INVESTIGATING THE POSSIBLE BENEFITS OF MATERNAL THIAMINE SUPPLEMENTATION FOR ENHANCING SOCIAL ALERTNESS IN INFANTS AT RISK FOR THIAMINE DEFICIENCY

by

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A THESIS

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

May 2021

An Abstract of the Thesis of

Jenna Rudolph for the degree of Bachelor of Science
in the Department of General Science to be taken June 2021

Title: Investigating the Possible Benefits of Maternal Thiamine Supplementation for Enhancing Social Alertness in Infants at Risk for Thiamine Deficiency

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Primary Thesis Advisor

Millions of Southeast Asian children are at risk for thiamine deficiency, which in turn puts their neuro-cognitive development in peril. Our study investigates the possibility that thiamine supplementation for Cambodian mothers protects infants' cognitive development. Specifically, we examined the extent to which thiamine supplementation enhances infants' alertness to caregivers' efforts to engage. Such alert responsiveness indexes neuro-cognitive well-being, while also supporting further neuro-cognitive progress. As part of a larger, double-blind, randomized controlled trial, lactating mothers (N = 335) received one of four levels of thiamine supplementation (0, 1.2, 2.4, or 10mg/day) beginning at 2 weeks post-partum. We assessed infants' alertness in relation to caregivers' efforts to interact via a new method, the Primary Engagement Task (PET), when infants were 2-, 12-, and 24-weeks. In the PET, mothers were asked to coax a smile from infants, and sustain a mutually positive interaction. As the PET progressed, mothers were cued to add, then remove, engagement modalities during six 30-second epochs. Video coding determined changes in infants' alertness across epochs. As predicted, infants' alertness increased, then decreased (remaining higher than baseline) as the PET unfolded. Moreover, these patterns tended to become more pronounced with increasing age and were influenced by maternal thiamine supplementation

Acknowledgements

There are so many people who made this thesis possible. I would first like to thank Dr. Dare Baldwin for her mentorship throughout this process. I feel so grateful to have been gifted her time and energy. I also feel so grateful to have learned from her expertise. Dare has showed me by example, not only how to conduct research, but also how to be a researcher who is curious, diligent, a connoisseur of open science, and a believer in the power of her own work. I would also like to thank Dr. Jeffrey Measelle for his support and friendship throughout this process. I have looked up to Jeff since hearing about his global health research during my first psychology class at the UO. I feel so lucky to have gotten to work for him in the Cambodia Project and to have contributed to the important work that he does. Furthermore, my thesis would not have been possible without the help of my fellow undergraduate research assistants who helped me code all of the behavioral videos for my thesis. Thank you for showing up for me when I needed you!

I also have to thank my support system outside of the Cambodia Project. To my sweet roommates (who are non-science majors), thank you for always trying to understand my thesis and for relating to the ups and downs of the writing process. To my family, thank you for getting excited about my research with me and for being there with an open ear when the process felt overwhelming or frustrating. Finally, to my partner, thank you for reading the many drafts of my thesis and for pretending that going over my thesis was the ideal date.

Table of Contents

List of Figures v

Prologue 1

Introduction 2

- Prevalence of MNM among Cambodian Mothers 4
- Factors Affecting Micronutrient Intake 5
- Factors Affecting Micronutrient Absorption 8
- Factors Affecting Micronutrient Utilization 9

Maternal MNM and Infant Cognitive Development 11

Thiamine 12

- Research Focus 12
- Biology of Thiamine 12
- Thiamine Deficiency in Infants 14
- Limited Early Access to Thiamine and Health and Development 14
- My Research 16

Methods 19

- Participants 19
- Procedure 21
- Measures 23

Results 25

- Validation of the Primary Engagement Task 25
- Age Differences in the Primary Engagement Task 26
- Effect of Maternal Thiamine Supplementation 28
- Exploratory analyses 32

Discussion 34

- Broader Implications 38
- Limitations 39
- Future Directions 41
- Conclusion 44

Bibliography 34

List of Figures

Figure 1. The Molecular Structure of Thiamine and Thiamine Pyrophosphate (TPP) .. 13
..... 21
Figure 2. Flow of Participants Through Study 21
Figure 3. Description of The Primary Engagement Task. 23
Figure 4. Pattern of Infant Alertness in the PET at 2-, 12-, and 24-weeks. 26
Figure 5. 12- and 24-week Infant Alertness by Thiamine Supplementation Group 29
Figure 6. Infant Alertness in Epoch 1 of the PET in Relation to Thiamine Supplementation Group. 30

List of Tables

Table 1. 4

Summary of Micronutrient Deficiencies among Cambodian Women of Child-Bearing Age 4

Table 2. 19

Baseline Characteristics of Participants 19

Prologue

My thesis investigates the extent to which maternal thiamine supplementation enhances social alertness in Cambodian infants at risk for thiamine deficiency. I came to this research through my work for the Cambodia Project in the Psychology Department at the University of Oregon. The primary investigators of the Cambodia Project, Dr. Dare Baldwin and Dr. Jefferey Measelle, are looking at the effects of maternal thiamine supplementation on Cambodian infant cognitive and socio-emotional development. Their work is part of a larger, international study led by researcher Kyly Whitfield of Mount Saint Vincent University. The larger study's overarching goal is to obtain the information necessary to formulate a thiamine fortified salt for future use in Cambodia (Whitfield, Kroeun, Green et al., 2019). This involves understanding both the optimal dose of maternal thiamine supplementation in order to protect infants from health and developmental assaults, and the typical salt intake of Cambodian mothers. In order to gain this understanding, the 4-armed, double-blind, randomized trial study followed a sample of 335 mother-infant pairs from the Kampong Thom Province of Cambodia. The study began when the infants were 2 weeks old and continued until they were 24 weeks old. The mother-infant pairs were assigned to one of four treatment groups, including a placebo control and one of three groups based on different daily levels of supplemental thiamine. Currently, the larger research team is using biological tests to determine how the differing levels of maternal thiamine supplementation affected the quantity of thiamine available to the infants through breast milk. They are also using salt disappearance patterns to fortify salt and assess the plausibility of it being introduced in Cambodia. Concurrently, my lab is using a number of behavioral tests to determine the extent to which the differing levels of maternal thiamine supplementation affected infant cognitive and socio-emotional development. My thesis, which investigates the degree that maternal thiamine supplementation enhanced infant social alertness, uses data from only one of our behavioral tests, the Primary Engagement Task. Thus, the findings I am presenting are just some of many that will come from our lab.

Introduction

Undernutrition is a form of malnutrition that includes stunting, wasting, and deficiencies of essential micronutrients. While stunting and wasting are visible, micronutrient malnutrition (MNM) often goes unseen until there are severe clinical manifestations. It is estimated that two billion people around the world suffer from the 'silent' epidemic of MNM (Tulchinsky, 2015). Akin to other forms of malnutrition, MNM disproportionately affects the most impoverished and underprivileged, contributing to the vicious cycle of underdevelopment (Allen et al., 2006).

According to the World Health Organization (WHO), the most common micronutrient deficiencies are iron, vitamin A, and iodine. There are, however, more than 15 additional micronutrient deficiencies of global health interest. While each deficit has a biologically specific outcome, micronutrient deficiencies in general lead to physiological impairments, reduced resistance to infections, metabolic disorders, and hindered physical and psychological development (Allen et al., 2006).

MNM is a significant public health issue in SouthEast Asia, disproportionately affecting the health of women and children in the region. In Cambodia, specifically, high rates of maternal MNM puts infants at risk of poor health and development. Without the proper micronutrients *in utero* and in breastmilk, Cambodian infants are unable to thrive during critical developmental periods. Unfortunately, the developmental delays that Cambodian children sustain during infancy continue to affect their health and ability to contribute to society in adulthood.

Due to the importance of human capital and the preventability of maternal MNM, the Cambodian government, philanthropic groups, and researchers (myself included) have been working in collaboration to protect Cambodian infants. Our collective goal necessitates a multifaceted approach – in which we understand the causes and effects of maternal MNM and make calculated steps forward.

My thesis aims to inform steps forward in regards to a specific form of maternal MNM, maternal thiamine deficiency. I will investigate the effects of differing levels of maternal thiamine supplementation on Cambodian infants' cognitive development, specifically in the domain of social alertness. My findings, along with those of my lab as a whole, will inform the Cambodian micronutrient fortification effort as to which dose of maternal thiamine supplementation best supports infant cognitive and socio-emotional development.

Before narrowing to focus in on thiamine, however, I will provide context to the micro-nutritional status of Cambodian mothers and their infants. Specifically, I will address the prevalence and causes of multiple forms of MNM among the at-risk population. Then, I will delineate how maternal MNM undercuts infant development. After addressing these issues more broadly, I will focus on the primary goal of my thesis research – investigating the extent to which infants' access to thiamine plays a significant role in their cognitive and socio-emotional development.

Prevalence of MNM among Cambodian Mothers

The most recent Cambodian Demographic and Health Survey (National Institute of Statistics, Directorate General for Health, and ICF International, 2015; Whitfield, et al., 2017) reported that child-bearing women in rural Cambodia show high rates of deficiencies in iron, vitamin A, vitamin B 12, vitamin B9, vitamin D, calcium, and iodine. The prevalence, as well as the cut off, of these micronutrient deficiencies appears in Table 1.

Table 1.

Summary of Micronutrient Deficiencies among Cambodian Women of Child-Bearing Age

Micronutrient	**Prevalence Deficiency**	**Measure and Cut off**
Iodine	44.8 %	(< 50 µg/L)
Iron (anemia)	43. 50 %	Pregnant mothers - Hemoglobin <11.0 g/dl Nonpregnant mothers Hemoglobin <12.0 g/dl
Vitamin D	31.6 %	(<50 nmol/L)
Thiamine	*27%*	(eThDP < 120 nmol/L)
Vitamin B 9	19.2 %	(<10 nmol/L)
Vitamin A	3.0 %	(RBP <0.70 µmol/L)
Vitamin B 12	1.0 %	(<150 pmol/L)
Calcium	.2 %	(< .90 mmol/L)

Many interconnected social, cultural, and biological factors contribute to the high rates of MNM among Cambodian women, affecting their intake, absorption, and utilization of micronutrients.

