MONITORING INFANT NEURODEVELOPMENT VIA THE HAMMERSMITH NEUROLOGICAL EXAMINATIONS IN CAMBODIAN INFANTS AT RISK FOR THIAMINE DEFICIENCY

by

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Thiamine deficiency affects millions of infants growing up in South and Southeast Asia due to heavy cultural reliance on thiamine-poor, polished white rice as a dietary staple. Recent evidence indicates that a thiamine-deficient diet not only endangers infants' health, but also hinders infants' neuro-cognitive development. As part of a larger, randomized controlled trial, my thesis investigated possible benefits of maternal thiamine supplementation for protecting breastfed Cambodian infants' neurological development. Lactating mothers were randomly assigned to four treatment groups (0, 1.2, 2.4, and 10mg daily thiamine supplement) when infants were between 2- and 24-weeks postnatal. Infants' neurological function was measured at 2-, 12-, 24-, and 52-weeks via the Hammersmith Neurological Examination, a field-standard clinical assessment tool. As expected, infants' Hammersmith scores improved significantly with age. However, maternal thiamine supplementation dose did not affect infants' Hammersmith scores. Above all, this research indicates that the basic neurological functions assessed by the Hammersmith in early infancy were relatively unaffected by maternal thiamine supplementation.

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Introduction

Thiamine, also known as vitamin B1, is an essential micronutrient for human health and well-being. Additionally, thiamine is crucial for human development – both survival and neurocognitive development. In some regions, such as Southeast Asia, thiamine deficiency is common, due to heavy reliance on polished white rice as a dietary staple. This puts infants at risk for delays and deficits in growth, health, and neurocognitive development.

The present thesis was part of a larger randomized controlled trial investigating the possibility that supplementing lactating mothers with daily thiamine might benefit their infants' growth and neurocognitive development. This study was undertaken in Cambodia, and the thesis specifically examined possible relationships between maternal thiamine supplementation dose and measures of infants' growth and neurocognitive functioning. In the following literature review, I will outline the role that thiamine plays in human physiological processes, how thiamine is typically obtained on the diet, and how much thiamine humans should consume. Then I will focus on the specific role of thiamine in neurological function and neurocognitive development, in particular motor skill, myelination, synapse formation, emotional stability, and cerebral metabolism.

Literature Review

Thiamine

Thiamine is present in the body as free thiamine, as well as in several phosphorylated forms: thiamine monophosphate (ThMP), thiamine diphosphate (ThDP), and thiamine triphosphate (ThTP). In addition, thiamine contributes to metabolic processes, such as carbohydrate and amino acid metabolism, and circulates to

cells with high metabolic demands (I.e., liver, brain, pancreas, heart, and skeletal and smooth muscles) (Whitfield et al., 2018). Moreover, thiamine acts as a cofactor of several enzymes, such as pyruvate dehydrogenase complex and ketoglutarate dehydrogenase. These enzymes are essential in Krebs's Cycle cell energy production (cell respiration). Not only does thiamine maintain and play a role in the energetic metabolism of the cell, but it also acts indirectly in lipid nucleotide formation (i.e., building DNA in fats). In the central nervous system (CNS), which consists of the brain and spinal cord, thiamine plays a fundamental role in neurophysiology. For instance, it participates in producing neurotransmitters underlying neural signaling, modifying ion channels to allow ions to enter cells for chemical or neural processes to occur, and playing a role in membrane structure and function stabilization (Dias, Silva, Doyle, & Ribeiro, 2013).

Since thiamine can only be stored in insignificant amounts and has a short half-life, it must be consumed frequently (Whitfield et al., 2016). Humans can obtain thiamine through their diet from whole grains, yeasts, meats, legumes, and nuts. However, in Southeast Asian countries, such as Cambodia, their dietary staple is thiamine-poor, polished, white rice. Additionally, there is a lack of dietary variation, which makes it challenging to consume enough thiamine for the body's demands. The recommended nutrient intake (RNI) of thiamine is 1.2 mg/day for men and 1.1 mg/day for women, which increases to 1.4 mg/day for pregnant and 1.5 mg/day for lactating women. In infancy, the adequate intake is thought to be 0.2 mg/day (0−6 months) and 0.3 mg/day (7−12 months) (Whitfield et al., 2018). When mothers are thiamine deficient, they produce thiamine-poor milk. As a result of low maternal milk thiamine

levels, the mother's offspring would be at considerable risk for thiamine deficiency and other adverse outcomes. Hence, 70–100% of infants and 27–100% of reproductive-age women in Cambodia are estimated to be thiamine deficient (Johnson, Fischer, Thacher, Topazian, Bourassa, & Combs, 2019).

Based on the diet in Cambodia, fortification -- a sustainable, cost-effective, and passive intervention -- is a possible solution for improving maternal thiamine intake (Whitfield et al., 2019). For instance, fish sauce – a widely and frequently utilized condiment -- could be used for thiamine fortification. In Prey Veng province, Cambodia, researchers performed two small, concurrent randomized controlled trials of thiamine-fortified fish sauces for over 6 months to determine whether thiamine-fortified fish sauce raised thiamine levels (Whitfield et al., 2018). Mothers consumed one sauce fortified at 2 g/L, one at 8 g/L, and one control sauce. As a result, maternal consumption of thiamine-fortified fish sauces significantly increased erythrocyte thiamine diphosphate concentrations (eTDP) in mothers and infants, and mothers had higher milk thiamine concentrations. The Whitfield et al. (2018) study indicated that thiamine fortified fish sauce could improve thiamine levels in Cambodians, particularly infants, to prevent adverse outcomes or thiamine deficiency. Namely, when infants had adequate thiamine levels, their bodies can grow and function properly. While fish sauce did enhance thiamine levels, commercially produced fish sauce may not be helpful for reducing thiamine deficiency in the poorest communities who make their own fish sauce, and consumption of this condiment is not universal in all regions where thiamine deficiency is common (Whitfield et al., 2019). For this reason, fish sauce is not an ideal vehicle for thiamine fortification.

Conversely, thiamine fortification of salt has the potential for combating thiamine deficiency because salt is low-cost (Whitfield et al., 2019), centrally manufactured, and widely utilized. However, both the salt intake of lactating women and the amount of thiamine added to salt that is required to optimize milk thiamine concentrations are unknown. Therefore, there is currently no fortification program for thiamine in Cambodia and thiamine deficiency remains a public health issue in Cambodia and Southeast Asia. One of the aims of the randomized controlled trial (of which the present study is one small part) was to investigate key background questions that set the stage for undertaking thiamine fortification in Cambodia.

Thiamine deficiency and neurological impairments

Beriberi

Beriberi, a serious and even fatal medical condition caused by thiamine deficiency, is prevalent in Southeast Asia. As a result, infantile beriberi-related mortality is still common. Beriberi is particularly serious in infants due to rapid physical and neurological growth and development during this time and high thiamine needs compared to body size. There are two categories of beriberi: Wet beriberi affects the cardiovascular system and is accompanied by edema, whereas dry beriberi affects the peripheral nervous system (Whitfield et al., 2018).

Wernicke's encephalopathy is another neurological disorder related to thiamine deficiency. Infants with this disorder would exhibit abnormal eye movement, gait ataxia, and cognitive impairment. The most severe form of Wernicke's encephalopathy is Korsakoff's psychosis, in which patients are profoundly confused, and display confabulation with little or no working memory (Whitfield et al., 2018).

The most famous case of infantile neurological deficits due to thiamine deficiency was the Israeli Outbreak in 2003. Several infants with encephalopathy were in pediatric intensive care units in Israel, and two died of cardiomyopathy. In addition, a 5.5-month-old infant arrived at the Sourasky Medical Center with rapid uncontrollable eye movement, weakness, paralysis of the eyes, vomiting, and Wernicke's encephalopathy. All these hospitalized infants had consumed a soy-based formula that had low thiamine levels due to thiamine having been inadvertently left out during manufacturing. Two years after the outbreak, Fattal-Valevski et al. (2005) compared neurocognitive progress between 20 infants who had been at risk of thiamine deficiency as a result of the defective formula and a control group of 20 infants who'd consumed other milk-related sources. The researchers found that, relative to control infants, infants who had consumed the thiamine-deficient formula displayed significant delays in expressive and receptive language development, mental development on the Bayley Scales of Infant Development mental motor development, and age at independent walking. These observational findings provided the first indication of how serious low thiamine intake may be for infants' neuro-cognitive development, which encompasses several domains: brain structure and neurology, cognition, motor and sensory skills, etc.

Neurological processes during infancy

One of the neurological processes that is affected by thiamine deficiency is coating axons with myelin (myelination). Myelin is a white, fatty matter that coats axons and increases nerve impulse speed. Prior to birth, myelination occurs in brain areas involved in orientation and balance. After birth, myelination rates in areas involving vision and hearing attain a peak before myelination of areas related to

language (Gilmore et al., 2018). Thus, infants would begin to show visual and auditory skills followed by language skills. In addition, oligodendrocytes are glial cells in the CNS that produce myelin. From birth to 3 years of age, the number of oligodendrocytes in cerebral white matter increases rapidly from approximately 7 billion to 28 billion at age 3 years (Gilmore et al., 2018).

