamine-deficient infants (24%) with a similar proportion of marginally-deficient infants (37%) as the 1.2 (41%) and 2.4 (35%) mg groups. Therefore, though the proportion of thiamine-deficient infants for the whole sample was 5.9%, there was a comparably higher proportion of thiamine deficiency within the placebo group (24%). This aligns with our original prediction that the placebo group would exhibit a higher level of thiamine deficiency compared to the other treatment groups and the sample as a whole.

Correlations Between Micronutrients

Next, we provide the correlation coefficients between each micronutrient and one another (both continuous and trichotomized variables) to show relationships among the three micronutrients (thiamine, iron, and vitamin A, see Table 3).

Table 3: Correlations between Thiamine, Iron, and Vitamin A status

	Thiamine Continuous	Thiamine Trichotomized	Iron Continuous	Iron Trichotomized	Vitamin A Continuous	Vitamin A Trichotomized
Thiamine Continuous	1	--	0.086	-0.057	-0.085	0.086
Thiamine Trichotomized		1	0.044	-0.042	-0.051	0.086
Iron Continuous			1	--	**0.160****	-0.113
Iron Trichotomized				1	-0.091	0.077
Vitamin A Continuous					1	--
Vitamin A Trichotomized						1

The bottom shaded half of this table would be a reflection of the top half of the table, so it has been shaded for clarity. The dashed lines (--) indicate correlations between the continuous and trichotomized variables of the same micronutrient. The numerical values aren't included as these were not independent measures (the trichotomized variable was created using the continuous variable for each micronutrient).

*P < 0.05; **P < 0.01; ***P < 0.001 (the significant result in this table is in bold).

As predicted thiamine levels were not significantly correlated with iron or vitamin A. The only correlation between different micronutrients in this sample, that met a $p < 0.01$ criterion level, was between iron (continuous) and vitamin A (continuous) as shown in Table 3. This positive association between iron and vitamin A replicates previous evidence of relations between vitamin A and iron status.

Correlations Between Micronutrient Status and MSEL Scores

In this section we provide the correlation coefficients between each micronutrient (for both continuous and trichotomized variables) and MSEL scores from each domain (gross motor, fine motor, visual reception, receptive language, and expressive language) at 24 weeks (see Table 4). In addition, correlational analyses were also run between micronutrient variables and MSEL scores of each domain at 52 weeks (see Table 5). This was conducted to see what

associations existed at 52 weeks as well as compare these associations with those seen at 24 weeks.

Table 4: Correlations Between Micronutrient Status and 24 Week MSEL Scores

	MSEL Gross Motor	MSEL Fine Motor	MSEL Visual Reception	MSEL Receptive Language	MSEL Expressive Language	Early Learning Composite
Thiamine Continuous	**0.144***	**0.128***	0.031	0.089	**0.187****	**0.138***
Thiamine Trichotomized	**0.150***	0.077	0.063	0.055	**0.127***	0.100
Iron Continuous	0.079	0.022	0.084	0.050	0.020	0.059
Iron Trichotomized	-0.074	-0.038	-0.010	0.002	0.015	-0.008
Vitamin A Continuous	0.060	-0.037	0.046	-0.045	0.002	-0.019
Vitamin Trichotomized	-0.023	0.014	0.029	0.019	0.000	0.021

*P < 0.05; **P < 0.01; ***P < 0.001 (the significant results in this table are in bold).

Table 5: Correlations Between Micronutrient Status and 52 Week MSEL Scores

	MSEL Gross Motor	MSEL Fine Motor	MSEL Visual Reception	MSEL Receptive Language	MSEL Expressive Language	Early Learning Composite
Thiamine Continuous	0.028	0.113	0.103	**0.123***	**0.192****	**0.160****
Thiamine Trichotomized	0.019	0.113	**0.129***	**0.150***	**0.212****	**0.182****
Iron Continuous	-0.113	**0.127***	0.093	0.080	**0.138***	**0.135***
Iron Trichotomized	0.064	-0.081	-0.102	-0.042	-0.111	-0.107
Vitamin A Continuous	-0.059	0.008	0.074	0.036	0.032	0.049
Vitamin A Trichotomized	0.012	-0.012	-0.073	-0.109	-0.056	-0.074

*P < 0.05; **P < 0.01; ***P < 0.001 (the significant results in this table are in bold).

