EXAMINING THE INFLUENCE OF MATERNAL THIAMINE SUPPLEMENTATION ON BREASTFED CAMBODIAN INFANT'S PERCEPTUAL AND COGNITIVE PROCESSING

by

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Thiamine deficiency affects millions of infants within South and Southeast Asia due to a cultural reliance on polished white rice, which is lacking in thiamine. Specifically, Cambodian infants between the ages of 0-6 months are at increased risk of suffering consequences of thiamine deficiency, such as impaired cognitive development and potentially fatal infantile beriberi (Whitfield et al., 2019). As a part of a larger, randomized controlled trial, my thesis investigated the extent to which maternal thiamine supplementation may protect infant's memory and perceptual abilities, as measured via the Visual Paired Comparison task (VPC). 335 lactating mothers were randomly assigned to four treatment groups (0mg, 1.2mg, 2.4mg, and 10mg daily thiamine supplement) when their infants were between 2- and 24-weeks postnatal. The VPC task was administered at 24- and 52-weeks postnatal; the task measures infants' ability to encode an image, such as a new face or geometric pattern, and then, during test trials, recognize the image and discriminate the now-familiar image from a novel image. Previous research documents that infants who readily succeed at these perceptual/cognitive skills tend to look longer at the novel than the familiar image

during test trials (Rose et al, 2008). We hypothesized that higher levels of maternal thiamine supplementation would be associated with higher novelty scores in infants' VPC looking times. We found that maternal thiamine supplementation indeed influenced infants' overall novelty scores; specifically, infants whose mothers received supplemental thiamine displayed higher average novelty scores than those whose mothers received a placebo. Above all, this research indicates that maternal thiamine supplementation benefits breastfed Cambodian infants' neurocognitive development.

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Introduction

In 2003, Israeli infants started to fill emergency rooms in critical condition, some of whom unexpectedly died. Upon urgent investigation, it was discovered that thousands of infants had been given a soy-based formula lacking in thiamine, or vitamin B1, which is an essential micronutrient required for energy metabolism. Observational research following the infants who survived has revealed that thiamine deficiency during early infancy may lead to neurocognitive developmental difficulties (Fattal-Valevski et al., 2005). Despite this catastrophe, thiamine deficiency has become a rare condition, as many countries supplement thiamine into processed grains. However, the addition of thiamine into processed grains is not a practice that occurs everywhere: infants in regions such as Southeast Asia, where thiamine is generally lacking, poses a major health risk because, even at sub-clinical levels, thiamine deficiency, may undercut neurocognitive development.

Due to the high prevalence of thiamine deficiency within Cambodia (Whitfield, et al., 2017), global efforts to combat this pressing issue are warranted**.** Although recent research indicates that thiamine deficiency has detrimental effects on infant neuro-cognitive development, the specific aspects of infants' neuro-cognitive function affected as well as the level of thiamine needed to protect neuro-cognitive development are not yet understood.

My thesis takes place within the context of a larger, double-blind, randomized, controlled trial (Whitefield, et al., 2019) that aims to determine both the dose of thiamine required to optimize human milk thiamine concentrations in lactating Cambodian women, and the level of maternal thiamine supplementation needed to

protect infants' neuro-cognitive development. In particular, I investigated the extent to which maternal thiamine supplementation protects the development of perceptual/cognitive skills that are foundational to infants' subsequent learning across a broad range of domains, as measured within the scope of the Visual Paired Comparison (VPC) task.

The Visual Paired Comparison (VPC) task assesses infants' fluency with foundational aspects of perceptual/cognitive processing. Previous research has documented that infants display individual differences in the tendency to look longer when simultaneously presented with a novel versus a familiar stimulus. A stronger novelty preference in this task positively predicts infants' subsequent cognitive functioning (Rose et al., 2008). Rose and colleagues have made a strong case that infants' scores on measures such as the VPC task are revealing of the trajectory of cognitive development due to the phenomenon of a cognitive cascade, which is how more primitive fundamental abilities underpin more complex ones that in turn affect overall cognition (Rose et al., 2008). The phenomenon of the cognitive cascade provides evidence that intervention in infancy may have profound effects on later outcomes. My thesis focused specifically on the possibility that the intervention of low-dose maternal thiamine supplementation might enhance breastfed infants' VPC task novelty scores, which in turn might herald cascading benefits for their long-term neurocognitive development.

Literature Review

The negative implication of clinical levels of thiamine deficiency is widely recognized as a public health issue among heavily rice-consuming groups and is thought to be a substantial cause of infant mortality. While it is well established that clinical levels of thiamine deficiency, especially during early infancy, may be fatal, the impact of sub-clinical levels of thiamine deficiency are less widely explored. The case of Israeli infants being given a thiamine deficient formula was influential because it allowed for a longitudinal study of the infants who survived the tragic event. As many as 600 to 1000 infants were impacted by this tragic event (Mimouni-Bloch et al., 2014). Many infants who'd received the formula were neurologically asymptomatic, but others were not as fortunate. Twenty infants were hospitalized due to their severe neurological symptoms, which included prolonged vomiting, nystagmus, seizures, lethargy, and coma. To investigate the long-term effects of thiamine deficiency, a longitudinal observational study was conducted, revealing that 57 out of the 59 thiamine-deficient children followed had language impairments compared to an age-matched control group of children who had not experienced thiamine deficiency (Mimouni-Bloch et al., 2014). Seven children exhibited mental retardation and motor abnormalities, two had early kyphoscoliosis, six had developed epilepsy, and one child had an atrioventricular block (Mimouni-Bloch et al., 2014).