During the first year of life, a host of neurodevelopmental processes occur, including neuron proliferation, axon and dendrite growth, synapse formation, pruning, and myelination. Neuron proliferation is the creation of new cells through cell division and this event starts in the 7th week of gestation to at least 4.5 months postpartum (Prado et al., 2014). Most neuron proliferation finishes at birth, but some neurons can be formed in adulthood. Dendrites are projections from cell bodies to make connections with other cells while axons are the portions of the neuron that transmit nerve signals and it can be myelinated. Axons and dendrites start to develop during gestation and continue to proliferate until at least 2 years after birth. Synapses are the ends of the neuron that release neurotransmitters to other neurons. Additionally, they connect between axons, dendrites, and cell bodies, and they begin to form around the 23rd week of gestation and persist throughout life (Prado et al., 2014). Synaptic density peaks at various times in different brain regions. For instance, the visual cortex attains its synaptic density peak between 4 and 12 months postpartum, while the prefrontal cortex reaches its peak after 15 months postpartum. Moreover, reduced synaptic density reflects synaptic pruning, which eliminates extra synapses and starts a year after birth and continues through adolescence.

When infants are developing under adverse circumstances, disruptions can occur in the neurological events described above. In association, infants may show delays in growth metrics (weight, head circumference, and length) and certain developmental milestones, such as grasping objects and walking (occurring normatively at about 52 weeks). For instance, head circumference changes with infants' rapid gray matter and white matter formation (Gilmore et al., 2018). Head circumference may provide a proxy for gray and white matter formation. Similarly, delays in growth, as measured by infants' weight and length, may serve as a proxy for malnutrition that infants experienced both prenatally and postnatally, which in turn may be associated with neurological delay.

In my thesis, I investigated how an infant's weight, head circumference, and length (anthropometrics), measured at 2 weeks (baseline) were associated with their Hammersmith scores and thiamine deficiency. These measures serve as an indicator for infant growth and development meaning they could provide essential information regarding the infant's health and neurological status. They are important because they reflect an infant's health and may relate to neurocognitive development. Additionally, Cambodian infants' growth metrics could demonstrate how they may exhibit delayed growth compared to WHO standards.

Impact of Thiamine Deficiency on Physiological Processes

There are some studies, primarily animal ones, that examined how nutrient deficiency (i.e., thiamine) could affect neurodevelopment. This section will explore both the animal and human literature to showcase what is known about the impact of

thiamine deficiency on neurodevelopment and physiological development more generally.

Animal Studies

Based on some animal studies, there is growing evidence that thiamine deficiency can also result in long-term cognitive impairments in milder forms. According to Dias et al. (2013), CNS development is influenced by psychosocial and biological factors, as well as genetic inheritance. These factors together increase or decrease cerebral development through neurogenesis, axonal and dendritic growth, synaptogenesis, cell death, synaptic pruning, myelination, and gliogenesis (Dias et al., 2013).

The period immediately after birth has the greatest cerebral vulnerability to maternal deficiency of thiamine. Namely, any deficiency that occurs during the postnatal period would negatively impact neurological function (i.e., reflexes, abilities, and gait). Dias and colleagues found that rats who were breastfed by thiamine-deficient mothers had defective cognitive function because thiamine deficiency hinders neurological processes, such as synaptogenesis and axogenesis. Therefore, low levels of thiamine resulted in disturbances in critical neurological processes in rats, leading to cognitive deficits such as memory loss, uncoordinated movement, and language impairment.

Furthermore, thiamine deficiency can cause disruptions to regions of the brain responsible for motor control. In a couple of studies that utilized rats (Dias et al., 2013; Zhao et al., 2009), researchers have found that thiamine deficiency during pregnancy and lactation causes atrophy and decreased density of pyramidal and granular cells of

the hippocampus of rat puppies. All these dysfunctions persist into later life and could hinder the rats' motor function.

Moreover, several animal studies showed that thiamine deficiency can cause defects in certain areas of the brain that are crucial for proper metabolic function. For instance, low levels of thiamine might cause brain tissue injury by inhibiting metabolism in cerebral regions with higher metabolic demands and high thiamine consumption. The high rate of thiamine uptake by the blood–brain barrier reflects high cerebral demand for thiamine and the demand for its continuous supply for brain activity (Hiffler et al., 2016). In a study by Dias et al. (2013), cerebral enzyme activities that participate in energy metabolism (i.e., pyruvate and ketoglutarate dehydrogenase complex) significantly decrease in puppies from deficient mothers compared to puppies of control mothers (Dias et al., 2013). Similarly, a study by Zhao et al. (2009) showed that thiamine deprivation for 14 days in mice led to various deficiencies in transketolase (TKT), pyruvate dehydrogenase (PDH), and alpha- ketoglutarate dehydrogenase (αKGDH) activities in the cortex and hippocampus (Zhao et al., 2009) and TKT activity had the greatest reduction. TKT is essential because it serves as a cofactor of transketolase enzymes, PDH, and αKGDH, which are key enzymes involved in cell respiration. If any of these enzymes show reduction in activity, CNS cells cannot produce enough energy to perform neurological mechanisms that allow for neuron communication or CNS growth (i.e., axon growth). Hence, thiamine deficiency can harm neurological function due to defects on energy metabolism.

Human Studies

There are also a few human studies that showed thiamine's impairments on infant neurodevelopment. For instance, Dias et al. (2013) claim that thiamine shortcoming during pregnancy and breastfeeding is directly related to cognitive impairment of the child. Based on demographic data from populations with underprivileged individuals, the researchers believe that poverty due to malnutrition is a crucial risk factor in the occurrence of thiamine shortcomings. In another study, Prado and Dewey (2014) focused on the effects of early nutrient deficiency on long-term brain function, cognition, and well-being in infants from low- and middle-income countries. The researchers found that certain types of nutritional deficiency, such as thiamine deficiency, can impair cognitive, motor, and socio-emotional abilities. Harel et al. (2017) also discovered that thiamine deficiency in infancy has long-term implications for gross and fine motor function and balance skills in childhood. Both Harel and Prado and their colleagues conclude that thiamine plays a crucial role in normal motor development.

Additionally, thiamine deficiency can alter certain brain regions that are responsible for motor control. In a study by Dhir et al. (2019), the researchers performed MRI studies and found that mammillary bodies, basal ganglia, and frontal lobes were affected in children with thiamine deficiency. The mamillary bodies are components of the brain that are associated with recollective memory and transmit information about memory via the mammillothalamic tract (Peterson et al., 2021). The basal ganglia functions in inhibition of muscle tone, coordination of slow and sustained movements, organization of motor behavior or movement, and suppression of useless

patterns of movement. Moreover, the frontal lobe is responsible for voluntary motor control.

Through animal studies, we could understand how thiamine deficiency plays a role in energy metabolism and neurological processes, such as axonogenesis, and relate that information to infants. With human studies, there are no studies that investigated thiamine's physiological role in infants, such as metabolism, but there is some evidence that showed thiamine deficiency affects brain structures responsible for motor development. While both animal and human studies demonstrated that low thiamine levels could hinder early neurodevelopment and body function, only a handful of studies thus far have directly assessed relationships in human infants between thiamine status and neurological development. Therefore, my overarching goal in this thesis is to contribute to the small existing literature regarding relationships between infants' thiamine status and both their physiological status shortly after birth, and their neurological development over the first year of life.

Hammersmith Neurological Examinations

Since nutrient deficiency appears capable of contributing to adverse effects on an infants' nervous system, neurological examinations can determine if there are delays in normal development and functions. Delays may be due to trauma during labor and delivery (I.e., decreased oxygen levels and high blood pressure), which result in neurological insults, or potentially to other causal factors such as thiamine deficiency.

Neurological examinations have been developed to understand the neurologic status of post-natal infants that were born at various times (full-term, late-term, etc.) and whether they had impairments in their nervous systems. There are several examinations

that are utilized in clinical and research settings, such as the Touwen Infant Neurological Examination (TINE), the Amiel-Tison Neurological Assessment at Term (ANAT), and Hammersmith (Maitre et al., 2016). TINE assesses infants' posture and motility during sitting, standing, and walking (Hadders-Algra et al., 2009). It also evaluates infants' brainstem reactions, visuomotor function, muscle tone, and different reflexes. Additionally, ANAT is a set of three different instruments that measures axial motor activity (active tone), passive tone in limbs and arms, head growth and cranial sutures (Gosselin et al., 2005). It is used in clinical and research settings to identify children from 32 weeks to 6 years of age with mild to severe neuro-cranial signs that may benefit from early intervention (Gosselin et al., 2005).

For my thesis, I will investigate the Hammersmith Neonatal Neurological Examination (HNNE) and the Hammersmith Infant Neurological Examination (I will collectively refer them as Hammersmith examinations). The Hammersmith examinations were originally created by Dr. Lilly Dubowitz and Professor Victor Dubowitz in 1981, and updated by Dr. Eugenio Mercuri, in 1998 (Mercuri et al., 2015). These assessments were meant to be fast, practical, easy to perform, and include standardized scoring columns that contained definitions along with diagrams (see Appendix A and B). The examination's approach to evaluate an infant's neurological status is to categorize tasks (i.e., head posture) into certain categories (i.e. posture) to comprehend specific aspects of neurodevelopment. The scores from the categories are combined to establish the infant's overall neurological status across different neurodevelopmental domains (Mercury et al., 2015). These examinations tell us about which areas in the brain may contribute to an infant's performance on a neurological

assessment. Namely, an infant's performance on these examinations can provide insight on how they are developing and where in the nervous system they exhibit defects in neurological development.

