

Mount Saint Vincent University
Department of Applied Human Nutrition

**Human milk total thiamine concentrations among rural Cambodian women on various
thiamine supplementation regimens in the exclusive breastfeeding period**

by
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Abstract

Introduction: Thiamine (vitamin B1) is a water-soluble vitamin essential in energy metabolism, neuronal functioning, and cognitive development. Thiamine deficiency remains a public health concern in Cambodia and other regions where thiamine-poor white rice is a staple food. The risk of deficiency is particularly high in exclusively breastfed infants when maternal thiamine intakes are low, resulting in thiamine-poor human milk. Strategies are needed to improve maternal thiamine intakes and, in turn human milk thiamine concentrations. However, there are limited data on the impact of long-term, low dose maternal thiamine supplementation on human milk thiamine concentrations.

Objective(s): To report, and assess differences in total thiamine concentrations in milk produced by Cambodian women consuming one oral capsule daily containing either 0, 1.2, 2.4, or 10 mg of thiamine at 2, 4, 12, and 24 weeks postpartum. Also to explore whether human milk total thiamine concentrations change over the exclusive breastfeeding period by examining potential lactation stage changes among un-supplemented women.

Methods: This study was part of a larger, 22-week double-blind, four-parallel arm, placebo-controlled randomized trial of thiamine supplementation in rural Kampong Thom, Cambodia (NCT03616288). Lactating women were randomized to consume one capsule containing 0 mg (placebo), 1.2 mg (Estimated Average Requirement), 2.4 mg, or 10 mg of thiamine daily from 2 through 24 weeks postpartum. At 2, 4, 12, and 24 weeks postpartum, a full breast expression was collected. Human milk thiamine concentrations were measured by HPLC-FLD. Human milk total thiamine concentrations are reported. Mean differences between treatment groups were assessed using linear mixed-effects models. An ANOVA repeated measures test was used to explore lactation stage changes in total thiamine concentrations in un-supplemented women (placebo group) between each timepoint.

Results: In total, 335 women were randomized: placebo ($n=83$), 1.2 mg ($n=86$), 2.4 mg ($n=81$), and 10 mg ($n=85$). At 24 weeks postpartum, the mean (SD) human milk total thiamine concentrations were significantly higher in all groups receiving thiamine (1.2 mg, 183 (91) $\mu\text{g/L}$; 2.4 mg, 190 (105) $\mu\text{g/L}$; 10 mg, 206 (89) $\mu\text{g/L}$) compared to the placebo group (153 (85) $\mu\text{g/L}$; $p<0.0001$), but groups receiving thiamine did not differ from one another. Among un-supplemented mothers, human milk total thiamine concentrations increased significantly from 116 (54) $\mu\text{g/L}$ at 4 weeks to 147 (74) $\mu\text{g/L}$ at 24 weeks postpartum ($p=0.008$). However, there were no significant differences in milk total thiamine concentrations at various timepoints, compared to 24 weeks (Δ 2 to 24 weeks, Δ 4 to 24 weeks, and Δ 12 to 24 weeks postpartum).

Conclusions and Implications: Thiamine supplementation of 1.2 mg/day in rural, lactating Cambodian women from 2 through 24 weeks postpartum was sufficient to increase milk total thiamine concentrations to levels reached by higher supplementation doses (2.4 mg and 10 mg/day) and comparable to those of adequately nourished mothers in regions where thiamine deficiency is not of concern. Among un-supplemented mothers, milk thiamine concentrations increased between 4 and 24 weeks postpartum. We hope these results can help to inform future interventions to combat maternal and infant thiamine deficiency.

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The entire journey of this masters thesis was an extraordinary privilege, and it would not have been possible without the time, support, and dedication from several individuals.

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List of Abbreviations

AI	adequate intake
ANOVA	analysis of variance
ATP	adenosine triphosphate
CDHS	Cambodia Demographic and Health Survey
CI	confidence interval
d	day
DRI	dietary reference intakes
EAR	estimated average requirement
EDTA	ethylenediaminetetraacetate
eThDP	erythrocyte thiamine diphosphate
FBS	food balance sheets
g	grams
h	hour(s)
HPLC-FLD	high-performance liquid chromatography with fluorescence detector
L	litres
LMIC	low- and middle-income countries
µg	micrograms
mg	milligrams
mL	millilitre
mo	month(s)
ng	nanogram
nmol	nanomole
NAPDH	nicotinamide adenine dinucleotide phosphate
RDA	recommended dietary allowance
SAM	severe acute malnutrition
SD	standard deviation
SDG	sustainable development goals
TDD	thiamine deficiency disorders
ThDP	thiamine diphosphate

ThMP	thiamine monophosphate
ThTP	thiamine triphosphate
THTR1	thiamine transporter 1
THTR2	thiamine transporter 2
UL	tolerable upper intake level
UNICEF	United Nations International Children's Emergency Fund
WEI	Wealth Equity Index
WHO	World Health Organization

Chapter 1: Introduction

Thiamine (vitamin B1) is a water-soluble vitamin essential in energy metabolism, neuronal functioning, and cognitive development. The richest dietary sources of thiamine include whole grains, yeasts, meats, legumes, and nuts. The human body does not store thiamine and requires frequent consumption to maintain adequate thiamine status. Thiamine deficiency can occur at any life-stage, although infants are particularly susceptible to the harmful effects of thiamine deficiency as rapid physical growth and cognitive development occurs during the first few months of life. In infancy, deficiency can lead to infantile beriberi, which can result in death within 24 hours of clinical presentation. The risk of deficiency is particularly high in exclusively breastfed infants when maternal thiamine intakes are low, resulting in low human milk thiamine concentrations. Low dietary thiamine intake predominately occurs in Southeast Asia, including Cambodia, as thiamine-poor foods, such as polished white rice and cassava, are dietary staples. We acknowledge that national data of infantile beriberi prevalence in Cambodia and other regions is unknown due to lack of agreed-upon biochemical cut-offs and its highly variable clinical symptoms. Regardless, the estimated prevalence of thiamine deficiency is highest in Southeast Asia, where research publications and clinical reports still frequently mention cases of infantile beriberi and suboptimal thiamine status.

As a global public health recommendation, infants should receive human milk exclusively for the first six months of life to ensure they receive adequate nutrition required for optimal growth and development and the benefits of protective effects to the infant and mother associated with breastfeeding. Since maternal thiamine consumption impacts milk thiamine, strategies are needed to improve maternal thiamine intake during the perinatal period to ensure that infants receive adequate thiamine to meet their needs for optimal growth and development. Although previous pharmacokinetic studies indicate that high-dose maternal thiamine supplements can improve milk thiamine concentrations acutely, there are limited data on the impact(s) of long-term, low dose supplementation on human milk thiamine concentrations during the exclusive breastfeeding period. We hope this research can elucidate the effects(s) of various maternal thiamine doses on milk thiamine content. This information could help inform future public health interventions that

could combat thiamine deficiency and reduce infant mortality in Cambodia and other beriberi-endemic regions.

This research was part of a larger, 22-week double-blind, four-parallel arm, placebo-controlled, randomized trial of thiamine supplementation among 335 lactating Cambodian women in Kampong Thom province. Participants were randomized to one of four treatment arms and asked to consume one oral capsule daily from 2 through 24 weeks postpartum. Treatment arms included: a negative control group (placebo; 0 mg thiamine), an Estimated Average Requirement (EAR) group (1.2 mg thiamine), a double EAR group (2.4 mg thiamine), and a positive control group (10 mg thiamine). At 2, 4, 12 and 24 weeks postpartum, a full breast expression was collected to assess human milk total thiamine concentrations. This study aimed to measure and assess differences between human milk total thiamine concentrations at 2, 4, 12, and 24 weeks postpartum among rural Cambodian women on various thiamine supplementation regimens and explore potential lactation stage changes to milk thiamine over this time period.

Chapter 2: Literature Review

2.1 Cambodia

The Kingdom of Cambodia is located in mainland Southeast Asia bordering Thailand, Laos, and Vietnam, and shares a coastline with the Gulf of Thailand (1). Cambodia is an agricultural country, with a long-standing history and comparative advantage in rice production (2,3), due to its tropical climate influenced by the annual monsoon cycle with two distinct seasons (1). Monsoon season with strong winds and rain occurs from May to November, followed by a dry season from December to April, with little temperature variation between seasons (1). The climate sets the rhythm for most Cambodians as 81% of the population still live in rural areas dependent on agriculture activities (2). However, recent rapid urbanization and internal migration have caused the population of the capital city, Phnom Penh, to more than double in the last 15 years (2).

Cambodia has an unfortunate history as war with widespread social, political and economic chaos overwhelmed the country throughout the 1970s and 1980s (2). In 1993, the United Nations Transitional Authority set out on what was then the largest international peacekeeping mission, to set up and supervise Cambodia's first free election. The Cambodian People's Party have been in power ever since (2). For many years following the conflicts, Cambodia was the poorest country in Asia (2). During the last two decades, Cambodia has made great progress, including a notable economic growth since 2002, with an average gross domestic product growth of 7 percent per annum (2).

2.1.1 Maternal, infant and young child malnutrition in Cambodia

In 2016, the United Nations General Assembly put into action 17 Sustainable Development Goals (SDG) to be achieved before the year 2030 (4). Zero Hunger is SDG number two, which includes ending hunger and all forms of malnutrition (4). To highlight their commitment, the Ministry of Health of the Kingdom of Cambodia, in collaboration with the World Health Organization (WHO), released its Country Cooperation Strategy in 2016 (5). Thus, this research is of particular importance as Health Development Goal number one in Cambodia's Country

Cooperation Strategy was to reduce maternal, newborn, and child mortality, and malnutrition among women and children (5).

Cambodia has made significant progress in improving maternal, infant, and young child health and nutrition outcomes. Mortality is one of the proxy indicators of health. From 2005 to 2014, the country's maternal mortality rate declined from 472 per 100,000 live births to 170; infant mortality rate decreased from 66 per 1,000 live births to 28, and the under-five mortality rate decreased from 83 per 1,000 live births to 35 (2). However, despite this progress, infant mortality still varies greatly between rural and urban areas, with rates in rural areas more than three times the rates in urban areas, with 42 versus 13 deaths per 1,000 live births, respectively (2).

Malnutrition is defined as deficiencies, excesses, or imbalances in a person's intake of energy and/or nutrients (6). Economically developing countries where malnutrition is present as undernutrition or overnutrition suffer from a "double-burden" of disease (7). Undernutrition can be in the form of 1) acute malnutrition, also known as wasting (with low weight-for-height and/or MUAC as indicators), 2) chronic malnutrition, also known as stunting (with low height-for-age as an indicator), and 3) micronutrient deficiencies (which manifest as "hidden hunger"). Malnutrition in the forms of chronic undernutrition and micronutrient deficiencies are of particular interest to this research, as overnutrition is not prevalent in rural Cambodian women and infants. The 2014 Cambodian Demographic and Health Survey (CDHS) reported that among Cambodian children under five years of age, 1 in 3 suffers from stunting and 1 in 10 from wasting (2). The Global Hunger Index scores countries on four indicators (undernutrition, and child wasting, stunting, and mortality) to measure a country's level of hunger on a 100-point scale (8). Cambodia scored 22.8 on the 2018 Global Hunger Index, indicating the country suffers from a level of hunger that is serious (8).

The consequences of malnutrition are often transgenerational, resulting in detrimental impacts on the next generation (8). In this intergenerational cycle of malnutrition, the infants of malnourished mothers are at higher risk of malnutrition (9). The consequences of malnutrition in the first 1,000 days (from conception to two years of age) substantially increase a child's vulnerability to poor health and impaired growth and cognitive development, which can lead to

reduced productivity and poorer economic outcomes in the later stages of life, as well as early mortality (10).

2.1.2 Food insecurity and the traditional Cambodian diet

Food insecurity and low intake of nutrient-dense foods are two of the leading factors that contribute to nutrient deficiencies and malnutrition, especially in women and children (10,11). In a study among 900 households in rural Prey Veng province, MacDonald *et al.* reported a high prevalence of food insecurity, where 82% of households were food insecure (12), as measured using the Household Food Insecurity and Access Scale (13). The same study highlighted low dietary diversity with a mean Household Dietary Diversity Scale score of 4.7 out of a possible 12 (12). Similarly, Vicheth Som and colleagues reported low dietary diversity in rural pregnant and lactating women, as measured through the Women's Dietary Diversity Score (14). Additionally, the Cambodia Ministry of Health has reported that the traditional Cambodian diet is primarily plant-based, with little dietary diversity, and low in energy and fat (15). Polished white rice is a staple food in Cambodian households (16), with daily intakes ranging from 302-823 g/person, contributing to upwards of 60% of daily energy intakes based on National Food Balance Sheets (FBS) (17).

This estimate is limited as FBS do not measure food consumption by household or individuals as it is a measurement of the food available for consumption at the national level and cannot be disaggregated to indicate the differences in food consumption by different population groups (e.g. different socioeconomic groups, geographical locations (urban/rural) within a country, etc.), seasonal variation in the food supply, or food wastage (18). High consumption of white, polished rice paired with limited dietary diversity is of particular concern as rice can provide adequate energy but lacks other essential micronutrients (10). Consequently, malnutrition in Cambodia often presents as “hidden hunger”, a term that characterizes malnutrition when energy intake is adequate, but the intake of micronutrients is severely lacking (19). Ergo, deficiency in the micronutrient thiamine in Cambodia is likely due to the traditional thiamine-poor diet based largely on polished white rice (20). There is limited data available on the current daily dietary thiamine intake in Cambodia. However, Gibson *et al.* estimated a daily per capita thiamine intake of 0.58 mg based on national FBS (21), which is approximately one-third of the

Recommended Dietary Allowance (1.4 mg/d) for lactating women (22). See *Section: 2.6.3* for the prevalence of thiamine deficiency in Cambodian mothers and their infants based on biochemical biomarkers.

The proposed research was conducted in rural Kampong Thom, Cambodia. Kampong Thom is located in central Cambodia (see **Figure 2-1**), and it is the second-largest province by area. Based on the 2014 CDHS, 63% of Kampong Thom's population falls within or below the middle wealth quintile (2). Recent unpublished data from the 2014 Cambodian National Micronutrient Survey showed women of reproductive age (16-49y) in Kampong Thom had among the lowest blood thiamine concentrations in the country.



Figure 2-1 Map of Cambodia (*public domain*).

2.3 Thiamine

Thiamine (vitamin B1) is an essential micronutrient that plays a crucial role in energy metabolism (23–25). Thiamine's chemical definition is 3-[(4-amino-2-methyl-5-pyrimidinyl)methyl]-5-(2-hydroxyethyl)-4-methylthiazolium (26); thiamine contains a pyrimidine ring and a thiazole ring connected by a methylene bridge. It was the first vitamin to be discovered, due to early research on the 'anti-beriberi factor' found in rice polishings (23). Additionally, thiamine was also the first vitamin to be isolated and synthesized (26). In 1911, Casimir Funk crystallized an amine substance from rice bran and called it 'vitamine' from 'vital amine', as he was convinced it contained the anti-beriberi factor (27). Jansen and Donath, in 1926, isolated thiamine from rice bran and named it aneurine (26). Unfortunately, the sulphur atom was missed in their published formula (26). In 1936, the first correct formula for thiamine was published by Williams and Cline (28), who also synthesized it and re-named it 'thiamin' (29). The American Medical Society added an 'e', to indicate its amine nature, and 'thiamine' is now the recognized term (26).

Thiamine-rich tissues include skeletal muscles, heart, liver, kidney, and brain (30). Thiamine exists in the human body as free thiamine, as well as in several phosphorylated forms: thiamine monophosphate (ThMP), thiamine diphosphate (ThDP), and thiamine triphosphate (ThTP) (23,25); see **Figure 2-2**. ThDP, also known as thiamine pyrophosphate, is the most abundant derivative in human tissue, making up nearly 90% of total body thiamine (31).

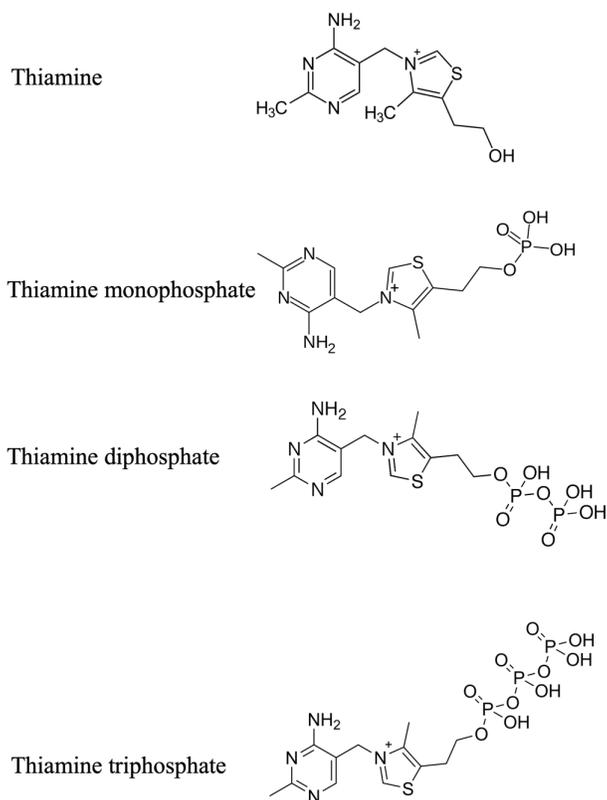


Figure 2-2. Chemical structures of thiamine vitamers: thiamine, thiamine monophosphate, thiamine diphosphate, and thiamine triphosphate (*public domain*).