Thiamine was significantly associated with MSEL gross motor, fine motor, expressive language, and early learning composite scores at 24 weeks (Table 4). At 52 weeks thiamine was no longer associated with gross or fine motor domains, but was then associated with receptive language in addition to expressive language and early learning composite scores. There were no significant associations between ferritin or RBP and MSEL scores at 24 weeks. However, at 52 weeks, ferritin (continuous) was correlated with fine motor, expressive language, and early learning composite scores (Table 5).

Micronutrient Status and MSEL Score Multivariate Regression Models

The last type of analysis provided is a set of multivariate regression models. The first two models compare the status of each micronutrient (thiamine, iron, and vitamin A) with MSEL scores from each domain (gross motor, fine motor, visual reception, receptive language, and expressive language) at 24 weeks. The models shown in Table 6 used continuous micronutrient variables and the models in Table 7 used the trichotomized micronutrient variables as measures of micronutrient status. Both models were first partially adjusted for maternal thiamine supplementation treatment group and then fully adjusted for treatment group, infant sex, infant birthweight, and MSEL baseline scores. See appendices C and D for R^2, F, and p values that summarize the models themselves.

Table 6: Micronutrient (continuous) Effect on 24 Week MSEL Scores

	Gross Motor				Fine Motor				Visual Reception				Receptive Language				Expressive Language				Early Learning Composite			
	B	**SE**	**b**	**t**	**B**	**SE**	**b**	**t**	**B**	**SE**	**b**	**t**	**B**	**SE**	**b**	**t**	**B**	**SE**	**b**	**t**	**B**	**SE**	**b**	**t**
Partially Adjusted																								
Thiamine Treatment Group	0.051	0.113	0.028	0.447	0.136	0.099	0.085	1.368	-0.135	0.102	-0.083	-1.324	0.373	0.175	0.133	2.127*	0.211	0.100	0.131	2.116*	0.585	0.363	0.100	1.611
Thiamine	17.267	7.440	0.146	2.321*	15.313	6.508	0.148	2.353*	-0.055	6.669	-0.001	-0.008	20.767	11.518	0.113	1.803	21.985	6.533	0.209	3.365**	58.010	23.846	0.152	2.433*
Iron	0.007	0.009	0.047	0.768	0.005	0.008	0.040	0.651	0.009	0.008	0.073	1.171	0.009	0.014	0.040	0.642	-0.001	0.008	-0.007	-0.122	0.022	0.028	0.048	0.782
Vitamin A	0.973	0.943	0.064	1.032	-0.414	0.825	-0.031	-0.502	0.382	0.845	0.028	0.452	-1.117	1.460	-0.047	-0.765	0.309	0.828	0.023	0.373	-0.840	3.023	-0.017	-0.278
Fully Adjusted																								
Thiamine Treatment Group	0.042	0.111	0.023	0.374	0.118	0.098	0.074	1.201	-0.143	0.102	-0.088	-1.408	0.332	0.174	0.118	1.911	0.215	0.100	0.133	2.148*	0.507	0.362	0.087	1.401
Thiamine	16.603	7.318	0.140	2.269*	16.567	6.497	0.160	2.550*	-1.259	6.685	-0.012	-0.188	11.757	11.756	0.064	1.000	21.641	6.597	0.206	3.280**	49.552	23.910	0.130	2.072*
Iron	0.006	0.009	0.039	0.603	0.000	0.008	0.001	0.017	0.004	0.009	0.029	0.428	0.001	0.015	0.003	0.042	-3.682E-5	0.008	0.000	-0.004	0.005	0.030	0.010	0.148
Vitamin A	0.750	0.925	0.049	0.811	-0.328	0.817	-0.025	-0.401	0.338	0.845	0.025	0.400	-1.222	1.442	-0.052	-0.848	0.278	0.830	0.021	0.335	-0.937	3.000	-0.019	-0.312
Infant Sex	-0.424	0.913	-0.029	-0.465	-0.267	0.806	-0.021	-0.332	0.869	0.833	0.067	1.043	1.594	1.424	0.071	1.119	-0.565	0.820	-0.044	-0.690	1.682	2.964	0.036	0.567
Infant Birth Weight	1.269	1.231	0.063	1.030	1.652	1.087	0.094	1.519	2.052	1.130	0.115	1.816	2.928	1.923	0.094	1.523	0.274	1.107	0.015	0.248	6.568	4.006	0.102	1.640
MSEL Baseline	0.213	0.059	0.216	3.619***	0.165	0.065	0.156	2.550*	0.004	0.009	0.029	0.428	0.219	0.086	0.157	2.550*	-0.327	0.277	-0.071	-1.179	0.259	0.129	0.122	2.002*

*P < 0.05; **P < 0.01; ***P < 0.001.

