PROTOCOL

Study Title: **Trial of thiamine supplementation in Cambodia**

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Statement of Compliance

This document is a protocol for a randomized controlled trial. The study will be conducted in compliance with all stipulations of this protocol and the conditions of ethics committee approval. I agree that the study will be conducted in accordance with the conditions outlined in the protocol (subject to any amendments). I have read and understood the protocol.

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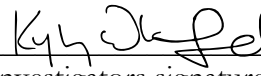
Investigator's name


Investigators signature

August 14, 2019.
Date

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1. Study Synopsis

1.1 Title

Trial of thiamine supplementation in Cambodia

Note that this study was funded using the title: 'Improving estimates of the global burden of thiamine deficiency disorders (TDDs) and approaches to their control - Objective 3: Trial of thiamine supplementation in Cambodia.'

1.2 Objectives

1. To conduct a dose response supplementation trial among lactating Cambodian women to determine the lowest dose of thiamine that yields the maximum increase in human milk thiamine.
2. To assess rural Cambodian households' usual salt intake using a household salt disappearance study.
3. To assess cognitive development of infants aged 2 weeks – 12 months to mothers consuming varying thiamine doses using a small battery of cognitive assessments.

1.3 Design

Double-blind, four-parallel arm, placebo-controlled randomized trial.

1.4 Outcomes and Analysis Objectives

The primary outcome of this study is human milk total thiamine concentration at 24 weeks postpartum. The primary objective of this study is to estimate the dose on the dose response curve where additional maternal intake of thiamine (oral dose) no longer meaningfully increases human milk total thiamine concentration at 24 weeks postpartum. A full list of outcomes and analysis objectives can be found in Section 4.

1.5 Study Duration

This is a two-year study, to be completed between 2018 and 2020. The intervention is 22 weeks, from 2 weeks through 24 weeks postnatal, with an additional follow-up at 12 months.

1.6 Interventions

Participants will be randomized to one of four treatment arms, and will be asked to consume one oral capsule daily. Treatment arms are:

- negative control group (placebo; 0 mg thiamine)
- EAR group (1.2 mg thiamine as thiamine hydrochloride)
- double EAR group (2.4 mg thiamine as thiamine hydrochloride)
- positive control group (10 mg thiamine as thiamine hydrochloride)

1.7 Sample size

320 women and their newborn infants.

1.8 Population

Participants must meet the following criteria:

- mothers of a newborn
- aged 18-45 years
- most recent pregnancy was normal (i.e. no known chronic conditions, preeclampsia, gestational diabetes etc.), and the singleton infant was born without complications (e.g. low birth weight (<2.5 kg), tongue tie, cleft palate)
- intends to exclusively breastfeed for six months
- resides in Kampong Thom province, Cambodia, and is not planning to move in the next six months
- is not currently taking, and hasn't taken any thiamine-containing supplements over the previous 4 months
- is not currently participating in any nutrition programs beyond normal care
- is willing to consume one capsule daily from 2 weeks through 24 weeks postpartum
- is willing for her entire household consume only salt provided by the study team
- is willing for the following biological samples to be collected: a maternal venous blood sample and human milk sample at 2 weeks postpartum, a human milk sample at 4 and 12 weeks postpartum, and maternal and infant blood samples and a human milk sample at 24 weeks postpartum.

2. Investigators, Scientific Advisory Board, and Study Locations

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2.4 Study Location

Households in Kampong Thom province, Cambodia within the catchment area of the following Health Centres: Tboung Kapoeur, Kampong Svay, Sankor, Chey, Salavisai, Prey Kuy, Prey Pros, and Srayov Health Centres.

2.5 Study Management

The Principal Investigator and Co-Investigators will oversee the coordination of the study; they will meet bi-monthly, or more often if required. Helen Keller International, Cambodia will be the primary implementer of the study, overseeing day-to-day operations, including recruitment, consent, data and biological sample collection, and data management. The Scientific Advisory Board will meet with the investigators annually.

2.6 Serious Adverse Events Committee / Data Safety Monitoring Board

There is no necessity for a Serious Adverse Events Committee or a Data Safety Monitoring Board (DSMB) in the current study because of the absence of potential adverse effects. There is no tolerable upper intake level (UL) for thiamine because there has never been an adverse event reported from high thiamine intake (1,2), even though over-the-counter supplements in Canada and the United States commonly contain 50 mg of thiamine or more. Hence, we are confident that there is a very low risk of serious adverse events in this study related to the intervention.