The impact of the thiamine deficient infant formula extended beyond severe cases of thiamine deficiency, however. Fattal Valevski and colleagues (e.g., 2005, 2009, Fattal, Friedman, & Fattal-Valevski, 2011) found significant negative effects of thiamine deficiency on infants' language and motor development some years later, even

when children were 5-7 years of age. Fattal-Valevski et al., (2005) found that relative to infants who had not received the thiamine-deficient soy formula, infants who had consumed the thiamine -deficient formula displayed lower scores on the Bayley Scales of Infant Development motor development scale. These findings have raised widespread concern, given that millions of infants, especially in regions such as Southeast Asia, currently remain at risk of thiamine deficiency which may undercut infant's neurocognitive development, which encompasses brain structure, motor and sensory skills, neurology, and cognition (National Institute of Statistics, 2011; Whitfield, et al., 2017).

Thiamine, commonly known as vitamin B1, is an essential nutrient that serves as a cofactor for enzymes that deal mostly with mitochondrial metabolism. Thiamine-dependent enzymes are critical for energy metabolism and the biosynthesis of nucleic acids. Thiamine also acts as a coenzyme in the process of carbohydrate and amino acid metabolism (Institute of Medicine, 1998). There are many important processes that thiamine is critical for, but most noticeably, it is essential in supporting cell growth, development, and aids to maintain nervous system functions. Thiamine circulates in the blood in erythrocytes and is delivered to cells with high metabolic needs, particularly cells within the brain.

Thiamine cannot be endogenously synthesized, so to obtain thiamine, it must be ingested (Shibani et al., 2019). Thiamine enters the body as free thiamine but mainly exists in the non-phosphorylated version called thiamine pyrophosphate (TPP) which is essential for metabolic pathways (Institute of Medicine, 1998). Thiamine has a short half-life, of around 1-12 hours, indicating that regular dietary supply is necessary to

maintain adequate thiamine levels (Whitfield et al., 2017). During the limited period in which thiamine may be physiologically stored, it is held within the liver while excess remains unchanged and is excreted through urine (Crook et al., 2018).

Since thiamine can only be stored in small amounts and has a relatively short half- life, it must be consumed regularly (Whitfield et al., 2016). This leads people to either rely on thiamine fortification in foods or natural sources of thiamine. Common sources of thiamine include, but are not limited to, beans, nuts, cereals, beef, fish, and poultry. Within western countries, thiamine is fortified in grains such as bread and cereal to ensure that adequate quantities of thiamine are being consumed. Thiamine does have some naturally occurring antagonists which include some tea leaves or betel nuts (Allen, 2006). Thiamine can be destroyed in heat, when cooked in water, and during the milling process (Guidelines on Food Fortification with Micronutrients). Due to the depletion of thiamine from processed grains such as bread, thiamine is commonly fortified in western countries such as within the United States (Dary, 2006).

Individuals at risk for thiamine deficiency include those experiencing malnutrition, aging, low economic status, eating disorders, medical conditions that impact the gastrointestinal tract, parental malnutrition, bariatric surgery, diabetes, and alcohol abuse (Dary, 2006). Deficiency may also result from genetic factors that prevent the uptake of thiamine, even in the presence of thiamine. Along with the previously stated risk factors, general malnutrition and lack of food variability may lead to thiamine deficiency (Whitfield et al., 2017).

In countries such as Cambodia, a lack of dietary variation and a high dependency on milled and polished white rice has made it difficult for generations of

people to have access to adequate quantities of thiamine. Along with a general reliance on polished white rice in Cambodia, there have not been widespread fortification efforts, which has left many people thiamine deficient (Whitefield, 2016). Specifically, it is estimated that 96% of Cambodian infants under the age of 6 months who are breastfed are at high risk of thiamine deficiency. In terms of the recommended intake of thiamine, Whitfield and colleagues (2017) stated that the quantity of thiamine needed varies depending on age and gender. For adults, 1.2 mg/day for men and 1.1mg/day for women is recommended. This increases to 1.4mg/day for pregnant women and 1.5 mg/day for lactating women. In infancy, the recommended intake is 0.2mg/day (0-6mo) and 0.3 mg/day (7-12mo). The recommended daily intake increases to 0.5 mg/day for children ages 1-3 yrs, 0.6 mg/day 4-6 yrs, and 0.9 mg/day 7-9yrs. After age 10, children's thiamine requirement is estimated to be the same as adults. Currently, there are no known adverse effects of high thiamine intake.

Thiamine appears to be especially critical in periods of rapid development, such as the perinatal period and childhood more generally, which is indicated by higher quantities of thiamine needed during infancy. Thiamine deficiency has been proven to contribute to neurological and psychiatric symptoms such as sleep disturbances, confusion, and reduced memory capacity. Severe cases of thiamine deficiency may lead to conditions such as beriberi and Wernicke- Korsakoff syndrome. There are two documented categories of beriberi which are defined based on symptoms present; wet beriberi mainly affects the cardiovascular system causing poor circulation and edema and dry beriberi affects the nervous system leading to nerve degeneration. In contrast, Wernicke - Korsakoff syndrome leads to severe neurological damage (Beriberi, 2020).

Infantile beriberi, often referred to as a 'forgotten disease' is caused by thiamine deficiency, and it is most serious in infants due to the rapid growth and development that occurs during this time, with an accompanying high requirement for thiamine. In severe cases of thiamine deficiency, infantile beriberi may lead to death within hours of clinical presentation of the disease if left untreated and undiagnosed. Kauffman et al., (2011) estimate that infantile beriberi may be responsible for 6% of overall infant mortality in Cambodia. Other severe cases of thiamine deficiency have also been shown to contribute to ataxia, congestive heart failure, muscle atrophy, and in extreme cases, death.

Thiamine deficiency in infants is difficult to diagnose because symptoms often mirror other diseases and are often misdiagnosed and remain untreated. Mild cases of thiamine deficiency result in generic symptoms such as constipation, vomiting, and restlessness. For a long time, the medical community has focused on infantile beriberi as the sole threat to thiamine-deficient infants. However, recent evidence suggests that subclinical levels of thiamine deficiency -- not severe enough to cause beriberi or other symptoms -- may have adverse effects on cognitive development, presumably because of thiamine's importance to the integrity of developing neural systems (Whitfield et al., 2017).