Table 7: Micronutrient (trichotomized variable) Effect on 24 Week MSEL Scores

	Gross Motor				Fine Motor				Visual Reception				Receptive Language				Expressive Language				Early Learning Composite			
	B	**SE**	**b**	**t**	**B**	**SE**	**b**	**t**	**B**	**SE**	**b**	**t**	**B**	**SE**	**b**	**t**	**B**	**SE**	**b**	**t**	**B**	**SE**	**b**	**t**
Partially Adjusted																								
Thiamine Treatment Group	0.061	0.114	0.034	0.535	0.127	0.100	0.080	1.262	-0.127	0.102	-0.079	-1.245	0.365	0.177	0.130	2.055*	0.193	0.101	0.120	1.918	0.557	0.367	0.096	1.518
Thiamine	1.821	0.758	0.151	2.402*	1.043	0.668	0.099	1.562	0.377	0.680	0.035	0.554	1.594	1.180	0.085	1.351	1.574	0.670	0.148	2.350*	4.653	2.439	0.121	1.908
Iron	-0.921	0.755	-0.074	-1.220	-0.438	0.665	-0.040	-0.659	0.033	0.677	0.003	0.049	0.317	1.175	0.017	0.270	0.378	0.667	0.035	0.566	0.201	2.432	0.005	0.083
Vitamin A	-0.520	0.830	-0.038	-0.627	0.170	0.731	0.014	0.233	0.328	0.744	0.027	0.440	0.518	1.291	0.025	0.401	-0.089	0.733	-0.007	-0.121	0.819	2.661	0.019	0.308
Fully Adjusted																								
Thiamine Treatment Group	0.048	0.112	0.026	0.428	0.105	0.100	0.066	1.059	-0.141	0.102	-0.088	-1.386	0.314	0.175	0.112	1.795	0.199	0.101	0.124	1.971	0.453	0.365	0.078	1.240
Thiamine	1.666	0.750	0.138	2.222*	1.091	0.667	0.104	1.635	0.182	0.683	0.017	0.266	0.686	1.191	0.037	0.576	1.573	0.676	0.148	2.327*	3.557	2.446	0.092	1.454
Iron	-0.768	0.779	-0.062	-0.986	-0.205	0.693	-0.019	-0.296	0.540	0.710	0.049	0.761	1.096	1.217	0.057	0.901	0.505	0.708	0.046	0.714	1.679	2.538	0.042	0.662
Vitamin A	-0.279	0.816	-0.021	-0.342	0.195	0.723	0.017	0.270	0.313	0.743	0.026	0.422	0.822	1.277	0.039	0.643	-0.121	0.734	-0.010	-0.165	1.350	2.652	0.031	0.509
Infant Sex	-0.426	0.894	-0.029	-0.476	-0.246	0.794	-0.019	-0.310	1.052	0.814	0.082	1.292	1.925	1.395	0.086	1.380	-0.314	0.806	-0.025	-0.389	2.335	2.902	0.050	0.805
Infant Birth Weight	1.222	1.231	0.061	0.993	1.638	1.095	0.093	1.496	2.327	1.125	0.131	2.067*	3.313	1.923	0.107	1.723	0.572	1.112	0.032	0.515	7.361	4.013	0.114	1.834
MSEL Baseline	0.209	0.059	0.212	3.547***	0.162	0.064	0.153	2.507*	0.038	0.106	0.022	0.359	0.233	0.086	0.167	2.720*	-0.414	0.279	-0.091	-1.482	0.277	0.130	0.130	2.127*

*P < 0.05; **P < 0.01; ***P < 0.001.