HNNE consists of 6 categories administered in the following order: tone, tone patterns, reflexes, spontaneous movements, abnormal signs, and behavior. It is performed on newborns from birth to 2 months old. Both assessments sum individual scores for each category to obtain both a subscore and an overall score representing an infant's neurological status. By comparison, HINE is a brief, standardized, and scorable clinical neurological examination administered to infants who are 2–24-months old. This examination consists of 34 items and six categories (tone, tone patterns, reflexes, spontaneous movements, abnormal signs, and behavior).

Many studies have utilized HINE and HNNE in different clinical groups of full-term and preterm infants at different ages within the neonatal period. These studies primarily assess neurodevelopmental disabilities in term and preterm infants with no underlying health conditions. Researchers have also utilized the HINE to distinguish babies with nervous system disorders, such as cerebral palsy, from healthy individuals (Romeo et al., 2020). Additionally, a study by Venkata et al. (2020) used HNNE to compare neurodevelopmental disabilities in term versus preterm infants. They also tried to determine if they could use HNNE to predict cognitive dysfunction subsequently, at one year of age. Furthermore, Chin et al. (2018) evaluated the neurological status of late-preterm infants using the HNNE. They found that these infants were neurologically more immature than their term counterparts.

Infant neurological examinations in relation to thiamine deficiency

Based on existing literature, Hammersmith examinations have been used to assess infants born at different terms and to detect high risk of neurological disorders, such as cerebral palsy, at an early age. It is also used to detect significant delay with respect to developmental milestones (i.e., independent walking) in children with this disorder (Maitre et al., 2016; Romeo et al., 2020; Venkata et al., 2020; Chin et al., 2018).

To date, there have been very few studies that utilized Hammersmith or other neurological assessments, such as the Touwen Infant Neurological Examination (Hadders-Algra et al., 2009) and the Amiel-Tison Neurological Assessment at Term (Gosselin et al., 2005), in the context of micronutrient deficiency. A study by Kumar et al. (2018) investigated the associations of iron and folic acid with developmental milestones and found that deficiencies in those nutrients resulted in delays in neurodevelopment. In particular, the researchers evaluated infants' neurological statuses using the Trivandrum Development Screening Chart, a test that assesses cognitive and motor milestones in children, and a full systemic neurological examination that assessed various aspects of neurodevelopment (i.e., motor skills, sensory skills, reflexes, and cranial nerves) (Kumar et al., 2018). However, very little is known about thiamine's role in human infant neurological development. Particularly, there are no studies that utilized Hammersmith in the context of thiamine deficiency.

Research Goal

As mentioned above, there are very few studies or clinical applications examining relationships between infants' thiamine status and their HNNE/HINE scores.

Hence, my thesis aimed to provide an initial attempt to fill this gap. In particular, the thesis research investigated the extent to which low-dose thiamine supplementation for lactating Cambodian mothers might affect their breastfed infants' neurological status, as measured by their Hammersmith scores on the HNNE and HINE.

This study took place in the context of a larger double-blind, four-parallel arm, randomized controlled trial. 335 healthy mother (18-45 years) and baby pairs from the Kampong Thom province of Cambodia were recruited as participants. Each mother-infant pair was assigned to one of four thiamine supplementation treatment groups: 0 mg (placebo, 1.2 mg (EAR), 2.4 mg (2 x EAR), and 10.0 mg (positive control). Mothers were asked to consume one capsule daily between 2 and 24 weeks postnatal. Additionally, maternal milk thiamine levels were assessed at various timepoints, the HNNE was performed when infants were 2 weeks post-natal, and the HINE was performed when infants were 12-, 24-, and 52-weeks post-natal.

For my thesis, I investigated several specific questions regarding possible relations between maternal thiamine supplementation and infants' developmental outcomes, using the HINE as the primary measure of infants' neurocognitive development at 24 weeks (Endline). My three main research questions were:

1) to what extent did infants display progress in their HINE scores from 12- to 52-weeks?
2) to what extent were infants' growth metrics at 2 weeks (weight, head circumference, length) associated with their Hammersmith scores?
3) to what extent did low-dose maternal thiamine supplementation affect infants' HINE/HNNE scores?

I predicted that infants' HINE scores would display evidence for developmental progress from 12- through 52-weeks, even while controlling for infants' baseline neurological status via their HNNE scores. Additionally, I hypothesized that infants' growth metrics at 2 weeks (weight, head circumference, length) would be associated with their Hammersmith scores, both concurrently and at subsequent timepoints. Specifically, I predicted that higher growth metrics at 2 weeks would be significantly correlated with higher Hammersmith scores at 2-, 12-, 24-, and 52-weeks post-natal. Finally, I hypothesized that low-dose maternal thiamine supplementation would benefit infants' neurological status based on previous evidence of thiamine's importance in neurological development. Thus, I predicted a dose-response relationship between maternal thiamine supplementation dose and infants' HINE scores at 24-weeks (Endline) and 52-weeks (One-Year Follow-up).

Methods

The research on which this thesis was based complied with all IRB-requirements to ensure the safety of the participants. Ethical approval was obtained from the National Ethics committee for Health Research, Cambodia; Mount Saint Vincent University Research Ethics Board, Canada; and the University of Oregon Institutional Review Board, USA.

Participants:

Participants (335 pregnant women) were recruited through antenatal care visits and consultations with local village chiefs, elders, and health center staff. The pregnant women were advised and provided with a general overview of the research study. To be eligible for the study, the mother's most recent pregnancy had to be normal (i.e., no known chronic conditions, preeclampsia, gestational diabetes, etc.), and the infant was born without complications (I.e., low birth weight, tongue tie, cleft palate). Other criteria included: not taking or having taken thiamine-containing supplements over the past 4 months and not currently participating in any nutrition programs beyond normal care. Furthermore, participants, research assistants, study investigators and data analysts were blinded to the randomized groups. Within each treatment group, women were asked to consume one capsule daily between 2 and 24 weeks postnatal.

Table 1: Treatment arm and thiamine dose administered to participants

Table 1 Treatment arms for the *Trial of thiamine supplementation in Cambodia*

Treatment arm	Thiamine dose	Rationale
Negative control	0 mg/day	Negative control (placebo)
EAR	1.2 mg/day	1xthiamine EAR for lactating women[19]
Double EAR	2.4 mg/day	2xthiamine EAR for lactating women[19]
Positive control	10 mg/day	Positive control (dose currently given in supplemental form in Myanmar)[4]

EAR, estimated average requirement.

Additionally, Table 2 presents the mothers' and infants' demographic information (reprinted from Measelle, et al, 2021).

Table 2: Infant and maternal sample characteristics by treatment group

	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%)
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%)

Hammersmith Neonatal Neurological Examination (HNNE)

The Hammersmith Neonatal Neurological Examination (HNNE) was only performed at 2 weeks, and it was modified and shortened relative to the original exam. The HNNE also provided insight about an infant's neurodevelopment by assaying infants' motor and sensory responses. In this study, the examination consisted of 5 categories that were administered in the following order (Mercuri, Ricci, Pane, & Baranello, 2005):

1. **Tone and posture:** posture, arm recoil, arm traction, leg recoil, leg traction, popliteal angle, head control (extensor and flexor tone), head lag, ventral suspension
2. **Reflexes:** tendon reflex, suck/gag, palmar grasp, plantar grasp, placing, moro reflex.
3. **Movements:** quantity and quality of spontaneous movement, head raising prone.

4. **Abnormal Signs:** abnormal hand or toe postures, tremor, startle.
5. **Orientation and Behavior:** eye appearances, auditory orientation, visual orientation, alertness, irritability, consolability, cry.

The HNNE yielded "optimality" scores on a 1-5 scale. Scores of 1 and 5 were considered non-optimal while scores of 2, 3, and 4 were considered degrees of "optimal." Since none of the infants scored a '5' on any of the tasks, their scores from 1-4 represented a linear scale, and could be analyzed in that fashion. The total score was summed from all 4 subscale scores (see below for clarification about subscales).

Hammersmith Infant Neurological Exam (HINE)

The Hammersmith Infant Neurological Examination (HINE) was performed at 2 weeks, 12 weeks, and 24 weeks postpartum. Both versions of the Hammersmith are brief, standardized, and scorable clinical neurological examinations that provide basic information about infants' neurological status through use of gentle touch and social interaction to evaluate infants' sensory and motor responses. Prior to the test, administrators were trained on how to perform the exam. See Appendices A and B for detailed information about the HINE and HNNE items and scoring.

This study performed a shortened version of the actual HINE. There were 12 items, and 4 categories with their corresponding items were performed in the following order:

1. **Posture:** head, trunk, hands
2. **Movements:** quantity and quality
3. **Tone:** scarf sign, passive shoulder, adductors, ankle dorsiflexion, and ventral suspension

4. **Reflex:** arm protection and vertical suspension

Each item was scored individually (0, 1, 2, or 3); items within each category were summed to create the subscore and all items were added to produce an overall score of all individual items, which ranged from 0 to 78. Motor milestones (e.g., walking) and behavior states (e.g., emotional state) were documented as part of the assessment but they were not scored. If any asymmetry was detected (i.e., different scores for an infant's right versus left side), each side was scored and averaged as one score for that item.