2.3.1 Thiamine absorption and transport

Thiamine-phosphorylated derivatives must undergo hydrolysis into free thiamine by phosphatases in the intestinal lumen before absorption into the enterocytes (16). The concentration of free thiamine dictates the absorption mechanism utilized (25): free thiamine absorption into the enterocyte occurs by active transport at concentrations $<2 \mu\text{mol/L}$ and passive diffusion at increased concentrations (24). Transportation of thiamine across membranes is primarily by-way-of two different solute carrier proteins, thiamine transporter 1 (THTR1) in systemic tissues and skeletal muscle and thiamine transporter 2 (THTR2) in the intestines (25). Intestinal transport of thiamine in humans is rate-limiting (24). At high doses, only a little thiamine is absorbed and even less retained (32). For example, a single oral dose of 2.5 to 5 mg thiamine is predominately unabsorbed in healthy adults (31).

Within the enterocyte, free thiamine is phosphorylated and then de-phosphorylated for transport to serosal cells and the bloodstream (16). In the blood, ThMP and free thiamine are found in low concentrations in the plasma or serum, whereas ThDP is present in high levels in the erythrocytes and leukocytes (16). Excess thiamine is rapidly cleared by the kidneys and excreted in the urine (24,25).

2.3.2 Intracellular thiamine metabolism

ThDP is the metabolically active derivative of thiamine (see **Figure 2-3** for the conversion of thiamine to ThDP) and is required as a cofactor in various enzyme complexes vital for the metabolism of carbohydrates and amino acids (20,24,26). These enzyme complexes include pyruvate dehydrogenase (which catalyzes the oxidative decarboxylation of pyruvate to form acetyl-coenzyme A (acetyl-CoA)), the α -ketoglutarate dehydrogenase complex (which catalyzes the oxidative decarboxylation of α -ketoglutarate to succinyl-CoA), and the branched-chain α -ketoacid dehydrogenase complex (which catalyzes the irreversible oxidative decarboxylation of branched-chain α -ketoacids to their corresponding acetyl-CoAs) (20,26).

Additionally, ThDP is a required cofactor for cytosolic transketolase in the pentose phosphate pathway (also referred to as the phosphogluconate pathway and the hexose monophosphate shunt) (20,26). The pentose phosphate pathway is an alternative to glycolysis, generating nicotinamide adenine dinucleotide phosphate (NADPH), pentoses, and ribose-5-phosphate. **Figure 2-4** outlines the summary of ThDP in the mentioned pathways. A deficiency in ThDP results in a reduction in the activity of these enzyme complexes, which results in: decreased adenosine triphosphate (ATP) and NADPH synthesis, oxidative damage, impaired mitochondrial function, and cell death (see **Figure 2-5**) (20,26). Independent of its function in carbohydrate metabolism, ThDP has additional roles in neuronal communication, immune system activation, signalling and maintenance processes in cells and tissues, and cell-membrane dynamics (24,25).

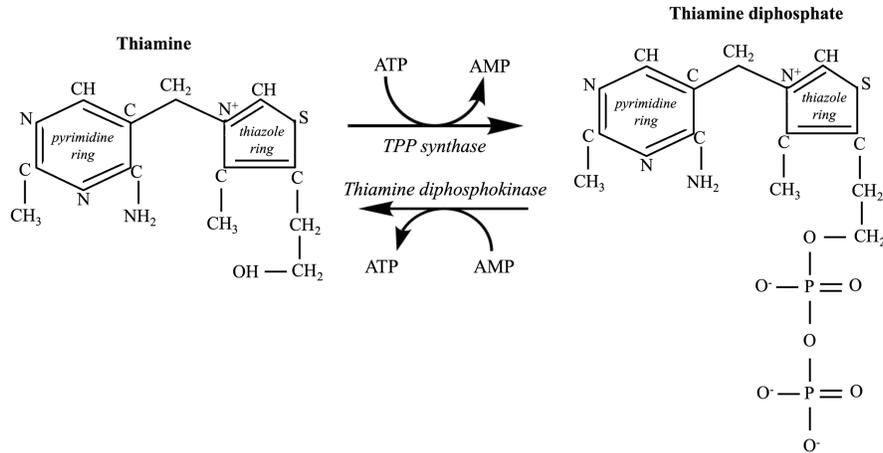


Figure 2-3. Conversion between thiamine and the biologically active derivative, thiamine diphosphate (ThDP).

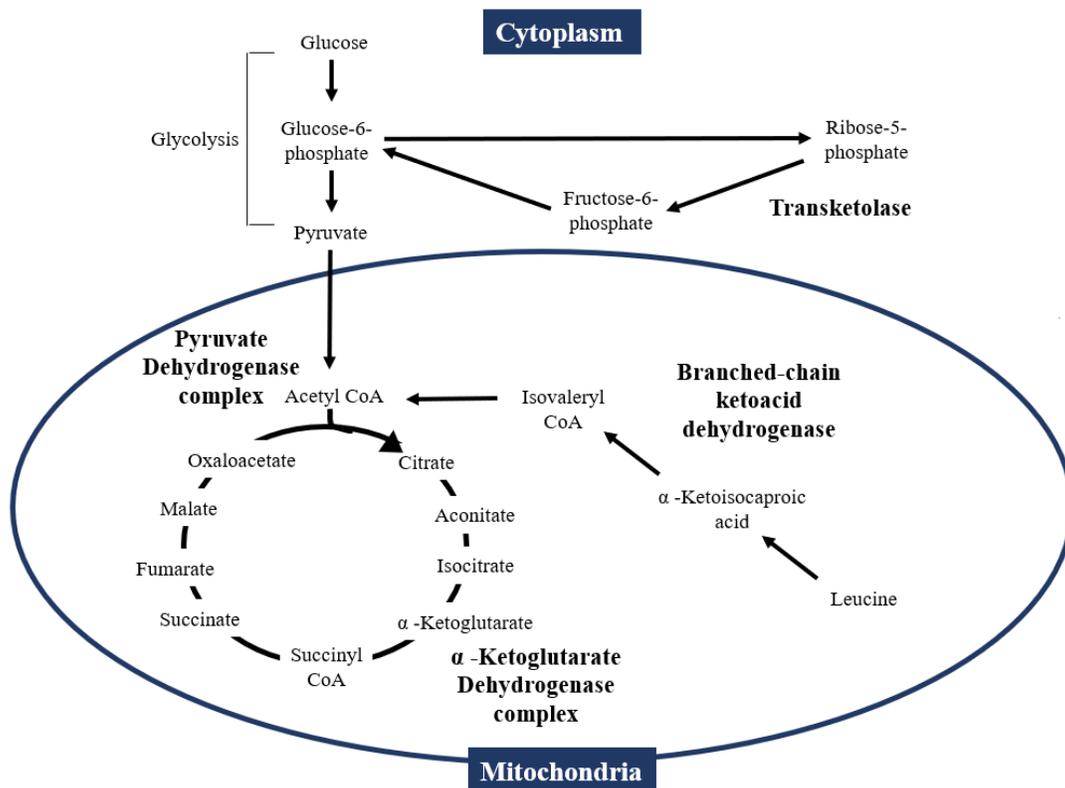


Figure 2-4. ThDP (as a cofactor required for several metabolic processes, displayed in bold text); adapted from (20).

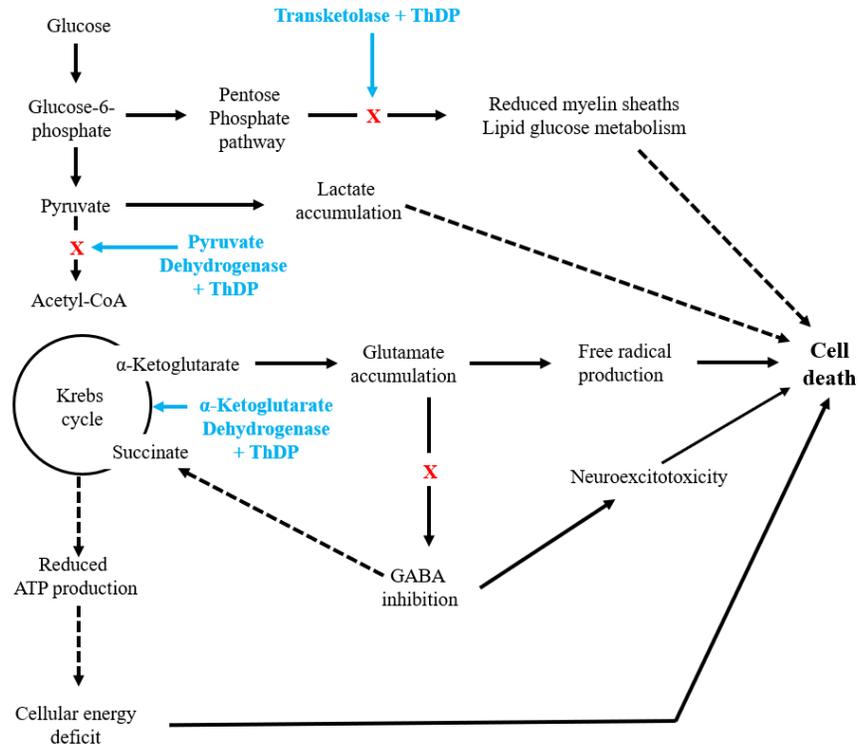


Figure 2-5. The three enzyme complexes ThDP is a cofactor for and their function in cell death pathogenesis in thiamine deficiency. Dashed lines show indirect pathways; adapted from (20).

2.4 Dietary thiamine sources

With a limited half-life of only 9-18 days (16), and the human body unable to store thiamine, frequent dietary intake is required to maintain sufficient thiamine status (24). Although human intestinal bacteria can synthesize thiamine in scant amounts, it is minimally/not absorbed (24).

The richest dietary sources of thiamine include whole grains, yeasts, meats, legumes, and nuts (20) (see **Table 2-1**). As mechanical milling and processing techniques replaced home-pounding grain techniques in the late 19th century, some countries began to enrich wheat flour to address micronutrient deficiencies caused by the loss of thiamine and other vitamins during processing (20,33). In high-income countries, thiamine-fortified and -enriched foods provide nearly half of the total thiamine consumed (34). Unfortunately, in low- and middle-income countries (LMIC) where dietary diversity is often limited, thiamine fortification is less common, therefore not contributing to thiamine intakes (20). Since thiamine is both water-soluble and heat-labile, considerable losses occur through cooking, and when cooking water is discarded (26).

Subsistence farming is also common in rural areas. Light, oxidation, moisture, pH, and the presence of sulphites also mediate thiamine degradation (20).

Table 2-1: Thiamine content of select foods.

Food	Thiamine content (mg/100g) ¹
Pork loin (roasted)	1.02
Beef	
<i>Liver (braised)</i>	0.194
<i>Kidney (simmered)</i>	0.160
Tofu (fried)	0.170
Kidney Beans	0.160
Lentils	0.169
Green Peas	0.259
Rice	
<i>Brown rice (cooked)</i>	0.102
<i>White rice (cooked)</i>	0.020
<i>Parboiled (cooked)</i>	0.074
<i>White rice - enriched (cooked)</i>	0.167
Fortified breakfast cereals	
<i>Cheerios</i>	1.30
Bread	
<i>Whole wheat bread</i>	0.522
<i>White bread (thiamine enriched)</i>	0.510

¹ Values derived from the National Nutrient Database for Standard Reference (Release 28, software v.3.9.5.3), United States Department of Agriculture Agricultural Research Service.

2.5 Dietary thiamine requirements

The Dietary Reference Intakes (DRI) for thiamine were established in 1998 (22); see **Table 2-2** for thiamine DRIs. DRI is the umbrella term used for four different reference values, which vary by life stage and sex group: Estimated Average Requirement (EAR), Recommended Dietary Allowance (RDA), Adequate Intake (AI), and Tolerable Upper Intake Level. The EAR is the estimated average level of daily intake to achieve the requirements of fifty percent of healthy individuals in a population sub-group (22). The EAR for thiamine was determined using data from multiple depletion-repletion studies designed to establish the minimum levels of thiamine intake required to prevent clinical signs of deficiency (35). However, these studies were conducted in the 1940s and 1950s with small sample sizes (36). The EAR was used to calculate the RDA ($RDA = EAR + 2SD$), which is the estimated average level of daily intake that fulfils the nutrient requirements of almost all (97.5%) healthy individuals in a population sub-group

(22). When there is insufficient evidence to establish an EAR and subsequently set an RDA, an Adequate intake (AI) is set (37). AIs reflect the recommended average daily intake level based on observed or experimentally obtained daily nutrient intake estimations by a group, or groups, assumed to be healthy and well-nourished individuals (22). The UL is the maximum daily nutrient intake level that is determined to likely present no risk of harmful health effects to the majority of individuals in a specific life-stage and sex-group (22). Currently, there is no UL for thiamine, as no negative health outcomes have been reported due to excessive intake (38). This is not uncommon, with approximately half of water-soluble vitamins lacking a set UL (i.e. thiamine, riboflavin, pantothenic acid, biotin, cobalamin). The DRIs of particular interest for this thesis is the EAR and RDA for pregnant and lactating women and the AI for infants 0-6 months.

Table 2-2: Criteria and dietary reference intake values for thiamine by life stage group (22).

Life Stage Group	EAR ^a (mg/d)		RDA ^b (mg/d)		AI ^c (mg/d)	
	Male	Female	Male	Female	Male	Female
0 - 6 months	-	-	-	-	0.2	0.2
7 - 12 months	-	-	-	-	0.3	0.3
1 - 3 years	0.4	0.4	0.5	0.5	-	-
4 - 8 years	0.5	0.5	0.6	0.6	-	-
9 - 13 years	0.7	0.7	0.9	0.9	-	-
14 – 18 years	1.0	0.9	1.2	1.0	-	-
19 - 30 years	1.0	0.9	1.2	1.1	-	-
31 - 50 years	1.0	0.9	1.2	1.1	-	-
> 50 years	1.0	0.9	1.2	1.1	-	-
Pregnancy (14-50 years)	-	1.2	-	1.4	-	-
Lactation (14-50 years)	-	1.2	-	1.4	-	-

^a Estimated Average Requirement (EAR), the nutrient intake level that meets the estimated needs of half the individuals in a population group.

^b Recommended Dietary Allowance (RDA), the nutrient intake level that meets the estimated needs of 97%-98% of the individual in a population group (mathematically derived as EAR + 2SD).

^c Adequate Intake (AI), the average intake of a nutrient that appears to meet the nutrient intake requirements of a group, is used in the absence of sufficient evidence to derive an EAR.

2.5.1 Pregnancy & lactation

During both pregnancy and lactation, thiamine DRIs for women increase (37). During pregnancy, a women’s thiamine requirements are increased by approximately 30% based on increased growth in both the mother and fetus and a slight increase in maternal energy requirements, resulting in an additional 0.27 mg/d (rounded up to 0.3 mg/d) for an EAR of 1.2 mg/d. During lactation, it is estimated that 0.16 mg/d of thiamine is transferred into maternal

milk when daily milk production is 780 mL/d. An additional 0.3 mg of thiamine was added to the EAR for non-pregnant, non-lactating women to account for the thiamine transferred into human milk (0.2 mg/d) and the increased maternal energy requirements associated with milk production (0.1 mg/d) (37).

2.5.2 Infants

For infants aged 0-6 months, the AI is set at 0.2 mg/d and increases to 0.3 mg/d for infants aged 7-12 mo (20). These AIs were established based on a 1985 report of human milk thiamine concentrations obtained from healthy, well-nourished mothers; where the mean (SD) for thiamine concentration of mature milk was 210 (40) µg/L and the estimated human milk intake of 780 mL/d (0-6 months) (39,40) and 600 mL/d in addition to the average daily intake of thiamine provided by complementary foods (7-12 months) (22,37). It is notable that the studies referenced in the 1985 report used to set the AIs for infants had small sample sizes ($n=24$ American women) and used potentially outdated laboratory assessment methods (39,40). A recent study by Hampel *et al.* used an ultra-performance liquid chromatography-tandem mass spectrometry method to analyze the human milk samples, which offers improved resolution, speed and sensitivity compared to previous methods (41). This study found a wide variation in mature human milk (≥ 2 weeks postpartum) thiamine concentrations by geographic location globally (41), and none of the milk samples achieved the concentrations used to set the AI (37,42), see **Table 2-3** (37,42). Although, the significant differences across geographic origins disregard that samples were non-representative and collected at different lactation stages. Nevertheless, consistent with these findings, others have also identified a low prevalence of infants meeting the AI from human milk alone in the first six months, suggesting that the AI may need to be reviewed (43).

Table 2-3 Median thiamine concentrations and range of human milk samples from different geographic origins; adapted from (41).

	Cameroon ($n=5$)	China ($n=5$)	India ($n=24$)	Malawi ($n=18$)	USA ($n=28$)
Milk total thiamine concentration (µg/L)	116 (86 – 221)	31 (15 – 127)	11 (4 – 75)	21 (2 – 152)	37 (5 – 66)

Cameroon, 13 – 104 weeks postpartum; China, 2 – 35 weeks postpartum; India, 12 – 24 weeks postpartum; Malawi, 2 – 24 weeks postpartum; USA, 4 – 12 weeks postpartum.

2.5.3 Maternal transfer of thiamine to the infant

During the third trimester, thiamine is diverted predominately to the fetus and placenta (44). Baker *et al.* found that thiamine concentrations in umbilical cord blood were more than three times that of maternal blood at birth (91 ng/mL vs. 27 ng/mL) (45). Infant ThDP status at birth is also usually higher than maternal status (44). In a recent study in Prey Veng, Cambodia, authors postulated that improved maternal thiamine intake in the third trimester of pregnancy likely resulted in improved fetal uptake *in utero* (43). During lactation, maternal transfer of thiamine into human milk is estimated at 0.16 mg/d (37). Additionally, McGready *et al.* observed that, despite the high incidence of thiamine deficiency among the Karen refugees on Thailand's western border, un-supplemented women ($n=9$, 3 months postpartum) had median milk thiamine concentrations of 117 $\mu\text{g/L}$ (46), which falls within the lower range of 100 – 200 $\mu\text{g/L}$ for European mothers previously reported by Dostálova (47). This finding led McGready and colleagues to suggest preferential delivery of thiamine to human milk at the expense of the mother (46).