To investigate the effects of subclinical effects of subclinical thiamine deficiency, Whitfield et al., (2017) studied and assessed the thiamine status among a sample of Cambodian women of childbearing age (15-49 years) and their children (6-69mo). Whitfield et al., (2017) found that despite variation in the prevalence of thiamine deficiency by cut-off, there is clear evidence of suboptimal thiamine status

among women and their children in Cambodia. Whitfield et al., (2017) stated that infants between the ages of 6-12 months are of highest concern regarding the effects of subclinical levels of thiamine deficiency, as a great deal of development occurs during this time.

Prior research conducted by Gallant et al., (2020) has indicated that there is ample evidence that maternal thiamine intake directly impacts the thiamine concentration of milk, and consequently the intake and status of breastfed infants. Gallant et al., (2021) examined the impact of low-dose maternal thiamine supplements of 1-2 mg/d and found that this dose was sufficient to improve thiamine status biomarkers to levels consistent with thiamine-replete populations that did not experience beriberi. Although Gallant et al., (2021) have demonstrated that maternal thiamine supplementation is beneficial for lactating infants, also in question is the extent to which maternal thiamine supplementation may benefit fundamental cognitive functioning such as information-processing abilities, including the ability to encode new information, discriminate newly learned information from novel information, and attend to novel information. These are foundational informational processing skills because they are critical to any type of learning that occurs downstream. If thiamine supplementation benefits infants' ability to encode, discriminate, and attend to novel information, it would have major cascading effects for infants' neurocognitive development.

My thesis takes place within the context of the larger randomized controlled trial and investigated the possible influence of maternal thiamine supplementation on breasted infants' ability to encode a new stimulus, recognize it when presented again,

and discriminate it from a novel stimulus. The larger clinical trial was a four-parallel-arm, double-blind, randomized, controlled trial provided daily thiamine supplementation (a capsule containing 0, 1.2, 2.4 or 10 mg of thiamine hydrochloride) was provided to 335 exclusively breastfeeding Cambodian mothers when their infants were between 2 and 24 weeks of age.

Within the scope of the larger randomized controlled trial, the Visual Paired Comparison (VPC) task was administered to tap into possible benefits of maternal thiamine supplementation for Cambodian infants' perceptual and cognitive processing. Specially, in the VPC task, infants became familiarized with an image- such as a face or geometric pattern- and subsequently were shown that same image paired with a novel image. Previous research has documented that infants who are able to encode features of a first image that is presented and then discriminate it from a novel image will tend to look longer at the novel image (Rose et al., 2004). Moreover, other research has documented that the magnitude of the infants' looking to the novel image (the size of their novelty score) is associated with other aspects of their cognitive functioning and predicts their subsequent cognitive functioning later in development. That is, infants who are born full term have shown higher novelty scores in the VPC task than infants born prematurely (Rose et al., 2004) and infants' novelty scores at 6-7 months of age have been shown to be positively correlated with measures of cognitive development at 11 years of age (Rose et al., 2004).

Hypothesis and Predictions

My thesis investigated the extent to which maternal thiamine supplementation enhanced breastfed Cambodian infants' novelty scores on the VPC task. Given evidence that subclinical levels of thiamine deficiency undercut neurological development, I hypothesized that access to thiamine supplementation from early infancy would protect the integrity of these neural systems, and thereby promote infants' ability to show stronger novelty scores on the VPC task. I predicted that, overall, 24- and 52-week-old Cambodian infants would show a preference for the novel items, with increasing novelty scores from 24- to 52-weeks. I also predicted higher levels of maternal thiamine supplementation would be associated with an increase in novelty scores.

Methods

In 2018 native Cambodian staff, employed by the organization Helen Keller International, collected VPC task video recordings of infants participating the larger study described in a recently registered report (Whitfield et al., 2019). The study utilized a community-based approach as data collection occurred within mother's homes within the Kampong Thom province in central Cambodia. This thesis focused on coding and analyzing the available video data.

Participants

Participants were recruited from the Kampong Thom province of Cambodia and were comprised of 335 mother-infant pairs. Participant recruitment began in August 2018 and data collection started on September 12, 2018. 48% of the infants were female and 98% of the mothers were married and 69% of them had more than one child. The mothers were required to be between 18-45 years old (M=28.1, SD=6.2) and healthy. Mothers were recruited through antenatal care visits along with consultations with healthcare staff, elders, and local village chiefs. Infants had to be born without complications such as low birth weight and cleft palate. Mother's pregnancy also had to be lacking any known chronic conditions, gestational diabetes, or preeclampsia. For involvement in the study, mothers must have intended to exclusively breastfeed for 6 months postnatal and had to be willing for her entire household to consume only salt fortified by the Cambodia research team. The mother also had to consent to a variety of biological samples to be collected (ie., blood and breastmilk). Infants and mother pairs had to have not participated in any other nutritional supplemental study. The mothers provided informed consent as well as written consent for themselves and their infant. Of

the 335 mother-infant pairs who participated in the larger randomized, controlled trial, analyzable VPC videos were available for coding and analysis for only 248. The ethical oversight for the research was provided by the National Ethics committee for Health Research Board, Canada (2017-1410; and the University of Oregon Institutional Review Board, USA (07052018.008). See table 1 for further demographic information on this study's sample.