Factors Affecting Micronutrient Intake

Perhaps the most prominent factor affecting the intake of micronutrients for Cambodian mothers is food insecurity - the inability to access consistently sufficient quantities of affordable and nutritious food (Merriam-Webster, n.d). When interviewed, women in the rural Kandal Province of Cambodia stated that their family's poor nutrition was caused by a lack of money (Wallace et al., 2013). With little money to spend on food, families rely primarily on items they can grow, catch, or buy inexpensively at the market. Depending on the season, these include some micronutrient rich foods such as papaya, mango, morning glory, ivy gourd, ginger leaf, chicken and small fish (Wallace et al., 2013). The issue is not always availability, but instead that families are forced to prioritize food in relation to its costs, not its nutritional value. Even if there are relatively inexpensive micronutrient dense foods available, poorer families are limiting their consumption in favor of rice, which they often can grow themselves, and even when purchased is considerably less expensive than many other food sources. This is in part why rice is consumed at every meal (Vilain, Baran, Gallego, Samadee, 2016). Unfortunately, the rice is highly polished, meaning that the

thiamine-rich envelope of the rice is removed, leaving behind a filling, but non-nutritious food (Barennes, Sengkhamyong, René, Phimmasane, 2015).

Within food insecure families, there are disparities in intrahousehold food allocation that disproportionately affect Cambodian mothers and put them more at risk of MNM. One study conducted by McDonald et al. (2014) found that food insecurity was not related to child undernutrition, but instead to maternal thinness and anemia in a dose-response manner. This pattern reflects inequitable intrahousehold food allocation, in which the nutritional needs of children and other family members are prioritized over those of the mother. Cambodian women sacrifice quantities of their food, particularly discretionary items, such as meat and eggs, to feed the men or children in the family under the assumption that their needs are greater (Gorman, 1999). They believe that their allocation of food will allow men and older children to work better and provide for the family (Tickner, 2019). Research suggests this phenomenon may also be related to beliefs about status in the household and sexual division of labor (Gorman, 1999). Although mothers are sacrificing their food for their current children, they are putting their breast fed and future children at risk of MNM by neglecting their own macro- and micro-nutritional needs.

Beyond food insecurity, there are pre- and post-natal practices that affect Cambodian mothers' intake of micronutrients. One Cambodian prenatal practice contributing to MNM is food avoidance in accordance with a prevalent Khmer folk medical theory: the hot vs. cold humoral theory. Cambodian women are believed to be

in a “hot” state during pregnancy and then a dangerously “cold” state after losing heat through the birthing process (Bazzono et al., 2020). In accordance to these principles, pregnant women avoid spicy or “hot” foods during pregnancy because they will turn the child ‘hot’ inside them (Wallace et al., 2013). On the other hand, postpartum women avoid ‘cold’ foods such as certain types of fish, banana, and salty foods like fermented fish paste (Wallace et al., 2013). Those ‘cold’ food items are thought to cause headaches, diarrhea, weakness, palpitations, abdominal pains and poor appetite (Raman et al., 2016). Unfortunately, these dietary restrictions during the pre- and post-natal periods diminish nutritional diversity (Vilain, Baran, Gallego, Samadee, 2016) and put Cambodian mothers at further risk of MNM.

A final practice impacting mothers’ intake of micronutrients is fetal growth restriction, when a neonate is kept smaller than expected for its gestational age. Some Cambodian women reduce their food intake and avoid prenatal vitamins during pregnancy in order to have smaller fetuses and thus, easier deliveries (Vilain, Baran, Gallego, Samadee 2016; Kelley, 1996). In addition, they sometimes drink a mixture of wine and herbs, including sesame seeds, at about seven months to keep the baby small and make the baby ‘slide out’ easier (Kelley, 1996). These efforts are most likely aimed at protecting mothers and infants during childbirth, as larger babies are associated with difficult deliveries for both young mothers, and mothers without the help of birthing practitioners. That being said, using fetal growth restriction as a means to protect

mothers and infants, actually makes both mothers (and of course their infants, as well) susceptible to MNM and other forms of malnutrition.

Factors Affecting Micronutrient Absorption

In addition to the inadequate consumption of micronutrients, there are also factors that affect micronutrient absorption. One of these is the consumption of foods that undercut specific nutrient absorption. Some micronutrients can act antagonistically with other micronutrients competing for uptake and/or hindering the digestion and assimilation of one another (Schoendorfer & Davies, 2012). For example, a potential cause of iron malabsorption in Cambodia mothers is the consumption of tea containing tannins and other compounds inhibits iron absorption. Tannins bind with iron forming mineral complexes that cannot be absorbed by the body (Delimont et al., 2017). In addition, a possible cause of thiamine malabsorption among Cambodian mothers is the consumption of staples such as fermennted fish paste, tea, and the betel nut, all of which contain thiaminases or thiamine antagonists (Coats et al., 2018) that affect thiamine absorption and bioavailability. Thiaminases split the thiamine molecule and render it inactive, while thiamine antagonists compete with thiamine derivatives.

Another factor that affects micronutrient absorption is infections with soil-transmitted helminths (STH) such as hookworm, whipworm, and roundworm (Vilain, Baran, Gallego, Samadee, 2016). STHs are intestinal worms transmitted through eggs in human feces. These infections predominate in areas with poor sanitation, making rural Cambodia, with more than 50% of households without improved sanitation facilities

(NIS, DGH, ICF, 2015), an easy target. STH eggs are deposited on soil when an infected person defecates in the open or if their excrement is used for fertilizer. Contact with infected soil, through touch or improperly prepared food, is enough to lead to ingestion and a new infection. STHs can lead to gastrointestinal inflammation and diarrhea (Wallace et al. 2013). This contributes to micronutrient malnutrition, because inflammation and diarrhea undercut the small intestine's ability absorb nutrients from food.

Factors Affecting Micronutrient Utilization

In addition to poor micronutrient intake and absorption, Cambodian mothers have an increased utilization of micronutrients that put them at risk of MNM. Their increase in utilization of micronutrients is due to biological processes that accompany pregnancy and lactation. In pregnancy, women have increased micronutrient utilization in order to adjust to the physical demands of child bearing, and to provide for the growing fetus. Interestingly, the micronutrient requirements of mothers increase more than their dietary energy requirements (Cetin et al., 2019). Their micronutrient stores must be mobilized and passed to the growing fetus through the placenta. Similar to pregnancy, lactating mothers' bodies utilize more micronutrients in order to produce micronutrient rich milk to support their infants. Womens' bodies prioritize the needs of their infants over their own. This means that micronutrients are excreted into breast milk in adequate and constant amounts, at the expense of maternal stores (Segura et al., 2000).

Clearly, there are many factors affecting Cambodian mothers' intake, absorption and utilization of micronutrients, including thiamine. Akin to many global health issues, MNM among Cambodian mothers is caused by a combination of social, cultural, environmental, and biological factors. Understanding the root causes of maternal MNM in Cambodia not only provides context to this research, but also demonstrates the complexity of the problem at hand.

The next section will speak to, in short, the effects of maternal MNM on infant cognitive and socio-emotional development, framing my investigation of possible benefits of maternal thiamine supplementation for infant development.

Maternal MNM and Infant Cognitive Development

The high rates of MNM among Cambodian women is of significant concern because maternal MNM affects infants' cognitive and socio-emotional development in a host of ways. A mother's diet and nutrient stores are the only source of nutrition for her growing fetus. Later, maternal nutritional status also affects the nutritional content of breast-milk, with obvious consequences for breast-fed infants. This means that a mother's micronutrient status has major implications for the micronutrient status of her child during the first 1,000 days of life, a critical period of brain development. Inadequate micronutrient access during this time can lead to irreversible changes in infants' brain structure and function. In addition, inadequate access to micronutrients during the first 1,000 days of life can hinder an infants' ability to enter into sustained positive social interactions with caregivers, which is a nexus within which learning and socio-emotional development occur.

Thiamine

Research Focus

The developmental importance of each micronutrient addressed thus far cannot be denied. The scope of my research, however, can only provide new insight on one. My thesis, and the broader study it is a part of, chose to focus on thiamine because of the increasingly high rates of infant mortality related to thiamine deficiency in South Asia and other low-to middle income countries (LMIC) (Whitfield et al., 2018). Thiamine has been a relatively neglected vitamin in recent years, with global research and interventions focused on more prominent micronutrients such as iron, vitamin A, and iodine. It wasn't until 2017, when the Sackler Institute of Nutrition Science at the New York Academy of Sciences (NYAS) and the Bill and Melinda Gates Foundation convened an expert panel, that thiamine became a main talking point for the global health community again. The panel's goal was to determine the best biomarkers of thiamine status, estimate the global prevalence of thiamine deficiency, devise strategies for increasing thiamine status, and identify research needs. Ultimately, the panel's determined research needs prompted the research coming from my lab, and the larger-international study as a whole.

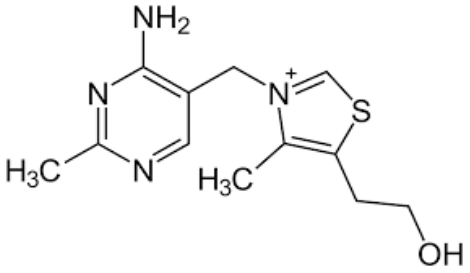
Biology of Thiamine

Thiamine (vitamin B1) is a water-soluble vitamin used by the body to generate energy from nutrients. The body's supply of thiamine depends almost entirely on dietary intake, as there is little to no endogenous synthesis. Because the vitamin has a short half-life ranging between 1- 12 hrs (Whitfield et al., 2018), and only 30 mg of it

can be stored in the body (Crook, 2019), regular consumption is required to maintain adequate levels. Thiamine can be consumed through whole grains, yeast, meat, legumes, and nuts. In high-income countries, it can also be consumed through thiamine-fortified foods such as flour, cereals, and baby formula.

When thiamine is ingested it is absorbed in the jejunum and the ileum through an active process at low concentrations and a passive process at high concentrations. After absorption in the gastrointestinal tract, thiamine enters the blood stream where it can circulate freely (Crook, 2019). There, the thiamine diphosphotransferase enzyme converts thiamine into its biologically active form, thiamine pyrophosphate (TPP) (Julianna et al., 2020). Figure 1. shows that the primary difference between the free form of thiamine and TPP is the presence of phosphate groups.