2.6 Thiamine deficiency

2.6.1 Historical context

In the seventeenth century on the island of Java, Jacobus Bonitus, a Dutch physician, described patients “with their knees shaking and legs raised up, walk[ing] like sheep. It is a kind of paralysis, or rather tremor...” (26). This was the first description of beriberi, which means sheep in the local language, to refer to patients' characteristic gaits. It was not until much later, in 1882-87, that the cause of beriberi being dietary in nature was first discovered (26). Kanehiro Takaki, a Japanese navy surgeon, concluded a significant reduction in the prevalence of kakké (Japanese term for beriberi, meaning leg disease) on voyages where some of the white rice was replaced with meat, dry milk, bread and vegetables (23,48,49). In 1886, Dr. Christian Eijkman strengthened the notion that beriberi resulted from a diet of white rice. He discovered that fowl fed polished rice developed thiamine deficiency symptoms such as polyneuritis; whereas feeding unpolished rice or rice polishings prevented - and even cured - the disease (50). Infantile beriberi was first referenced in the literature in 1888 when Hirota observed Japanese infants breastfed by women experiencing beriberi developed thiamine deficiency symptoms (51). Following this discovery, he also described similar symptoms in infants breastfed by assumed healthy mothers

and/or wet nurses and called this condition “breast milk intoxication” (51). Today, thiamine deficiency is recognized as a cause of peripheral, central, and autonomic nervous system dysfunction (dry beriberi), cardiomyopathy with congestive cardiac failure (wet beriberi), and severe lactic acidosis (49).

2.6.2 Disorders associated with thiamine deficiency

A technical report by Whitfield *et al.* proposed adopting the collective term ‘thiamine deficiency disorders (TDDs)’ to portray the broad scope of overlapping clinical presentations attributable to thiamine deficiency throughout the various life stages (20). See **Table 2-4** for various clinical presentations of TDDs.

Amongst populations at risk for thiamine deficiency, exclusively breastfed infants in the first three months of life appear to be at the highest risk of developing TDD (52). In infants, TDD typically presents during the exclusive breastfeeding period among infants whose mothers have suboptimal thiamine intake and/or status, as they produce milk low in thiamine (53). Mothers often remain asymptomatic (54,55), while infants develop beriberi, likely due to an extremely high metabolic rate compared to body size (55), with subsequent infantile mortality peaking at three months of age (56). Among infants under four months of age, thiamine deficiency often manifests with nonspecific indications, including “irritability, refusal to breastfeed, tachycardia and tachypnea, vomiting and incessant crying that is often described as “loud” or “piercing” and in some cases evolves into a “silent cry,” or aphonia...” (20). The advancement of the disease results in indications and symptoms of congestive heart failure (tachypnea, tachycardia, pulmonary edema, hepatomegaly, and possibly cyanosis). Once an infant has reached this symptomatic stage, their clinical condition often declines rapidly (20). This broad spectrum of general clinical features confounds diagnosis, and infantile beriberi often goes wrongly diagnosed as a viral infection, pneumonia, typhus, or malaria (49,57,58). Infants often die before the correct diagnosis of infantile beriberi, as it can be fatal within 24 hours of the onset of clinical symptoms without rapid thiamine administration (55,59).

TDD is the most damaging and life-threatening in infants because this is a life stage of rapid growth and development (24). For example, an outbreak of thiamine deficiency in Israel, caused

by the routine consumption of thiamine-deficient soy infant formula, resulted in infant mortality. However, infants who survived have shown long-term medical, neurodevelopmental, and gross motor impairments (60,61). This unfortunate ‘natural experiment’ emphasizes the importance of adequate thiamine in early life, as sub-clinical thiamine deficiency can still have detrimental impacts.

Table 2-4. Clinical spectrum of thiamine deficiency disorders; adapted from (20).

Clinical Presentation		Life stage
Acute Cardiologic Form	<ul style="list-style-type: none"> - Colic - Restlessness - Anorexia - Vomiting - Edema - Cyanosis and breathlessness with signs of heart failure - Pernicious form or Shoshin Beriberi (rapid onset) - Sudden cardiogenic shock 	Peak prevalence among breastfed infants, 1 – 3 mo of age
Aphonic Form	<ul style="list-style-type: none"> - Initially hoarse cry until no sound is produced while crying - Restlessness - Edema - Breathlessness 	Peak prevalence at 4 – 6 mo of age
Pseudo Meningitic Form	<ul style="list-style-type: none"> - Nystagmus (involuntary eye movement) - Muscle twitching - Bulging fontanelle - Convulsions - Unconsciousness 	Peak prevalence at 7 – 9 mo of age
Encephalopathy	<ul style="list-style-type: none"> - Psychomotor slowing or apathy - Nystagmus or ophthalmoplegia - Ataxia - Impaired consciousness - Coma 	Generally older children or adults, but also seen in infants
Peripheral Neuropathies	<ul style="list-style-type: none"> - Pain - Tingling or loss of sensation in hands and feet (peripheral neuropathy) - Muscle wasting with loss of function or paralysis of lower extremities - Loss of ankle and knee reflexes - Cranial nerve impairment 	Older children or adults

2.6.3 Prevalence of thiamine deficiency: mothers and their infants

Thiamine deficiency still occurs endemically in rural areas of Southeast Asia, particularly Myanmar, Laos, and Cambodia (54,59,62,63). Various factors complicate the accurate assessment of the prevalence of low-thiamine status, including the lack of agreed-upon biochemical cut-offs (56,63) and numerous cut-offs available in the literature (56).

A recent study by Whitfield *et al.* used the most conservative cut-off (eThDP<120 nmol/L (64,65)) and found that 27% of Cambodian women of childbearing age and 38% of Cambodian infants aged 6-12 mo were thiamine deficient (56). However, in the same study, prevalence rates of thiamine deficiency increased to 78% and 72 % in Cambodian women of childbearing age and infants (6-12 months) when the most liberal cut-off (eThDP<180 nmol/L (66)) was used (56), respectively. In a recent, small, cross-sectional study, women of reproductive age in both rural and urban Cambodia had significantly lower erythrocyte ThDP compared to a sample of purportedly thiamine-adequate women from urban Canada (63,67). These studies highlight that even though a universal thiamine status cut-off has yet to be established, there is evidence of low blood thiamine concentrations among Cambodian mothers and their infants, with infants <12 mo at the highest risk (56,63,67).

It is equally challenging to assess clinical cases of thiamine deficiency accurately. Cases of infantile beriberi have been identified in regions of South and Southeast Asia, specifically Myanmar (46,49), Laos (55,59,62), and Cambodia (54,68). Cases have also been observed in West Africa, Angola, Mayotte Island, Kiribati, Cuba, and some Caribbean areas (20). However, TDD, including infantile beriberi, present with a broad spectrum of general clinical features that overlap other conditions, often causing thiamine deficiency disorders to go undiagnosed and subsequently unreported, making prevalence estimates challenging (20,35,57).

2.7 Risk factors for thiamine deficiency

2.7.1 White rice in Southeast Asia

In Southeast Asia, thiamine deficiency remains a public health issue largely due to the high consumption of non-parboiled, unfortified, polished, white rice (56,57,69). Polished white rice

continues to be the favoured type of rice in high rice intake countries like Cambodia for its: ideal organoleptic qualities, the symbolism of economic status (48), increased shelf-life with the removal of the lipid- and thiamine-rich bran (70), and decreased fuel and water resource needs to cook (70,71).

While rice naturally contains thiamine (24), it is found mostly in the outer husk and bran (only ~2 mg of thiamine per kg in the endosperm of the rice kernel), which is removed through the milling process, resulting in the low-thiamine content of polished white rice (72). Parboiling rice before milling allows some influx of thiamine from the aleurone layer (outermost layer) into the endosperm (inner layer), reducing thiamine losses (20). However, parboiling rice is not commonly practised in Southeast Asia: traditionally, rice underwent long-term solar drying that resulted in a musty aftertaste caused by mould growth from the water migration into the endosperm (20,72), as well as a darkening of the rice grain (20). While modern parboiling processes have addressed the taste issue, the colour change remains and adversely impacts consumption patterns (20). Thiamine fortification of rice would not be viable in rural Cambodia as thiamine is both water-soluble and heat-labile, resulting in considerable losses through cooking and discarding the cooking water (26). In addition, rice fortification in Cambodian would be infeasible as rice is grown and milled at the community level, lacking a 'central production' essential for fortification programs. Therefore, rice fortification in Cambodia would be extensively decentralized, which would result in increased cost of fortification programs and convoluted quality control measures (20).

In addition to polished, white rice being thiamine-poor, increased consumption can further exacerbate thiamine deficiency in another respect. Increased dietary intake of carbohydrates (such as white rice) increases thiamine requirements as thiamine is required for carbohydrate metabolism. For example, in a short-term trial among 12 healthy Austrians ($n=6$ women, 6 men), diets that increased from 55% carbohydrate (control diet) to 65% and 75% carbohydrates resulted in significantly lower plasma and urine thiamine concentrations, suggesting higher thiamine utilization at increased carbohydrate intakes (73). Thus thiamine deficiency in Cambodia is likely a result of increased needs due to a traditional diet where upwards of 60% of the daily energy intake is from carbohydrate sources such as white-polished rice (17).

2.7.2 Co-morbidities & drug therapies

Children with severe acute malnutrition (SAM) have various risk factors for thiamine insufficiency. Yet, Hiffler *et al.* highlighted that the thiamine content of F-75, a therapeutic refeeding milk formula that is used for several days upon a critically ill child's admission to hospital for SAM, possibly has insufficient amounts of thiamine (0.5–1.7 mg/d depending on the total daily F-75 intake) (74). Co-morbidities such as shock, fever, and sepsis in tandem with SAM may further perpetuate decreased thiamine levels and/or may increase thiamine requirements (75). Overall, in critically ill individuals, particularly children, thiamine deficiency can be worsened by inadequate absorption, increased digestive losses, and systemic inflammation (57). Similar to many other micronutrients, thiamine status/absorption can be adversely impacted by any medications that accelerate gastric emptying or induce intestinal malabsorption (57). Loop diuretic therapy, commonly employed in managing congestive heart failure, may lead to excessive thiamine losses post-absorption (76). However, neither of these are of major concern in the context of this thesis.

2.7.3 Food insecurity, thiamine antagonists, and thiaminases

Thiamine deficiency is rare in food-secure settings with access to diverse diets (20). Increased risk of thiamine deficiency typically occurs under conditions that contribute to food deficits or limited dietary diversity, such as conflict or displacement due to war, famine, drought, or a natural disaster (49,77–79). Issues with low thiamine intakes can be exacerbated when local diets also incorporate the frequent consumption of tea leaves or betel nut (which contain thiamine antagonists) or raw/fermented fish or African silkworm larvae (which have thiaminases) (20,59,80). Thiamine antagonists compete with thiamine and its derivatives in enzymatic reactions, whereas thiaminases are enzymes that cleave thiamine, rendering it inactive (81). Vimokesant *et al.* found that daily supplementation with 10 mg of thiamine effectively reduced the effect of raw fermented fish consumption on thiamine degradation but was not sufficient to neutralize the effect of betel nut chewing (82). In contrast, Coats *et al.* reported that consumption of foods previously referenced in the literature to contain anti-thiamine compounds (including betel nut leaves and fish paste) were not associated with decreased thiamine levels among lactating Cambodian mothers ($n=54$) (54).

2.7.4 Breastfed infants

As noted in *Section 2.6.2 Disorders associated with thiamine deficiency*, thiamine deficiency can occur at any life-stage, although infants are particularly susceptible to the harmful effects of thiamine deficiency as rapid physical growth and cognitive development occurs during the first few months of life (24). The risk of deficiency is particularly high in exclusively breastfed infants when maternal thiamine intakes are low, resulting in low human milk thiamine concentrations (53).

2.8 Human milk

Human milk is a biological fluid produced by the mammary glands (85), with its nutritional components sourced from the maternal diet, maternal nutrient stores, and lactocyte nutrient production. Human milk is comprised of macronutrients, micronutrients, and various non-nutritive protective factors (e.g. cytokines, growth factors, oligosaccharides, antibodies, and hormones) (85). Human milk composition is not uniform; it is subject to variability within a single feeding, time of day, throughout the stages of lactation, and changes in maternal status, diet, and supplementation (85–87).

The composition of human milk changes throughout lactation, which characterizes the three different lactation stages (colostrum, transition milk, and mature milk) (85). Colostrum is the first fluid produced by the mammary glands post-delivery and is unique in appearance, composition and volume. Colostrum is typically referred to as “liquid gold” as it is thick and yellow in appearance and is rich in developmental factors. The tight junction closure occurs in the mammary epithelium results in a decline in sodium-potassium ratio and an increase in lactose concentration, indicating secretory activation and the beginning of transitional milk production. Transitional milk is produced between five days to two weeks postpartum when there is a significant increase in milk production in order to support the increased milk intake of the rapidly growing infants’ nutritional and developmental requirements. The production of mature milk starts around two weeks postpartum and is solely expressed by four to six weeks postpartum. Mature milk composition in healthy women is thought to remain relatively content through the rest of lactation. (85).

Within a single feeding, foremilk is the first milk to exit the breast, followed by hindmilk, closer to the feed's end (88). Fore- and hind-milk are considerably different in fat content, as it is well known that fat content is higher in hindmilk. For instance, Saarela *et al.* reported that hindmilk contains two to three times more fat than foremilk (89). The micronutrient content of milk, including thiamine, will vary between fore- and hindmilk (86); see *Section: 2.9.2 Non-dietary factors affecting milk thiamine concentrations*. In addition, human milk composition also differs between mothers and populations (41) due to maternal genetic and environmental factors, such as maternal lifestyle and dietary habits (90,91).

2.9 Thiamine in human milk

In human milk, thiamine is present as free thiamine, ThMP, and ThDP (87). The primary derivatives of thiamine in human milk are free thiamine (~30%) and ThMP (~70%) (92,93), whereas ThDP was only first identified in milk in 2016 and is proportionately lower (~1.9-4.5%) (94). Little is known about the transport mechanisms and regulation of thiamine secretion into human milk. However, thiamine concentrations are higher in human milk than plasma levels, suggesting active secretion into milk (95).

2.9.1 Methods for measuring thiamine in human milk

Free thiamine, ThMP, and ThDP in human milk are commonly analyzed via HPLC-FLD, with precolumn derivatization of thiamine vitamers to their thiochrome esters (43). This method allows for precise and highly repeatable measurements (96). However, there are currently no cut-offs for human milk total thiamine concentrations (43).

2.9.2 Non-dietary factors affecting human milk thiamine concentrations

Thiamine concentrations vary in human milk with time within a feed, stage of lactation, and supplementation. A study by Hampel *et al.* found that the thiamine concentrations in milk produced by Bangladeshi mothers differed within a single feed, with significantly higher thiamine concentrations in aliquot II (milk expressed after 2 minutes until breast emptied), compared to aliquot I (milk expressed within the first two minutes) (86). However, the thiamine concentration in the full breast expression aliquot III (aliquot I and aliquot II combined) was not

significantly different from aliquot I (milk expressed within the first two minutes) (86). This finding highlights the importance of assessing a full breast expression when evaluating thiamine concentrations in human milk. A full breast expression of human milk is required as it is a better reflection of infant intake. Thiamine concentrations of human milk increase throughout lactation (39,41,94,97,98). The 1998 report on DRIs by the Institute of Medicine indicated that thiamine concentration was low in colostrum (approximately 0.01 µg/L), whereas in mature human milk, the mean (SD) thiamine concentration was 210 (40) µg/L (37). In 2016, Hampel and colleagues found human milk total thiamine concentrations significantly increased between 2 weeks and 24 weeks postpartum ($p < 0.001$) (94). However, human milk thiamine concentrations seem to plateau at 6 weeks postpartum and are maintained until 6 months postpartum; this same study found human milk total thiamine concentrations did not change from 6 to 24 weeks postpartum (94). These factors have to be considered when collecting human milk samples for thiamine analysis. It is important to collect a full breast expression of mature human milk (> 2 weeks postpartum).

2.9.3 Factors affecting human milk thiamine concentrations

Maternal thiamine intake has been reported to be positively associated with human milk concentrations in both well-nourished and unnourished populations (44,99,100). Human milk thiamine concentrations respond rapidly to supplementation in maternal populations where deficiency is prevalent (101,102) but not in healthy, well-nourished women (39,40). Human milk concentrations, and subsequently infant thiamine status, are dependent on maternal intake (53), suggesting that we need to focus efforts to increase maternal thiamine intake when intake is low during the postpartum period to optimize exclusively breastfed infants' health outcomes.

2.10 Effectiveness of supplementation in lactating women

As noted in *Section 2.9 Thiamine in human milk*, thiamine levels in human milk are directly influenced by maternal thiamine intakes; low thiamine intakes among lactating women can rapidly result in the production of low-thiamine milk (103). It is well-established that maternal supplementation in women with sub-optimal thiamine status improves human milk thiamine concentrations (46,49,101,104,105) and, in turn, infant thiamine status (53). Coats and colleagues found thiamine supplementation increased in human milk from 54 µg/L to 150 µg/L

(179.5–502.7 nmol/L) in severely deficient lactating Cambodian women (101). Whitfield *et al.* reported that maternal consumption of thiamine-fortified fish sauce over six months resulted in higher human milk thiamine concentrations in rural Prey Veng, Cambodia (106). Hampel *et al.* found acute supplementation effects on human milk thiamine concentrations at 2 – 4 hours post supplementation among fasting lactating Bangladeshi women ($n=18$, 2 – 4 months postpartum), highlighting the acute effect of thiamine supplementation on human milk (86). However, maternal supplementation increases milk thiamine levels to a certain extent, indicating a limit in the amount that can be transferred into milk (107).