Table 1: Demographic information on mother participants within the sample

Characteristic	Dose = 0 mg n = 83	Dose = 1.2 mg n = 86	Dose = 2.4 mg n = 81	Dose = 10 mg n = 86	Total n = 335
Mean age of mother (years): Mean (SD)	28.3 (6.1)	27.9 (6.7)	28.1 (6.1)	28.1 (5.9)	28.1 (6.2)
Mother's whose highest level of education attended was primary school: N(%)	43 (51.8 %)	37 (43.0 %)	40 (49.4 %)	41 (48.2 %)	161 (48.1%)
Income for the entire household in the past 12 months in US dollars: Median (IQ range)	1800.0 (950.0-3000.0)	2050.0 (962.5-3500.0)	1600.0 (1000.0- 3000.0)	2000.0 (1200.0-3500.0)	2000.0 (1000.0-3000.0)
Mother's household does not have electricity: N(%)	44 (53.0%)	33 (38.4%)	40 (49.4%)	40 (47.1%)	157 (46.9%)
Mother's household does not have a refrigerator: N(%)	82 (98.8)	82 (95.3)	81 (100.0)	85 (100.0)	390 (98.5)
No member of the Mother's household has a bank account: N(%)	76 (91.6)	75 (87.2)	76 (93.8)	76 (89.4)	303 (90.4)

Randomization to Supplementation Groups

Lactating mothers were randomly assigned to one of four treatment groups (placebo: 0mg; estimated average requirement [EAR]: 1.2mg, double the EAR: 2.4mg; and a positive control group: 10mg). The mothers were asked to consume one capsule containing thiamine per day from 2-24 weeks postnatal. Compliance was relatively high (Gallant, et al, 2021). Participants, research assistants, and study investigators were blinded to the randomized groups.

Procedures

Women were asked to consume one capsule daily between 2- and 24-weeks postnatal. The capsules contained varying amounts of thiamine hydrochloride and a cellulose filter. Compliance was assessed fortnightly by research assistants visiting participants' homes to collect and count capsules and to deliver new capsules. A wide array of measures that assessed demographics, health, thiamine status of breast milk, and infant growth were included within the scope of the project (Whitfield, et al., 2019). Infant's neurological and cognitive development were measured at all four timepoints: [2-weeks (baseline), 12-weeks (midline), 24-weeks (endline), and 52-weeks (follow-up)]. Video recordings of some of these measures occurred at all timepoints, as well as for several mother-infant interaction tasks at various time points. However, this thesis reports on the findings in relation to the VPC task.

The Visual Paired Comparison Task

Infants, with their mother's consent, participated in the Visual Paired Comparison task at 24- and 52- weeks postnatal. The purpose of the Visual Paired Comparison task was to record when infants were looking at a particular image (face or pattern) to discover the extent to which infants could discriminate a novel face or pattern from a face or pattern they had been briefly habituated to. Stimuli were derived from the Chicago Face Project (faces), from Rose et al., (2009), and from the internet.

The faces and patterns utilized in the Visual Paired Comparison (VPC) task were chosen specifically by Baldwin; some were adapted from Rose et al., (2009). The Asian-heritage faces selected for the task were chosen to be moderately similar to the kinds of faces that the Cambodian infants might encounter.

What is referred to as the VPC task within the scope of the thesis was coined the immediate recognition task, shift rate task, and look duration task by Rose et al., (2009). Rose et al., (2009) developed the immediate recognition task to tap into different aspects of infants' perceptual and memory capabilities. The investigation of infant's perceptual and memory capacities is revealing of the trajectory of cognitive development due to the phenomenon of the cognitive cascade, which is how more primitive fundamental abilities underpin more complex ones that in turn affect overall cognition (Rose et al., 2008). Rose et al., (2008) further demonstrated that the cognitive cascade is relatively stable over time, which indicates that examinations of fundamental cognitive abilities in infancy may reveal insights about cognitive processes later in life, thereby providing evidence that intervention at lower levels of cognition in infancy may affect later outcomes. The VPC task originated from the immediate recognition task

(Rose et al., 2008) and was modified for implementation in the field in rural Cambodian homes.

It is assumed that if the infants were able to discriminate between a novel and familiar item, they would look longer at the novel item. The VPC task particularly requires infants to encode the features of the first familiarization phase, remember it, discriminate between the familiar item and a novel item by looking longer at the novel item, and then look longer at the novel item once the location of the items is switched. This task aims to provide information about the integrity of key information processing skills that are fundamental for infant's cognitive functioning and learning more generally.

Structure of the VPC task

Prior to beginning the VPC task, but after the camera was set to record, the researcher shook a rattle behind the camera and indicated the left look, too far, right, and too far. This gaze calibration provided a frame of reference by which video coders could subsequently identify the location of the stimuli (to either the left or right side) in relation to the infant. The pictures below depict Dr. Measelle teaching the Cambodian staff how to begin administering the VPC task.

Shortly after calibration was completed, the researcher initiated the VPC Task by stating "One!" to indicated the start of the first trial, with the beginning of each subsequent trial announced with its trial number. Two versions of the VPC task were administered, a face stimuli version, and a geometric pattern variation. Together, the two versions yielded 18 total trials that were stated aloud by the researcher behind the camera. In other words, the VPC task consisted of 6 blocks of trials in which each block

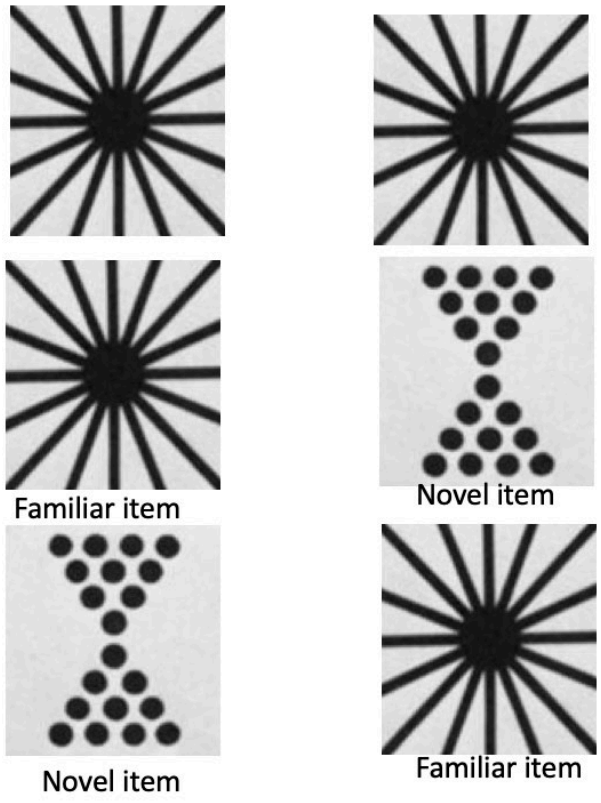
was comprised of three trials: 1) a familiarization trial which was 15 seconds in length, in which two identical copies of the same image were presented to infants on a white posterboard background at roughly 2 feet apart, 2) a first test trial (roughly 10 seconds) in which one of the images were replaced with a novel image, and 3) a second test trial (roughly 10 seconds) in which the novel image and the familiar image locations were switched relative to the previous trial. This process then occurred over the span of 18 trials, which was on average a little over five minutes. The task consisted of 12 test trials, and 6 familiarity trials.