At 24 weeks thiamine status was significantly associated with gross- and fine-motor, expressive-language, and early learning composite MSEL scores in both partially and fully adjusted models (Tables 6 & 7). Though this is intriguing, the directionality of these associations between our measure of thiamine availability -- ETKac -- and MSEL outcome measures is puzzling. The analysis shows a positive association between ETKac and MSEL scores, but we had expected ETKac to be negatively associated with MSEL scores, because thiamine hydrochloride levels and ETKac are typically negatively associated. In other words, higher ETKac would indicate more severe thiamine deficiency, and we'd expect that increased severity of deficiency would be associated with lower MSEL scores. Thus, the directionality of the relationship between ETKac and MSEL outcomes in our findings prompts further investigation in future studies.

Furthermore, MSEL baseline scores were included in these models (Tables 6 & 7) as autoregressive variables in order to see the change in MSEL scores over time as a result of micronutrient factors. MSEL baseline scores were significantly associated with gross- and fine-

motor and receptive language at 24 weeks (Tables 6 & 7). Moreover, thiamine was significantly associated with motor and language scores in the fully adjusted models (that include MSEL baseline) showing that thiamine status accounts for a significant amount of the change in those scores from baseline to 24 weeks. Lastly, infant birthweight and visual reception were significantly associated at 24 weeks.

The next two models compare the status of each micronutrient (thiamine, iron, and vitamin A) with MSEL scores from each domain (gross motor, fine motor, visual reception, receptive language, and expressive language) at 52 weeks. The models shown in Table 8 used continuous micronutrient variables and the models in Table 9 used trichotomized micronutrient variables as measures of micronutrient status. Both models were first partially adjusted for maternal thiamine supplementation treatment group and then fully adjusted for treatment group, infant sex, infant birthweight, and MSEL endline (24 week) scores. See appendices E and F for R^2, F, and p values that summarize the models themselves.

Table 8: Micronutrient (continuous variable) Effect on 52 Week MSEL Scores

	Gross Motor				Fine Motor				Visual Reception				Receptive Language				Expressive Language				Early Learning Composite			
	B	**SE**	**b**	**t**	**B**	**SE**	**b**	**t**	**B**	**SE**	**b**	**t**	**B**	**SE**	**b**	**t**	**B**	**SE**	**b**	**t**	**B**	**SE**	**b**	**t**
Partially Adjusted																								
Thiamine Treatment Group	-0.069	0.176	-0.025	-0.392	0.184	0.194	0.061	0.946	-0.056	0.224	-0.016	-0.250	-0.041	0.139	-0.019	-0.294	-0.007	0.150	-0.003	-0.049	0.079	0.566	0.009	0.140
Thiamine	3.768	11.417	0.021	0.330	20.172	12.572	0.104	1.605	20.500	14.535	0.091	1.410	15.985	9.033	0.114	1.770	28.051	9.697	0.184	2.893**	84.708	36.664	0.148	2.310*
Iron	-0.019	0.014	-0.088	-1.390	0.024	0.015	0.100	1.587	0.019	0.017	0.069	1.097	0.011	0.011	0.068	1.068	0.022	0.012	0.118	1.894	0.076	0.044	0.109	1.743
Vitamin A	-1.083	1.428	-0.048	-0.759	-0.036	1.573	-0.001	-0.023	2.143	1.818	0.075	1.178	0.480	1.130	0.027	0.425	0.489	1.213	0.025	0.404	3.077	4.586	0.042	0.671
Fully Adjusted																								
Thiamine Treatment Group	-0.100	0.170	-0.037	-0.586	0.147	0.192	0.049	0.765	-0.014	0.224	-0.004	-0.063	-0.071	0.140	-0.033	-0.504	-0.053	0.150	-0.022	-0.353	-0.098	0.559	-0.011	-0.176
Thiamine	-4.027	11.184	-0.023	-0.360	15.328	12.593	0.079	1.217	21.350	14.568	0.095	1.466*	14.098	9.111	0.101	1.547	22.634	9.881	0.149	2.291*	66.753	36.563	0.117	1.826
Iron	-0.024	0.014	-0.111	-1.682	0.022	0.016	0.091	1.354	0.020	0.019	0.075	1.097	0.008	0.012	0.048	0.698	0.012	0.012	0.067	1.012	0.062	0.046	0.089	1.334
Vitamin A	-1.453	1.380	-0.065	-1.053	0.093	1.554	0.004	0.060	2.056	1.813	0.072	1.134	0.562	1.131	0.032	0.497	0.411	1.204	0.021	0.341	3.300	4.510	0.045	0.732
Infant Sex	0.898	1.386	0.041	0.648	-0.105	1.564	-0.004	-0.067	-0.358	1.825	-0.013	-0.196	0.882	1.139	0.051	0.775	2.488	1.213	0.132	2.052*	2.648	4.538	0.038	0.583
Infant Birth Weight	-0.756	1.861	-0.025	-0.406	-0.464	2.100	-0.014	-0.221	-1.932	2.458	-0.051	-0.786	-0.089	1.529	-0.004	-0.058	1.218	1.620	0.047	0.752	-2.101	6.112	-0.022	-0.344
MSEL Endline	0.436	0.093	0.286	4.661***	0.366	0.120	0.192	3.061**	0.289	0.136	0.134	2.125*	0.077	0.049	0.100	1.581	0.154	0.091	0.106	1.693	0.318	0.094	0.209	3.368***