Conversely, Allen *et al.* (108) found no influence of a lipid-based nutrient supplement (LNS) containing 1.6 mg/d on maternal human milk thiamine concentrations in HIV-infected Malawi women (19-30y). However, the Malawian women in this study were likely already receiving sufficient thiamine in their diet without additional supplementation. Women in the control group had human milk thiamine concentrations of 199 $\mu\text{g/L}$ at 24 weeks postpartum. Similar milk thiamine concentrations have been reported in both thiamine-replete American mothers $n=16$ (6-28 weeks postpartum), with median milk thiamine concentrations of 173 $\mu\text{g/L}$ (101); and in Finnish mothers ($n=57$) consuming a daily 2 mg thiamine supplement with a mean (SD) of 199 (45) $\mu\text{g/L}$ at 6 months postpartum (47).

While it is clear that supplementation of women with sub-optimal thiamine status can increase human milk thiamine concentrations, there are still limited data on the impact of long-term, low dose thiamine supplementation. Further research is needed to investigate how much supplemental thiamine is required to increase human milk thiamine concentrations in lactating Cambodian mothers to those comparable to adequately nourished mothers in regions where thiamine deficiency and infantile beriberi is not of concern. Investigating the impact of various supplementation regimens and potential lactation stage differences may inform future interventions to combat maternal and infantile deficiency during the exclusive breastfeeding period when human milk is the sole source of infant nutrition.

Chapter 3: Rationale and research objectives

3.1 Rationale

Thiamine deficiency and potentially fatal infantile beriberi remain public health concerns in Cambodia and regions where thiamine-poor white rice is the staple food (20,54,59,62,63,67,68). Low maternal thiamine intake (43) results in low milk thiamine concentrations (72), placing exclusively breastfed infants at high risk of developing TDD, including infantile beriberi (53). In addition, even short-term exposure to low thiamine intake in early life may have life-long impacts on cognition (60,61). Improving maternal thiamine intakes can prevent thiamine deficiency among breastfed infants (24,53). However, little is known about the impact of long-term, low dose supplementation on human milk thiamine concentrations. This research aims to address this knowledge gap by determining milk total thiamine concentrations among lactating women who consumed one of three thiamine supplementation doses or a placebo between 2-24 weeks postpartum. In addition, we will assess lactation stage differences over the exclusive breastfeeding period. These results may inform future public health interventions that could target maternal and infantile TDD in Cambodia and other at-risk countries.

3.2 Research Objectives

The objectives of this study are as follows:

1. To report total thiamine concentrations at 2, 4, 12, and 24 weeks postpartum in milk produced by lactating Cambodian women consuming one oral capsule daily containing either 0, 1.2, 2.4, or 10 mg thiamine over a 22-week period between 2 and 24 weeks postpartum.
2. To assess whether there are differences in total thiamine concentrations in milk produced by lactating Cambodian women at 4, 12, and 24 weeks postpartum, by treatment group.
3. To explore whether human milk total thiamine concentrations change over the exclusive breastfeeding period by examining potential lactation stage changes among un-supplemented Cambodian women from 2 through 24 weeks postpartum.



3.3 Hypotheses

1. a) Null hypothesis (H_0): There will be *no difference* in human milk total thiamine concentrations between any of the treatment groups.
b) Research hypothesis (H_A): The total thiamine concentration in milk produced by women in the 10 mg group will be the highest, and milk from women in the 0 mg group will contain the lowest total thiamine concentrations.
2. a) Null hypothesis (H_0): There will be *no difference* in human milk total thiamine concentrations between un-supplemented mothers between collection timepoints.
b) Research hypothesis (H_A): The total thiamine concentrations in milk produced by women at 24 weeks postpartum will be the highest, and milk produced at 2 weeks postpartum will contain the lowest total thiamine concentrations.

Chapter 4: Methodology

4.1 Study Design

This research was a secondary outcome of the larger *Trial of thiamine supplementation in Cambodia* (ClinicalTrials.gov Identifier: NCT0361628) (109). The details below specifically relate to this thesis: a community-based, double-blind, four-parallel arm, placebo-controlled randomized trial. Each mother-infant dyad participated in the study for 22 weeks, from 2 through 24 weeks postpartum (see **Figure 4-1**) (109).

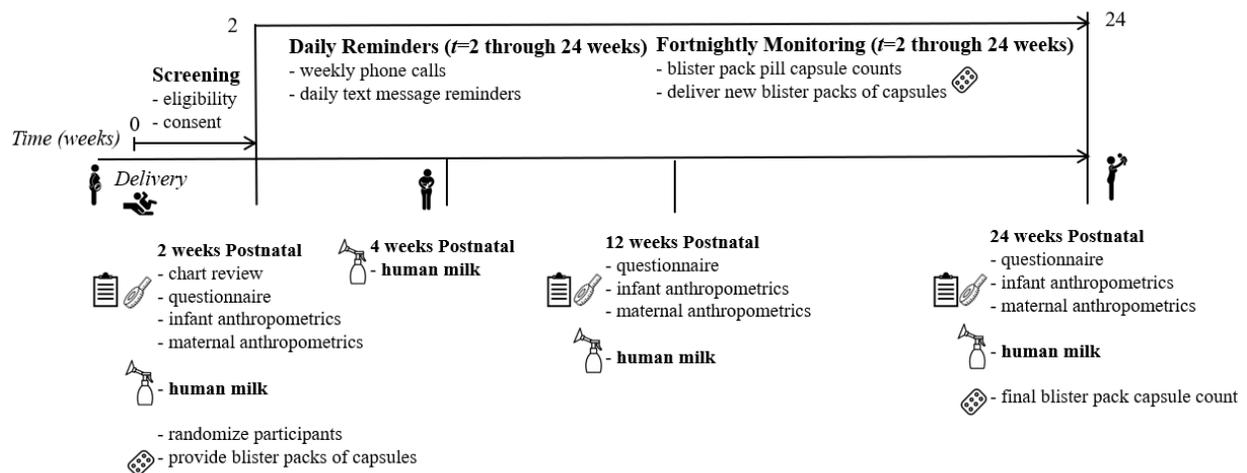


Figure 4-1: Study timeline and data collection schedule for *Trial of thiamine supplementation in Cambodia*; Adapted from *Whitfield et al.* (109).

4.2 Study Treatments

4.2.1 Treatment arms

Participants were randomized to one of four treatment arms and asked to consume one oral capsule daily. The EAR dose aligned with the National Academy of Medicine (previously the Institute of Medicine) EAR of 1.2 mg/d, and the Double EAR group received 2.4 mg/d. The positive control group dose was set at 10 mg, as this is the current dosing regimen in Myanmar for women during the perinatal period (20). Supplements were opaque capsules containing varying amounts of thiamine hydrochloride and cellulose filler, as indicated in **Table 4-1**. All thiamine was delivered as thiamine hydrochloride, calculated using a 1.271 correction factor (ratio of molecular weights of thiamine hydrochloride and thiamine). The thiamine capsules were produced and packed in two batches (in July and in September 2018) at the Quinpool Wellness

Centre in Halifax, Nova Scotia, Canada, with codes assigned by an independent scientist not involved in this study. The safety data sheet for thiamine hydrochloride is located in **Appendix A**.

Women were visited fortnightly to distribute new blister packs (containing 14 capsules) and collect used blister packs for capsule counts (see **Appendix B**). All participants were provided with a mobile phone and received weekly phone calls and daily text messages to assist in women’s capsule consumption adherence. Women were considered compliant if they consumed $\geq 80\%$ of their capsules over the 22-week intervention period.

Table 4-1: Treatment arms for *Trial of thiamine supplementation in Cambodia*; adapted from (109).

Treatment Group	Reasoning	Dose of thiamine
Placebo	Negative Control (placebo)	0 mg/d
1.2 mg	1 x thiamine EAR for lactating women (37)	1.2 mg/d
2.4 mg	2 x thiamine EAR for lactating women (37)	2.4 mg/d
10 mg	Positive Control (dose currently given in supplemental form in Myanmar) (20)	10 mg/d

4.2.2 Recruitment

Convenience sampling was used to recruit women from antenatal care programs situated in the catchment areas of the eight selected Kampong Thom health centres (Tboung Kapoeur, Kampong Svay, Sankor, Chey, Salavisai, Prey Kuy, Prey Pros, and Srayov). These eight health centres were selected for high birth rates through consultation with the National Nutrition Programme, Cambodian Ministry of Health.

A general overview of the study was provided to women, and the names and contact information of interested women were then shared with the data collection team. The data collection team contacted interested women after delivery, screened for eligibility, obtained consent, and enrolled women. Recruitment continued on a rolling basis until 335 mother-infant dyads were enrolled. See **Figure 5-1** for participant recruitment and enrolment.

Written, informed consent was obtained from all participants. Women were reminded of consent and their ability to withdraw without repercussions before collecting data or human milk samples at 2, 4, 12, and 24 weeks postpartum. See **Appendix C** for the consent form.

4.2.3 Randomization

The randomized groups were blinded to participants, field staff, and study investigators. The randomization was stratified via study centre and utilized randomly permuted blocks of size 8 within strata to distribute participants to one of either treatment codes in the ratio 1:1:1:1:1:1:1:1 (2 treatment codes for each treatment group). Statisticians at the South Australian Health and Medical Research Institute (SAHMRI) generated a total of 8 alpha-numeric codes that consisted of 3 letters followed by 8 numbers to label study supplements. There were two sets of codes per treatment arm so that complete unblinding need not occur in the event of an adverse event.

4.3 Data Collection

A questionnaire was administered by health centre staff at delivery and by trained field officers in participants' homes at 2, 12, and 24 weeks postpartum to collect participant demographic, socioeconomic, and health information (see **Appendix D**, **Appendix E**, and **Appendix F**; ODK software on Samsung Galaxy Tablet). Anthropometric data including infants: length (UNICEF, portable wooden length board), weight (Seca, model 878) and head circumference (Seca, model 201); and mothers: height (microtoise, StaturMeter) and weight (Seca, model 878) were collected with calibrated instruments and standard protocols as per (110). All initial infant measurements were taken at the Health Centre at delivery, and all other measurements were collected at participants' homes (2, 12, and 24 weeks postpartum).

4.4 Human milk collection, processing, and analysis

At 2, 4, 12, and 24 weeks postpartum, human milk samples were collected using a battery-powered single breast pump (Swing Breast pump, Medela, Bar, Switzerland). A full, single breast expression was collected from the breast that participants self-identified as more 'full'. Breast side, time of mother's last meal, and time of sample collection were recorded. Samples were transported on ice from the collection site (usually the women's homes) to the field lab in

Kampong Thom within 5 hours of collection. At the field lab, milk volume was measured by weighing the milk to 1 g (KD-810, Tanita) and then converted to mL using the specific gravity of human milk (1.032 g/mL) (111). Samples were gently mixed prior to being transferred by transfer pipettes into 1.5 mL amber screwcap cryovials. Samples were stored at -20°C for ≤ 2 weeks before being transported to the National Institute for Public Health Laboratory in Phnom Penh for storage at -80°C. Following this study's completion, all human milk samples were batch shipped on dry ice to the US Department of Agriculture, ARS Western Human Nutrition Research Center, University of California, Davis, for human milk thiamine concentrations assessment (41).

Human milk analyses were performed via an Agilent 1200 HPLC System equipped with a fluorescence detector using a Phenomenex Kinetex C18 column, 150 \times 4.6 mm, 5 μ m, protected by a C18 precolumn, 4 \times 3 mm (Phenomenex Security- Guard), and operated by Chemstation Rev. B.02.01. SR1 (Santa Clara, CA, USA); methods have been published elsewhere (94). A pooled human milk sample with established thiamine concentrations was utilized as an internal control (43). Free thiamine, ThMP, and ThDP concentrations (controlling for molecular weights) were then used to calculate total thiamine concentrations based on molecular weights: total thiamine = free thiamine + (ThMP \times 0.871) + (ThDP \times 0.707) (43).

4.5 Participants

4.5.1 Inclusion and exclusion criteria

To participate in this study, women had to be 18-45 years old, healthy with a recent normal pregnancy (i.e. no known chronic conditions, no prior history of preeclampsia, pre-term delivery, etc.), with singleton infant born without complications (e.g. no low birth weight (<2.5 kg), tongue tie, cleft palate, addition appendage), and had to intend to breastfeed for the first 6 months exclusively. Women had to reside in Kampong Thom province and did not intend to move within six months. Women had to be willing to consume one capsule daily beginning at 2 weeks until 24 weeks postpartum, and provide human milk samples at 2, 4, 12, and 24 weeks postpartum.

Women were excluded if they were currently taking or had taken thiamine-containing supplements over the past 4 months, including consumption of ‘Mama Milks’ or had participated in nutrition programs beyond standard care.

4.5.2 Sample Size

The sample size for this sub-study aligns with the larger trial; for detailed information, see Whitfield *et al.* (109). The overall trial's primary objective was to estimate the dose on an Emax dose-response curve where the additional maternal intake of thiamine (oral dose) no longer meaningfully increased human milk total thiamine concentration at 24 weeks postpartum. Briefly, an overall sample size of 320 women was established, assuming a clinically meaningful difference of 40 µg/L in human milk total thiamine concentrations between any two treatment groups with 90% power, a SD of 43 µg/L (43), 20% attrition, and use of a two-sided alpha of 0.0083 for each of the 6 pairwise comparisons between the four treatment groups to control the familywise error rate at the 0.05 level using a Bonferroni adjustment for multiple comparisons. Given that this thesis was based on a secondary outcome of the larger trial, we used the same sample size.

4.5.3 Ethics

This study's ethics approvals were obtained from the following: Mount Saint Vincent University Research Ethics Committee Canada (MSVU UREB #2017-141) and Research Ethics Boards: National Ethics Committee for Health Research Cambodia (#112NECHR). See **Appendix G and H**, respectively. The overall study is registered at ClinicalTrials.gov Identifier: NCT03616288.

4.5.4 Confidentiality and data security

All participants were assigned a unique alpha-numeric study ID code, which was not generated from personal identifiers. The only key that connected participant study ID codes and participant information are kept on a password-protected computer in a secure area at the Helen Keller International (HKI) office in Phnom Penh, Cambodia.

All electronic data files are saved on password-protected computers and/or secure servers accessible only to the research team members. All paper (e.g. consent forms) and archived electronic data will be stored for 25 years following the publication of research findings (112). After this time, they will be physically destroyed (e.g., paper copies will be shredded), and electronic files will be permanently deleted.

4.9 Statistical analyses

Descriptive statistics for demographic characteristics and human milk total thiamine concentrations were computed and summarized as mean (SD) or *n* (%), while the mean differences in human milk total thiamine concentrations by treatment group are presented as mean (95% CI).

Analyses to assess potential differences in human milk total thiamine concentration by treatment group were completed using intent-to-treat analyses, using multiple imputations to create 100 complete datasets for analysis (113). Differences in human milk total thiamine concentrations between treatment groups (pairwise comparisons; Tukey post-hoc tests used to adjust p-values for multiple comparisons) were assessed using a linear mixed-effects model, adjusted for human milk total thiamine concentrations at 2 weeks postpartum and health centre. This linear mixed-effects model was used to analyze the data at 4, 12 and 24 weeks postpartum with a treatment by timepoint interaction model. Mean differences between treatment groups are collapsed across all timepoints (4, 12 and 24 weeks postpartum) because the treatment by time interaction p-value was > 0.05 (and hence was dropped from the analysis model). These analyses were completed by trial statisticians using R version 3.6.3 (R Foundation for Statistical Computing, Vienna, Austria).

In exploratory analyses, lactation stage changes in total thiamine concentrations in un-supplemented women's (placebo group) milk were assessed. Here, an ANOVA repeated measures test with Mauchly's test of sphericity (using Bonferroni post hoc test) was used to assess potential differences in mean human milk total thiamine concentrations between collection timepoints. Potential lactation stage differences were also assessed by assessing the change in milk thiamine at various timepoints against 24 weeks postpartum: (Δ 2 to 24 week,

Δ 4 to 24 weeks, Δ 12 to 24). The changes as compared to 24 weeks were selected to compare against a measure of mature milk, as thiamine levels should theoretically remain similar until the mother finishes lactating (94). Deltas were computed, and then an ANOVA repeated measures test with Mauchly's test of sphericity (using Bonferroni post hoc test) was run to assess differences. Only women with milk samples available at all of the collection timepoints (2, 4, 12, and 24 weeks postpartum) were included in the analysis ($n=73$ included, $n=10$ excluded). These analyses were performed on SPSS for Macintosh version 25.0 (IBM Corp., Armonk, NY), with a significance level of $p<0.05$.

4.10 Dissemination of study findings

The results of this research will be disseminated in various ways. Results will be presented at academic nutrition or public health conferences (i.e. International Society for Research in Human Milk and Lactation Meeting, Canadian Nutrition Society etc.). Results will be submitted for publication in peer-reviewed journals that offer open access (e.g. American Journal of Clinical Nutrition, Pediatrics, etc.). Lastly, we will hold a Dissemination Workshop in Phnom Penh open to all relevant stakeholders (NGOs, government, researchers, media, clinicians, public health, all sectors).

Chapter 5: Results

5.1 Participant Characteristics

The flow chart is shown in **Figure 5-1**. Rolling recruitment occurred between August 28 and December 24, 2018. 516 women were assessed for eligibility, 335 mother-infant dyads met the inclusion criteria, provided informed consent, and were randomized (placebo, $n=83$; 1.2 mg, $n=86$; 2.4 mg, $n=81$; 10 mg, $n=85$). At 2, 4, 12 and 24 weeks postpartum, milk samples were available for analysis from 335, 331, 309, and 295 mothers, respectively. The study had an overall attrition rate of 12% (placebo, $n=10$; 1.2 mg, $n=9$; 2.4 mg, $n=9$; 10 mg, $n=12$), with no differences by treatment group. Migration out of Kampong Thom province of mother-infant dyads was the most common reason for loss to follow-up.