Video coders were given videos of the infants at 24 and 52-weeks participating in the VPC task. Video coders were blind to the stimuli as well as whether it was a familiarization or test trial. Using Audacity, video coders documented the onset and offset of trial times as well as the durations of looks to either the indicated left or right sides where the stimuli were presumably located at. After audacity was used to find the onset and offset of trial lengths, trial times were manually inputted into a datavyu file. Look durations, whether they were to the left or right, were manually documented on datavyu. R code was utilized to process data from Datavyu files to derive the dependent measure, which was a novelty score, and to organize novelty scores in .csv files for data analysis. The dependent measure, the novelty score, was calculated for test trials by taking the looking time of the novel image and dividing it by the sum of looking times to both the familiar and the novel image. Novelty scores were calculated for 24-week infants for faces and patterns by averaging across all test trials that involved either faces or patterns. An overall novelty score was calculated for each infant by averaging across all 12 test trials.

The first three VPC blocks involved pairs of faces, and the last three involved pairs of geometric stimuli. Figure 1 below presents the actual face stimuli presented for one set of faces in the VPC task, as well as the trial order structure for that set of faces. Figure 2 presents one set of the geometric patterns as well as the trial order structure for that set of patterns.

Figure 1: Face stimuli presented during the VPC task

Figure 2: Geometric pattern stimuli presented in the VPC task



Faces depict individuals of Asian heritage which were selected for the task to increase the overall level of familiarity of the face stimuli for Cambodian infants. The specific pairs of faces were selected to be moderately challenging to discriminate. Geometric patterns were also selected with the goal of being moderately challenging to discriminate.

Approach to Answering Research Questions

Here I outline the plan for analyses. First, I planned to investigate infants' novelty preference for face and pattern stimuli by using a one-sample t-test against chance levels (.5). Infants' novelty scores for face and pattern stimuli were examined at both timepoints (24 and 52 weeks postnatal). Then, using correlational analyses, I

investigated possible relationships between novelty scores for faces and patterns within the same timepoint and across timepoints. Lastly, I used a mixed design repeated-measures analysis of variance with two repeated measures factors (timepoint: 24 and 52 weeks; and type of stimulus: faces and patterns) and one between-subjects factor: maternal thiamine supplementation group with 4 levels (0mg, 1.2mg, 2.4mg, 10mg to examine the influence of timepoint, stimulus type, and maternal thiamine supplementation on infants' novelty scores.

Results

The primary aim of this thesis was to investigate the extent to which maternal thiamine supplementation influenced breastfed infants' tendency to look longer at novel test items. Since the Visual Paired Comparison Task had never been conducted in a field setting, we also asked if Cambodian Infants within the context of the study displayed a novelty preference that was different from chance with respect to the two different types of stimuli presented (faces and patterns). We predicted that infants would display a preference for novel faces and patterns, with infants at 52 weeks displaying a stronger novelty preference than at 24 weeks.

For all data analyses, a novelty score (looking time at the novel test item divided by the sum of both familiar and novel test items) was computed for each infant, with scores ranging from 0 to 1. Scores of less than .5 indicated a preference for the familiar stimuli, and a score of greater than .5 indicated that the infant showed a preference for the novel stimuli. Means and standard deviations of novelty scores were computed for infants at 24 and 52 weeks. The overall mean outcome for novelty scores at 24 weeks was .52, SD=.08 (N=230). Similarly, the overall mean novelty score at 52 weeks was .51, SD=.08 (N=230).

Novelty Preference for Faces and Patterns

An important preliminary issue concerned the extent to which infants tended to display a novelty preference in the Visual Paired Comparison task. Generally as predicted, when both faces and patterns across both time points were examined, an overall marginal novelty preference was detected $t(\text{df}) = 1.94$, $p = .054$. However, this overall tendency to prefer novelty masked a difference in infants' pattern of looking in

relation to faces versus patterns. Means and standard deviations for each timepoint and stimulus type appear in Table 2.

Table 2: Mean novelty scores for face and pattern stimuli at 24 and 52 weeks postnatal (along with sample size and standard deviations)

	N	**Mean**	**SD**
24 weeks: All	260	.51	.08
24 weeks: Faces	260	.46	.10
24 weeks: Patterns	254	.57	.13
52 weeks: All	283	.51	.08
52weeks: Faces	283	.48	.10
52 weeks: Patterns	281	.54	.13
Both timepoints: All	313	.51	.07

To examine if novelty scores were different from chance levels (.5), one-sample t-tests were conducted. As is apparent in Table 3, infants at both timepoints (24 and 52weeks) displayed significantly less-than-chance novelty scores to faces (t's > 4.06, p's <.001), but greater-than-chance novelty scores to patterns (t's > 4.59, p's < .001), meaning that infants tended to display a familiarity preference to faces but a novelty preference to patterns. We had predicted that infants would display greater-than-chance novelty scores to both faces and patterns; thus, the finding of a familiarity preference to faces was unexpected. As we discuss further below, one possible explanation was that the pairs of faces we selected to present to infants may have been challenging for infants to discriminate.