*P < 0.05; **P < 0.01; ***P < 0.001.

Table 9: Micronutrient (trichotomized variable) Effect on 52 Week MSEL Scores

	Gross Motor				Fine Motor				Visual Reception				Receptive Language				Expressive Language				Early Learning Composite			
	B	**SE**	**b**	**t**	**B**	**SE**	**b**	**t**	**B**	**SE**	**b**	**t**	**B**	**SE**	**b**	**t**	**B**	**SE**	**b**	**t**	**B**	**SE**	**b**	**t**
Partially Adjusted																								
Thiamine Treatment Group	-0.082	0.177	-0.030	-0.464	0.202	0.195	0.067	1.039	-0.017	0.224	-0.005	-0.077	-0.006	0.139	-0.003	-0.040	0.026	0.150	0.011	0.171	0.207	0.564	0.023	0.366
Thiamine	-0.037	1.168	-0.002	-0.032	2.068	1.283	0.105	1.611	2.793	1.477	0.122	1.891	2.265	0.914	0.159	2.477*	3.294	0.987	0.212	3.336***	10.494	3.717	0.180	2.823**
Iron	0.759	1.162	0.041	0.653	-1.277	1.277	-0.063	-1.000	-1.809	1.470	-0.077	-1.231	-0.296	0.910	-0.020	-0.325	-1.415	0.983	-0.089	-1.440	-4.641	3.706	-0.077	-1.253
Vitamin A	0.358	1.270	0.018	0.282	-0.268	1.396	-0.012	-0.192	-2.285	1.607	-0.089	-1.422	-1.830	0.995	-0.115	-1.840	-1.037	1.074	-0.060	-0.965	-5.599	4.031	-0.086	-1.389
Fully Adjusted																								
Thiamine Treatment Group	-0.112	0.171	-0.041	-0.652	0.164	0.193	0.054	0.851	0.028	0.224	0.008	0.123	-0.071	0.140	-0.033	-0.504	-0.025	0.149	-0.011	-0.168	0.024	0.556	0.003	0.043
Thiamine	-0.776	1.149	-0.043	-0.675	1.669	1.283	0.085	1.301	2.783	1.484	0.122	1.876	14.098	9.111	0.101	1.547	2.749	0.996	0.177	2.761**	8.874	3.693	0.153	2.403*
Iron	1.125	1.185	0.061	0.949	-0.257	1.378	-0.012	-0.186	-2.189	1.542	-0.093	-1.419	0.008	0.012	0.048	0.698	-0.818	1.024	-0.051	-0.799	-4.229	3.834	-0.071	-1.103
Vitamin A	0.524	1.228	0.026	0.427	-0.257	1.378	-0.012	-0.186	-2.391	1.599	-0.093	-1.495	0.562	1.131	0.032	0.497	-1.056	1.063	-0.061	-0.993	-5.781	3.955	-0.089	-1.462
Infant Sex	0.507	1.361	0.023	0.372	0.397	1.529	0.017	0.260	-0.232	1.776	-0.008	-0.131	0.882	1.139	0.051	0.775	2.641	1.179	0.140	2.240*	3.375	4.401	0.048	0.767
Infant Birth Weight	-1.092	1.873	-0.037	-0.583	-0.138	2.107	-0.004	-0.066	-2.109	2.457	-0.056	-0.859	-0.089	1.529	-0.004	-0.058	1.262	1.618	0.049	0.780	-1.908	6.098	-0.020	-0.313
MSEL Endline	0.431	0.094	0.284	4.584***	0.371	0.119	0.195	3.115**	0.315	0.136	0.146	2.315*	0.077	0.049	0.100	1.581	0.161	0.090	0.110	1.792	0.328	0.094	0.215	3.506***

*P < 0.05; **P < 0.01; ***P < 0.001.