Thiamine



Thiamine Pyrophosphate (TPP)

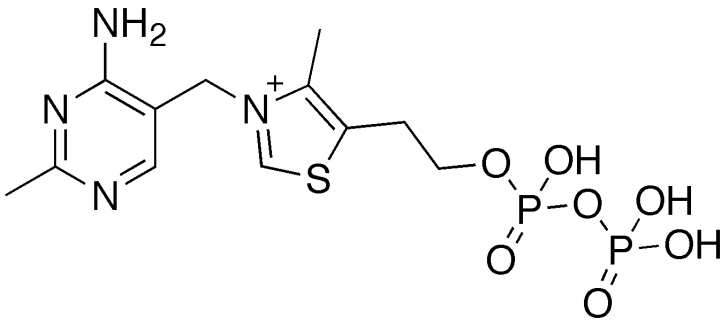


Figure 1. The Molecular Structure of Thiamine and Thiamine Pyrophosphate (TPP)

TTP is transported throughout the body in erythrocytes also known as red blood cells (Crook, 2019). It goes on to play a critical role in energy metabolism by serving as a cofactor for enzymes that catalyze reactions supporting energy production, cell viability and proper neuronal functioning.

Thiamine Deficiency in Infants

Due to the biological importance of thiamine, thiamine deficiency effects multiple systems in the body including the cardiovascular, nervous, and immune systems. Severe thiamine deficiency in infants, called Infantile Beriberi, is often fatal. The illness has a rapid onset, and death occurs after a few days of the onset of detected symptoms. As the World Health Organization (1999) describes, initially, an infant will have a relatively normal appearance and varying severities of constipation, vomiting, crying, and restlessness. As the disease progresses, however, an infant begins to have difficulty breathing, cardiac disturbances, gastrointestinal issues, and diminished urine output. At this point, an infant will die if it does not receive treatment of intramuscular or slow intravenous injection of thiamine.

Limited Early Access to Thiamine and Health and Development

Clinical Infantile Beriberi is in some ways just the tip of the iceberg. Less severe degrees of thiamine deficiency in infants can be clinically unapparent. Research has found that this overt form of thiamine deficiency in infants and mothers is endemic in southeastern rural Cambodia (Coats et al., 2012). For example, Whitfield et al. (2017) found that 27 percent of mothers and 15 percent of children of a representative

Cambodian sample were thiamine deficient using the most conservative cut off (eThDP < 120 nmol/L). When assessing infants aged 6-24 months alone, the researcher found that 38 percent were thiamine deficient.

There is growing evidence that subclinical thiamine deficiency has negative physical and cognitive implications for infants and children. Khounnorath et al. (2011) found latent thiamine deficiency is common among sick infants admitted to hospitals in Laos (a neighboring country to Cambodia). This study showed that subclinical thiamine deficiency may contribute to other illnesses.

As to the cognitive-developmental implications of subclinical thiamine deficiency, a devastating milk formula manufacturing mistake in Germany in 2003 provided a sample of Israeli infants with subclinical thiamine deficiency. Researchers have conducted a number of studies on this sample of children, in order to investigate the extent to which access to thiamine in infancy affected their cognitive development. In 2009, Fattal-Valevski et al., published a study investigating the language development of 20 of the Israeli children who had received the thiamine deficient formula. They found significant deficits in expressive communication, auditory comprehension language subscales, the Mental Development Index, and age at independent walking in comparison to a healthy control group. In 2011, Fattal, Friedmann, and Fattal-Valeyski published a study investigating the development of syntax and lexical retrieval in 59 of the Israeli children when they were aged 5-7. They found that 57 of the 59 children had a language impairment compared to healthy

controls. Finally, in 2017, Yael et al., (2017) published a study that had tested a sub-sample of the Israeli children when they were aged 5-6 years old. They found significant motor difficulties, particularly in balance and fine motor skills.

Beyond these seminal studies, not much is known about the relationship between early access to thiamine and cognitive development. Further, there are no studies to this date assessing the extent to which access to thiamine effects socio-emotional functioning. My research aims to partially fill that void by addressing the effects of maternal thiamine supplementation on infant social alertness.

My Research

I will utilize the Primary Engagement Task (PET). The nature of this newly designed task, is to have mothers partake in mutual and positive engagement with their infants. Dr. Dare Baldwin and Dr. Jefferey Measelle created the PET specifically for their work in Cambodia. They needed a behavioral task that would be both suited to cross cultural research in low- income countries and sensitive to nutritional assaults. Since caregivers' interactions with their infants differ globally, many tasks in the field of developmental psychology do not directly translate to other cultures. The PET is intended to be an exception, because it capitalizes on the universal tendency of caregivers to interact with their infants in positive social ways. The PET is also intended to be sensitive enough to reveal the effects of thiamine access on different domains of infant cognition. We hope that the stepwise progression of the task, will provide information on an infant's ability to sense and respond to each engagement modality.

During the PET, a mother's overall goal is to elicit a smile from her infant. She must do this in a relatively scripted manner, by adding and then removing different modalities of engagement as the task progresses. The stepping up and stepping down pattern of the task spans 6 epochs (30 second periods). During epoch 1 (the baseline), the mother looks completely away from her infant. At the start of epoch 2, the mother can look at her infant and use her facial expressions to elicit a smile. For epoch 3, she can now use her voice along with her facial expressions. During epoch 4, the mother can add touch. Epoch 5 marks the beginning of stepping down so the mother removes touch, but maintains facial expressions and voice. Finally, for epoch 6, the mother removes voice and uses solely her facial expressions.

In order assess infant social alertness across the six epochs of the PET, a state coding system developed by Tronick (2004) will be used. The system is part of the Network of Neurobehavioral Scale (NNNS), a standard, clinical measure to assess infant neurological status. The component of the NNNS I am using (the 6-state scale) rates infants as either in a quiet sleep, active sleep, drowsy, quiet awake, active awake, or crying state.

I have a number of research questions. The first question relates to the newly designed measure being used: Does the Primary Engagement Task (PET) elicit systematic changes in infants' alertness state? More specifically, does an infant's state increase as the mother adds additional modes of engagement (facial expressions, voice, touch, etc.) and does it decrease as she takes those cues away? This question seeks to

expand on a previous undergraduate thesis with a larger and more diverse sample of infant ages (Steeves, 2019). Once the PET task is validated, subsequent research questions can be addressed. These include, to what extent does increasing age lead to a more predictive infant response pattern during the PET? And to what extent does thiamine supplementation lead to a more predictive infant response pattern during the PET? An additional, unexpected follow up question of this thesis was to what extent does an infants' pre-supplementation alertness in the PET predict their end line cognitive functioning.

I hypothesize that the PET will elicit the predicted changes in infant state. In addition, I hypothesize that age and thiamine supplementation will lead to a more predictive infant response pattern in the PET.

Methods

Participants

Participants were mother infant dyads from the Kampong Thom province of Cambodia. The complete sample was 335 dyads. As is shown in Table 2, the mothers were between 18 to 45 years of age (M = 28.1, SD = 6.2). In addition, 98% of the mothers were married and 69% of them had more than one child. In regard to the infants, 48% were female. The mothers were recruited via antenatal care visits and consultation with local village chiefs, elders, and health center staff. The mothers' most recent pregnancy had to have been normal, meaning they had no known chronic conditions, preeclampsia, or gestational diabetes. In addition, their infant had to have been born without any complications such as low birth weight or cleft palate. Other criteria included: not having taken thiamine supplementation and not currently participating in nutritional programs.

Table 2.

Baseline Characteristics of Participants

	TOTAL (N = 335)
Mother	
Age, *years*	28.1 (6.2)
Parity	
>1 child	57 (69%)
Marital Status	
Married	330 (98%)
Divorced/Separated/Widowed	5 (1%)
Infant	
Sex	
female	161 (48%)
Household	

Household size	
≤2 persons	27 (8%)
3−6 persons	242 (73%)
≥7 persons	62 (19%)
Daily per capita income	
≤$3.20 USD	284 (86%)
>$3.20 USD	47 (14%)

Adapted from: Chan et al. 2020. (Full table in Appendix B)

Participation in the PET wasn't always possible for all mother/infant dyads at all relevant time points (2, 12, and 24 weeks postnatal). 25 mother/infant dyads left the larger study before participating in the PET at 12 weeks and 12 mother/infant dyads left the larger study before participating in the PET at 24 weeks. Their reasons for leaving included family migration, illness, extended visits to relatives, and withdrawal of consent. There was one adverse event, in which one infant died due to causes unrelated to study involvement. For the mother/infant dyads who remained during the entirety of the larger study, there was sometimes infant tiredness and/or recording equipment failure that made the PET impossible to conduct. Other factors such as mothers being off task, or poor filming quality made some of the PET videos impossible to code later on. The final sample in the PET was 335, 310, and 293 dyads at 2, 12, and 24 weeks respectively. This is summarized in Figure 2.