Table 3. One Sample T-Test statistics for tests against chance regarding face and pattern stimuli at 24 and 52 weeks postnatal

	t	**df**	**Two-sided *p*-value**	**95% Confidence Interval**
24 weeks: All	2.70	259	.008	[.003, .022]
24 weeks: Faces	-7.0	259	< .001	[-.054, -.030]
24 weeks: Patterns	8.80	253	< .001	[.054, .085]
52 weeks: All	1.17	282	.242	[-.004, .015]
52 weeks: Faces	-4.06	282	< .001	[-.034, -.012]
52 weeks: Patterns	4.59	280	< .001	[.021, .052]
Both timepoints: All	1.94	312	.054	[-.0001, .015]

Correlational Analyses

Also of interest was the extent to which novelty scores for face and pattern stimuli at 24 and 52 weeks were related (Table 4). Pearson correlations revealed a statistically significant positive relationship between infant's novelty preference at 24 weeks for faces and their novelty preference for patterns at 52 weeks. No other significant correlations emerged in the analyses. The general sparsity of systematic relationships was somewhat unexpected and will be explored in the discussion below.

Table 4. Correlations for face and pattern novelty scores at 24 and 52 weeks

	24 weeks: Faces	**52 weeks: Faces**	**24 weeks: Patterns**	**52 weeks: Patterns**
24 weeks: Faces	**1**	**---**	**---**	**---**
52 weeks: Faces	**.036**	**1**	**---**	**---**
24 weeks: Patterns	**-.011**	**.024**	**1**	**---**
52 weeks: Patterns	**.138***	**.005**	**.098**	**1**

Note: *p < .05

Influence of Timepoint, Type of Stimulus, and Maternal Thiamine Supplementation on VPC Task Novelty Scores

To investigate the question of the extent to which maternal thiamine supplementation influenced breastfed infants' tendency to look longer at novel test items at 24 and 52 weeks, we used a mixed-design repeated measures analysis of variance with two repeated measures variables (timepoint: 24 and 52 weeks: and type of stimulus: faces and patterns) and maternal thiamine supplementation group with 4 levels as the between-subjects factor (0mg, 1.2mg, 2.4mg, 10mg). We predicted that infants would show higher novelty scores at 52 weeks, indicating a higher novelty score with age. We expected that infants would show a higher novelty score with patterns due to the patterns appearing to be easier to discriminate than the faces. Finally, we expected that infants whose mothers received thiamine in their capsules would have higher novelty scores.

The ANOVA revealed a non-significant main effect of time point on infant's novelty scores, which went against prediction $F(1,220) = 1.80$, $p = .2181$, partial eta squared=.008. This indicates that with age, novelty preference did not increase. In contrast, the analysis of variance revealed a significant main effect of the type of stimulus presented (faces or patterns) $F(1,220) = 139.690$, $p < .001$, partial eta squared=.388 (see Figure 3). Infants tended to display a higher novelty preference towards patterns (M=.49, std error=.004, [.459, .476] than faces (M=.55, std error=.006, [.542, .567]. Means and standard deviations for novelty scores for each stimulus type at each timepoint appear in Table 5.

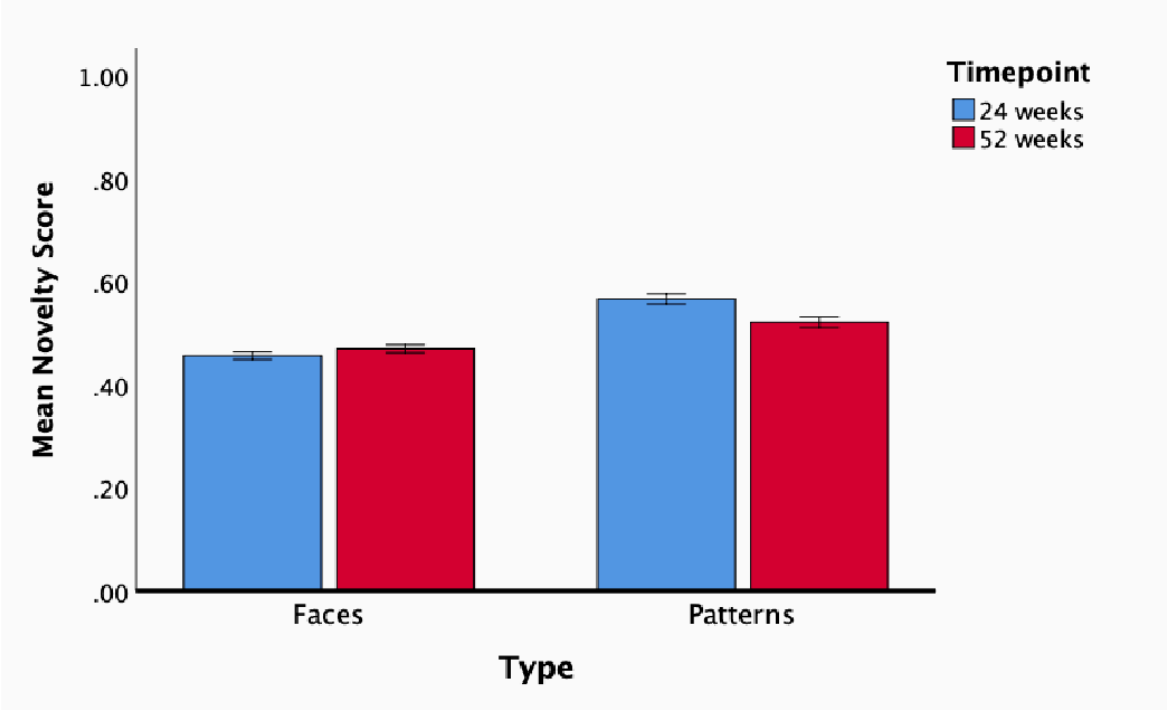
Table 5: Means, standard deviations, and sample size for the four treatment groups (0mg, 1.2mg, 2.4mg, 10.0mg) at 24 and 52-weeks postnatal