Maternal Thiamine-status Measurements:

Maternal blood samples were collected at 2 and 24 weeks whereas infant blood samples were obtained at 24-weeks postnatal. Both samples were collected using EDTA-coated tubes. Human milk samples were collected using a battery-powered single breast pump at 2-, 4-, 12-, and 24-weeks postnatal. For analysis, mothers' venous blood samples were measured for erythrocyte transketolase, a marker for thiamine deficiency, and thiamine diphosphate concentrations (ThDP) at 2 weeks and 24 weeks postnatal. Milk samples were measured at the same ages for total milk thiamine concentrations. For infants, venous blood samples were also assayed for whole blood ThDP and erythrocyte transketolase at 24-weeks. In the thesis, only mothers' 2-week total milk thiamine levels were analyzed.

Approach to Answering Research Questions

Here I outline the plan for analyses. First, I planned to investigate infants' progress in their HINE scores from 12- to 52-weeks by performing a one-way ANOVA to determine if there are differences between groups.

Next my plan was to run a 3 X 4 mixed-design ANCOVA (with baseline HNNE scores as the covariate) to analyze infants' total raw HINE scores to determine the relationship between low-dose maternal thiamine supplementation and infants' HNNE/HINE scores. Additionally, I planned to perform Pearson correlations between HNNE scores and HINE scores at each time point.

Finally, I planned to examine infants' z-scored growth metrics (in relation to World Health Organization norms) at the baseline measurement timepoint (HNNE 2 weeks) to determine how infants were faring at baseline. I then planned to examine the association of infants' anthropometrics at 2 weeks with their Hammersmith scores by conducting Pearson correlations between each growth metric (length, head circumference, and weight) and HNNE score.

Lastly, I planned to utilize two multiple regressions to explore the collective association of four predictors -- baseline Z-scored weight, mothers' total milk thiamine levels measured at baseline (a proxy for infants' prenatal access to thiamine), baseline HNNE scores, and maternal thiamine supplementation dose (0, 1.2, 2.4, 10mg) -- with infants' 24-week HINE scores, and with infants' 52-week HINE scores. These multiple regression models had the advantage of providing a) a potentially more sensitive test of maternal thiamine supplementation because they treated it as a scalar variable (as opposed to ANCOVA, which treated it as a categorical variable), and b) information regarding possible associations of other variables (e.g., 2-week Z-scored weight, mothers' 2-week total milk thiamine level, maternal thiamine supplementation dosage) with neurological change (assessed at 24- and 52-weeks), given that HNNE baseline scores were auto-regressed in the analyses.

Results

This study investigated several key questions, including possible relationships between maternal thiamine supplementation and indices of infants' growth, such as their weight, height, and head circumference, as well as possible benefits of maternal thiamine supplementation for infants' Hammersmith scores.

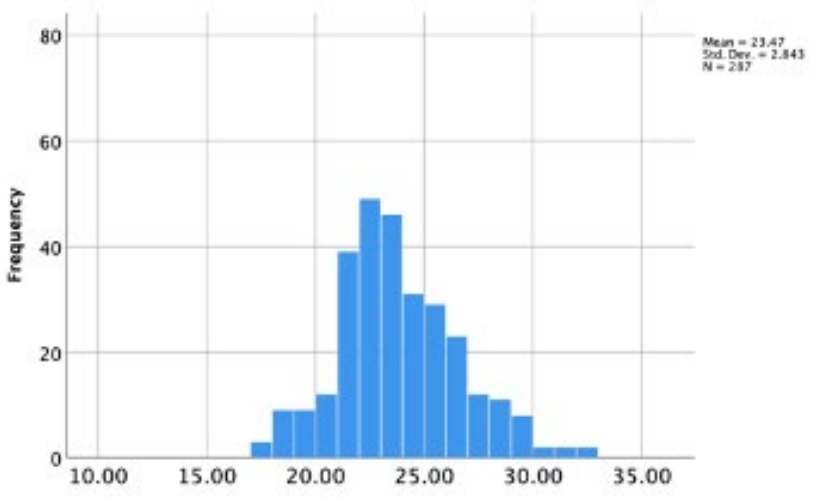
Preliminary Analyses

An important preliminary issue concerned the possibility that infants in the four thiamine treatment groups might have shown differences in their neurocognitive function, as measured by the HNNE, already at the baseline measurement timepoint (despite randomization to treatment group). However, no such differences were observed: A one-way ANOVA examining 2-week HNNE scores revealed no significant main effect of treatment group, $F(3,331) = 0.55$, $p=0.65$.

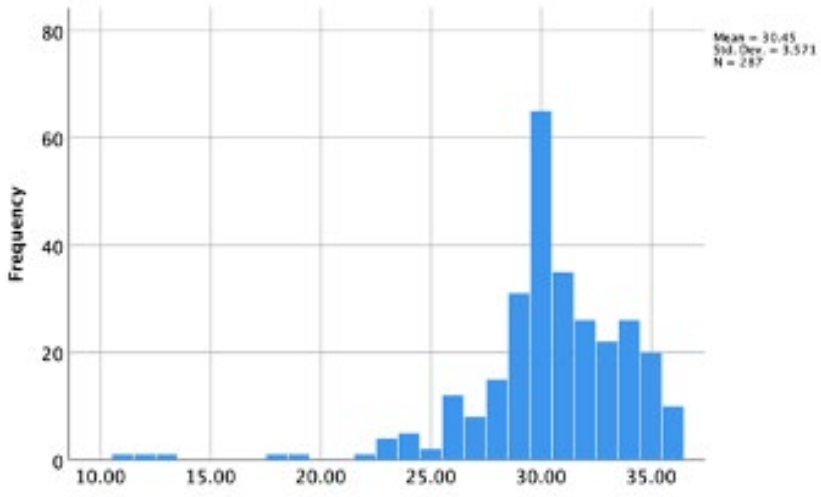
We examined distributions of the Hammersmith scores at each timepoint (Figure 1). In Figure 1, the 2-week HNNE baseline showed a normal distribution with scores in the middle of the scoring range. Most of the infants had moderate HNNE scores while a few of them had fairly high or low scores (i.e., 31 or 17) meaning the majority of the infants had mid-range scores, indicating moderate neurological health. HINE scores increasingly shifted towards the high end of the Hammersmith scale from 12 weeks to 52 weeks, indicating neurological function improving with age for infants in the sample, as a whole. At the same time, HINE distributions became increasingly negatively skewed as infants' age increased. In particular, departures from normality were obvious when infants were 24 and 52 weeks, with a few infants displaying HINE scores that

were much lower than average. This pattern points to a handful of infants within the sample increasingly falling behind in neurological development.

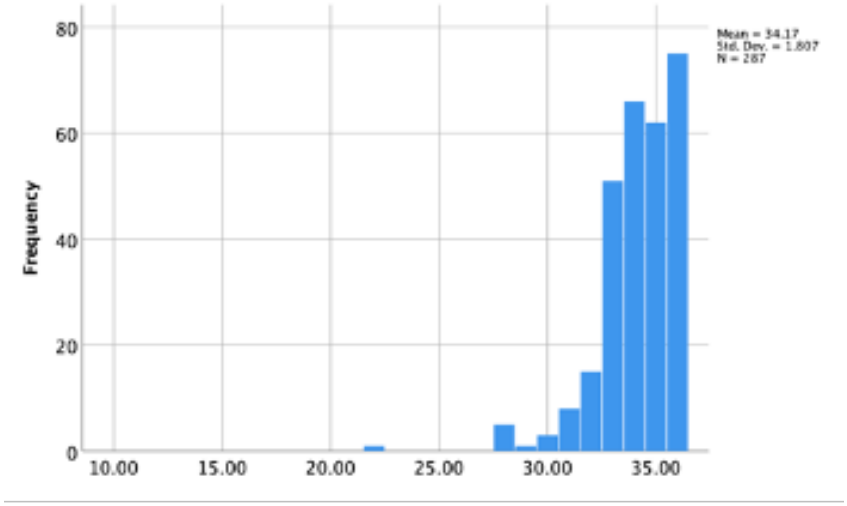
Baseline (2 weeks)



Midline (12 weeks)



Endline (24 weeks)



Follow-up (52 weeks)

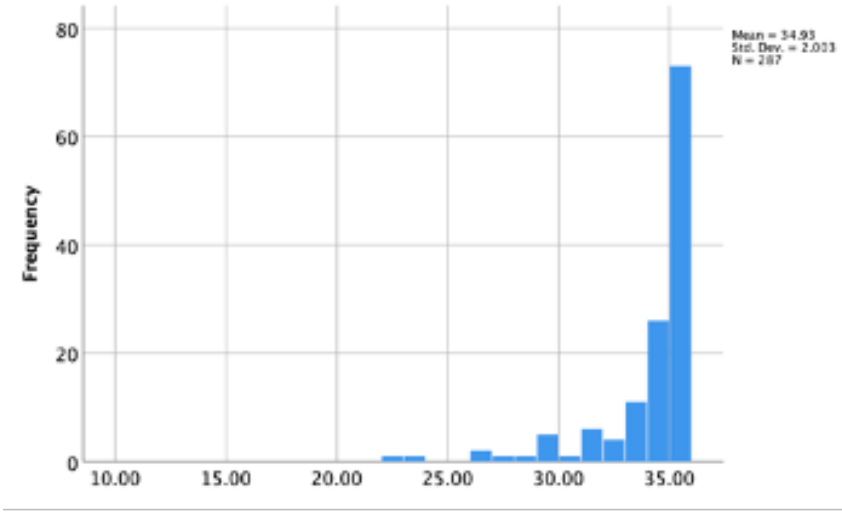


Figure 1. The frequency of HNNE (2-week baseline) and HINE (12-, 24-, and 52-weeks) scores at the four measurement timepoints.