At 52 weeks, significant associations were observed between thiamine and visual reception, receptive language, expressive language, and early learning composite scores in both the partially and fully adjusted models (Tables 8 & 9). This is interesting because thiamine was no longer associated with gross- and fine-motor as it was at 24 weeks (Tables 6 & 7), but it was associated with the domains that didn't have a significant relationship to thiamine at 24 weeks (visual reception and receptive language). At both 24 and 52 weeks, thiamine had a significant relationship to expressive language and early learning composite scores.

Similar to MSEL baseline scores being autoregressive variables in Tables 6 & 7, MSEL endline (24 week) scores were autoregressive variables in Tables 8 & 9 to assess the change in MSEL scores from 24 to 52 weeks as a result of micronutrient factors. Gross- and fine-motor endline scores were significantly associated with 52 weeks MSEL scores as gross- and fine-motor baseline scores were associated with 24 week MSEL scores. MSEL endlines scores were

also positively associated with visual reception MSEL scores at 52 weeks. Since thiamine was significantly associated with vision and receptive language at 52 weeks in the fully adjusted models (with MSEL endline scores as a control), thiamine status accounts for a significant amount of change in those scores from 24 to 52 weeks. In addition, associations were seen between infant sex and expressive language at 52 weeks.

Regarding iron and vitamin A, though there was a significant correlations between iron and MSEL scores in the correlational analysis, when adding the controls into these regression models, ferritin as well as RBP were not significantly associated with any of the MSEL scores at either 24 or 52 weeks. This shows that thiamine and previous MSEL scores may be more influential factors in predicting MSEL scores than iron or vitamin A, at least in this rural Cambodian sample.

Discussion

Micronutrient Status of Infants in Cambodia

We analyzed biomarkers for thiamine, iron, and vitamin A to characterize micronutrient status of infants in Cambodia as well as assess the prevalence and severity of these deficiencies. The majority of this sample was not "deficient" for thiamine, iron, or vitamin A based on the cut-offs used. However, a sizeable proportion of the sample was marginally deficient for each micronutrient. In particular, the majority of infants were marginally deficient for vitamin A. Moreover, when thiamine status was broken out by treatment groups, the placebo group had a much higher proportion of deficient infants compared to the other treatment groups. These results align with our original hypothesis that thiamine status would trend towards sufficiency for all treatment groups that received thiamine and that the placebo group would have a higher percentage of marginally deficient/deficient infants. Also, we predicted prenatal supplementation of iron in Cambodia would likely result in low prevalence of iron deficiency in the sample compared to vitamin A.

Micronutrient Status and Cognitive Development

We adopted a multiple regression approach to examine links between micronutrient status and MSEL scores at 24 and 52 weeks. Of particular interest was to discover which micronutrients were significantly associated with MSEL outcomes while controlling for the contribution of other micronutrients. In these models, we also included infant birthweight, sex, and baseline or endline MSEL scores as control variables. The inclusion of baseline MSEL scores into models regarding 24-week outcomes, in particular, meant that findings spoke to micronutrient factors that were significantly associated with change in infant MSEL performance

from baseline to 24 weeks. With regard to 52-week outcomes, the models included 24-week MSEL scores as predictors, meaning that findings spoke to micronutrient factors that were significantly associated with change in infant MSEL performance from endline to 52 weeks. As it turned out, thiamine was the only micronutrient displaying a significant relationship to MSEL scores in both the partially and fully adjusted regression models, at both 24 and 52 weeks. Thiamine was associated with gross and fine motor at 24 weeks as well as expressive language and early learning composite scores at 24 and 52 weeks. These findings indicate that thiamine status can predict cognitive development both concurrently and several months into the future. This exemplifies the importance of adequate thiamine status for long term cognitive development.