24 Weeks				**52 Weeks**			
Treatment Group	**Mean**	**SD**	**N**	**Treatment Group**	**Mean**	**SD**	**N**
0 mg	.50	.08	50	0 mg	.48	.08	50
1.2 mg	.53	.08	63	1.2 mg	.52	.07	63
2.4 mg	.51	.071	53	2.4 mg	.51	.08	53
10.0 mg	.53	.08	64	10.0 mg	.52	.08	64
Overall	.52	.08	230	Overall	.51	.08	230

When the interaction between type of stimulus presented and timepoint was examined, a statistically significant interaction emerged, $F(1,220) = 15.17$, $p < .001$ (See Figure 3). In follow up simple effects analyses, I found that with faces, infants displayed a statistically significant increase in novelty preference between 24 and 52 weeks postnatal, $p = .023$. In contrast, when patterns were examined across the two timepoints, a significant decrease in novelty score was observed, $p < .001$. The observed

decrease in novelty preference between timepoints for pattern stimuli disconfirmed our prediction.

Figure 3. Infants' mean novelty scores for faces and patterns at both the 24- and 52-week timepoints (error bars: +/- 1 standard error of the mean)

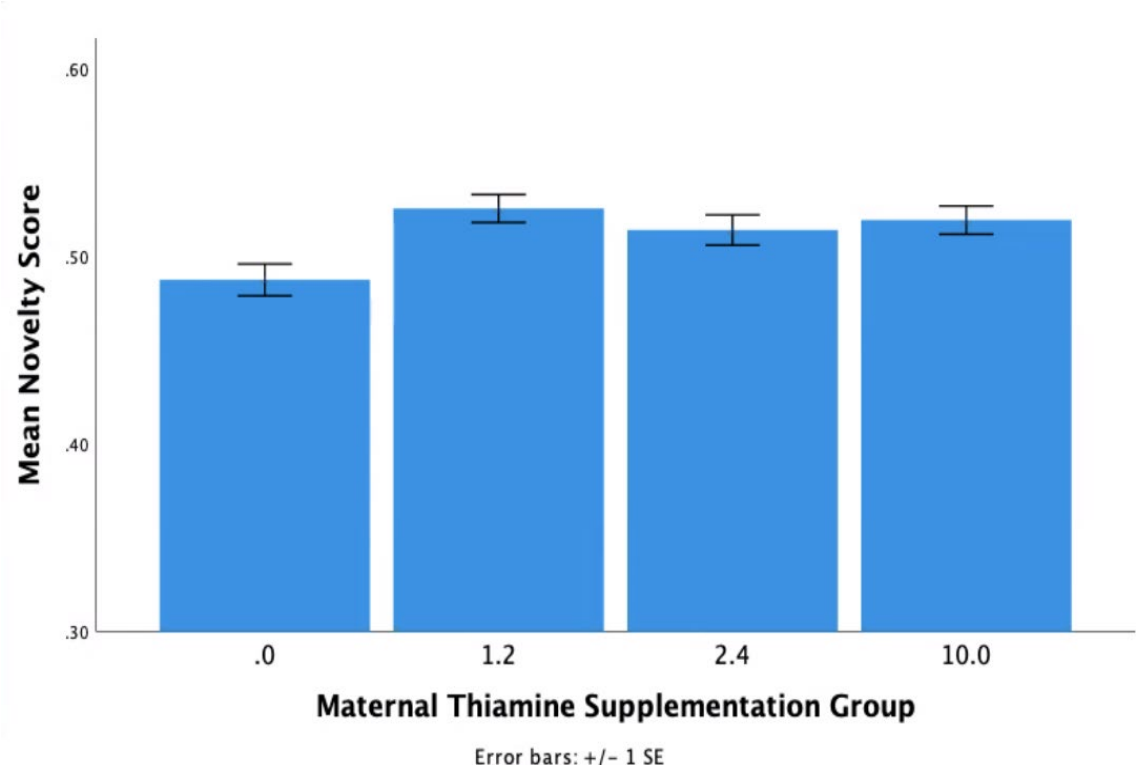


Effect of Maternal Thiamine Supplementation on Infant's Mean Novelty Preference

Notably, there was a main effect of the maternal supplementation group on novelty preference $F(3, 220) = 4.188$, $p = .007$, partial eta squared=.054 (placebo group: M=.487, N=48 std. error=.008; 1.2mg: M=.53, N=62, std error=.007; 2.4mg: M=514, N=53, std. error=.008; 10.0mg: M=5.2, N=61, std. error= .008). Specifically, planned comparisons between the placebo group (0mg) and each of the other three groups revealed significant differences in all cases (0 mg versus: 1.2mg, p <.001; 2.4mg, p =.024; 10.0mg, p =.005). Together these findings suggest that 1.2 mg of thiamine may have been an adequate quantity to protect infants' ability to discriminate between a novel and familiar stimulus (See Figure 4). However, no difference for novelty

preference was detected when comparing different levels of thiamine supplementation (p's > .05). Along with this, all two-way and three-way interactions involving maternal thiamine supplementation were statistically non-significant (All F's < .99 and all p's > .397).

Figure 4. Infants' mean novelty scores in relation to their treatment group (0mg, 1.2mg, 2.4mg, 10.0mg)



Discussion

The primary purpose of this thesis was to investigate the extent to which maternal thiamine supplementation benefitted exclusively breastfed, rural Cambodian infants' neurological development, as measured by the Visual Paired Comparison Task. I predicted infants would exhibit a dose-response relationship between maternal thiamine supplementation group and novelty score, with 10mg of supplementation resulting in the highest novelty scores.

Our first prediction, that higher levels of maternal thiamine supplementation would be associated with increased novelty scores, was partially supported. That is, infants whose mothers received supplemental thiamine displayed significantly higher novelty scores than those whose mothers received a placebo. However, higher levels of thiamine supplementation did not produce higher novelty scores. In other words, contrary to prediction, while beneficial, maternal thiamine supplementation did not display a dose-response relationship to infants' novelty scores.