We also examined infants' anthropometrics (Z-scored weight, head circumference, and length in relation to World Health Organization norms) at the

baseline measurement timepoint to gain a sense of how infants were faring in terms of growth at baseline, and in relation to possible differences at baseline among treatment groups (see Table 3).

Table 3: Infants' mean Z-scored weight, head circumference, and length at 2 weeks in relation to WHO norms (standard deviations in parentheses)

	Total	**0 mg**	**1.2 mg**	**2.4 mg**	**10 mg**
	N=335	**n=83**	**n=86**	**n=81**	**n=85**
Z-weight	-0.52(0.97)	-0.40(0.95)	-0.50(0.96)	-0.59(0.88)	-0.58(1.07)
Z-head circumference	-0.72(1.02)	-0.59(1.14)	-0.77(0.98)	-0.81(0.99)	-0.73(0.96)
Z-length	-0.62(1.02)	-0.52 (0.98)	-0.66(1.11)	-0.69(1.01)	-0.63(1.01)

As is clear from Table 3, infants' Z-scored growth metrics indicated substantial growth reductions relative to WHO norms when measured at 2 weeks. This was not unexpected but underscores a range of factors affecting infants' growth in Cambodia. A series of one-sample t-tests comparing the three growth metrics at 2 weeks to 0 (the expected mean for WHO norms) revealed that infants in the sample scored significantly below the WHO-normed mean on all three metrics, t's < -9.76, p's < .000. In addition, I conducted a series of one-way between-subject ANOVAs examining possible treatment group differences for the three different growth metrics (Z-scored weight, head circumference, length) at 2-weeks, with the expectation that, due to random assignment to treatment groups, no such differences would be statistically significant. These

analyses confirmed (all p's > 0.165) that no statistically significant differences occurred in any of the three growth metrics for infants in the four different treatment groups.

Developmental Progress in Hammersmith Scores

Our first analysis of Hammersmith scores focused on two questions: a) the extent to which infants' HINE scores displayed increases with development, and b) the extent to which maternal thiamine supplementation dosage influenced HINE scores. We investigated these questions via a 3 X 4 mixed-design ANCOVA examining infants' total raw HINE scores with timepoint (12, 24, and 52-weeks) as the within-subjects, repeated-measures variable, treatment group (0, 1.2, 2.4, and 10mg daily thiamine) as the between-subjects variable, and baseline (2 week) HNNE score as a covariate (to control for starting differences in infants' neurological status). Means and standard deviations can be viewed in Table 4. This analysis revealed a significant main effect of timepoint, F (2, 564) =23.96, p =.000, indicating that infants' HINE scores showed systematic development with age.

Table 4: Mean HNNE scores at 2 weeks and mean HINE scores at other timepoints in the study (standard deviations in parentheses)

	Total	**0 mg**	**1.2 mg**	**2.4 mg**	**10 mg**
	N=335	**n=83**	**n=86**	**n=81**	**n=85**
HNNE 2 weeks	9.42 (1.71)	9.62 (1.53)	9.29 (1.79)	9.25 (9.88)	9.52 (1.62)
HINE 12 weeks	30.41(3.54)	30.89 (2.93)	30.47 (2.94)	30.03 (4.22)	30.24 (3.94)

HINE 24 weeks	34.18 (1.80)	34.26 (1.68)	34.22 (1.82)	33.86 (2.17)	34.38 (1.47)
HINE 52 weeks	34.96 (1.95)	35.04 (1.77)	35.06 (1.88)	34.91(2.12)	34.85 (2.03)

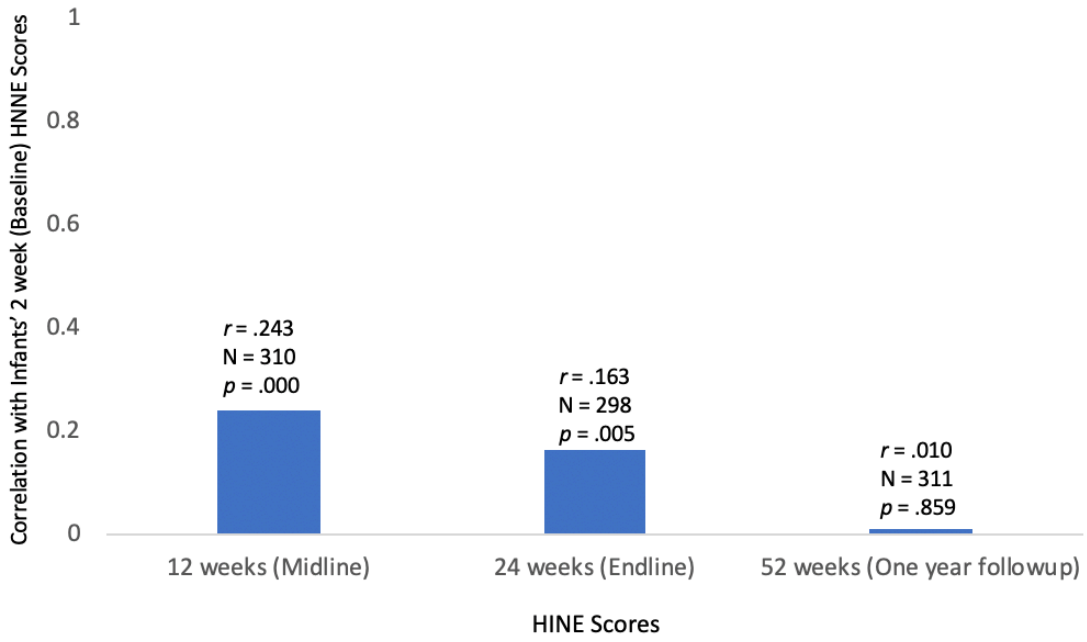
Maternal Thiamine Supplementation on Hammersmith Scores

The ANCOVA revealed neither a significant main effect of treatment group, F (3, 282) = 1.12, p = .34, nor a significant timepoint by treatment group interaction, F (6,564) = .45, p =.84, indicating that maternal thiamine supplementation had no systematic effect on infants' HINE scores at any of the timepoints measured. However, the ANCOVA did reveal a significant effect of the covariate - baseline (2 week) HNNE scores – on infants' average HINE score across the three timepoints, F (1,282) = 15.20, p = .000. At the same time, a significant timepoint by covariate interaction indicated that this relationship depended on timepoint, F (2,564) = 8.46, p = .000. Figure 2 displays the timepoint-related changes with respect to baseline HNNE scores, with the strongest relationship occurring at earlier timepoints (e.g., 12-weeks) and waning at later timepoints (e.g., 24- and 52-weeks), with a non-significant relationship at the 52-week follow-up. These analyses show HNNE was associated with neurocognitive status only at earlier ages (12 and 24 weeks postpartum). Conversely, HNNE scores were not related with neurodevelopment of older infants (52 weeks postpartum).

The ANCOVA just reported revealed no effect of thiamine on infants' Hammersmith scores. I opted to undertake an additional analysis as a potentially more sensitive test to examine this issue. In this new ANCOVA, I collapsed across all three

thiamine supplementation conditions (i.e., 1.2, 2.4, and 10mg) to compare effects of thiamine supplementation (at any dose) relative to placebo (0mg). Other variables in the analysis were the same as the previously reported ANCOVA (i.e., timepoint and HNNE baseline as covariate). However, consistent with the pattern of findings from the previous ANCOVA, no significant main effect nor interactions emerged involving maternal thiamine supplementation.

Figure 2. Correlations between infants' 2-week (Baseline) HNNE scores and infants' HINE scores at subsequent timepoints



Correlational Analyses

Also of interest was the extent to which infants' baseline anthropometrics (e.g., Z-scored weight, head circumference, and length) and their Hammersmith scores at 2-, 12-, 24-, and 52-weeks were related (Tables 5 and 6). Pearson correlations revealed

statistically significant positive relationships among all anthropometric measures at all timepoints. HNNE at 2 weeks (Baseline) and HINE scores at 12- and 24-weeks were each significantly positive related, but none of these were significantly related to 52-week (One Year Follow-up) HINE scores. Lastly, 2-week (Baseline) anthropometrics (Z-scored weight, head circumference, and length) were significantly (or marginally, in one case) positively associated with 2-week (Baseline), 12-week (Midline), and 24-week (Endline) Hammersmith scores, but none of the anthropometrics was significantly associated with HINE scores at 52-weeks (One Year Follow-up).

Table 5: Correlations between infants' neurological scores on the Hammersmith at 2-, 12-, 24-, and 52-weeks and infants' anthropometric growth scores at 2-weeks as measured by standardized length, weight and head circumference.