That said, the positive directionality of this relationship (between ETKac and MSEL scores) is surprising, and particularly interesting. It has been hypothesized that chronic early thiamine deficiency in infancy may result in lower ETKac ratios due to a reduction of apoenzyme levels in response to a lack of thiamine (Jones et al., 2021). Our findings are consistent with this hypothesis that low ETKac may, at least in some infants, be an indication of reduced access to thiamine. A study conducted in 2020 reported a stronger relationship between basal ETK activity and erythrocyte ThDP than ETKac (Taylor et al., 2020). However, in the present RCT, basal ETK activity was not assayed, so using basal ETK activity as a measure of thiamine was not an option. Further research is needed to see if the positive relationships between thiamine and MSEL scores change if basal ETK activity is used as the measure for thiamine.

Another limitation of this RCT was that infant blood samples were only collected at 24 weeks meaning no infant blood samples were taken at 2 or 52 weeks. Therefore, we were not able to see if micronutrient status at baseline predicted cognitive development at 24 or 52 weeks

(or thiamine status at 52 weeks). However, we were still able to examine the extent to which micronutrient status earlier in life (at 24 weeks) predicted MSEL scores later in life (52 weeks, Tables 8 & 9). What emerged clearly was that ETKac levels at 24 weeks significantly predicted gross- and fine-motor as well as visual reception at 52 weeks. However, it would be interesting to investigate further if micronutrient status assayed via ETK closer to birth would be a stronger predictor for cognitive outcomes than micronutrient status at 24 weeks. This has implications for potential prenatal thiamine supplementation and thiamine fortification.

Though we were not able to assay micronutrient status at baseline via blood ETKac, we were able to compare MSEL scores at baseline with MSEL scores at 24 and 52 weeks. The associations seen were similar to the results seen in Measelle et al. (2021). Baseline gross motor, fine motor, and receptive language scores were significantly associated with 24-week MSEL scores in their corresponding domain. This indicates that cognitive development (specifically gross/fine motor and receptive language development) at 2 weeks postpartum is a predictor of cognitive outcomes at 24 weeks. Baseline gross and fine motor remained predictors of cognitive development at 52 weeks, but baseline receptive language was not associated with 52-week MSEL scores. Instead, baseline visual reception was significantly associated with 52-week MSEL scores. Further research should be done to determine if infant receptive language development, shortly after birth, is a short-term predictor for cognitive outcomes (up to 24 weeks post-partum), and if visual reception development, shortly after birth, predicts long-term cognitive outcomes (after 52 weeks post-partum).

I undertook a sizeable number of exploratory analyses in this thesis. However, one additional limitation is that I haven't undertaken any statistical correction for the possibility that so many analyses may have inflated the Type 1 error rate (i.e., incorrectly rejecting the null hypothesis).

This presents a reason to view the findings, in their present form, with additional caution. In future, the analyses I've presented here will be reconducted with correction for multiple comparisons, such as the Bonferroni correction.

Future Directions

Cambodia is one of many countries that lacks access to food and/or has a lack of dietary diversity. In order to understand the scope of micronutrient deficiency in the world more data needs to be collected on the prevalence of thiamine, iron, and vitamin A deficiencies in other LMICs. The present study adds to accumulating evidence that access to adequate thiamine is important for cognitive development. It is of particular interest to understand the prevalence and severity of thiamine deficiency in infants in other countries. Then, more analysis can be done to examine the relationships between thiamine and cognitive outcomes, and we can determine regions that might benefit from thiamine supplementation. Furthermore, it is possible that prenatal thiamine supplementation and supplementing thiamine beyond 24 weeks could likely benefit cognitive development based on the findings presented. However, evidence to this effect awaits further research.

Conclusion

In this sample of Cambodian infants, levels of micronutrient deficiency seemed to be highest for vitamin A, and for thiamine among infants whose mothers were in the placebo group, and thus did not receive supplemental thiamine. Vitamin A and iron status were significantly associated, though the mechanism for how this happens is not fully understood. Thiamine and MSEL baseline scores were found to be related to motor and language development, which replicates previous findings (e.g., Fattal et al., 2011; Measelle, et al. 2021). In terms of thiamine measures, we've found that ETKac levels matter in terms of cognitive outcomes, but the specific

causal pathway is not yet fully understood, given that the directionality of association (higher levels of ETKac were associated with better MSEL scores) was unexpected. That said, these findings provide further evidence that thiamine plays a pivotal role in infant cognitive development. In all, ensuring adequate nutrition to infants in all parts of the world is not only important to the global mission of ending hidden hunger, but also to providing children with what they need to develop to their fullest potential.