Our findings add to previously reported findings to provide further support for the benefit of maternal thiamine supplementation for protecting infants' neuro-cognitive development. Within the same larger clinical trial, Measelle and colleagues documented that infants whose mothers received supplemental thiamine displayed significantly enhanced language and fine-motor skills on the Mullen Scales of Early Learning (MSEL) at 24 weeks. Similarly, Baldwin and colleagues (under revision) found that infants whose mothers received higher levels of supplemental thiamine displayed enhanced attention to infant-directed speech at 24 weeks, suggesting benefits to language processing. The present findings from the Visual Paired Comparison task add

to this growing body of evidence that infants' thiamine status plays an important role in their neurocognitive development.

Challenges, Limitations, and Future Directions

As with any study, this thesis confronted challenges and limitations throughout the research process. To our knowledge, this is the first time that the VPC task has been mounted in a field setting, as well as the first time that the task has been utilized to investigate the effects of a micro-nutrient intervention. Conducting the task in the field indeed presented some challenges. In an ever-changing field setting, it was difficult at times to sufficiently signal left and right looks during the calibration period, as well as ensure that the infant did not get distracted by what was going on in the background. This made it challenging for coders to reliably determine when infants were, and were not, fixating the actual images during the VPC task. These factors may have increased variability in infants' novelty scores, rendering the task potentially less sensitive to effects of the thiamine intervention. An assessment of coding reliability will be essential for determining the extent to which these factors indeed affected VPC novelty scores. Despite these potential issues, however, the task yielded findings that conformed to predictions, in the sense that maternal thiamine supplementation significantly enhanced infants' novelty scores on the task relative to placebo.

Certain findings were unexpected and even puzzling. For example, at both 24- and 52-weeks infants showed a stronger-than-chance familiarity preference for the face stimuli during test trials, rather than a novelty preference as predicted. This suggests that the face stimuli pairs may have tended to appear quite similar to infants, making it challenging for them to encode the unique facial features of each face, and discriminate

between familiar and novel faces. A pattern of preferring familiarity when stimuli are complex and difficult to discriminate has been extensively documented in previous research and is known to shift toward novelty preference as infants' perceptual skills develop (Hunter & Ames, 1988). Along these lines, it is interesting that infants' familiarity preference for faces decreased between 24 and 52 weeks; that is, at the later timepoint infants displayed an expected shift in the direction of novelty. Likewise, the influence of maternal thiamine supplementation was to reduce infants' familiarity preference, meaning that supplemental thiamine appeared to enhance infants' attention to the novel faces. In these senses, the findings conformed to what we predicted.

Lastly, it is important to acknowledge the possibility that maternal thiamine supplementation may have resulted in a significant novelty preference for face stimuli if the randomized controlled trial had a) included higher doses of thiamine than the low-dose supplementation undertaken in this research (with the highest daily dose being just 10mg thiamine), b) begun either during or before pregnancy, and/or c) continued beyond 24-weeks post-natal. To investigate these possibilities, future research is needed.

In contrast to faces, with patterns infants displayed a greater-than-chance preference for the novel items during test trials, as predicted. Also as expected, infants whose mothers received supplemental thiamine tended to display stronger novelty preferences than those whose mothers received a placebo. However, given previous findings that novelty preference tends to increase with age, it was unexpected that infants' preference for novel patterns during test trials instead decreased between 24 and 52 weeks. The fact that thiamine supplementation ceased in the clinical trial at 24 weeks

might be a factor in this decline in novelty preference between 24 and 52 weeks. Along these lines, it is worth noting that similarly unexpected declines in cognitive functioning were observed in infants' MSEL language sub-scale scores in this same period between 24 and 52 weeks. The extent to which the conclusion of thiamine supplementation at 24 weeks was related to these declines is an important question for further investigation.

When we examined relationships between infants' novelty scores for faces and patterns, we found a surprising set of outcomes. Infants' face and pattern novelty scores were not significantly correlated with one another at either 24 or 52 weeks. Likewise, novelty scores to faces at 24 weeks were not significantly related to 52-week novelty scores to faces; nor were novelty scores to patterns at 24 weeks related to 52-week novelty scores to patterns. Similarly, 24-week novelty scores to patterns were not significantly related to 52-week novelty scores to faces. Surprisingly, however, 24-week novelty scores to faces were significantly related to 52-week novelty scores for patterns. On the one hand, this relationship between novelty scores across age and stimulus type points to some potentially interesting developmental stability in the findings. On the other hand, that such developmental stability emerged only across stimulus types (faces versus patterns) but not within stimulus types is puzzling and not easily explained.

Another somewhat surprising result within the VPC findings was the absence of a dose-response relationship between levels of maternal thiamine supplementation and infants' novelty scores. This was surprising given that maternal thiamine supplementation displayed a significant dose-response relation to both infants' MSEL language sub-scale scores (Measelle, et al., 2021) and their enhanced attention to infant-directed speech (Baldwin, et al., under revision). In the VPC task, in contrast, maternal

thiamine supplementation at any level was associated with a comparable degree of benefit to infants' novelty scores. These different outcomes might have arisen because the MSEL language subscales and IDS task index functioning of distinct neural subsystems relative to the VPC task, with these two subsystems varying in regard to how they are affected by thiamine. However, a variety of other possible explanations should be considered and addressed in future research.

Conclusions

In the present study, breast-fed Cambodian infants whose mothers received supplemental thiamine of at least 1.2 mg showed enhanced novelty scores on the Visual Paired Comparison task, when compared to infants whose mothers received a placebo. Previous findings indicate that the perceptual and cognitive skills underlying novelty scores on the VPC task participate in a neuro-cognitive cascade (Rose et al., 2008). With this in mind, the present findings suggest that infants' early access to adequate thiamine is important for protecting perceptual and cognitive skills that are foundational to learning and thus have a cascading influence on infants' subsequent neuro-cognitive development.

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