Standardized infant growth measures at 2-weeks

	Weight	Head circumference	Length
2 weeks HNNE (Baseline)	0.152**	0.119*	0.178**
12 weeks HINE (Midline)	0.225***	0.155**	0.216***
24 weeks HINE (Endline)	0.139*	0.104	0.147*
52 weeks HINE (One Year Follow-up)	0.05	0.04	0.062

Note. Standardized (z-score) measures of infant growth are based on World Health Organization norms.
*$p < .05$; **$p < .01$; ***$p < .001$

Table 6: Correlations between infant's 2-week HNNE baseline and 12 weeks, 24 weeks, and 52 weeks HINE. (denotes significance at the 0.01 level)**

	2 weeks HNNE (Baseline)	12 weeks HINE (Midline)	24 weeks HINE (Endline)
2 weeks HNNE (Baseline)	1	---	---
12 weeks HINE (Midline)	0.243**	1	---
24 weeks HINE (Endline)	0.163**	0.329**	1
52 weeks HINE (One Year Follow-up)	0.010	0.041	0.053

Note. Standardized (z-score) measures of infant growth are based on World Health Organization norms.
*$p < .05$; **$p < .01$; ***$p < .001$*

Regression Models

Lastly, I conducted two multiple regression analyses to examine the extent to which several predictors were systematically associated with a) 24-week HINE scores, and b) 52-week HINE scores. These regressions had the advantage of making it possible to test for a dose-response relationship between thiamine treatment group and Hammersmith scores, given that supplementation dose was treated as a scalar variable (0, 1.2, 2.4, or 10mg) in the model. As well, in these regression models, I was controlling an earlier timepoint (2-week HNNE) of my dependent variable (24 weeks HINE), meaning the models were testing change in neurological level from baseline to the later timepoint.

The predictor variables we were interested in included a) infants' z-scored baseline weight (a proxy for the quality of infants' intra-uterine environment), b) mothers' total milk thiamine levels at baseline (a proxy for infants' access to thiamine

prenatally), c) baseline HNNE scores, and d) the thiamine supplementation treatment group to which mothers and infants had been assigned.

Regarding the 24-week timepoint, the multiple regression shown in Table 7 revealed an overall significant model ($F = 3.236$, $p = 0.013$), with $R^2 = 4.2\%$. Individual beta coefficients were statistically significant for baseline z-scored weight (beta = 0.123, $t = 2.086$, $p = 0.013$) and baseline HNNE (beta = 0.135, $t = 2.312$, $p = .013$). These findings indicated that baseline weight was associated with 24-week Hammersmith scores, even while controlling for baseline Hammersmith, and that baseline Hammersmith was associated with 24-week Hammersmith, further confirming correlational findings reported earlier. None of the other individual beta-coefficients were statistically significant. Thus, regarding infants' access to thiamine, neither maternal thiamine dosage during the clinical trial nor mothers' baseline total milk thiamine level were significantly associated with infants' 24-week neurological performance on the Hammersmith.

The same multiple regression model yielded a different outcome for infants at the 52-week follow-up timepoint. As seen in Table 7, the regression model was non-significant ($F = 1.45$, $p = 0.219$, $R^2 = 1.7\%$). The only predictor variable with an individually significant beta-coefficient was maternal total milk thiamine level at baseline (beta = 0.12, $t = 2.05$, $p = 0.042$). This finding suggested that infants' pre-natal access to thiamine was significantly associated with change in their neurological function as assessed by Hammersmith at 52 weeks. This stood out as the one finding within this dataset hinting at a possible relationship between infants' thiamine status and their developing neurological function (as measured at 52 weeks).

Table 7: Regression analyses of 2 week HNNE, Z-weight 2 weeks, treatment group thiamine levels, and 2 wees milk thiamine levels at 24 weeks and 52 weeks HINE

	24 Week Outcomes					52 Week Outcomes				
Predictors	B	SE	*β*	*t*	95% CI	B	SE	*β*	*t*	95% CI
HNNE-2 weeks	.09	.04	.14	2.31*	[.01, .16]	.01	.04	.01	.14	[-.07, .08]
Z-weight-2 weeks	.23	.11	.12	2.09*	[.01, .44]	.10	.12	.05	.88	[-.13, .33]
Treatment group thiamine levels	.03	.03	.05	.87	[-.03, .08]	-.02	.03	-.03	-.53	[-.07, .04]
Milk thiamine-2 weeks	.00	.00	.00	.09	[-.00, .00]	-.003	.002	.12	2.05*	[.00, .01]
	R= 0.042, F (4, 293) = 3.24, p=0.013					R=0.017, F (4, 306) = 1.45, p=0.219				

Additionally, the regression models were rerun with maternal thiamine supplementation groups collapsed together (thus the variable was dummy-coded as 0 for placebo control group vs. 1 for any level of thiamine supplementation) to increase the sensitivity of detecting a possible thiamine effect. The pattern of results from these models was the same as the findings from the previously reported regressions, meaning that recoding the maternal thiamine supplementation did not change the outcome of these analyses.

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 Hammersmith Neurological Examinations (HNNE and HINE). I predicted infants would exhibit developmental

progress on the Hammersmith from 12 to 52 weeks, and that maternal thiamine supplementation would have a dose-response effect on infants' neurological function. Specifically, I anticipated that infants whose mothers received higher levels of thiamine supplementation would show higher Hammersmith scores. Additionally, I examined the extent to which infants' growth at 2 weeks post-natal (baseline) was associated with infants' Hammersmith scores as they developed, hypothesizing that infants with higher growth metrics at 2 weeks would display higher Hammersmith scores at subsequent developmental timepoints. Furthermore, I explored other predictors, such as maternal milk thiamine at 2 weeks, that were associated with 24 weeks HINE and 52 weeks HINE. Since most of my analyses showed a nonsignificant relationship between thiamine group and infants' Hammersmith scoring, the finding about 2 weeks maternal milk thiamine predicting 52 weeks HINE was surprising.

Anthropometrics at 2 weeks and Hammersmith Scores

Infants' 2-week growth metrics showed delays relative to WHO growth norms, which was not unexpected given previous findings for infants born in rural Cambodia (Whitfield, et al., 2017). I undertook correlational analyses to investigate the extent to which 2-week growth metrics were positively associated with subsequent Hammersmith scores. These analyses confirmed that infants' growth, as measured by Z-scored weight, head circumference, and length, were significantly (and in one case marginally) positively correlated with infants' Hammersmith scores at 2-, 12-, and 24-weeks. That is, when infants were larger in regard to their growth metrics, they tended to display higher Hammersmith scores at the first three measurement timepoints. By the 52-week follow-up timepoint, these relationships had waned and were no longer statistically

significant. In any case, however, these findings underscore the potential value of early growth metrics for predicting infants' neurocognitive trajectories in their first 6 months of life.

Maternal Thiamine and Hammersmith Scores

Analysis of variance revealed that infants' Hammersmith scores showed significant improvements with development. That said, although most infants performed increasingly well on the HINE, there were a handful who had HINE scores that were strikingly lower than average. Examination of distributions at each timepoint revealed that some infants had HINE scores below 25 at 12 weeks (the average HINE score at that time point was 30.41). When timepoint progressed to 52 weeks, the frequency of infants who had low HINE scores became even more obvious. The negatively skewed distributions indicated that a handful of infants were displaying increasingly obvious delays in neurological development.

In the analysis of variance, the thiamine supplementation treatment group to which infants had been randomly assigned had no significant effect on their Hammersmith scores at any developmental timepoint. Thus, my hypothesis regarding maternal thiamine supplementation benefitting infants' neurological development – as measured by the Hammersmith -- was disconfirmed.

I opted to carry out some additional exploratory analyses in relation to the dose-response hypothesis. Particularly, I examined the hypothesis using a regression approach in addition to the analysis of variance approach already described, because a regression model considers the scalar level of maternal thiamine supplementation dosage. The regression approach thus provides a more sensitive test of the hypothesized

dose-response relationship of maternal thiamine supplementation to infants' Hammersmith outcomes than analysis of variance, which only treats thiamine supplementation group as a categorical variable. I opted to include several predictor variables in addition to maternal thiamine supplementation treatment group to the regression model, including infants' 2-week Z-scored weight (as a proxy for the quality of their intra-uterine environment), their 2-week HNNE score (to control for baseline Hammersmith scores), and maternal 2-week total milk thiamine levels (as a proxy for infants' prenatal access to thiamine). When this regression model was conducted to examine 24-week Hammersmith scores, I found that the model overall was statistically significant, and that both infants' 2-week Z-scored weight and their 2-week HNNE scores were significantly associated with their 24-week HINE scores. However, neither maternal supplementation treatment group nor 2-week maternal total milk thiamine levels were significantly associated with their 24-week HINE scores. These findings again disconfirmed my hypothesis that maternal thiamine supplementation would show a dose-response relationship to infants' neurological function as measured by the Hammersmith.