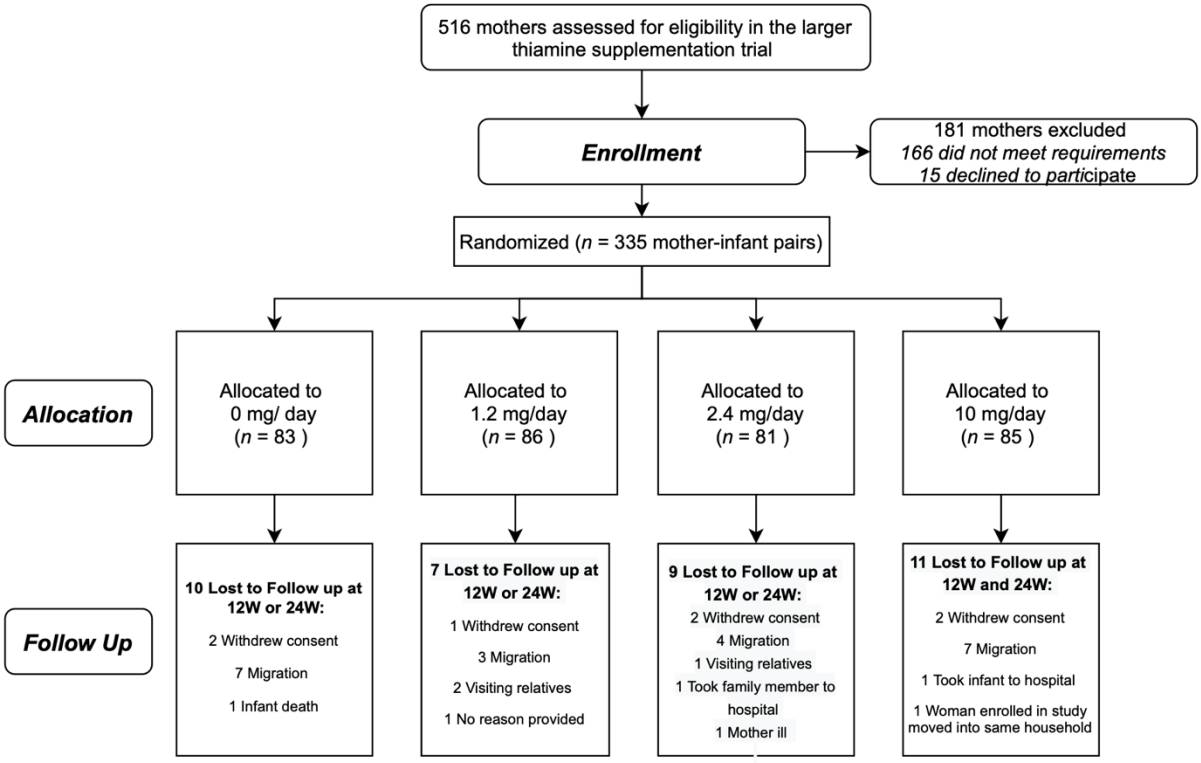


Figure 2. Flow of Participants Through Study

Procedure

As part of their participation in the larger, four-parallel-arm, double-blind, randomized, controlled trial (Whitfield, et al., 2017), infant/mother dyads were video-recorded as they participated in the Primary Engagement Task (PET) at 2, 12, and 24 weeks. The structure of the PET is depicted in Table 1. The PET was designed to provide an opportunity to observe and quantify the degree to which caregiver and infant establish and sustain mutually positive interaction. Caregivers were given the overarching goal during the PET to elicit a smile from their infant and sustain a positive interaction throughout the task. They are asked to achieve this in a relatively scripted manner across 6 epochs (successive periods of 30 seconds) displayed in Table 1. The

interaction that unfolds during the PET can be coded to derive information about the quality of a) caregivers' attempts to elicit positive engagement from infants, b) infants' response to the caregivers' efforts, and c) the dyads' ability to establish and sustain positive engagement. My thesis focused specifically on coding infants' response to caregiver's efforts to engage them, and in particular, I used a state coding system to quantify changes in infants' alertness over the course of the PET.

Immediately before the PET, mothers were given instructions in Khmer by trained Cambodian researchers who were native Khmer speakers. The task began with mothers cradling their infants or with infants lying on a pillow on the ground. Epoch 1 of the task was a baseline where the mothers were completely looking away from their infants. After 30 seconds the mothers were verbally instructed to start epoch 2, where they could look at their babies and use their facial expressions to elicit a smile. After another 30 seconds the mothers were told to start epoch 3 where they could begin to use their voice along with their facial expressions. When it was time for epoch 4, the mothers could also use touch as a tool. After 30 seconds, the mother was instructed to start epoch 5 in which they removed touch but maintained voice and facial expressions. Finally, for epoch 6, the mothers removed voice but continued to look at their babies.

PHASES	Epoch 1 (Baseline)	Epoch 2	Epoch 3	Epoch 4	Epoch 5	Epoch 6
	30 sec	30 sec	30 sec	30 sec	30 sec	30 sec
PRIMARY ENGAGEMENT BEHAVIORS	Caregiver silently looking away from infant	Caregiver orients to infant and produces elicitation attempts via facial expression alone	Voice added	Touch/body manipulation added	Touch/body manipulation removed (voice and gaze/ facial expression remain)	Voice removed (gaze/ facial expression remain)
Touch baby	X	X	X	✓	X	X
Talk to baby	X	X	✓	✓	✓	X
Look at baby	X	✓	✓	✓	✓	✓

Figure 3. Description of The Primary Engagement Task.

Measures

Thiamine status – Mothers. Breast milk and venous blood samples were collected from mothers at 2 and 24 weeks post-natal. Both kinds of samples underwent analysis for total thiamine diphosphate concentration (ThDP), and venous blood samples were also assayed for erythrocyte transketolase, an enzyme involved in thiamine metabolization (which tends to be found in complementary distribution to thiamine diphosphate concentration).

Thiamine-status – Infants. Venous blood samples were collected from infants at 24 weeks. The samples underwent analysis for total ThDP concentration and erythrocyte transketolase activity.

Primary Engagement Task (PET) – Infant State. Video recordings of mothers and infants participating in the PET were first segmented with respect to the onset time of each epoch in the task, and subsequently coded to determine changes in infants' state

of alertness as the task unfolded. Segmentation coding to identify epoch onsets was accomplished via open-source Datavyu software (Datavyu Team, 2014). State coding was conducted in which infants' state changes were marked across the epochs defined by segmentation. A modified version of the Network of Neurobehavioral Scale (NNNS) developed by Tronick (2004) was used. The values for infant state included: 1 – quiet sleep, 2 – active sleep, 3 – drowsy, 4 – quiet awake, 5 – active awake, and 6 – crying. The detailed state coding guide appearing in Appendix A was used in order to ensure consistency and accuracy.

Mullen Scales of Early Learning (MSEL). In addition to the PET, trained field staff administered an adapted version of the Mullen Scales of Early Learning (MSEL) at 2, 12, 24-weeks. The MSEL is a standardized, performance-based developmental test used on children age 0 to 68 months. It assesses five developmental domains including gross motor, fine motor, visual reception, receptive language, and expressive language. Each subscale of the MSEL has a set of performance-based items that are presented in order of difficulty. Children are scored on whether they successfully complete task items to establish performance ranges that can be used to determine raw scores. Final T-scores are obtained by summing a child's raw points on each MSEL scale, and then converting them to age-normed T-scores (Measelle et al., 2020).

Results

Validation of the Primary Engagement Task

First, we wanted to assess the extent to which the newly designed Primary Engagement Task (PET) was functioning as expected. The task was designed to enable observation of mother and infant as the mother sought to engage the infant in mutual positive interaction. We predicted that infant state would increase as mothers added interaction tools, and then decrease as interaction modalities were removed; thus, we expected a quadratic pattern in infants' alertness state over the course of the PET. At the same time, we predicted that infant state would be sustained at a level higher than when the task began; thus, we also expected a linear trend in the pattern of infants' state over the course of the PET. To examine these predictions, we conducted a univariate repeated measures ANOVA on infants' state across the PET (collapsing across the 2-, 12-, and 24-week age points), with epoch (epochs 1-6) as the repeated measure. The pattern of infants' responses in the PET can be seen in Figure 4. The analysis revealed a significant main effect of epoch, $F(5,1670) = 70.058$, $p = .000$, partial-eta-squared = .173, and as predicted, polynomial contrasts on the epoch variable revealed significant linear, $F(1,334) = 122.698$, $p = .000$, and quadratic, $F(1,334) = 104.451$, $p = .000$, trends. These findings indicated that infants' patterns of alertness across the PET conformed to expectations.

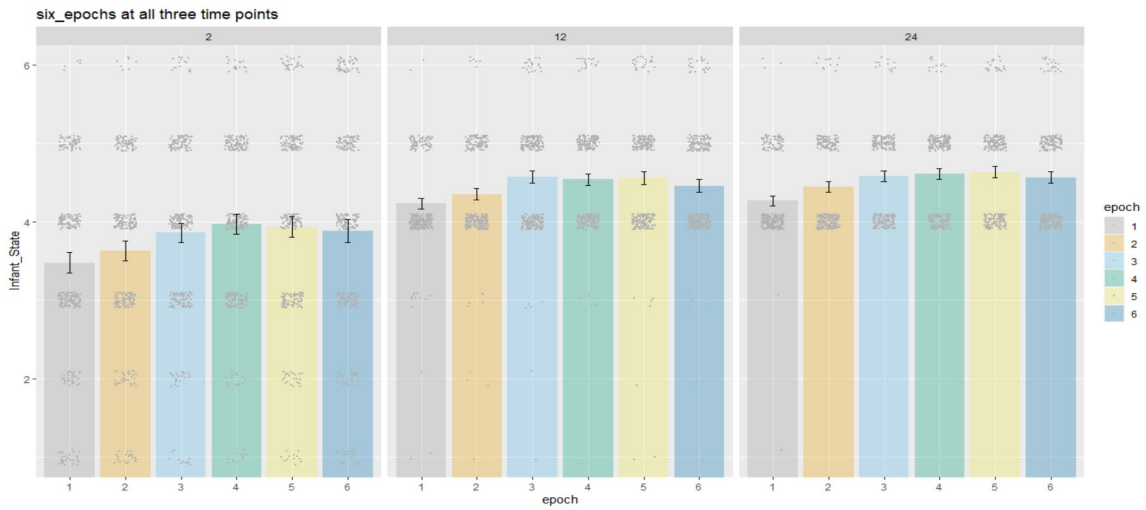


Figure 4. Pattern of Infant Alertness in the PET at 2-, 12-, and 24-weeks.