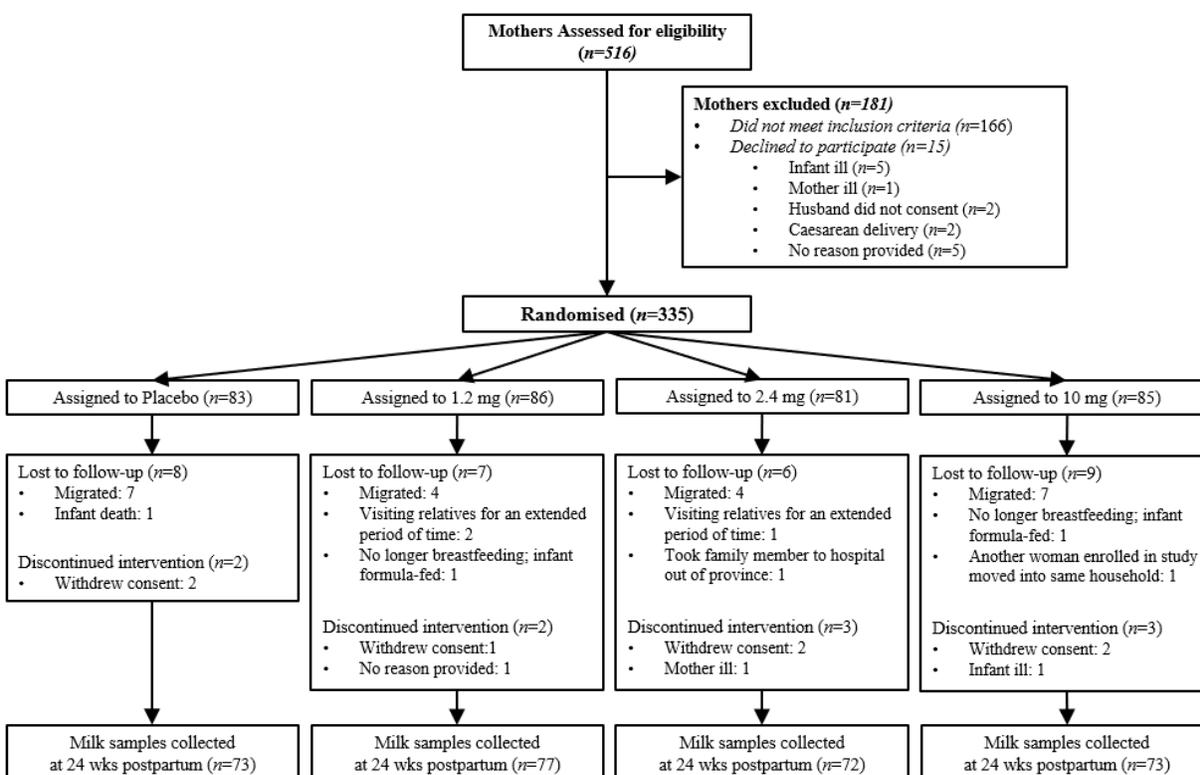


Figure 5-1: Flow chart for mother-infant dyads in Kampong Thom, Cambodia; wks, weeks.

Baseline characteristics of mothers, infants, and their households are outlined in **Table 5-1**. The mean (SD) maternal age at enrollment was 28 (6) years, and all women self-reported to be of Khmer ethnicity. Two-thirds of women were multiparous, and nearly 50% of infants were female. The majority of women were married, and greater than two-thirds of women and their husbands both had an education level of lower primary school or less. On average, household size was 4 persons, with only 14% of households daily per capita income above the Cambodian poverty line. Compliance was high, with 89% of women consuming at least 80% of their capsules (data not shown).

5.2 Human milk total thiamine concentrations

Human milk total thiamine concentrations are presented by treatment group in **Table 5-2**. At 24 weeks postpartum (endline), human milk total thiamine concentrations were 153 (85), 183 (91), 190 (105), and 206 (89) $\mu\text{g/L}$ in the placebo, 1.2 mg, 2.4 mg, and 10 mg groups, respectively. The mean differences in human milk total thiamine concentrations between each treatment group, shown as pairwise comparisons, can be found in **Table 5-3**. In adjusted analyses (adjusted for human milk total thiamine at 2 weeks postpartum, and health center), the mean difference in human milk total thiamine concentration was significantly higher in all of the thiamine groups (1.2 mg, 2.4 mg, and 10 mg) as compared to the placebo group ($p < 0.0001$). None of the thiamine groups differed significantly from one another. Results from raw data were similar to the results based on the analysis of 100 imputed datasets (data not shown).

Mean total thiamine concentrations in un-supplemented (placebo group) over time is shown in **Figure 5-2**. In un-supplemented women, mean total milk concentrations significantly increased from 4 to 24 weeks postpartum ($p = 0.008$). There were no significant differences seen in Δ mean differences of human milk total thiamine concentrations at various timepoints against 24 weeks postpartum; see **Figure 5-3**.

Table 5-1: Baseline characteristics of randomized participants by treatment group.

	Placebo			
	(0 mg) <i>n</i> =83	1.2 mg <i>n</i> =86	2.4 mg <i>n</i> =81	10 mg <i>n</i> =85
Mother				
Age, years	28.3 (6.1)	27.9 (6.7)	28.1 (6.1)	28.1 (5.9)
Parity, multiparous	54 (65%)	54 (63%)	58 (72%)	64 (75%)
Ethnicity, Khmer	83 (100%)	86 (100%)	81 (100%)	85 (100%)
Marital status, married	79 (95%)	86 (100%)	81 (100%)	84 (99%)
Education				
None	10 (12%)	8 (9%)	13 (16%)	9 (11%)
Primary (1-6 years)	43 (52%)	37 (43%)	40 (49%)	41 (48%)
Lower Secondary (7-9 years)	16 (19%)	29 (34%)	19 (24%)	19 (22%)
Upper Secondary (10-12 years)	12 (15%)	9 (11%)	8 (10%)	14 (17%)
Higher education	2 (2%)	3 (3%)	1 (1%)	2 (2%)
Household				
Husband education				
None	10 (12%)	9 (10%)	9 (11%)	10 (12%)
Primary (1-6 years)	42 (51%)	37 (43%)	39 (48%)	33 (39%)
Lower Secondary (7-9 years)	21 (25%)	24 (28%)	23 (28%)	29 (34%)
Upper Secondary (10-12 years)	5 (6%)	13 (15%)	8 (10%)	8 (9%)
Higher education	5 (6%)	3 (3%)	2 (3%)	5 (6%)
Household size, number of people	3.7 (1.7)	3.6 (1.8)	4.0 (2.1)	4.1 (2.0)
Median Annual household income, US\$ (IQR)	1800 (950-3000)	2050 (963-3500)	1600 (1000-3000)	2000 (1200-3500)
Wealth Index Score ^a				
Poorest	22 (27%)	12 (15%)	21 (26%)	25 (29%)
Second	16 (19%)	14 (16%)	20 (25%)	19 (22%)
Middle	26 (31%)	31 (36%)	24 (30%)	27 (32%)
Fourth	14 (17%)	20 (23%)	11 (13%)	9 (11%)
Wealthiest	5 (6%)	8 (10%)	5 (6%)	5 (6%)
Infant				
Sex, female	43 (52%)	43 (50%)	33 (41%)	42 (49%)
Length-for-age (Z-score)	-0.52 (0.98)	-0.66 (1.11)	-0.69(1.01)	-0.63 (1.01)
Weight-for-age (Z-score)	-0.40 (0.95)	-0.50 (0.96)	-0.59 (0.88)	-0.58 (1.07)
Weight-for-length (Z-score)	-0.30 (1.16)	-0.26 (1.15)	-0.42 (1.45)	-0.46 (1.34)
Head circumference-for-age (Z-score)	-0.59 (1.14)	-0.77 (0.98)	-0.81 (0.99)	-0.73 (0.96)

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

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

Table 5-2: Human milk total thiamine concentrations ($\mu\text{g/L}$)^a, by treatment group.

	Placebo (0mg) <i>n</i> =83	1.2 mg <i>n</i> =86	2.4 mg <i>n</i> =81	10 mg <i>n</i> =85
2 weeks postpartum	135.5 (77.7)	129.3 (71.4)	126.2 (77.2)	125.4 (72.3)
4 weeks postpartum	118.8 (59.8)	145.1 (55.7)	168.0 (76.9)	162.0 (76.6)
12 weeks postpartum	119.0 (69.8)	149.8 (63.5)	148.4 (62.7)	142.2 (62.0)
24 weeks postpartum	152.5 (84.8)	183.2 (90.6)	190.1 (105.1)	205.6 (89.4)

Values are mean (SD). Results are based on analysis of 100 imputed datasets.

^a Human milk total thiamine concentrations calculated as free thiamine + (thiamine monophosphate x 0.871) + (thiamine diphosphate x 0.707).

Table 5-3: Mean differences in human milk total thiamine concentrations ($\mu\text{g/L}$)^a, between treatment groups.

	Unadjusted Mean difference ($\mu\text{g/L}$) (95% CI)	P*	Adjusted^b Mean difference ($\mu\text{g/L}$) (95% CI)	P*
1.2 mg – Placebo (0mg)	29.05 (12.17, 45.94)	<0.0001	30.09 (13.30, 46.88)	<0.0001
2.4 mg – Placebo (0mg)	38.66 (19.88, 57.45)	<0.0001	39.84 (21.21, 58.47)	<0.0001
2.4 mg – 1.2 mg	9.61 (-8.32, 27.54)	0.5	9.75 (-7.99, 27.49)	0.5
10 mg – Placebo (0mg)	37.25 (18.71, 55.79)	<0.0001	38.65 (20.35, 56.95)	<0.0001
10 mg – 1.2 mg	8.19 (-9.30, 25.68)	0.6	8.56 (-8.85, 25.97)	0.6
10 mg – 2.4 mg	-1.42 (-20.93, 18.10)	1.0	-1.19 (-20.20, 17.93)	1.0
Overall		<0.0001		<0.0001

Data are mean differences (95% CI). Results are based on analysis of 100 imputed datasets.

* Post-hoc (Tukey) adjusted p-values for multiple comparisons.

^a Linear mixed-effects model was used to analyze the data at 4, 12 and 24 weeks postpartum with a treatment by timepoint interaction model. Mean differences between treatment groups collapsed across all timepoints (4, 12 and 24 weeks) are reported, as the treatment by time interaction p-value was >0.05 and hence was dropped from the analysis model.

^b Human milk concentrations adjusted for human milk total thiamine at 2 weeks, and health center.

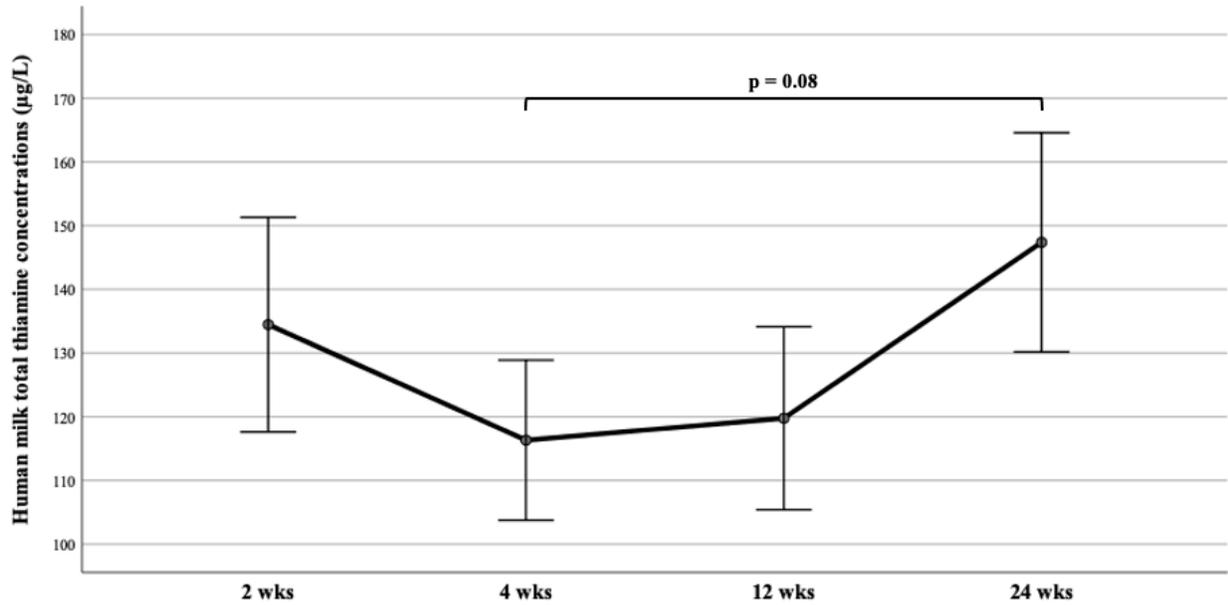


Figure 5-2: Mean (95% CI) human milk total thiamine concentrations ($\mu\text{g/L}$) in un-supplemented women (placebo group, $n=73$) from 2 weeks postpartum to 24 weeks postpartum. Evaluated using ANOVA repeated measures with Mauchly's test of sphericity and Bonferroni post-hoc.

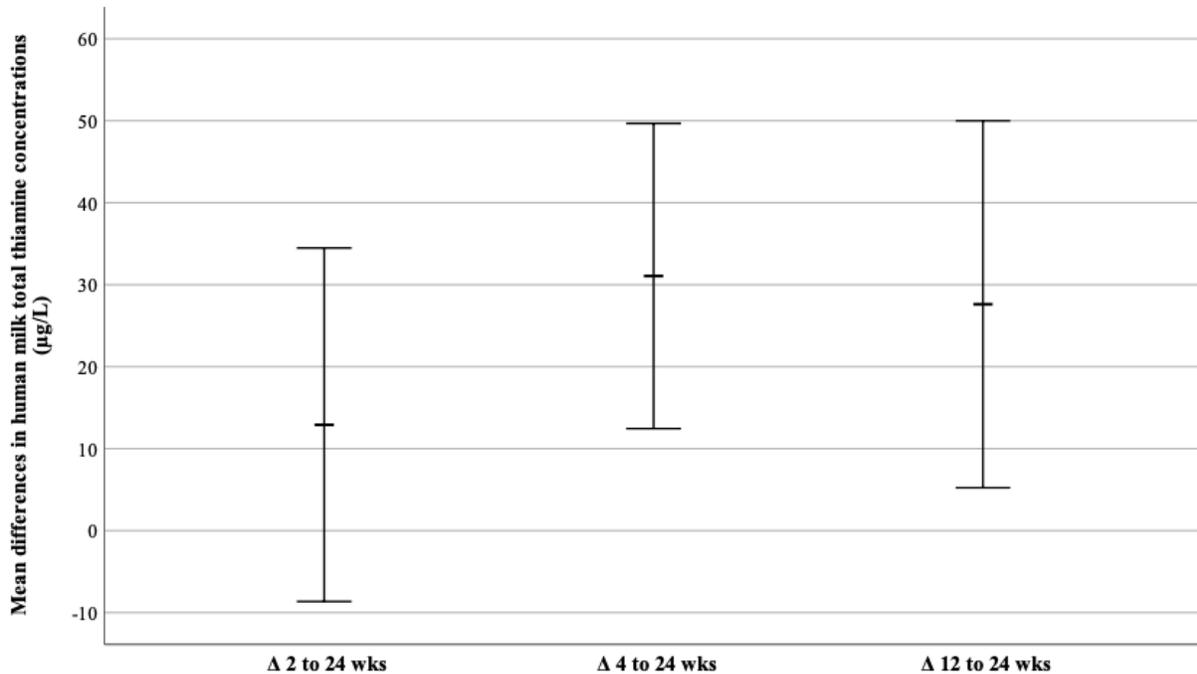


Figure 5-3: Mean differences (95% CI) in human milk total thiamine concentrations ($\mu\text{g/L}$), at various timepoints against 24 weeks postpartum, in un-supplemented women (placebo group, $n=73$); wks, weeks. Evaluated using ANOVA repeated measures with Mauchly's test of sphericity and Bonferroni post-hoc. For all, $p\text{-value} > 0.05$.

Chapter 6: Discussion

We found that human milk total thiamine concentrations at 24 weeks postpartum adjusted for baseline total thiamine concentrations at 2 weeks postpartum were significantly higher in supplemented lactating women (1.2 mg, 2.4 mg, and 10 mg) compared to placebo group women ($p < 0.001$). None of the thiamine-containing groups differed significantly from one another. As such, 1.2 mg/d was sufficient to increase the total thiamine in human milk to both those achieved by the higher supplementation doses (2.4 and 10 mg/d) and those of adequately nourished mothers.

6.1 Impact of supplemental thiamine

In the present study, mothers in the 1.2 mg group had mean (SD) total thiamine concentrations of 183 (91) $\mu\text{g/L}$ at 24 weeks postpartum, which is comparable to mean concentrations of 199 (45) $\mu\text{g/L}$ in milk collected at 6 months postpartum from 57 Helsinki mothers consuming 2 mg of thiamine per day (47). More recently, Coats *et al.* found median human milk thiamine concentrations of 173 $\mu\text{g/L}$ (converted from 500 nmol/L) in thiamine-replete American mothers ($n=16$; 6 – 28 weeks postpartum) (101).

It is well established that supplementation effectively increases milk thiamine concentrations among thiamine deficient women. For example, median human milk thiamine concentrations increased significantly from 62 to 174 $\mu\text{g/L}$ (converted from 180 and 503 nmol/L, respectively; $p < 0.001$) in Cambodian mothers ($n=16$; 1-7 months postpartum) who consumed a supplement containing 100 mg/d of thiamine hydrochloride for five days (101). Whitfield and colleagues reported similar results: Cambodian women had higher mean human milk total thiamine concentrations of 207 and 177 $\mu\text{g/L}$, after consuming thiamine-fortified fish sauce (2 or 8g/L, respectively) *ad libitum* over 6 months in late pregnancy and early postpartum, as compared to women who consumed a control fish sauce containing no thiamine (144 $\mu\text{g/L}$; $p < 0.05$) (43). Conversely, studies have shown that human milk produced by American mothers with adequate thiamine intakes was not impacted by thiamine supplementation (39,40). Similarly, Allen and colleagues (108) found no effect of a supplement containing 1.6 mg/d on maternal human milk

concentrations in Malawi women (108). However, likely because these women already had adequate thiamine status.