Appendices

Appendix A: Ferritin Correction Factors

Inflammation Group	Correction Factor
1 (no inflammation)	1.00
2 (incubation)	1.30
3 (early convalescence)	1.90
4 (late convalescence)	1.36

Appendix B: RBP Correction Factors

Inflammation Group	Correction Factor
1 (no inflammation)	1.00
2 (incubation)	1.15
3 (early convalescence)	1.32
4 (late convalescence)	1.12

Appendix C: Model Summary for Table 6 (Micronutrient Continuous vs 24 week MSEL)

	Gross Motor	Fine Motor	Visual Reception	Receptive Language	Expressive Language
Partially Adjusted Model	R^2= 0.027 F(4,264)= 1.802 p= 0.129	R^2= 0.028 F(4,264)= 1.868 p= 0.116	R^2= 0.014 F(4,264)= 0.928 p= 0.448	R^2= 0.029 F(4,264)= 1.979 p= 0.098	R^2= 0.048 F(4,264)= 3.320 p= 0.011*
Fully Adjusted Model	R^2= 0.078 F(3,261)= 3.162 p= 0.003**	R^2= 0.060 F(3,261)= 2.395 p= 0.022*	R^2= 0.030 F(3,261)= 1.143 p= 0.337	R^2= 0.064 F(3,261)= 2.569 p= 0.014*	R^2= 0.055 F(3,261)= 2.169 p= 0.037

Appendix D: Model Summary for Table 7 (Micronutrient Trichotomized vs 24 week MSEL)

	Gross Motor	Fine Motor	Visual Reception	Receptive Language	Expressive Language
Partially Adjusted Model	R^2= 0.027 F(4,264)= 1.857 p= 0.118	R^2= 0.015 F(4,264)= 0.981 p= 0.418	R^2= 0.009 F(4,264)= 0.630 p= 0.641	R^2= 0.020 F(4,264)= 1.365 p= 0.246	R^2= 0.030 F(4,264)= 2.016 p= 0.092
Fully Adjusted Model	R^2= 0.077 F(3,261)= 3.121 p= 0.004**	R^2= 0.046 F(3,261)= 1.810 p= 0.086	R^2= 0.032 F(3,261)= 1.243 p= 0.279	R^2= 0.063 F(3,261)= 2.522 p= 0.016*	R^2= 0.039 F(3,261)= 1.531 p= 0.157

Appendix E: Model Summary for Table 8 (Micronutrient Continuous vs 52 week MSEL)

	Gross Motor	Fine Motor	Visual Reception	Receptive Language	Expressive Language
Partially Adjusted Model	R^2= 0.013 F(4,252)= 0.808 p= 0.521	R^2= 0.023 F(4,252)= 1.492 p= 0.205	R^2= 0.021 F(4,252)= 1.346 p= 0.253	R^2= 0.021 F(4,252)= 1.337 p= 0.257	R^2= 0.052 F(4,252)= 3.429 p= 0.009**
Fully Adjusted Model	R^2= 0.093 F(4,249)= 3.642 p= <0.001***	R^2= 0.059 F(3,249)= 2.219 p= 0.033*	R^2= 0.039 F(3,249)= 1.460 p= 0.182	R^2= 0.033 F(3,249)= 1.229 p= 0.287	R^2= 0.078 F(3,249)= 3.004 p= 0.005**

Appendix F: Model Summary for Table 9 (Micronutrient Trichotomized vs 52 week MSEL)

	Gross Motor	Fine Motor	Visual Reception	Receptive Language	Expressive Language
Partially Adjusted Model	R^2= 0.004 F(4,252)= 0.228 p= 0.923	R^2= 0.017 F(4,252)= 1.076 p= 0.369	R^2= 0.029 F(4,252)= 1.857 p= 0.119	R^2= 0.036 F(4,252)= 2.329 p= 0.056	R^2= 0.056 F(4,252)= 3.768 p= 0.005**
Fully Adjusted Model	R^2= 0.082 F(3,249)= 3.173 p= 0.003**	R^2= 0.055 F(3,249)= 2.067 p= 0.048	R^2= 0.050 F(3,249)= 1.876 p= 0.074	R^2= 0.051 F(3,249)= 1.902 p= 0.070	R^2= 0.087 F(3,249)= 3.398 p= 0.002**

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