Age Differences in the Primary Engagement Task

A second question of interest was the extent to which age affected infants' patterns of alertness in the PET. We predicted that the linear and quadratic trends in infant alertness across the PET would become more pronounced with increasing age. For this reason, we expected that timepoint might interact with epoch, and in particular, we expected that timepoint would interact with linear and quadratic patterns in the polynomial contrasts on the epoch variable. Changes in infants' alertness during the PET can be seen in Figure 4. A 2 X 2 repeated measures ANOVA with timepoint (2-, 12-, and 24-weeks) and epoch (epochs 1-6) provided partial support for these predictions. The ANOVA revealed a significant main effect of timepoint, $F(2, 572) = 98.047$, $p = .000$, partial eta-squared = .255, with higher average infant alertness levels at the older timepoints than the youngest timepoints. The ANOVA also revealed a significant main effect of epoch, $F(5, 1430) = 72.498$, $p = .000$, partial eta-squared = .202. As can be

seen in Figure 4, infant alertness tended to peak in the middle of the PET and then decline to some degree thereafter. The ANOVA also revealed a significant timepoint X epoch interaction, $F(10, 2860) = 2.412$, $p = .007$, partial eta-squared = .008, indicating that alertness patterns across epochs depended to some degree on the timepoint at which participation occurred. Consistent with what was reported above, polynomial contrasts on the epoch variable revealed the predicted significant linear and quadratic trends, $F(1, 286) = 126.347$, $p = .000$, partial eta-squared = .306 and $F(1, 286) = 107.679$, $p = .000$, partial eta-squared = .274 respectively. This analysis also examined the timepoint by epoch interaction with respect to linear and quadratic trends, revealing that 2-week olds differed significantly from both 12- and 24-week olds with respect to only the linear trend (F's $(1, 286) > 4.127$, p's $< .043$, partial eta-squared's $> .014$). Twelve- and 24-week-olds did not significantly differ with respect to either trend. Also noteworthy, however, was that follow-up repeated measures ANOVAs examining PET state ratings at each timepoint separately revealed that significant linear and quadratic trends on the epoch variable were present at all three ages (2-weeks: linear trend $F(1,329) = 57.600$, $p = .000$, partial eta-squared = .149 and quadratic trend $F(1,329) = 36.222$, $p = .000$, partial eta-squared = .099 ; 12 weeks: linear trend $F(1,309) = 35.279$, $p = .000$, partial eta-squared = .102, and quadratic trend, $F(1,309) = 46.022$, $p = .000$, partial eta-squared = .130; and 24 weeks: linear trend $F(1,290) = 57.183$, $p = .000$, partial eta-squared = .165, and quadratic trend $F(1,290) = 32.807$, $p = .000$, partial eta-squared = .102). Taken altogether, these findings indicate that infants at all timepoints tended to display a pattern of peaking in alertness midway through the PET, with a decline thereafter but with some sustained alertness relative to the beginning of the task. At the same time,

however, there was a systematic tendency for the linear trend in response across the PET to be amplified in the two older timepoints.

Effect of Maternal Thiamine Supplementation

Thiamine supplementation dosage. A question of central interest was the extent to which low-dose maternal thiamine supplementation influenced infants' alertness patterns in the PET. We hypothesized that the predicted linear and quadratic trends in infant response across the PET would become more pronounced with increasing levels of thiamine supplementation. This analysis focused specifically on infants PET responding at the 12- and 24-week timepoints, because at the 2-week timepoint, maternal thiamine supplementation did not begin until after the PET was administered. Infants' alertness in the PET at these two timepoints for the four maternal thiamine supplementation groups appears in Figure 5. A mixed-design ANOVA with epoch (epochs 1-6) and timepoint (12- and 24- weeks) as repeated measures factors and maternal thiamine supplementation dosage (0, 1.2, 2.4, and 10mg/day) as a between-subjects factor partially confirmed our hypothesis. With only the older two timepoints included in the analysis, the timepoint main effect was only marginally significant, $F(1, 287) = 3.779$, $p = .053$, partial eta-squared = .013. Consistent with the findings reported earlier, the ANOVA revealed a significant main effect of epoch, $F(5, 1435) = 46.806$, $p = .000$, partial eta-squared = .140, with polynomial contrasts revealing significant linear, $F(1, 287) = 85.812$, $p = .000$, partial eta-squared = .230, and quadratic, $F(1, 287) = 78.719$, $p = .000$, partial eta-squared = .215, trends in infants' alertness across epochs. As well, the analysis revealed no significant main effect of maternal thiamine supplementation dosage on infants' alertness, $F(3, 287) = .137$, $p = .938$, partial eta-

squared = .001. Both the time point X epoch interaction and the time point X maternal thiamine supplementation interaction were non-significant [F(5,1435) = .965, p = .438, partial eta-squared = .003 ; F(3,287) = 1.738, p = .159, partial eta-squared = .018, respectively]. However, a significant epoch X maternal thiamine supplementation dosage interaction did emerge, F(15, 1435) = 1.725, p = .041, partial eta-squared = .018. Lastly, no significant three-way interaction between epoch, timepoint, and maternal thiamine supplementation emerged in the analysis, F(15, 1435) =1.474, p = .107, partial eta-squared = .015.

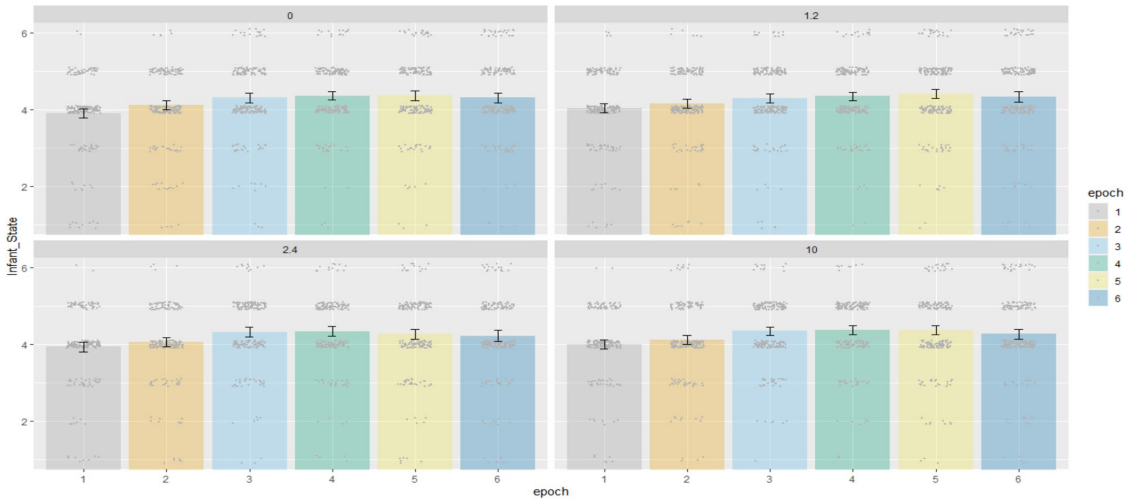


Figure 5. 12- and 24-week Infant Alertness by Thiamine Supplementation Group

Follow up simple effects analyses exploring the locus of the epoch X supplementation dosage interaction revealed significant differences in infant alertness were related to maternal thiamine supplementation dosage for the first epoch (F (3, 306) = 2.966, p = .032, partial eta-squared = .028) but not for any other epochs F's(3, 306) < .998, p's > .394, partial eta-squared's < .010. A univariate analysis of variance focusing on infant state in only epoch 1 with maternal thiamine supplementation dosage as a

between subjects factor, confirmed the significant impact of maternal thiamine supplementation on epoch 1 state $F(3,306) = 2.966$, $p = .032$, partial eta-squared = .02. Pair wise comparisons clarified that the impact of thiamine supplementation dosage on epoch 1 infant state did not follow a dose response pattern. As seen in Figure 6, infant state during epoch 1 was highest in 1.2 mg which differed significantly from both the placebo and the 2.4 mg group. The 10 mg group did not differ significantly in infant alertness during epoch 1 from any other dosage.

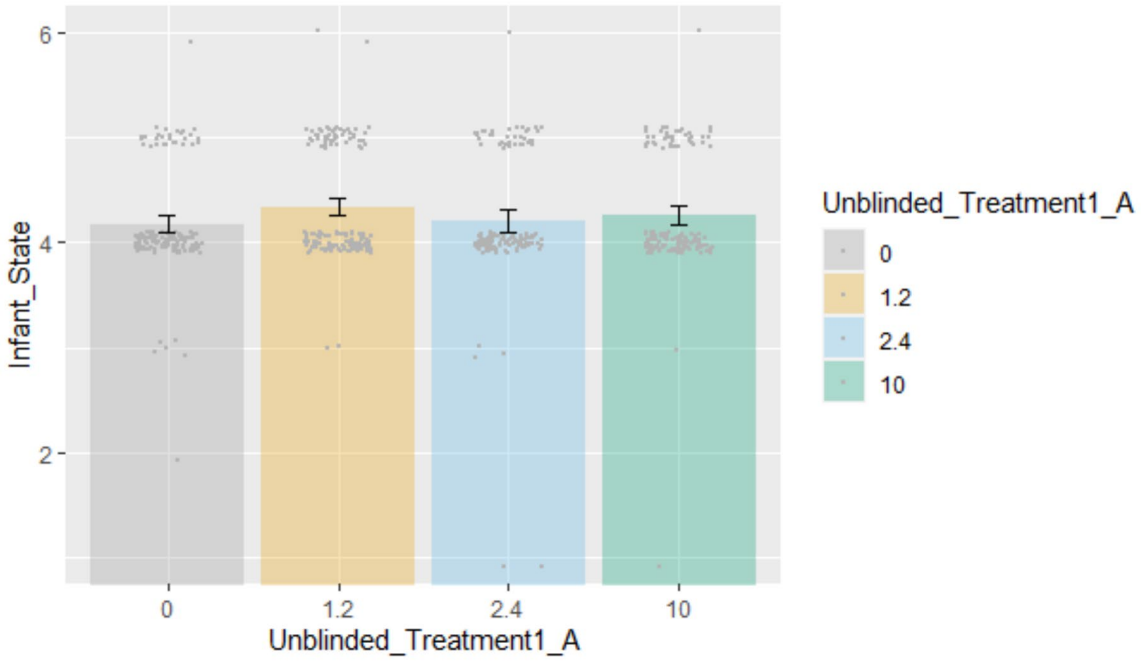


Figure 6. Infant Alertness in Epoch 1 of the PET in Relation to Thiamine Supplementation Group.

Milk total thiamine levels. We took an alternative route to examining possible relationships between thiamine and infant state. We used the same ANOVA models

just reported while introducing maternal milk thiamine levels as a covariate (2W, 4W, 12W, 24W). These models did reveal some significant relationships between milk thiamine levels and infant alertness in the PET. Appendix D, provides the detailed outcomes from these models. To summarize, 2-week and 4-week milk thiamine levels interacted significantly with infant pattern of alertness across epochs. Twelve-week milk thiamine levels no longer showed this interaction, but they did display a significant tendency to predict infant state overall. Twenty-four-week milk thiamine levels failed to systematically predict infant state.

Summary. We took two different strategies for examining relationships between thiamine status (maternal thiamine supplementation dosage on the one hand and maternal milk thiamine levels on the other) and infant alertness in the PET. Taken together, they revealed some systematic relationships. However, the nature of these relationships did not conform to our predictions. We predicted that linear and quadratic trends would be more pronounce with higher thiamine status. This was not the case; instead, thiamine supplementation dose influenced infant alertness only in epoch 1 and not in a dose response fashion. Milk thiamine levels also systematically predicted infant alertness in the PET. There were hints that the linear trend across epochs was stronger in infants whose mothers had higher milk thiamine levels at 2- and 4-weeks, however, these relationships were only marginally significant and thus their robustness is subject to question.