At 24 weeks postpartum, women in the placebo group produced milk containing on average 153 (85) $\mu\text{g/L}$ total thiamine. Although this concentration was significantly lower than the supplemented groups in this study, without cut-offs, we are unable to speculate whether milk thiamine concentrations at this level would confer risk of TDD to infants. In fact, much lower milk total thiamine concentrations have been reported in populations that do not report cases of beriberi. In 2012, Hampel *et al.* reported a mean milk total thiamine concentration of 37 (range 5-66) $\mu\text{g/L}$ among milk samples collected from 28 American mothers, although collected between 4-12 weeks postpartum (41). Although this report is lower than those of other American women (e.g. 173 $\mu\text{g/L}$ from 16 Minnesota mothers between 6 – 28 weeks postpartum (101)), these samples were collected from reportedly healthy mothers. Therefore, while the milk collected from women in the placebo group was significantly lower than the supplemented group in the current study, research is needed to establish clinically meaningful cut-offs in order to assess risk.

6.2 Impact of lactation stage on human milk thiamine

We examined milk thiamine concentrations across timepoints among women who were not receiving any supplemental thiamine ($n=73$) and found that milk total thiamine concentrations increased significantly from 116 (54) $\mu\text{g/L}$ to 147 (74) $\mu\text{g/L}$ between 4 and 24 weeks postpartum ($p=0.008$). Similarly, Hampel *et al.* found that in un-supplemented women, median total thiamine concentrations significantly increased from 2 weeks to 24 weeks postpartum (154 to 196 $\mu\text{g/L}$; $p<0.001$) (94). Although the pharmacokinetics of thiamine in human milk are not well understood, concentrations of some water-soluble vitamins, including thiamine, have been reported to increase as lactation progresses from the production of colostrum to that of mature milk (41,88).

We speculate that seasonal variation in dietary thiamine intake was likely not a factor in the significant increase we report in un-supplemented mothers between 4 and 24 weeks postpartum, as rolling recruitment took over 4 months (August 28th – December 24th, 2018). Changes in the

seasonal diet are unlikely to impact human milk total thiamine concentrations as women were entering the different seasons (rainy season, May to October; dry season, December – April) at different timepoints during lactation. However, this cannot be confirmed; although it is well-established that dietary thiamine intake in Cambodia is generally low (21) and unlikely to vary with seasonality, we did not collect dietary thiamine intake data. Given the evidence impact of lactational stage on human milk total thiamine concentrations shown here, further investigation is warranted.

6.3 EAR for lactating women

The National Academy of Medicine established the EAR during lactation of 1.2 mg/d for women in Canada and the United States, with an assumed macronutrient distribution of 45-60% carbohydrates (22). However, more thiamine is likely required by Cambodian women because of their higher dietary carbohydrate intakes and physically laborious lifestyles, both of which increase thiamine requirements (20,73). We found that thiamine supplementation of 1.2 mg/d, in addition to the estimated 0.58 mg/d thiamine in the Cambodian diet (FBS estimate) (20), achieved human milk total thiamine concentrations in lactating rural Cambodian women that were not different from higher supplementation doses. These findings align with the current intake recommendations: since the lactating women in this study received 1.2 mg/d of supplemental thiamine and thus had an average intake of 1.7 mg/d, they exceeded the thiamine RDA (which assumes an adequate intake in 97.5% of a population sub-group) of 1.4 mg/d (22).

The EAR of 1.2 mg thiamine is thought to be easily met through usual diets in Canada and the US as thiamine-fortified and -enriched foods are common and provide nearly half of the daily total thiamine consumed (34). For example, a lactating American mother could meet the thiamine EAR by consuming either: 1 ¾ cup of sunflower seeds, 2 medium (5.5oz) baked pork chops, ~1.5 cups of thiamine-fortified breakfast cereal, or 5 slices of thiamine-enriched white bread (114). However, supplement use in the peripartum period is also common; 62% of pregnant women involved in the US National Health and Nutrition Examination Survey 2007–2014 took prenatal supplements, which commonly contain 1.4 mg/d of thiamine (115). As such, American women likely consume more than 1.2 mg/d in lactation. Taken overall, supplemental

thiamine intakes of 1.2 mg above routine dietary thiamine intakes could likely be beneficial for lactating women in Cambodia and other high rice-consuming regions, like Myanmar, Laos, and areas of India, including Assam (20).

6.4 Adequate intakes in infants

None of the infants in this study showed clinical symptoms of TDD; however, a lack of human milk total thiamine cut-offs hinders assessment of thiamine adequacy. The WHO recommends human milk as the sole source of nutrition for the first 6 months of life (53,116); therefore, adequate vitamin content of human milk is essential to infant health and development. The adequate intake (AI) of 0.2 mg/d for infants during the exclusive breastfeeding period (0 – 6 months) is based on observed milk thiamine concentration of 210 µg/L of thiamine in healthy mothers and a reference milk intake of 780 mL/d (36). Yet, in this study, we found that only a small proportion (< 16%) of supplemented and (< 8 %) of un-supplemented women produced milk ≥ 210 µg/L thiamine at any timepoint or dose. Similarly, Whitfield *et al.* found only 10% of infants would have achieved the AI when thiamine-fortified fish sauce was consumed by breastfeeding mothers during pregnancy and in early lactation (43). Additionally, Hampel and colleagues reported a wide variation in mature human milk (≥ 2 weeks postpartum) thiamine concentrations by region, with few milk samples achieving the AI (41).

The current infant thiamine AI was set based on data from a few small studies conducted in the 1980s with only 24 American women, and thiamine values were measured via the thiochrome method (37,39,40). Over the past 35 years, there have been notable improvements in analytical techniques for quantifying thiamine in biological samples, which may be the reason for the contradiction of recently published human milk values of thiamine and the AI (43). Today, human milk is commonly analyzed using HPLC-FLD (43). This approach is now preferred as it allows for precise and highly reproducible separation of the analytes from the matrix (87). Although no cut-offs have been established to determine the adequacy of thiamine in human milk, this research adds to the growing literature that the AI may be set too high and is likely not a valid proxy indicator of thiamine sufficiency of human milk (86,94).

6.5 Inter-individual variation in milk thiamine concentrations

We found large standard deviations associated with human milk thiamine concentrations in all groups, at all timepoints. In the present study, at 24 weeks postpartum, human milk total thiamine concentrations were 153 (85), 183 (91), 190 (105), and 206 (89) $\mu\text{g/L}$ in the 0 mg, 1.2 mg, 2.4 mg, and 10 mg groups, respectively. This large variation in milk thiamine concentrations is also found in the ranges, even among women receiving supplemental thiamine: The ranges of human milk total thiamine concentrations were 24 – 331, 37 – 432, and 51 – 488 $\mu\text{g/L}$ in the 1.2 mg, 2.4 mg, and 10mg groups, respectively. We speculate that the large variation in human milk total thiamine concentrations is likely attributable to large inter-individual variability in maternal biological ability to transfer thiamine circulation to milk; however, little is known about thiamine milk biology. Similarly, large inter-individual variations in human milk thiamine concentrations have been reported elsewhere. Coats *et al.* found that human milk thiamine concentrations varied considerably among both thiamine-replete American mothers (range 39-215 $\mu\text{g/L}$), and Cambodian mothers before (29-124 $\mu\text{g/L}$) and after (125-280 $\mu\text{g/L}$) consuming high dose thiamine supplements ($n=16$; all values converted to $\mu\text{g/L}$ from nmol/L) (101). The Breast Milk Quality study also reported high inter-individual variability ($n=18$, Bangladeshi women) (86). This study investigated how the vitamin concentrations in human milk were affected by acute maternal supplementation at 2 – 4 months of lactation, and found that over the 3-day study, inter-individual variability attributed for between 70 – 85% of the variability in milk thiamine concentrations (86). Given the wide range in response to thiamine supplementation, mechanistic studies of thiamine sequestration into human milk are warranted.

6.6 Recommendations for future research

Based on, 1.2 mg/d thiamine is sufficient human milk total thiamine concentrations to levels consistent with thiamine-replete populations, which has the potential to prevent potentially fatal infantile beriberi. We would encourage policymakers in regions where thiamine deficiency is considered a concern to assess the feasibility and efficacy of programs such as the United Nations International Multiple Micronutrient Antenatal Preparation supplement (UNIMMAP; 1.4 mg thiamine) for the perinatal period. However, the women potentially even at higher risk of thiamine deficiency than the lactating moms in this study would be women that did not have healthy pregnancies or access to health care. Considering these women are not being followed by

health care staff or giving birth in health care centres, which often distribute peripartum supplementation, food fortification programs could be a more pragmatic intervention, potentially reaching the most vulnerable lactating women and the wider population.

We would encourage future research to focus on food fortification programs. Food fortification can be a sustainable, cost-effective, and passive means of improving thiamine and other micronutrient intakes in a population (117). Previous research in Cambodia showed that thiamine-fortified fish sauce to households with lactating women increased milk thiamine concentrations and blood biomarkers of both women and their breastfeeding infants (43). However, because fish sauce is not consumed in other areas where thiamine deficiency is of concern (e.g. Assam (118), Kashmir (119), Kiribati (120)), and often households with lower economic status make their fish sauce, this vehicle for thiamine would not always reach the intended targeted populations. Future fortification programs that focus on salt, which is a ubiquitous condiment globally, have already been utilized successfully to fortify other micronutrients (i.e. iron and iodine). The larger Trial of Thiamine Supplementation (109), which looked at salt as a fortification vehicle for thiamine in Cambodia, concluded that mass fortification with thiamine-fortified salt could help. However, the study suggested that future research should explore the technical and cost-related aspects, as well as the feasibility and efficacy of salt fortification.

6.7 Strengths and limitations

This research had several strengths, the main one being that, to our knowledge, this is the first long-term (22 weeks) study during the exclusive breastfeeding period (0 – 6 months) to investigate the impact of various low-dose thiamine doses on milk thiamine concentrations. Past research investigated high therapeutic doses (e.g. 50 or 100 mg/d) over shorter time-periods (days to weeks) (46,101), which are less informative for understanding biological mechanisms and the potential impacts of programmatic interventions such as peripartum multiple micronutrient supplementation or mass fortification. Additional strengths included low attrition and strong compliance, likely due to daily SMS messages, weekly phone calls, and fortnightly follow-ups that helped build rapport between field staff and study participants. We collected a full breast expression, therefore capturing both fore- and hindmilk, to account for thiamine

concentration variations within a single feed. We also collected milk samples at four different timepoints throughout 2 to 24 weeks postpartum. We had a placebo group, which was particularly important as we saw a significant lactation stage change among non-supplemented women in milk thiamine at 4 to 24 weeks postpartum.

The main limitation of this research is the lack of human milk cut-offs for thiamine. While we have learned that supplementation at a dose of 1.2 mg/d results in human milk thiamine concentrations similar to higher supplementation doses (2.4 and 10 mg/d), and similar to milk of thiamine-replete women in high-income countries, there are no cut-offs linking human milk concentrations to clinically meaningful outcomes in infants. More research is needed to set clinically meaningful cut-offs to assess the risk of TDD. Other limitations include that data collection was restricted to one rural province in Cambodia. However, the study sample size was large for rural lactating women, a generally hard to reach population. Also, 81% of the Cambodian population lives in rural areas (2). Lastly, although it is well-established that dietary thiamine intakes are low (21), and unlikely to change seasonally, we did not collect dietary thiamine intake data in this study.

Chapter 7: Conclusion

Thiamine deficiency continues to be a persistent public health concern in Cambodia and other regions where thiamine-poor white rice is a staple food (49,53,54,59). In this study, we found that 1.2 mg/d supplemental thiamine was sufficient to increase human milk total thiamine concentrations to levels achieved by higher doses (2.4 mg and 10mg) and comparable to those of adequately nourished mothers in regions where thiamine deficiency is not of concern. We also found that, among un-supplemented women, milk thiamine concentrations increased significantly between 4 and 24 weeks postpartum, suggesting a lactation stage impact on milk thiamine concentrations. We hope these results can help to inform future interventions to combat maternal thiamine deficiency and subsequently, infantile beriberi.

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 120. Nilles EJ, Manaia A, Ruaia B, Huppatz C, Ward C, George P, Sies C, Cangiano A, Sejvar J, Reiffer A, et al. Re-emergence of thiamine deficiency disease in the Pacific islands (2014–15): A case-control study. *PLoS ONE*. 2018. p. e0198590.

Appendices

Appendix A: Thiamine hydrochloride safety data sheet



SAFETY DATA SHEET

SECTION 1: PRODUCT IDENTIFICATION

PRODUCT NAME	THIAMINE HYDROCHLORIDE, USP (Vitamin B1)
PRODUCT CODE	1963
SUPPLIER	MEDISCA Inc. Tel.: 1.800.932.1039 Fax.: 1.855.850.5855 661 Route 3, Unit C, Plattsburgh, NY, 12901 3955 W. Mesa Vista Ave., Unit A-10, Las Vegas, NV, 89118 6641 N. Belt Line Road, Suite 130, Irving, TX, 75063 MEDISCA Pharmaceutique Inc. Tel.: 1.800.665.6334 Fax.: 514.338.1693 4509 Rue Dobrin, St. Laurent, QC, H4R 2L8 21300 Gordon Way, Unit 153/158, Richmond, BC V6W 1M2 MEDISCA Australia PTY LTD Tel.: 1.300.786.392 Fax.: 61.2.9700.9047 Unit 7, Heritage Business Park 5-9 Ricketty Street, Mascot, NSW 2020
EMERGENCY PHONE	CHEMTREC Day or Night Within USA and Canada: 1-800-424-9300 NSW Poisons Information Centre: 131 126
USES	Supplement

SECTION 2: HAZARDS IDENTIFICATION

GHS CLASSIFICATION	Based on available data, the classification criteria are not met.			
PICTOGRAM	Not Applicable			
SIGNAL WORD	Not Applicable			
HAZARD STATEMENT(S)	Not Applicable			
AUSTRALIA-ONLY HAZARDS	Not Applicable.			
PRECAUTIONARY STATEMENT(S)	Prevention	Not Applicable	Response	Not Applicable
	Storage	Not Applicable	Disposal	
HMS CLASSIFICATION	Health Hazard	0	Flammability	0
	Reactivity	0	Personal Protection	B

SECTION 3: COMPOSITION/INFORMATION ON INGREDIENTS

CHEMICAL NAME	Thiazolium, 3-[(4-amino-2-methyl-5-pyrimidinyl) methyl]-5-(2-hydroxyethyl)-4-methyl-, chloride, monohydrochloride
BOTANICAL NAME	Not applicable
SYNONYM	Vitamin B1 hydrochloride
CHEMICAL FORMULA	C ₁₂ H ₁₇ ClN ₄ OS.HCl
CAS NUMBER	67-03-8



SAFETY DATA SHEET

ALTERNATE CAS NUMBER	Not applicable						
MOLECULAR WEIGHT	337.3						
COMPOSITION	<table border="1"> <thead> <tr> <th>CHEMICAL NAME</th> <th>CAS NUMBER</th> <th>% BY WEIGHT</th> </tr> </thead> <tbody> <tr> <td>THIAMINE HYDROCHLORIDE</td> <td>67-03-8</td> <td>100</td> </tr> </tbody> </table>	CHEMICAL NAME	CAS NUMBER	% BY WEIGHT	THIAMINE HYDROCHLORIDE	67-03-8	100
CHEMICAL NAME	CAS NUMBER	% BY WEIGHT					
THIAMINE HYDROCHLORIDE	67-03-8	100					

There are no additional ingredients present which, within the current knowledge of the supplier and in the concentrations applicable, are classified as health hazards and hence require reporting in this section.

SECTION 4: FIRST-AID MEASURES

IN CASE OF EYE CONTACT	Flush with copious amounts of water for 15 minutes, separating eyelids with fingers. If irritation persists seek medical aid.
IN CASE OF SKIN CONTACT	Wash with soap & water for 15 minutes. If irritation persists seek medical aid.
IF SWALLOWED	Call a physician. Wash out mouth with water. Do not induce vomiting without medical advice.
IF INHALED	Remove to fresh air. If not breathing, give artificial respiration. If breathing is difficult, give oxygen. Call a physician
SYMPTOMS AND EFFECTS	Not expected to present a significant hazard under anticipated conditions of normal use.

SECTION 5: FIREFIGHTING MEASURES

SPECIFIC HAZARDS ARISING FROM THE CHEMICAL	Not applicable
FLAMMABLE PROPERTIES	May be combustible at high temperature
HAZARDOUS COMBUSTION PRODUCTS	Under fire conditions, hazardous fumes will be present.
EXTINGUISHING MEDIA	Small fire: dry chemical, CO ₂ or water spray. Large fire: dry chemical, CO ₂ , alcohol resistant foam or water spray. Do not get water inside containers.
PROTECTIVE EQUIPMENT AND PRECAUTIONS FOR FIREFIGHTERS	Wear self-contained breathing apparatus and protective clothing to prevent contact with skin and eyes.

SECTION 6: ACCIDENTAL RELEASE MEASURES

PERSONAL PRECAUTIONS	Wear respiratory protection. Avoid dust formation. Avoid breathing vapours, mist or gas. Ensure adequate ventilation. Evacuate personnel to safe areas. Avoid breathing dust.
METHODS & MATERIAL FOR CONTAINMENT	On land, sweep or shovel into suitable containers. Minimize generation of dust.
CLEANUP PROCEDURE	Do not touch damaged containers or spilled material unless wearing appropriate protective clothing. Wear respirator, chemical safety goggles, rubber boots and heavy rubber gloves. Stop leak if you can do it without risk. Prevent entry into waterways, sewers, basements or confined areas. Shut off all sources of ignition. Evacuate the area. If necessary, employ water fog to disperse the vapors. Absorb the matter with compatible vermiculite or other absorbing material. Place in a suitable container and retain for disposal. Ventilate and clean the affected area. Do not flush into sewerage system or to drains.

SECTION 7: HANDLING AND STORAGE

PRECAUTIONS FOR SAFE HANDLING

Do not inhale. Avoid contact with eyes, skin and clothing. Avoid prolonged or repeated exposure. Wash thoroughly after handling.