In contrast to these findings, the same regression model conducted in relation to Hammersmith scores from the 52-week follow-up measurement timepoint revealed a quite different picture. Unlike at 24-weeks, neither 2-week Z-scored weight, nor 2-week HNNE scores were systematically associated with 52-week Hammersmith scores. However, treatment group again was not significantly associated with 52-week Hammersmith scores. Among other things, these findings further disconfirmed the dose-response hypothesis regarding maternal thiamine supplementation. Yet strikingly,

in this analysis, maternal 2-week total milk thiamine levels displayed a significant relationship to infants' 52-week HINE scores. This, then, was one sign – albeit the only sign – within my thesis dataset that infants' early, pre-supplementation access to thiamine (prenatally and/or during their first two weeks of life) mattered for their subsequent neurological development. Maternal milk thiamine at 2 weeks baseline may have predicted 52-week HINE, but not 24-week HINE, because certain relevant neurological functions might not emerge in Hammersmith testing until 52 weeks. However, it is important to underscore that this regression analysis did not reach statistical significance. Given this, it is important to interpret this finding with caution.

Broader Implications

These thesis findings build on previous studies utilizing the Hammersmith Neurological Examinations by applying these neurological assessments in a nutritional setting. Furthermore, my results add to the literature regarding thiamine's physiological effects on human development.

My findings were consistent with previous evidence (Whitfield et al., 2017) that many infants growing up in rural Cambodia experience delayed growth (at least in relation to WHO norms). This is a concern for numerous reasons, but based on the findings from some statistical analyses, early growth parameters are associated with neurocognitive development. I found that infants' early growth metrics (operationalized as Z-scored weight, head circumference, and length measured at 2 weeks postnatal in the present study) were associated with their subsequent Hammersmith scores up through 24-weeks of age. These findings point to the importance of continuing efforts to better support maternal and fetal health in the larger region of which Cambodia is a part.

Moreover, this thesis provided the first investigation ever to examine possible relationships between infants' access to thiamine via maternal supplementation and their neurological function as measured by the Hammersmith. For this reason, my finding that Hammersmith scores were apparently unaffected by maternal thiamine supplementation is noteworthy. Yet in some respects this finding is also somewhat puzzling, as I discuss below. Further research will be necessary to investigate the many unanswered questions that arise in relation to my findings.

Limitations

I have considerable confidence that the Hammersmith was an appropriate instrument to utilize as a measure of infant neurological function for the present study. For example, previous research (e.g., Romeo, et al., 2020) indicates that the Hammersmith Neurological Examination is informative about neurological delay in early infancy even when infants do not have severe neurological dysfunction, such as cerebral palsy. At the same time, one of the possible limitations in this thesis was that modified versions of the two forms (HNNE and HINE) of the Hammersmith Neurological Examination were used. For instance, the cranial nerve function test was not included in the HINE. Some of these items could indicate an infant's sensory (i.e., smell, hearing, taste) and motor (i.e., eye and facial movement) skills. Since cranial nerves and some other tests, such as lateral tilting, were excluded from the HINE measures utilized in this study, the infant's overall HINE scores may not have reflected their entire neurological status. To the extent that the excluded items are important to overall HINE scores, my tests of thiamine supplementation's influence on HINE scores might have been insensitive to such effects.

Another possible limitation of the present study was the fact that it wasn't possible to ascertain the degree of reliability in the researchers' Hammersmith scoring. As is typical in the field, the Hammersmith was scored live by an observing researcher while another researcher worked directly with infants, and these sessions were not videotaped. Thus, the degree to which Hammersmith scores in this study may have been affected by researcher implementation variability and other factors affecting scoring reliability isn't known.

Lastly, it is important to recognize the possibility that maternal thiamine supplementation might have revealed an influence on infants' neurological function had the randomized controlled trial a) included higher dosages 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. Future research will be necessary to provide information on these important issues.

Future Directions

The findings from the thesis raise a number of questions for future investigation. For one, to some degree there is a disconnect between the thesis findings and other findings recently reported for the results of the larger dataset arising from the same randomized, controlled trial. These previous studies documented a statistically significant dose-response relationship between maternal thiamine supplementation and a) infants' scores on the language subscales of the Mullen Scales of Early Learning (MSEL) (Measelle, et al., 2021) and b) infants' attentional response to infant-directed speech (Baldwin, et al., under review). These findings point to benefits of such

supplementation for infants' neurocognitive development. Given these findings, it might seem surprising that maternal thiamine supplementation did not appear to have a similar beneficial effect on infants' performance on the Hammersmith measure of neurological development.

One possible explanation for what might appear to be a discrepancy in findings could be that benefits of thiamine supplementation are especially pronounced for language development, which the Hammersmith does not measure. Consistent with this possibility, Measelle and colleagues found that maternal thiamine supplementation did not show a dose-response benefit for infants' performance on the MSEL fine- and gross-motor subscales; that is, the supplementation benefit was largely limited to the MSEL language subscales. In other words, differences between my findings and others from the same sample may have occurred because the Hammersmith primarily assesses movement and motor skills whereas MSEL and the infant-directed speech task measure language abilities. Taken together, the prior findings and the present findings appear to provide growing evidence that thiamine supplementation during early infancy is particularly impactful for infants' developing language functions.

Although my analyses disconfirmed the hypothesis that maternal thiamine supplementation would show a dose-response relationship to neurological development, as measured by Hammersmith scores, a lone finding hinted that infants' prenatal access to thiamine might matter for Hammersmith scores downstream at 52-weeks. Specifically, maternal total milk thiamine levels assayed at 2 weeks postnatal – a proxy for infants' prenatal access to thiamine -- were significantly associated with infants' 52-

week HINE scores, while controlling for treatment group, Z-scored weight, and 2-week baseline HNNE scores.

On the one hand, there is strong reason for caution in the interpretation of this finding. The finding arose from an exploratory regression analysis that revealed a non-significant model overall and is the only finding within this thesis that pointed to a possible impact of maternal thiamine on infants' Hammersmith scores. The finding warrants further investigation, however, both to discover whether it replicates, and to examine in a more direct way the extent to which infants' prenatal access to thiamine impacts their subsequent neurological development.

Having noted the need for interpretive caution, it is also worthy of note that the finding in this thesis of a significant relationship between maternal total milk thiamine levels at 2 weeks and Hammersmith scores at 52 weeks meshes to some degree with findings from Measelle, et al. (2021) for this same sample. Measelle and colleagues documented a statistically significant association between 2-week maternal total milk thiamine levels and infants' fine-motor MSEL sub-scale scores, as well as their MSEL language sub-scale scores (although in the case of their findings the relationship was statistically significant at 24 weeks but not at the 52-week follow-up timepoint). Moreover, the association between 2-week total milk thiamine levels and 52-week Hammersmith scores also echoes a finding reported by Fattal-Valevski and colleagues (2009). They observed that Israeli infants who had earlier been affected by thiamine-deficient formula subsequently displayed delays in independent walking, which is a skill that tends to emerge normatively at about 52 weeks of age (Fattal-Valevski et al., 2009). All in all, these small convergences in findings across studies further underscore

the need for continued investigation of the implications of infants' prenatal thiamine access for their subsequent neuro-cognitive development, especially in relation to motor and language development. Such investigation will benefit from initiation of new research in which maternal thiamine supplementation is begun during the prenatal period, and potentially continued beyond the 24-week timepoint (at which supplementation ceased in the present study).

Lastly, an important future direction will be to include additional neurological probes beyond the Hammersmith. Inclusion of other neurological measures will make it possible to assess the degree to which the present findings are robust across multiple neurological instruments.

Conclusions

In the present study, breast-fed Cambodian infants showed marked delays in growth metrics relative to WHO norms. Moreover, their growth metrics were associated with their neurological function during early infancy, as measured by the Hammersmith Neurological Examination. This underscores the importance of interventions to protect infants' health and growth in the Southeast Asia region.

Low-dose maternal thiamine supplementation did not appear to affect infants' Hammersmith scores in this study. Given that the Hammersmith primarily probes motor function, this may indicate that motor development is not a primary locus of benefit from maternal thiamine supplementation. Other accounts of the findings are also possible, however, raising the need for further investigation of these important developmental issues.

Appendices

Appendix A: HNNE Scoring Rubric and Procedure

Tone and Posture

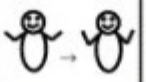
NAME:_____, CODE:_____, SEX:_____, RACE_____,