Exploratory analyses

Predicting 24-week MSEL Scores

An additional question of interest in this research, was the extent to which infants' pre-supplementation alertness in the PET was predictive of their end line cognitive functioning. Given the finding reported earlier, that epoch 1 of the PET was a locus at which maternal thiamine supplementation dosage influenced infant alertness, we opted to use infant state during epoch 1 of the PET as the predictor variable in examining this question. Of course, it was also important to examine such a relationship while taking into account other variables that might well be predicting infant cognitive functioning at end line. Previous analyses from this data set had already revealed that MSEL scores at 24-weeks (our field standard estimate of cognitive functioning at end line) were associated with maternal thiamine supplementation dosage, 2-week maternal total milk thiamine levels, and baseline MSEL scores. For this reason, we opted to control all of these variables as we tested for a possible relationship between infants' 2-week state in the PET and 24-week MSEL scores. In addition, we included infant birthweight into the model as a proxy for the quality of their intrauterine environment. We ran a series of multiple regression analyses instantiating this model with each of the MSEL sub-scales as the dependent variable (also shifting the baseline MSEL predictor sub-scale in accordance). The results of these regression models appear in Appendix E. To summarize briefly, when predicting end line MSEL composite the model was statistically significant with R square = .081, and beta weights of all predictor variables – including infant state during epoch 1 of the PET – were statistically significant at the .05 level, with the exception of maternal thiamine supplementation dosage, which was

only marginally significant. Interestingly, infant epoch 1 state in these models predicted end line MSEL scores in the negative direction, meaning that lower epoch 1 state ratings were associated with higher endline MSEL performance. Analogous models on MSEL sub-scales (gross motor, fine motor, visual reception, receptive language, expressive language) revealed that the model accounted for the most variance for the receptive language scale compared to any other scale, but the model was statistically significant for the fine motor sub-scale and marginally significant for the expressive language sub-scale. Infants epoch 1 state significantly predicted the receptive language sub-scale and the fine motor subscale, and it was a marginally significant predictor of the expressive language sub-scale. Together, these findings point to infant alertness state providing systematic, long range prediction of cognitive functioning some months later even while controlling for a range of other meaningful predictors of subsequent cognitive functioning.

Discussion

In this thesis, I investigated the extent to which low-dose maternal thiamine supplementation enhanced social alertness in Cambodian infants at risk for thiamine deficiency. In order to do so, I used behavioral and biological data from a larger, international study led by Dr. Kyly Whitfield of Mount Saint Vincent University. In the study, Cambodian mothers were supplemented with four different thiamine doses (0, 1.2, 2.4, 10 mg/day) starting at 2 weeks postpartum. Infant cognitive development was assessed by Dr. Dare Baldwin and Dr. Jeffery Measelle of the University of Oregon at select points during the first year of life. My investigation was contingent on their newly designed Primary Engagement Task that mother-infant pairs participated in at 2, 12, and 24 weeks postpartum.

Validation of the PET

The first point of this thesis was to validate the newly designed PET as a task that produces systematic changes in infants' alertness as mothers make graduated efforts to engage with them. Our results showed that infants displayed predictable responding in the innovative new task. These findings expand on a previous undergraduate thesis written by Steeves (2019), that found congruent patterns of infant response across the PET in a sub-sample of 44 mother-infant pairs at 2-weeks post-partum.

Age differences

Another objective of this thesis was to explore how alertness (and patterns in alertness) in the PET changed with age. As to be expected, we found that 12-week- and 24-week-

old infants had higher average levels of alertness in the PET compared to the 2-week-old infants. This result can be explained by the course of cognitive development, as infants at 12- and 24-week infants are more attuned to their surroundings and the social cues within them. In addition, we found that infants at all time points (2-, 12-, 24-weeks) displayed both quadratic and linear trends in their response across the PET. In other words, infants at all age points peaked in alertness midway through the PET, and declined thereafter but sustained some alertness relative to the beginning of the task. This result can be explained by the stepping up, stepping down nature of the PET that is shaping infant alertness as the task unfolds.

While each age group displayed both a quadratic and linear trend in response across the PET, there was an age difference such that infants at 12-weeks and 24-weeks displayed a significantly more pronounced linear trend in their response during the PET, meaning they had more sustained alertness as the task ended compared to infants at 2-weeks. There are a number of possible explanations for this difference. Perhaps infants at 12- and 24-weeks are continuing to stay more alert as mothers remove engagement tools in the PET as a bid to continue social engagement with their caregiver. This would be true if infants were staying in quiet awake or active awake state as the PET came to a close. Another explanation is that infants at 12- and 24- weeks are beginning to get fussy or dysregulated as mothers remove engagement tools in the PET. This would be true if infants were advancing to active awake or crying state as the PET came to a close. As yet it isn't possible to empirically distinguish between these possible explanations for the observed age difference; this is an interesting topic for future research.

Thiamine Effects

Another topic of central interest in this thesis research was the extent to which low-dose maternal thiamine supplementation was related to infant response patterns across the PET. It seemed plausible that infants might display higher overall levels of alertness in the PET in relation to higher levels of maternal thiamine supplementation. Contrary to this prediction, however, maternal thiamine supplementation had no significant overall impact on infants' level of alertness in the PET.

However, maternal thiamine dosage did display an interaction with the pattern of infants' alertness across epochs. Follow-up analyses revealed that the locus of this interaction was centered on the first epoch, during which infants' baseline level of alertness was assessed while mothers' attention was focused elsewhere. Interestingly, however, the effect of thiamine on infants' alertness levels in the first epoch did not display a dose-response pattern. If anything, the pattern of findings suggested that 1.2 mg and higher levels of supplementation may be associated with increased levels of alertness in the first epoch of the PET; in our data this was true for both infants whose mothers received either 1.2 or 10mg daily thiamine supplementation, although it was not true for infants whose mothers received 2.4 mg. All in all, this pattern of findings was both unexpected and somewhat inconsistent, and thus warrants further investigation.

Notably, analysis showed that 2-week and 4-week maternal milk thiamine levels were associated with 2 and 12-week infants' pattern of alertness across epochs. Higher levels of maternal milk thiamine were related to more sustained 2- and 12- week infant alertness as mothers removed engagement tools and the PET came to a close. Again, as

these findings were not predicted a priori, thus they warrant replication and further investigation.

Exploratory Analyses

A final point of this thesis was to examine the extent to which infants' pre-supplementation alertness in the PET was predictive of their endline (24 week) cognitive functioning. We found that infants' state of alertness in epoch 1 of the PET at 2-weeks, was predictive of their 24-week Mullen Scales of Early Learning (MSEL) scores in the composite scale, the receptive language sub-scale, and the fine motor sub-scale. It was also marginally predictive of their 24-week scores in the expressive language sub-scale. These relationships held true even when controlling for other significant predictors of subsequent cognitive functioning such as maternal thiamine supplementation dosage, 2-week maternal milk thiamine levels, baseline MSEL scores, and infant birthweight.

What is particularly interesting is that infant epoch 1 state ratings predicted the MSEL scores in the negative direction, meaning the lower the infant state of alertness during the baseline epoch, the higher their endline MSEL performance. The directionality of this relationship could be explained in a number of ways. One is that lower baseline states – such as active asleep, drowsy awake, or quiet awake -- are normative levels of alertness for 2-week infants when caregivers are not using engagement tools such as their facial expression, voice, or touch. This would mean that 2-week infants in higher states such as active awake or crying during epoch 1 of the PET are atypical. Thus 2-week infant state in epoch 1 of PET might serve as a potential

diagnostic of dysregulated babies and a potential indicator of lower future cognitive functioning. Another explanation of the directionality rests on recognition that there sees to have been a restricted range of state in the PET. The staff who conducted the PET in the field were instructed to begin the task when infants were in a quiet awake state. This hypothetically limits the states of alertness that infants could be in from quiet awake to crying. Within that small range, if infants were scoring high in alertness, they would be crying or in a fussy state. In these dysregulated states, infants do not have the capacity to engage in a mutually positive interaction with their caregivers, and thus are not reaping the benefits and the potential learning that comes from primary engagement. On the other hand, if infants are scoring on the lower end of the scale, they are in a quiet awake state, primed and ready to positively engage with and learn from their caregiver, supporting their future cognitive functioning.

Broader Implications

Validation of the PET

The first important implication of this study is its validation of the Primary Engagement Task (PET). The PET's ability to produce predictable infant responding indicates that it is a valuable new tool to observe caregivers and infants as caregivers make efforts to engage with their infants in mutually positive ways. The PET's design, with its addition and then removal of different interaction modalities (facial expression, voice and touch), has the potential to provide researchers with insight into developmental domains related to an infants' ability to sense and respond to the changes in their caregivers' efforts. In addition, this thesis demonstrates that the PET can be successfully used in cross-cultural field research settings and that it produces systematic

changes in infant alertness that are stable despite considerable variability in the rural home settings in which it was administered

Importance of the NNNS 6-state scale

An additional implication of this study is the importance of measuring infant alertness, as a cognitive domain that predicts future developmental outcomes. The connection between infant alertness at 2-weeks postpartum and their scores on the Mullen Scales of Early Learning at 24-weeks is robust. The findings also demonstrate that the NNNS 6-state scale, on which the state coding of the PET was based, provides valuable information, including long range prediction of cognitive functioning some months later even while controlling for a range of other meaningful predictors of subsequent cognitive functioning.

Limitations

Behavioral Task and Coding System

As with any study, this thesis was presented with challenges and limitations throughout the research process. One set of limitations stems from the behavioral task and the coding system used in this study. In regard to the behavioral task, although the Primary Engagement Task was carefully crafted with cross-cultural research in mind, it is important to note that it was developed from a western understanding of primary engagement. In this study, it was used in a country with different parenting norms. This means the task may not have fully captured the dynamics o primary engagement (positive social interaction between infant and caregiver) that is typical in Cambodian

culture. Beyond the conceptualization of the task itself, there are more general potential sources of error associated with the use of a behavioral task in human research. One possible source of error is the extent to which researchers gave mothers clear and timely directions and the extent to which mothers faithfully followed directions during the six-part task. There were instances where mothers did not add or remove interaction modalities in a timely manner or at all. This could be related to researcher error in giving directions or to misunderstandings on the mothers' parts. Finally, there is potential that the abnormality of having researchers observe and record the Primary Engagement Task on their cameras influenced mothers' behaviors throughout the task.