STORAGE CONDITIONS

Store in original container, tightly sealed, protected from direct sunlight, in a dry, room temperature and well-ventilated area, away from incompatible materials. Store in accordance with local regulations. Eliminate all ignition sources. Separate from oxidizing materials. Containers that have been opened must be carefully resealed and kept upright to prevent leakage. Do not store in unlabelled containers. Use appropriate containment to avoid environmental contamination. Use non-metallic containers. Preserve in tight, light-resistant containers.

SECTION 8: EXPOSURE CONTROLS/ PERSONAL PROTECTION

Chemical Name: THIAMINE HYDROCHLORIDE CAS #: 67-03-8

	TWA	Ceiling	STEL	REL	IDLH	Remarks
NIOSH	N/L	N/L	N/L	N/L	N/L	-
AIHA WEEL	N/L	N/L	N/L	-	-	-
Safe Work Australia HSIS	N/L	N/L	N/L	-	-	-
HSE	N/L	N/L	N/L	-	-	-
OSHA PEL	N/L	N/L	-	-	-	-
ACGIH TLV	N/L	N/L	N/L	-	-	-

N/L = Not Listed

EXPOSURE GUIDELINES

Consult local authorities for provincial or state exposure limits. Particulates not otherwise regulated, respirable fraction: 5 mg/m³.

PERSONAL PROTECTIVE EQUIPMENT

Eyes: Wear appropriate protective eyeglasses or chemical safety goggles as described by WHMIS or OSHA's eye and face protection regulations in 29 CFR 1910.133 or European Standard EN166. Skin: Wear appropriate gloves to prevent skin exposure. Clothing: Wear appropriate protective clothing to minimize contact with skin. Respirators: Follow WHMIS or OSHA respirator regulations found in 29 CFR 1910.134 or European Standard EN 149. Use a NIOSH/MSHA or European Standard EN 149 approved respirator if exposure limits are exceeded or if irritation or other symptoms are experienced.

SPECIFIC ENGINEERING CONTROLS

Adequate mechanical ventilation. Fumehood, eye wash station, and safety shower.

SECTION 9: PHYSICAL AND CHEMICAL PROPERTIES

PHYSICAL STATE	Solid				
DESCRIPTION	White to off-white crystals or crystalline powder, usually having a slight, characteristic odor. Is hygroscopic.				
SOLUBILITY	Freely soluble in water; soluble in glycerin; slightly soluble in alcohol; insoluble in ether and in benzene.				
ODOR	Characteristic nut-like odor.				
FLAMMABILITY	May be combustible at high temperature				
ODOR THRESHOLD	Not available	pH	2.7 - 3.4 (1%)	MELTING POINT	248 °C, 478.4 °F (decomposes)
BOILING POINT	Not available	FREEZING POINT	Not available	FLASH POINT	Not available
SPECIFIC GRAVITY	1.4 (30 °C)	EVAPORATION RATE	Not available	EXPLOSIVE LIMIT	Not available



SAFETY DATA SHEET

UPPER FLAMMABLE/ EXPLOSIVE LIMIT(S)	Not available	LOWER FLAMMABLE/ EXPLOSIVE LIMIT(S)	Not available	VAPOR PRESSURE	Not available
VAPOR DENSITY (AIR = 1)	Not available	RELATIVE DENSITY (WATER = 1)	Not available	log P (OCTANOL-WATER)	-3.930
AUTO-IGNITION TEMPERATURE	Not available	DECOMPOSITION TEMPERATURE	Not available	VISCOSITY	Not available

SECTION 10: STABILITY AND REACTIVITY

REACTIVITY	Not established
STABILITY	Stable under recommended storage conditions
MATERIALS TO AVOID	Alkalis. Oxidizing agents. Reducing agents.
HAZARDOUS DECOMPOSITION PRODUCTS	Toxic fumes of carbon monoxide, carbon dioxide, nitrogen oxides and other gases may occur
HAZARDOUS POLYMERIZATION	Will not occur
POSSIBILITY OF HAZARDOUS REACTION	Not established
CONDITIONS TO AVOID	Moisture, sunlight and extreme temperatures

SECTION 11: TOXICOLOGICAL INFORMATION

ACUTE TOXICITY	Oral: Rat: LD50: (mg/kg): 3710 Dermal: Rabbit: LD50: (mg/kg): Not available Inhalation: Rat: LD50: (mg/L/4hr): Not available
SKIN CORROSION/IRRITATION	Due to lack of data the classification is not possible.
SERIOUS EYE DAMAGE/EYE IRRITATION	Due to lack of data the classification is not possible.
RESPIRATORY OR SKIN SENSITIZATION	Due to lack of data the classification is not possible.
GERM CELL MUTAGENICITY	Due to lack of data the classification is not possible. Data from germ cell mutagenicity tests were not found.
CARCINOGENICITY	OSHA THIAMINE HYDROCHLORIDE is not listed. NTP THIAMINE HYDROCHLORIDE is not listed. IARC THIAMINE HYDROCHLORIDE is not evaluated. California This product does not contain any chemicals known to the State of California to cause Proposition 65 cancer, birth defects, or any other reproductive harm. Not available
ADDITIONAL CARCINOGENICITY INFORMATION	Not available
REPRODUCTIVE TOXICITY	Due to lack of data the classification is not possible.
SPECIFIC TARGET ORGAN TOXICITY - SINGLE EXPOSURE	Due to lack of data the classification is not possible.
SPECIFIC TARGET ORGAN TOXICITY - REPEATED EXPOSURE	Due to lack of data the classification is not possible.



YOUR TRUSTED PARTNER IN COMPOUNDING

SAFETY DATA SHEET

ASPIRATION HAZARDS	Based on available data, the classification criteria are not met.								
SIGNS AND SYMPTOMS OF EXPOSURE	Not expected to present a significant hazard under anticipated conditions of normal use. Adverse effects with thiamine are rare, but hypersensitivity reactions have occurred, mainly after parenteral doses. These reactions have ranged in severity from very mild to, very rarely, fatal anaphylactic shock.								
POTENTIAL HEALTH EFFECTS	<table border="0"> <tr> <td style="padding-right: 20px;">Inhalation</td> <td>May be harmful if inhaled. May cause respiratory tract irritation.</td> </tr> <tr> <td>Ingestion</td> <td>May be harmful if swallowed.</td> </tr> <tr> <td>Skin</td> <td>May be harmful if absorbed through skin. May cause skin irritation.</td> </tr> <tr> <td>Eyes</td> <td>May cause eye irritation.</td> </tr> </table>	Inhalation	May be harmful if inhaled. May cause respiratory tract irritation.	Ingestion	May be harmful if swallowed.	Skin	May be harmful if absorbed through skin. May cause skin irritation.	Eyes	May cause eye irritation.
Inhalation	May be harmful if inhaled. May cause respiratory tract irritation.								
Ingestion	May be harmful if swallowed.								
Skin	May be harmful if absorbed through skin. May cause skin irritation.								
Eyes	May cause eye irritation.								

SECTION 12: ECOLOGICAL INFORMATION

TOXICITY	EC50: 48 Hr: Daphnia magna: (mg/L): Not available LC50: 96 Hr: Fish: (mg/L): Not available IC50: 72 Hr: Algae: (mg/L): Not available
PERSISTENCE AND DEGRADABILITY	Not available
BIOACCUMULATIVE POTENTIAL	Not available
MOBILITY IN SOIL	Freely soluble in water.
OTHER ADVERSE EFFECTS	Not available
	This product is not intended to be released into the environment

SECTION 13: DISPOSAL CONSIDERATIONS

WASTE DISPOSAL	Dispose of in accordance with federal / local laws and regulations. Avoid release into the environment.
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SECTION 14: TRANSPORT INFORMATION

UNITED STATES & CANADA

UN PROPER SHIPPING NAME	Not dangerous good
UN NUMBER	Not applicable
CLASS	Not applicable
PACKING GROUP	Not applicable

AUSTRALIA

UN PROPER SHIPPING NAME	Not dangerous good
UN NUMBER	Not applicable
CLASS	Not applicable
PACKING GROUP	Not applicable
HAZCHEM	Not applicable

ENVIRONMENTAL HAZARDS	Not available
SPECIAL SHIPPING INFORMATION	Not applicable



SAFETY DATA SHEET

SECTION 15: REGULATORY INFORMATION

Chemical Name & CAS	CERCLA 40 CFR Part 302.4	SARA (Title III) 40 CFR Part 372.65	EPA 40 CFR Part 366		Right-to-know			California Prop 65
			Appendix A	Appendix B	Pennsylvania	New Jersey	Massachusetts	
THIAMINE HYDROCHLORIDE 67-03-8	N/L	N/L	N/L	N/L	N/L	N/L	N/L	N/L

N/L = Not Listed; X = Listed

AUSTRALIAN REGULATIONS

Chemical Name & CAS	Poisons and Therapeutic Goods Regulation	Therapeutic Goods Act	Code of Practices - Illicit Drug Precursors
THIAMINE HYDROCHLORIDE 67-03-8	N/L	N/L	N/L

SECTION 16: OTHER INFORMATION

REFERENCES

Available upon request

ABBREVIATIONS AND ACRONYMS

CAS – Chemical Abstract Service; GHS – Global Harmonized System; OSHA PEL – Occupational Safety & Health Administration Permissible Exposure Limits; TWA – Time Weighted Average; HSIS – Hazardous Substances Information System; STEL – Short Term Exposure Limit; AIHA WEEL – American Industrial Hygiene Association Workplace Environment Exposure Levels; LD50 – Lethal Dose, 50%; IARC – International Agency for Research on Cancer; NTP – National Toxicology Program; WHMIS – Workplace Hazardous Materials Information System; SARA – Superfund Amendments and Reauthorization Act; EPA – Environmental Protection Agency; CERCLA – Comprehensive Environmental Response, Compensation, and Liability Act; HMIS – Hazardous Materials Information System; NIOSH – National Institute for Occupational Safety and Health; MSHA - Mine Safety and Health Administration; ACGIH - American Conference of Governmental Industrial Hygienists; IDHL - Immediately Dangerous to Health or Life; TLV – Threshold Limit Value; HSE – Health and Safety Executive; REL - Recommended Exposure Limit

LAST REVISION

12/2015

SUPERSEDES

09/2013

DISCLAIMER

This document was created in accordance with OSHA, Safe Work Australia and WHMIS regulations. The above information is believed to be correct but does not purport to be all inclusive and shall be used only as a guide. The information in this document is based on the present state of our knowledge and is applicable to the product with regard to appropriate safety precautions. It does not represent any guarantee of the properties of the product. MEDISCA® shall not be held liable for any damage resulting from handling or from contact with the above product. Recipients of the product must take responsibility for observing existing laws and regulations.

Appendix B: Abridged fortnightly questionnaire

Trial of thiamine supplementation in Cambodia



FORTNIGHTLY MONITORING DATA COLLECTION SHEET

IDENTIFICATION INFORMATION	
Subject ID	_____
Date of Monitoring Visit	____ / ____ / _____
Field Staff Name	_____
Phase	1. Week 4 2. Week 6 3. Week 8 4. Week 10 5. Week 12 6. Week 14 7. Week 16 8. Week 18 9. Week 20 10. Week 22

MODULE 1: SUPPLEMENT ADHERENCE	
17. ID code on supplement blister pack.	_____
18. Number of pills consumed from 14-pill blister pack.	____
19. Were any pills missed/skipped in the past fortnight?	1. Yes 2. No → skip to Q5
20. If yes, why were pills missed/skipped?	1. forgot 2. felt ill 3. other: _____
21. Did the participant consume more than one pill any day during the last fortnight?	1. Yes 2. No → skip to Q7
22. If yes, why did the participant consume more than one pill on the same day?	1. wanted to 'make up' for missing a pill 2. accidental consumption of 2+ pills in one day 3. other: _____

Appendix C: English version of consent form for *Trial of Thiamine Supplementation in Cambodia*

Trial of thiamine supplementation in Cambodia

CONSENT FORM

Principal Investigator: **Kyly Whitfield**, Department of Applied Human Nutrition, Mount Saint Vincent University

Co-Investigators: **Hou Kroeun**, Helen Keller International Cambodia
Keith Porter, Helen Keller International Cambodia
Tim Green, South Australia Health and Medical Research Institute
Frank Wieringa, Institut de recherche pour le développement
Mam Borath, National Sub-Committee for Food Fortification, Ministry of Planning, Cambodia
Prak Sophonneary, National Nutrition Program, Ministry of Health, Cambodia
Jeff Measelle, Department of Psychology, University of Oregon
Dare Baldwin, Department of Psychology, University of Oregon
Geraldine Richmond, Presidential Chair in Science, University of Oregon

Contact If you have questions you can ask the interviewer, or contact a Khmer speaking co-investigator, Hou Kroeun

Site Kampong Thom province, Cambodia

Granting Agency Bill and Melinda Gates Foundation; Sackler Institute for Nutrition Science, New York Academy of Sciences



Introduction

Thiamine, also known as vitamin B₁, is found in some foods. When we don't have enough thiamine we can develop a disease called beriberi. Beriberi is common in Southeast Asia because the dietary staple, rice, contains almost no thiamine. We recently found that up to three quarters of women and half of children in Cambodia had low levels of thiamine in their blood.

Babies usually develop beriberi when they are three months old. It can lead to heart problems and sometimes death. We've also recently learned that babies who don't eat enough thiamine can have trouble learning later in life, even if they don't have the symptoms of beriberi as a baby. If a mother doesn't eat enough thiamine, her milk is low in this vitamin, and the baby doesn't get enough thiamine. By giving more thiamine to mothers we hope to increase the amount of thiamine in her breast milk, preventing the disease in babies.

Adding thiamine to salt in the factory (fortification), in the same way that all salt currently contains iodine, may be a good way to prevent beriberi. Although we know that eating thiamine can prevent beriberi, we don't know how much thiamine mothers need to eat to ensure there is enough thiamine in their milk. Also, we don't know how much salt breastfeeding mothers in Cambodia usually eat. The purpose of this study is to determine the correct dose of thiamine required by mothers, and determine how much salt they usually eat. We also want to watch babies' milestones to determine if they are learning at the right pace.

Three hundred and twenty breastfeeding women in Kampong Thom will be invited to participate in this study. Women will be randomized (like flipping a coin) to one of four groups and take one pill each day for six months. Three groups will consume a thiamine supplement (1.2 mg/day, 2.4 mg/day, and 10 mg/day) and one group will have a placebo (no thiamine). We will also provide salt to your household to determine how much you usually eat.

Participation

Your participation in this study is entirely voluntary, so it is up to you to decide whether you would like to take part in this study. Before you decide, it is important for you to understand what the research involves. This consent form will tell you about the study, why the research is being done, what participation in the study will look like, and the possible benefits, risks, and discomforts.

If you wish to take part in the study, you will be asked to provide consent to the interviewer by stamping your thumbprint on this consent form. If you do decide to take part in this study, you are still free to withdraw at any time and without giving any reasons for your decision. If you do not wish to take part, you do not have to provide any reason for your decision not to participate.

Please take time to read the following information carefully and to discuss it with your family, friends, and Village Chief before you decide.

Who is conducting the study?

Researchers from the Department of Applied Human Nutrition at Mount Saint Vincent University in Canada are conducting this study in collaboration with Helen Keller International, Cambodia and a number of other researchers listed on the first page. You are entitled to request further details from the investigators. If you have questions you can ask the interviewer, or contact a Khmer speaking co-investigator, Hou Kroeun, at XXX XXX.

Who Can Participate?

In order to participate in this study, you must:

- be the mother of a newborn
- be between 18-45 years of age,
- have had a normal most recent pregnancy (i.e. no known chronic conditions, no preeclampsia, gestational diabetes etc), and your singleton infant must have been born without complications (e.g. low birth weight (<2.5 kg), tongue tie, cleft palate)
- intend to exclusively breastfeed for the first six months,
- reside in Kampong Thom province, Cambodia, and not be planning to move in the next six months,
- be willing to consume one pill daily from 2 weeks through 24 weeks postpartum,
- be willing for your entire household consume only salt provided by the study team, and,
- be willing for following biological samples to be collected: a maternal venous blood sample and breast milk sample at 2 weeks postpartum, a breast milk sample at 12 weeks postpartum, and maternal and infant blood samples and a breast milk sample at 24 weeks postpartum.

Who Should Not Participate in This Study?

You should **not** participate in this study if you:

- do not meet the criteria above,
- are currently participating in any nutrition programs beyond normal care,
are currently taking, or have taken any thiamine-containing supplements over the previous 4 months, or
are unable to provide informed consent.

What will participation in this study look like?

If you agree to participate in this research study, you will be asked to complete three study visits, one 2 weeks after delivery, and one each when your baby is 1, 3, and 6 months old. You will first be asked to complete a short questionnaire at your home, which should take about half an hour to complete. The questionnaire will ask questions about you, such as your age, and education level, as well as questions about the food you regularly eat. After you finish the survey in your home, we will measure your height and weight, and the length, weight, and head circumference of your baby. At each session, the researcher will interact with your baby to get a sense of his or her developmental progress. For example, the

researcher will see how babies respond to sights and sounds and social interaction, and how easily they move their bodies. Also, the researcher will ask you to interact with your baby in particular ways, such as trying to coax a smile, or encourage interest in a new toy; if you consent, these interactions will be videotaped to help us with scoring. The researcher will also ask you a range of questions about what you are observing about your baby's development. These activities will take about 45-50 minutes.

The next day you will be asked to travel to a central village location such as the Village Chief's home, where a trained phlebotomist will collect blood and breast milk samples so we can measure thiamine (vitamin B₁). The blood sample will involve having a needle poke to your inner elbow to collect half a tablespoon of blood. For the milk sample, we will use an electronic breast pump to collect a full breast expression from the breast you have not most recently fed from. Once we have taken these samples, we will randomize you (like flipping a coin) to one of four groups and ask you to take one pill each day. Three groups will consume a thiamine supplement (1.2 mg/day, 2.4 mg/day, or 10 mg/day) and one group will have a placebo (no thiamine). You will not know which supplement you are receiving. We will also provide salt to your household to determine how much you usually eat. Field staff will visit your home every two weeks to provide more salt as needed, to pick up used supplement packs, and deliver new supplements.