D.O.B.:_____, D.O.E.:_____, AGE:_____, G.A.:_____, BW:_____,

Tone						STATE	ASYMM.
POSTURE Infant supine, look mainly at position of legs but also note arms. *score predominant posture*	Arms and legs extended or very slightly flexed	Legs slightly flexed	Leg well-flexed but not adducted	Leg well flexed and adducted near abdomen	Abnormal posture: a) opistotonus		
ARM RECOIL Take both hands, quickly extend arms parallel to the body. Count to three. Release. Repeat ×3	Arms do not flex	Arms flex slowly, not always; not completely	Arms flex slowly; more complete	Arms flex quickly and completely	Arms difficult to extend; snap back forcefully		
ARM TRACTION Hold wrist and pull arm upwards. Note flexion at elbow and resistance while shoulder lifts off table. *Test each side separately*	Arms remain straight; no resistance R L	Arms flex slightly or some resistance felt R L	Arms flex well till shoulder lifts, then straighten R L	Arms flex at approx 100° and mantained as shoulder lifts R L	Flexion of arms <100°; mantained when body lifts up R L		
LEG RECOIL Take *both* ankles in one hand, flex hip+knees. Quickly extend. Release. Repeat ×3	No flexion	Incomplete or variable flexion	Complete but slow flexion	Complete fast flexion	Legs difficult to extend; snap back forcefully		
LEG TRACTION Grasp ankle and slowly pull leg upwards. Note flexion at knees and resistance as buttocks lift. *Test each side separately*	Legs straight – no resistance R L	Legs flex slightly or some resistance felt R L	Legs flex well till bottom lifts up R L	Knee flexes remains flexed when bottom up R L	Flexion stays when back + bottom up R L		
POPLITEAL ANGLE Fix knee on abdomen, extend leg by gentle pressure with index finger behind the ankle. Note angle at knee. *Test each side separately*	180° R L	~150° R L	~110° R L	~90° R L	<90° R L		
HEAD CONTROL (1) ***(extensor tone)*** Infant sitting upright; hold at shoulder. Let head drop forward.	No attempt to raise head	Infant tries: effort better felt than seen	Raises head but drops forward or back	Raises head: remains vertical; it may wobble			
HEAD CONTROL (2) ***(flexor tone)*** Infant sitting upright; hold at shoulder. Let head drop backward.	No attempt to raise head	Infant tries: effort better felt than seen	Raises head but drops forward or back	Raises head: remains vertical; it may wobble	Head upright or extended; cannot be passively flexed		
HEAD LAG Pull infant to towards sitting posture by traction on both wrists and support head slightly. Also note arm flexion	Head drops & stays back	Tries to lift head but it drops back	Able to lift head slightly	Lifts head in line with body	Head in front of body		
VENTRAL SUSPENSION Hold infant in ventral suspension; observe curvature of back, flexion of limbs and relation of head to trunk.	Back curved, head and limbs hang straight	Back curved, head ↓, limbs slightly flexed	Back slightly curved, limbs	Back straight, head in line, limbs flexed	Back straight, limbs above body		

Reflexes

Reflexes

TENDON REFLEX Test biceps, knee and ankle jerks.	Absent	Felt, not seen	Seen	' Exaggeratedi	Clonus		
SUCK/GAG Little finger into mouth with pulp of finger upwards.	No gag/no suck	Weak irregular suck only: No stripping	Weak regular suck Some stripping	Strong suck: (a) irregular (b) regular Good stripping	No suck but strong clenching		
PALMAR GRASP Put index finger into the hand and gently press palmar surface. Do not touch dorsal surface. *Test each side separately*	No response R L	Short, weak flexion of fingers R L	Strong flexion of fingers R L	Strong finger flexion, shoulder ↑ R L	Very strong grasp; infant can be lifted off couch R L		
PLANTAR GRASP Press thumb on the sole below the toes. *Test each side separately*	No response R L	Partial plantar flexion of toes R L	Toes curve around the examiner's finger R L				
PLACING Lift infant in an upright position and stroke the dorsum of the foot against a protruding edge of a flat surface. *Test each side separately*	No response R L	Forsiflexion of ankle only R L	Full placing response with flexion of hip, knee & placing sole on surface R L				
MORO One hand supports infantis head in midline, the other the back. Raise infant to 45° and when relaxed let his head fall through 10°. Note if jerky. Repeat ×3	No response or opening of hands only	Full abduction at shoulder and extension of the arms; no adduction 	Full abduction but only delayed or partial adduction 	Partial abduction at shoulder and extension of arms followed by smooth adduction 	• No abduction or adduction; • Only forward extension of arms from the shoulders • Marked adduction only  or		

Movements

SPONTANEOUS MOVEMENT (quantity) Watch infant lying supine.	no movement	sporadic and short isolated movements	frequent isolated movements	frequent generalized movements	continuous exaggerated movements		
SPONTANEOUS MOVEMENT (quality) Watch infant lying supine.	only stretches	stretches and random abrupt movements; some smooth movements	fluent movements but monotonous	fluent alternating movements of arms + legs; good variability	• cramped, synchronized; • mouthing • jerky or other abnormal movements		
HEAD RAISING PRONE Infant in prone, head in midline.	no response	infant rolls head over, chin not raised	infant raises chin, rolls head over	infant brings head and chin up	infant brings head up and keeps it up		

Abnormal Signs

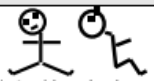
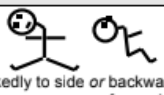
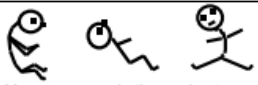
ABNORMAL HAND OR TOE POSTURES		hands open, toes straight most of the time	intermittent fisting or thumb adduction	continuous fisting or thumb adduction; index finger flexion, thumb opposition	continuous big toe extension or flexion of all toes		
TREMOR		no tremor, or tremor only when crying or only after Moro reflex	tremor occasionally when awake	frequent tremors when awake	continuous tremors		
STARTLE	no startle even to sudden noise	no spontaneous startle but reacts to sudden noise	2-3 spontaneous startles	more than 3 spontaneous startles	continuous startles		

Orientation and Behavior

EYE APPEARANCES	does not open eyes		full conjugated eye movements	*transient* • nystagmus • strabismus • roving eye movements • sunset sign	*persistent* • nystagmus • strabismus • roving eye movements abnormal pupils		
AUDITORY ORIENTATION Infant awake. Wrap infant. Hold rattle 10 to 15 cm from ear.	no reaction	auditory startle; brightens and stills; no true orientation	shifting of eyes, head might turn towards source	prolonged head turn to stimulus; search with eyes; smooth	turns head (jerkily, abruptly) & eyes towards noise every time		
VISUAL ORIENTATION Wrap infant, wake up with rattle if needed or rock gently. Note if baby can see and follow red ball (B) or target (T).	does not follow or focus on stimuli B T	stills, focuses, follows briefly to the side but loses stimuli B T	follows horizontally and vertically; no head turn B T	follows horizontally and vertically; turns head B T	follows in a circle B T		
ALERTNESS *Tested as response to visual stimuli* (B or T).	will not respond to stimuli	when awake, looks only briefly	when awake, looks at stimuli but loses them	keeps interest in stimuli	does not tire (hyper-reactive)		
IRRITABILITY In response to stimuli.	quiet all the time, not irritable to any stimuli	awakes, cries sometimes when handled	cries often when handled	cries always when handled	cries even when not handled		
CONSOLABILITY Ease to quiet infant.	not crying; consoling not needed	cries briefly; consoling not needed	cries; becomes quiet when talked to	cries; needs picking up to be consoled	cries; cannot be consoled		
CRY	no cry at all	whimpering cry only	cries to stimuli but normal pitch		High-pitched cry; often continuous		

Appendix B: HINE Scoring Rubric and Procedure

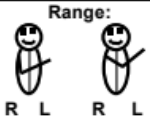
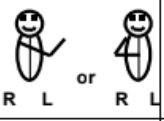
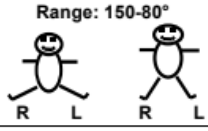
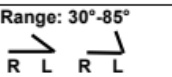
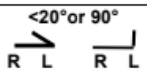
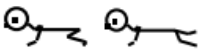
Posture

	score 3	score 2	score 1	score 0	sc	Comment / asymmetry
Head in sitting	 Straight; in midline		 Slightly to side *or* backward *or* forward	 Markedly to side *or* backward *or* forward		
Trunk in sitting	 Straight		 Slightly curved or bent to side	 Very rounded rocketing back bent sideways		
Hands	Hands open		**Intermittent** adducted thumb *or* fisting	**Persistent** adducted thumb *or* fisting		

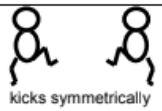
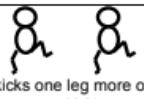
Movements

	Score 3	Score 2	Score 1	Score 0	sc	asymmetry
Quantity Watch infant lying in supine	Normal		Excessive or sluggish	Minimal or none		
Quality Observe infant's spontaneous voluntary motor activity during the course of the assessment	Free, alternating, and smooth		Jerky Slight tremor	• Cramped & synchronous • Extensor spasms • Athetoid • Ataxic • Very tremulous • Myoclonic spasm • Dystonic movement		

Tone

	Score 3	Score 2	Score 1	Score 0	sc	comment
Scarf sign Take the infant's hand and pull the arm across the chest until there is resistance. Note the position of the elbow in relation to the midline.	**Range:** 					
Passive shoulder elevation Lift arm up alongside infant's head. Note resistance at shoulder and elbow.	Resistance overcomeable 	Resistance difficult to overcome R L	No resistance 	Resistance, not over comeable 		
Adductors With both the infant's legs extended, abduct them as far as possible. The angle formed by the legs is noted.	**Range: 150-80°** 	**150-160°** 	**>170°** 	**<80°** 		
Ankle dorsiflexion With knee extended, dorsiflex the ankle. Note the angle between foot and leg.	**Range: 30°-85°** 	**20-30°** 	**<20°or 90°** 	**> 90°** 		
Ventral suspension Hold infant horizontally around trunk in ventral suspension; note position of back, limbs and head.						

Reflex

Arm protection Pull the infant by one arm from the supine position (steady the contralateral hip) and note the reaction of arm on opposite side.	 arm & hand extend R L		 arm semi flexed R L	 arm fully flexed R L		
Vertical suspension hold infant under axilla making sure legs do not touch any surface – you may "tickle" feet to stimulate kicking.	 kicks symmetrically		 kicks one leg more or poor kicking	 no kicking even if stimulated or scissoring		

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