In regard to the coding system, there is the general concern of reliability across coders. There is also potential for imprecision in the coding scale itself. The scale worked well for 2-week and 12-week infants, because it captured a range of their alertness states. In the two younger age groups, infants started the task at lower alertness states, namely active asleep (2), drowsy asleep (3), or quiet awake (4). As the task progressed their alertness state increased and then decreased slightly, allowing the coders to use the full range of the state coding scale. For the 24-week infants, the infants began the task at the higher states such as quiet awake (4), or active awake (5). As the task progressed, their alertness state increased but the changes in their state could not be fully captured with the 6-state scale because there was only one other state for infants to be categorized in.

Possible Sources of Error Variance in the Findings

Another set of limitations for this study are related to variables that may have acted antagonistically to thiamine supplementations' benefits. Two of these variables are maternal consumption of foods that contain thiamine antagonists, and maternal betel leaf chewing. Another variable is maternal depression. To the extent that a caregiver is depressed, this may impede the quality and or frequency of primary engagement with her infant. This could have the potential to affect both a mother's ability to participate in the PET and an infant's response to their mother's efforts to engage in a mutually positive way. A final concern is that the Cambodian sample of mother-infant pairs was at risk for malnutrition more generally. This means that the benefits of thiamine supplementation could have been masked or amplified depending on levels of malnutrition across supplementation groups.

Future Directions

Other Components of Infant Development. One clear future direction is to assess other aspects of infant socio-cognitive development in relation to thiamine supplementation. This thesis has provided insight into the extent to which thiamine supplementation affects infant social alertness in relation to a caregivers' efforts to engage, but the question still remains, how does access to thiamine affect the development of other components of infant social cognition?

There is more research to come from the Cambodia Project at the University of Oregon using data from the Primary Engagement Task (PET). Soon we will know the extent to which thiamine supplementation affected the ability of caregivers and infants

to connect and/or "gel" during the PET. In addition, we will understand the extent to which thiamine supplementation affected the ability of infants to respond appropriately to their caregivers' bids of engagement during the PET. There is also more information about infant social cognition to come from a different behavioral task used later in infant development called the Secondary Engagement Task (SET). The task focuses on examining the nuances of secondary engagement (social connection between caregiver and infant in relation to a third thing). Beyond the engagement tasks, future research from the Cambodia Project will also address the extent to which early thiamine access is related to outcomes for other infant developmental modalities such as a) the development of infant vision, and b) foundational discrimination and memory skills as measured the Visual Preference Comparison Task (VPC).

Longitudinal Assessments. Another critical future direction is to examine the extent to which thiamine supplementation affects later cognitive outcomes in childhood and adolescence. The larger randomized, controlled trial of which this study is a part is just beginning to probe how early access to thiamine is related to infant developmental outcomes at 2-, 12, 24-, and 52-weeks. But this research is unable to address the extent to which thiamine supplementation or early access to thiamine affects developmental outcomes after the first year of life. There is reason to believe that early detriments to the development of critical pathways in the brain, due to inadequate access to thiamine, will compound with time. For example, if inadequate access to thiamine impairs an infant's ability to sense and respond to their caregivers' cues at 2-, 12- and 24-weeks, they will miss out on critical social engagement that, when functioning properly,

supports their subsequent learning. By this token, an infants' early ability to interact with and learn from their caregiver will directly affect their learning, and thus their subsequent cognitive development. Such possible long-term developmental effects are an important topic for future investigation.

Mothers' ability to provide sensitive care. Much of the focus of the Cambodia Project thus far has been the extent to which thiamine supplementation affects infant developmental outcomes. Equally as important as thiamine supplementation, however, is considering how an infant's environment and the ways in which infants interact with the primary caregiver operate together or on their own to affect an infant's development. This is why future research should access the extent to which thiamine supplementation affects mothers' behaviors and well-being. Something of particular interest would be the extent to which access to thiamine (and down the line other micronutrients) affects parental sensitivity during the postnatal period. Sensitive parental care, defined by prompt and adequate response to the child's signals and needs predicts a more secure attachment relationship, higher levels of cognitive competence, and fewer psychological problems (Kok et al., 2015). When a mother has inadequate access to thiamine (or other micronutrients), she may not have the physical or psychological energy to respond appropriately to her child. Without stimulation provided by a sensitive caregiver, an infant does not have a supportive environment necessary for brain development – particularly experience-dependent processes that impact synaptogenesis and pruning (Kok et al., 2015). These issues are another important focus for further investigation.

Conclusion

Foremost, this thesis demonstrated that the Primary Engagement Task (PET) is a valuable new tool to observe caregivers and infants as caregivers make efforts to engage with their infants in mutually positive ways. It also showed that the NNNS 6-state scale approach to coding infant alertness in the PET can provide long range prediction of cognitive functioning when controlling for other meaningful predictors of subsequent cognitive functioning. Although relationships between a) thiamine supplementation dosage and milk thiamine levels with b) infant alertness in the PET did emerge, the specific patterns obtained were not predicted. Thus, final conclusions about the benefits of maternal thiamine supplementation on infant social alertness await further investigation.

Moving forward, it is our responsibility as researchers and as world citizens to work diligently as we continue to study the cognitive development of infants at risk of micronutrient malnutrition and malnutrition more generally. Impaired cognitive, motor, and socio-emotional development is an epidemic in itself, with an estimated 200 million children under five years not fulling their developmental potential (Grantham-McGregor et al., 2007). The consequences of impaired development – poor school outcomes, low income, high fertility, and poorer child rearing - contribute to the cycle of poverty and disease in many parts of the world (Rosenberg, 2007). Eliminating obstacles to child development gives children the opportunity to thrive and grow into citizens who work towards making their nations thrive as well.

Appendix A: Synopsis of Cognitive Development

Cognitive development is a complex process that never truly ends. Even as adults, our brains are constantly evolving to accommodate new information, experiences, and demands. Although cognitive development is endless, it does not stay the same. Instead, it evolves with age, following a timeline of different processes. The simplest timeline of cognitive development in the first 1,000 days of life, begins with the creation, proliferation and migration of neurons – the cells of the brain that give rise to all of our senses, emotions and thoughts. Neurons are created when a fetus' neural stem cells replicate themselves. In the first five weeks of gestation, this gives rise to the neural tube (Nyaradi et al., 2013), a group of cells that will eventually form the entire central nervous system. During the first 12 weeks of gestation, the neural tube cells replicate themselves exponentially, rapidly increasing in number.

Once more neurons are created, they migrate to the location where they are destined to function. When the neurons reach their location, they evolve to become specialized for their function in a process called differentiation. At the same time, the neurons also begin forming synapses, or connections between one another so they can relay messages. Both differentiation, and the formation of synapses occur after 15 weeks' gestation. Those processes, along with the removal of unnecessary synapses during the second trimester (Newville, Debenham, Monroy, & Jolles, 2018), create the foundation of the specialized structures of the brain. In the third trimester, a fetus' brain has a growth spurt nearly doubling in size. This occurs as a result of a continuation of

the processes described above and because of the start of myelination, when the axons of neurons are covered to increase signal speed and provide protection.

All of the above processes including - neurogenesis, migration, differentiation, synaptogenesis, synaptic pruning, and myelination - continue at varying rates into infancy (and across an individual's life span). Rapid brain growth continues for the first two years of life (Nyaradi et al., 2013). During that period, structural development in the form of increased cortical thickness and surface area occurs (Haartsen, Jones, & Johnson, 2016). This means that the network of neurons that makes up the cerebral cortex expands to continue to develop the frontal, temporal, parietal, and occipital lobes and give the brain its characteristic outer folding. Functional development such as the formation of resting state networks and cortical specialization to social stimuli also takes place during the first two years of life (Haartsen, Jones, & Johnson, 2016). This means that networks that coordinate multiple regions of the brain are formed to facilitate neural systems such as visual processing, and memory and attention (Asis-Cruz, Bouyssi-Kobar, Evangelou, Vezina,& Limperopoulos, 2015). It also means that an infant's brain is learning and adapting (i.e. creating new neurons and connections, as well as pruning unused neurons) based on its social interactions with its caregiver(s).

Appendix B: State Coding Guide

Code	Description
1	**Quiet Sleep (State 1):** Sleep with regular breathing, eyes closed, no spontaneous activity except startles or jerky movements at quite regular intervals; external stimuli produce startles with some delay; suppression of startles is rapid; state changes are less likely than from other states; and no eye movements.
2	**Active Sleep (State 2):** Sleep with eyes closed; rapid eye movements often can be observed under closed lids; low activity level, with random movements and startles or startle equivalents; movements are likely to be smoother and more monitored than in State 1; responds to internal and external stimuli with startle equivalents, often with a resulting change of state. Respiration is irregular; sucking movements occur on and off. Eye opening may occur briefly at intervals.
3	**Drowsy (State 3):** Eyes may be open but dull and heavy lidded, or closed, eyelids fluttering; activity level minimal, may be reactive to sensory stimuli, but response often delayed. Movements are usually smooth though there may be startles. Infant has a dazed appearance and is minimally reactive even when his or her eyes are open. This is also considered a "transitional" state and is sometimes difficult to score. Some infants may also show fuss/cry vocalizations in this state. When this happens, State 3 may be difficult to distinguish from State 5. The minimal movement in State 3 and considerable movement in State 5 is what distinguishes State 3 from State 5 when both are accompanied by fuss/cry vocalizations.
4	**Quiet Awake (State 4):** Alert, eyes open with bright look and appropriate changes in facial expression as stimulation is varied; focuses attention on source of stimulation, or a visual or auditory stimulus. Motor activity is minimal. There can be a glazed look that is easily changed into a brighter look with appropriate stimulation.
5	**Active Awake (State 5):** Eyes likely to be open, considerable motor activity, with thrusting movements of the extremities, and even a few spontaneous startles; reactive to external stimulation with increase in startles or motor activity, but discrete reactions difficult to distinguish because of general activity level. Brief fussy vocalizations can occur in this state. Some infants may transition directly from lower states (1, 2, or 3) directly to State 5. These often are the cases described above in which fuss/cry vocalizations occur at States 5 and 3 are difficult to distinguish unless the difference in motor activity is considered.
6	**Crying (State 6):** Characterized by intense, loud, rhythmic, and sustained cry vocalizations, which are difficult to break through with stimulation; motor activity is high. It is important to distinguish between cry as a state from fuss/cry vocalizations that can occur in State 5 and even State 3. Some infants have shown repeated episodes of fuss/cry vocalization in State 5 but may not reach State 6. This may also be a maturational issue, as some preterm infants may not have the energy reserves to sustain State 6. In general, State 6 can be distinguished from State 5 by the intensity and sustained quality of the crying (at least 15 seconds) and unavailability of the infant in State 6. Repeated brief episodes of fuss/cry in State 5 do not mean that the infant has moved into State 6. Coders need to give the infant the opportunity to show State 6. Premature administration of consolability and cuddling maneuvers may prevent the infant from reaching State 6 and provide an inaccurate assessment of the infant.