When your baby is 1 month old we will collect another breast milk sample. When your baby is 3 months old we will return to complete another questionnaire, cognitive assessments, conduct a non-invasive physical exam of your baby, and will collect another breast milk sample. This visit will take place in your home, and should take about an hour.

At the end of the study, when your baby is 6 months old, we will ask you to complete another questionnaire, take body measurements from you and your baby, and collect blood and breast milk samples from you, as well as a blood sample from your baby (we will collect less than a third of a tablespoon of blood). Again, we will conduct cognitive assessments and a physical exam.

Risks

The blood collection procedure may cause some discomfort and slight bruising or, very rarely, an infection at the site of the needle poke. After the blood draw you will immediately be given a bandage to cover the spot where the blood was taken.

Benefits

If you agree to take part in this study, there may or may not be a direct benefit to you. Three out of four women will be taking a pill that contains thiamine, but no one, not even the field staff, will know which pill is which until after the study is completed. All women participating in this study will receive a free 22-week supply of iodized salt for her entire household, and you can keep the salt container provided. At the end of the study we will return to your village to inform you of your thiamine status. Since we will need to be able to

contact you by phone, you will receive a mobile phone as well as \$1/month in mobile credit during the study. You can feel free to keep this mobile phone after the study finishes.

We understand this survey will take some time away from your work and family, so after each study visit (2 weeks, 1 month, 3 months, and 6 months postpartum) we will provide you with a small gift (e.g. sarong, or laundry soap) to thank you for taking the time to participate.

Confidentiality

Your confidentiality will be respected. You will be assigned a unique study number as a participant in this study. This number will not include any personal information that could identify you (e.g., it will not include your initials, date of birth, etc.). Only this number will be used on any research-related information collected about you during the course of this study, so that your identity will be kept confidential. Information that contains your identity will remain only with the Principal Investigator and/or designate

All data from this study will be sent electronically from Cambodia to Canada in an encrypted file that requires a secret code to be accessed. Only members of the research team will know this code.

Participation and Withdrawal from this Study

Taking part in this study is voluntary. You may choose not to take part or may leave the study at any time and do not have to give a reason for your decision. If you decide not to take part or decide to leave the study, you may do so at any time without any consequences. All data collected about you up to the point of withdrawal will be retained for analysis. You will be given a copy of this signed and dated consent form. Throughout the study (at 1, 3, and 6 months) we will remind you about this visit and consent so that you remember that you are free to withdraw at any time and without giving any reasons for your decision.

What will information collected be used for?

All of the information collected from you and your child will be used to inform a future salt fortification program. We will measure thiamine in the blood and breast milk samples to determine the best dose of thiamine to give to future breastfeeding mothers to help prevent against beriberi. Information about how much salt you and your household usually eats will help us to formulate a recipe for thiamine-fortified salt. The cognitive assessments of your baby will help us understand how much thiamine babies need to develop normally.

Although the main purpose of this study is to understand thiamine and beriberi, if blood and breast milk samples remain after thiamine assessment, we will keep these samples in a freezer in Canada, and may analyze these samples for other nutrition-related research. These samples will only be labelled with the unique study ID, and will not be able to be traced back to you.

What happens after the study finishes?

We will return to your village after the study is complete to share the results of the study. If you want, we will also provide you with you and your baby's blood and breast milk results.

We may want to stay in touch with you and continue to follow your child and track his/her development. When you sign this consent form, the field staff person will ask you whether we can keep your contact information so we can be in touch about future research studies.

Trial of thiamine supplementation in Cambodia: Consent Form

- I have listened to, or read, and understood the information provided on this consent form.
- I have had sufficient time to consider the information provided and to ask for advice (if needed).
- I have had the opportunity to ask questions and have received a satisfactory response to my questions.
- I understand that all of the information collected will be kept confidential and that the results of this study will only be used for scientific objectives.
- I understand that participation in this study is voluntary and that I am completely free to refuse to participate or to withdraw from this study at any time without changing in any way the quality of care that I receive.
- I understand that I am not waiving any of my legal rights as a result of signing this consent form.
- I have listened to, or read, the information on this form and I freely consent to participate in this study.
- I have been told that I will receive a dated and signed copy of this form.

I consent to myself and my infant participating in this study.

Participant's name

Child's name

Do you give consent for videotaping? We will not distribute this video or use it for any commercial purpose.

_____ No. _____ Yes → Participant's thumbprint:

Can we keep your contact information to contact you about future studies? _____

Participant's thumbprint:

Date:_____

Name of person obtaining consent

Signature of person obtaining consent



Appendix D: Abridged baseline questionnaire

Trial of thiamine supplementation in Cambodia



BASELINE QUESTIONNAIRE

MODULE 2: DEMOGRAPHIC INFORMATION		
2. What is your date of birth? (DD/MM/YYYY)	__ __ / __ __ / __ __ __ __	
3. What is your marital status?	1. Married 2. Divorced/Separated 3. Widowed 4. Single	
4. What is your ethnicity?	1. Khmer 2. Chinese 3. Vietnamese 4. Thai 5. Cham 6. other: _____	
5. How many pregnancies have you had?	__	
6. How many live births have you had?	__ → if 1, code and skip to Q8	
1. Please describe the sex and ages of the all people living in your household who eat from the household common pot (excluding yourself).	Sex	Age (circle m/y)
	1. Male	_____ months / years
	2. Female _____	_____ months / years
	1. Male	_____ months / years
	2. Female _____	_____ months / years
	1. Male	_____ months / years
	2. Female _____	_____ months / years
2. Have you attended school?	1. Yes 2. No → code, then proceed to Q11	
3. What is the highest level of school you attended?	1. Primary school 2. Lower Secondary school 3. Upper Secondary school 4. Higher education	

4. Has your husband/partner attended school?	1. Yes 2. No → code, then proceed to Q13
5. What is the highest level of schooling your husband/partner attended?	1. Primary school 2. Lower Secondary school 3. Upper Secondary school 4. Higher education
6. What is your occupation?	1. Homemaker 2. Unemployed 3. Seller 4. Garment Factory 5. other: _____
7. What was the income for your household last month ?	US\$ _____
8. What was the income for your household in the past 12 months ?	US\$ _____
1. How often do you chew betel nut/betel leaf?	1. Daily 2. Several times per week 3. Once per week 4. Several times per month 5. Once per month 6. Less than once per month 7. Never
1. How many episodes of diarrhea did you experience in the last week?	_____
2. Was this typical for you?	1. Yes 2. No

MODULE 3: EQUITY INDEX

1. Does your household have electricity?	1. Yes 2. No
2. Does your household have a television?	1. Yes 2. No
3. Does your household have a refrigerator?	1. Yes 2. No
4. Does your household have a CD / DVD player?	1. Yes 2. No
5. Does your household have a wardrobe?	1. Yes 2. No
6. Does your household have a generator / battery / solar panel?	1. Yes 2. No
7. Does any member of your household own a motorcycle / scooter?	1. Yes 2. No
8. Does any member of your household own a watch?	1. Yes 2. No
9. Does any member of this household have a bank account?	1. Yes 2. No

<p>OBSERVATION ONLY: What is the main material of the floor of the living house?</p> <p>10. RECORD ONLY ONE OBSERVATION.</p>	<p>Natural floor:</p> <ol style="list-style-type: none"> 1. Earth/sand 2. Dung <p>Rudimentary Floor:</p> <ol style="list-style-type: none"> 3. Bamboo/palm 4. Wood planks <p>Finished floor:</p> <ol style="list-style-type: none"> 5. Parquet or polished wood 6. Ceramic tiles 7. Cement 8. Carpet <p>99. Other – Specify _____</p>
<p>OBSERVATION ONLY: What is the main material of the exterior walls of the living house?</p> <p>11. RECORD ONLY ONE OBSERVATION</p>	<p>0. No Walls</p> <p>Natural walls:</p> <ol style="list-style-type: none"> 1. Earth/sand 2. Dung <p>Rudimentary walls:</p> <ol style="list-style-type: none"> 1. Bamboo/palm with mud 2. Stone with mud 3. Uncovered adobe 4. Plywood 5. Carboard 6. Re-used wood <p>Finished Walls:</p> <ol style="list-style-type: none"> 1. Metal 2. Cement 3. Stone with lime / cement 4. Bricks 5. Cement blocks 6. Covered adobe 7. Wood planks / shingles <p>99. Other – Specify _____</p>
<p>12. What type of fuel does your household mainly use for cooking?</p>	<ol style="list-style-type: none"> 1. Charcoal 2. Wood 3. Electricity 4. LPG (natural gas) 5. Biogas 6. Straw/shrubs/grass 7. Animal dung <p>99. Other – Specify _____</p>
<p>What is the main source of drinking water during the rainy season for members of your household?</p> <p>13.</p>	<ol style="list-style-type: none"> 1. Piped into dwelling 2. Open well 3. Covered well 4. Drilled Borehole (with hand pump or other type of pumping system) 5. Surface water (e.g. spring, river/stream, pond/lake/dam)

	6. Rainwater 7. Bottled water 99. Other – Specify: _____
What kind of toilet facility do members of your household usually use? 14. ASK TO SEE.	0. No facility—bush, field 1. Flush to piped sewer system (not shared with other households) 2. Flush to septic tank (not shared with other households) 3. Flush or pour toilet piped sewer system (shared with other households) 4. Flush or pour toilet to septic tank (shared with other households) 5. Traditional pit latrine 6. Ventilated Improved Pit (VIP) latrine 7. Pit latrine without slab 8. Composting toilet 9. Bucket 10. No permission to see 99. Other – Specify: _____
11. Do you share this toilet facility with other households?	1. Yes 2. No

MODULE 6: ANTHROPOMETRIC MEASUREMENTS	
1. Height of Mother	1. ___ ___ . ___ cm 2. ___ ___ . ___ cm 3. ___ ___ . ___ cm
2. Weight of Mother	1. ___ ___ . ___ kg 2. ___ ___ . ___ kg 3. ___ ___ . ___ kg
3. Length of Infant	1. ___ ___ . ___ cm 2. ___ ___ . ___ cm 3. ___ ___ . ___ cm
4. Weight of Infant	1. ___ ___ . ___ kg 2. ___ ___ . ___ kg 3. ___ ___ . ___ kg
5. Head circumference of Infant	1. ___ ___ . ___ cm 2. ___ ___ . ___ cm 3. ___ ___ . ___ cm

Appendix E: Abridged midline questionnaire

Trial of thiamine supplementation in Cambodia



MIDLINE QUESTIONNAIRE

MODULE 1: DEMOGRAPHIC INFORMATION	
1. What was the income for your household last month ?	US\$ _____
1. How often do you chew betel nut/betel leaf?	1. Daily 2. Several times per week 3. Once per week 4. Several times per month 5. Once per month 6. Less than once per month 7. Never
2. How many episodes of diarrhea did you experience in the last week?	_____
3. Do you typically have diarrhea?	1. Yes 2. No

MODULE 6: ANTHROPOMETRIC MEASUREMENTS	
1. Weight of Mother	4. ___ ___ . ___ kg 5. ___ ___ . ___ kg 6. ___ ___ . ___ kg
2. Length of Infant	4. ___ ___ . ___ cm 5. ___ ___ . ___ cm 6. ___ ___ . ___ cm
3. Weight of Infant	4. ___ ___ . ___ kg 5. ___ ___ . ___ kg 6. ___ ___ . ___ kg
4. Head circumference of Infant	4. ___ ___ . ___ cm 5. ___ ___ . ___ cm 6. ___ ___ . ___ cm

Appendix F: Abridged endline questionnaire

Trial of thiamine supplementation in Cambodia



ENDLINE QUESTIONNAIRE

MODULE 1: DEMOGRAPHIC INFORMATION	
1. What was the income for your household last month ?	US\$ _____
3. How often do you chew betel nut/betel leaf?	1. Daily 2. Several times per week 3. Once per week 4. Several times per month 5. Once per month 6. Less than once per month 7. Never
1. How many episodes of diarrhea did you experience in the last week?	_____
2. Do you typically have diarrhea?	1. Yes 2. No

MODULE 6: ANTHROPOMETRIC MEASUREMENTS	
1. Weight of Mother	1. ___ ___ . ___ kg 2. ___ ___ . ___ kg 3. ___ ___ . ___ kg
4. Length of Infant	1. ___ ___ . ___ cm 2. ___ ___ . ___ cm 3. ___ ___ . ___ cm
4. Weight of Infant	1. ___ ___ . ___ kg 2. ___ ___ . ___ kg 3. ___ ___ . ___ kg
5. Head circumference of Infant	1. ___ ___ . ___ cm 2. ___ ___ . ___ cm 3. ___ ___ . ___ cm

Appendix G: MSVU Certificate of research ethics clearance



University Research Ethics Board (UREB)

Certificate of Research Ethics Clearance

<input checked="" type="checkbox"/> Clearance	<input type="checkbox"/> Secondary Data Clearance	<input type="checkbox"/> Renewal	<input type="checkbox"/> Modification	<input type="checkbox"/> Change to Study Personnel
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Effective Date	April 27, 2018	Expiry Date	April 26, 2019
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File #:	2017-141
Title of project:	Trial of thiamine supplementation in Cambodia
Researcher(s):	Kyly Whitfield
Supervisor (if applicable):	n/a
Co-Investigators:	n/a
Version :	1

The University Research Ethics Board (UREB) has reviewed the above named research proposal and confirms that it respects the *Tri-Council Policy Statement: Ethical Conduct for Research Involving Humans* and Mount Saint Vincent University's policies, procedures and guidelines regarding the ethics of research involving human participants. This certificate of research ethics clearance is valid for a period of **one year** from the date of issue.

Researchers are reminded of the following requirements:	
Changes to Protocol	Any changes to approved protocol must be reviewed and approved by the UREB prior to their implementation. Form: REB.FORM.002 Info: REB.SOP.113 Policy: REB.POL.003
Changes to Research Personnel	Any changes to approved persons with access to research data must be reported to the UREB immediately. Form: REB.FORM.002 Info: REB.SOP.113 Policy: REB.POL.003
Annual Renewal	Annual renewals are contingent upon an annual report submitted to the UREB prior to the expiry date as listed above. You may renew up to four times, at which point the file must be closed and a new application submitted for review. Form: REB.FORM.003 Info: REB.SOP.116 Policy: REB.POL.003
Final Report	A final report is due on or before the expiry date. Form: REB.FORM.004 Info: REB.SOP.116 Policy: REB.POL.003
Privacy Breach	Researchers must inform the UREB immediately and submit the Privacy Breach form. The breach will be investigated by the REB and the FOIPOP Officer. Form: REB.FORM.015
Unanticipated Research Event	Researchers must inform the UREB immediately and submit a report to the UREB within seven (7) working days of the event. Form: REB.FORM.008 Info: REB.SOP.115 Policy: REB.POL.003
Adverse Research Event	Researchers must inform the UREB immediately and submit a report to the UREB within two (2) working days of the event. Form: REB.FORM.007 Info: REB.SOP.114 Policy: REB.POL.003

*For more information: <http://www.msvu.ca/ethics>



Daniel Seguin, Chair
University Research Ethics Board

Halifax Nova Scotia B3M 2J6 Canada
Tel 902 457 6350 • msvu.ca/ethics

Appendix H: NECHR certificate of research ethics clearance



ក្រសួងសុខាភិបាល
MINISTRY OF HEALTH
គណៈកម្មាធិការជាតិរក្សាសីលធម៌
សំរាប់ការស្រាវជ្រាវសុខភាពដែលទាក់ទងនឹងមនុស្ស
National Ethics Committee for Health Research

លេខ.....118.....NECHR.....

ព្រះរាជាណាចក្រកម្ពុជា
KINGDOM OF CAMBODIA
ជាតិ សាសនា ព្រះមហាក្សត្រ
NATION RELIGION KING

រាជធានីភ្នំពេញ, ថ្ងៃទី...០១...ខែ...០៤...ឆ្នាំ2018.....

Dr. Kyly Whitfield

Project: Trial of thiamine supplementation in Cambodia. Version N° 1, dated 1st March 2018

Reference: 26th April 2018 NECHR meeting minute

Dear Dr. Kyly Whitfield,

I am pleased to inform you that your study of the protocol entitled “Trial of thiamine supplementation in Cambodia. Version N° 1, dated 1st March 2018” has been approved by National Ethics Committee for Health Research (NECHR) in the meeting on 26th April 2018. This approval is valid for twelve months after the approval date.

The Principal Investigator of the project shall submit following document to the committee’s secretariat at the National Institute of Public Health at #80 Samdach Penn Nouth Blvd, Sangkat Boeungkok2, Khan Tuol Kork, Phnom Penh. (Tel: 855-23-880345, Fax: 855-23-881949):

- Annual progress report
- Final scientific report
- Patient/participant feedback (if any)
- Analyzing serious adverse events report (if applicable)

The Principal Investigator should be aware that there might be site monitoring visits at any time from NECHR team during the project implementation and should provide full cooperation to the team.

Regards,

Chairman

Prof. ENG HUOT

ភ្នំពេញលេខ៨០, វិទ្យាស្ថាន បឹង ឆ្នក (២៨៩) សង្កាត់បឹងកក់២ ខណ្ឌ ទួលគោក រាជធានីភ្នំពេញ, កម្ពុជា (៨៥៥-២៣) ៨៨០ ៣៤៥, ទូរស័ព្ទលេខ (៨៥៥-១២) ២៨០ ៧៩០, (៨៥៥-១២) ៨៤២ ៤៤២
 Lot #80, Samdach Penn Nouth Blvd (289), Sangkat Boeungkok2, Khan Tuol Kork, Phnom Penh, Cambodia. Tel: (855-23) 880 345, Mobile Phone: (855-12) 280 790, (855 12) 842 442