*The state coding system for was developed by Tronick (2004) and is included as part of the Network Neurobehavioral Scale (NNNS) that is a standard measure of infant neurological status used by clinicians.

Appendix C: Participant Demographics

	TOTAL *N*=335	Placebo (0 mg) *n*=83	1.2 mg *n*=86	2.4 mg *n*=81	10 mg *n*=85
Infant					
Sex, *female*	161 (48%)	43 (52%)	43 (50%)	33 (41%)	42 (49%)
Length-for-age (Z-score) at 2-weeks	-.62 (1.02)	-0.52 (0.98)	-0.66 (1.11)	-0.69(1.01)	-0.63 (1.01)
MSEL[1] at 2-weeks					
Gross Motor	36.91 (7.18)	37.60 (6.45)	37.36 (6.97)	35.38 (7.40)	37.21 (7.78)
Fine Motor	33.89 (5.96)	34.41 (6.10)	33.79 (5.52)	32.72 (5.84)	34.60 (6.33)
Visual Reception	22.97 (3.70)	31.91 (8.11)	31.90 (7.51)	30.38 (8.02)	31.86 (8.73)
Receptive Language	31.53 (8.07)	31.91 (8.11)	31.90 (7.51)	30.38 (8.02)	31.86 (8.73)
Expressive Language	38.10 (1.36)	38.00 (.93)	38.24 (1.98)	37.88 (.97)	38.26 (1.51)
Mother					
Age, *years*	28.1 (6.2)	28.3 (6.1)	27.9 (6.7)	28.1 (6.1)	28.1 (5.9)
Parity, *multiparous*	57 (69%)	54 (65%)	54 (63%)	58 (72%)	64 (75%)
Ethnicity, *Khmer*	335 (100%)	83 (100%)	86 (100%)	81 (100%)	85 (100%)
Marital status					
Married	330 (98%)	79 (95%)	86 (100%)	81 (100%)	84 (99%)
Divorced/Separated/Widowed	5 (<1%)	4 (5%)	0 (0%)	0 (0%)	1 (1%)
Education					
None	40 (12%)	10 (12%)	8 (9%)	13 (16%)	9 (11%)
Primary (1-6 years)	161 (48%)	43 (52%)	37 (43%)	40 (49%)	41 (48%)
Lower Secondary (7-9 years)	83 (25%)	16 (19%)	29 (34%)	19 (24%)	19 (22%)
Upper Secondary (10-12 years)	43 (13%)	12 (15%)	9 (11%)	8 (10%)	14 (17%)
Higher education	8 (2%)	2 (2%)	3 (3%)	1 (1%)	2 (2%)
Milk total thiamine concentrations (µg/L) at 2-weeks	129.1 (74.4)	135.5 (77.7)	129.3 (71.4)	126.3 (77.3)	125.4 (72.3)
Household					
Husband education					
None	38 (11%)	10 (12%)	9 (10%)	9 (11%)	10 (12%)
Primary (1-6 years)	151 (45%)	42 (51%)	37 (43%)	39 (48%)	33 (39%)
Lower Secondary (7-9 years)	97 (29%)	21 (25%)	24 (28%)	23 (28%)	29 (34%)
Upper Secondary (10-12 years)	34 (10%)	5 (6%)	13 (15%)	8 (10%)	8 (9%)
Higher education	15 (4%)	5 (6%)	3 (3%)	2 (3%)	5 (6%)
Household size, *number of people*	3.9 (1.9)	3.7 (1.7)	3.6 (1.8)	4.0 (2.1)	4.1 (2.0)
Median Annual household income, *US$ (IQR)*	1620 (950-3500)	1800 (950-3000)	2050 (963-3500)	1600 (1000-3000)	2000 (1200-3500)
Wealth Index Score*					
Poorest	81 (24%)	22 (27%)	12 (15%)	21 (26%)	25 (29%)
Second	69 (21%)	16 (19%)	14 (16%)	20 (25%)	19 (22%)
Middle	108 (32%)	26 (31%)	31 (36%)	24 (30%)	27 (32%)
Fourth	54 (16%)	14 (17%)	20 (23%)	11 (13%)	9 (11%)
Wealthiest	23 (7%)	5 (6%)	8 (10%)	5 (6%)	5 (6%)

Data are mean (SD) or n (%), except household income, shown as median (IQR). Percentages may not add to 100% due to rounding.

[1] MSEL= Mullen Scales of Early Learning

* Wealth equity index (WEI) quintiles calculated based on the Demographic Health Survey Program guidelines (USAID); Cambodian WEI developed using 2014 DHS data.

Appendix D: Maternal Milk Thiamine Analyses

Within Subjects Contrasts

	PREDICTORS	df	F	*p*
2-week epoch linear	2-week maternal milk total thiamine	1	2.985	.085
2-week epoch quadratic	2-weel maternal milk total thiamine	1	.072	.788

	PREDICTORS	df	F	*p*
12-week epoch linear	2-week maternal milk total thiamine	1	1.170	.280
	4-week maternal milk total thiamine	1	3.876	.050
	12-week maternal milk total thiamine	1	.745	.361
12-week epoch quadratic	2-week maternal milk total thiamine	1	.803	.371
	4-week maternal milk total thiamine	1	.044	.834
	12-week maternal milk total thiamine	1	2.023	.155

	PREDICTORS	df	F	*p*
24-week epoch linear	2-week maternal milk total thiamine	1	.210	.647
	4-week maternal milk total thiamine	1	2.707	.101
	12-week maternal milk total thiamine	1	.040	.841
	24-week maternal milk total thiamine	1	.009	.924
24-week epoch quadratic	2-week maternal milk total thiamine	1	3.280	.071
	4-week maternal milk total thiamine	1	.002	.962
	12-week maternal milk total thiamine	1	.695	.405
	24-week maternal milk total thiamine	1	.000	.990

Appendix E: Mullen Scales of Early Learning

		24 Week Outcomes					
PREDICTORS		B	SEB	β	*t*	*p*	95% CI
Endline MSEL Composite	Maternal thiamine supplementation dosage	.643	.344	.105	1.867	.063	[-.035, 1.321]
	Maternal milk thiamine at 2 weeks	.047	.019	.137	2.430	.016	[.009, .085]
	Baseline MSEL composite	.245	.123	.113	1.992	.047	[.003, .487]
	Birth weight	9.310	3.649	.145	2.552	.011	[2.129, 16.491]
	Infant 2-week state in epoch 1 of the PET	-2.267	1.144	-.133	-1.982	.048	[-4.517, -.016]
Endline MSEL Gross Motor	Maternal thiamine supplementation dosage	.025	.106	.013	.233	.816	[-.184, .234]
	Maternal milk thiamine at 2 weeks	-.009	.006	-.086	-1.500	.135	[-.021, .003]
	Baseline MSEL gross motor	.192	.058	.192	3.333	.001	[.079, .306]
	Birth weight	2.191	1.120	.112	1.956	.051	[-.013, 4.396]
	Infant 2-week state in epoch 1 of the PET	-.311	.356	-.051	-.874	.383	[-1.011, .389]
Endline MSEL Fine Motor	Maternal thiamine supplementation dosage	.124	.094	.075	1.317	.189	[-.061, .308]
	Maternal milk thiamine at 2 weeks	.012	.005	.126	2.219	.027	[.001, .022]
	Baseline MSEL fine motor	.149	.062	.138	2.424	.016	[.028, .271]
	Birth Weight	2.155	.989	.124	2.180	.030	[.210, 4.101]
	Infant 2-week state in epoch 1 of the PET	-.630	.313	-.116	-2.011	.045	[-1.246, -.013]
Endline MSEL Visual Reception	Maternal thiamine supplementation dosage	-.077	.097	-.046	-.793	.439	[-.269, .114]
	Maternal milk thiamine at 2 weeks	-.001	.005	-.007	-.115	.909	[-.011, 0.10]
	Baseline MSEL visual reception	.050	.105	.028	.474	.636	[-.156, .256]
	Birth Weight	1.653	1.030	.094	1.605	.110	[-.374, 3.680]
	Infant 2-week state in epoch 1 of the PET	.328	.323	.059	1.015	.311	[-.308, .963]
Endline MSEL Receptive Language	Maternal thiamine supplementation dosage	.421	.163	.143	2.587	.010	[.101, .742]
	Maternal milk thiamine at 2 weeks	.025	.009	.155	2.775	.006	[.007, .043]
	Baseline MSEL receptive language	.200	.079	.141	2.543	.011	[.045, .356]
	Birth Weight	3.917	1.725	.127	2.271	.024	[.523, 7.312]
	Infant 2-week state in epoch 1 of the PET	-1.379	.541	-.143	-2.551	.011	[-2.443, -.315]
Endline MSEL Expressive Language	Maternal thiamine supplementation dosage	.194	.100	.111	1.944	.053	[-.002, .390]
	Maternal milk thiamine at 2 weeks	.013	.006	.129	2.245	.025	[.002, .024]
	Baseline MSEL expressive language	-.475	.273	-.100	-1.741	.083	[-1.012, .062]
	Birth Weight	1.908	1.051	.104	1.816	.070	[-.160, 3.976]
	Infant 2-week state in epoch 1 of the PET	-.572	.330	-.100	-1.730	.085	[-1.222, .079]

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