However, we do expect some infant deaths in our cohort due to other causes. As per the 2014 Cambodian Demographic and Health Survey (CDHS), the rate of neonatal (birth to one month) and infant (birth to one year) mortality was 29 and 11 deaths per 1,000 live births, respectively (3). The majority of neonatal deaths occur in the first 6 days postnatal: in the 4 years preceding the 2014 CDHS data collection, 82% of neonatal deaths occurred in the first 6 days after birth (3). Since this study starts at 2 weeks postnatally, we would expect the mortality rate in our study to lie between 1.67 (neonatal mortality > 6 days postnatal for Kampong Thom = 0.029*0.18*320) and 3.68 (infant mortality in Kampong Thom = 0.011*320) infant deaths.

2.7 Funding

This study is funded through the Bill & Melinda Gates Foundation and the Sackler Institute for Nutrition Science, New York Academy of Sciences (Opportunity ID OPP1176128) as 'Objective 3: Trial of thiamine supplementation in Cambodia', as part of the larger program grant entitled 'Improving estimates of the global burden of thiamine deficiency disorders (TDDs) and approaches to their control'. The cognitive follow-up assessment at 12 months postnatal is funded by the New York Academy of Sciences.

3. Introduction, Background, and Rationale

3.1 Thiamine, and thiamine deficiency

Thiamine (vitamin B_1) is an essential water-soluble micronutrient required for energy metabolism (4). Thiamine deficiency remains an under-studied public health issue, recently being called the forgotten disease of Asia (5). There is evidence of suboptimal thiamine intake or status throughout Southeast

Asia, notably in Cambodia (6–11), Laos (5,12–14), and among Karen refugees during their early arrival in the Mae La Camp on the Thai-Myanmar border (15,16).

Infantile beriberi, a disease caused by thiamine deficiency, presents during the exclusive breastfeeding period and without treatment can result in death within hours of clinical presentation (15). The dietary staple of Cambodia is B-vitamin poor, white, polished rice; it makes up upwards of 60% of daily energy intake (17). Women with poor dietary thiamine intake produce milk low in thiamine, putting their exclusively breastfed infants at a high risk of developing infantile beriberi (18). We recently demonstrated that maternal consumption of a thiamine-fortified fish sauce significantly increased maternal, human milk, and infant thiamine status (10).

There is also a growing body of evidence suggesting thiamine deficiency not severe enough to cause clinical symptoms has a negative effect on cognitive development and functioning (19–22), though the precise pathways and mechanisms linking these two factors remain poorly understood. Important questions remain about the timing of deficiency, levels of deficiency, and the ability of prophylactic interventions to prevent or therapeutic interventions to remediate the effects of suboptimal thiamine status on cognitive outcomes in humans (23).

3.2 Dietary thiamine requirements

The Institute of Medicine's Dietary Reference Intakes (DRI) can be used to assess dietary thiamine adequacy (24). Thiamine has an estimated average requirement (EAR), the median requirement for a given lifestage group, and a statistically derived recommended dietary allowance (RDA), an estimate of the daily average dietary intake that meets the nutrient needs of 97.5% of healthy members of the group (24). There is no UL for thiamine because there have been no reports of adverse effects of excess thiamine intake (1,2,25). Lactating women secrete an estimated 0.16 mg thiamine per day into human milk, and additional thiamine is required by the mother herself to meet increased energy needs for milk production (1). For lactating women, the EAR and RDA are 1.2 and 1.4 mg/day, respectively (1).

For infants, an adequate intake (AI) was derived due to insufficient evidence to set an EAR and, in turn, a RDA (1). The thiamine concentrations of milk from healthy, well-nourished mothers are extrapolated, assuming a daily milk intake of 780 mL (1). The thiamine AI for infants aged 0-6 months is 0.2 mg/day, however, the evidence base used for setting this AI is poor due to the small sample size and the outdated analytical techniques to assess human milk thiamine concentrations (26).

3.3 Biomarkers of thiamine status

Two biomarkers are used to assess thiamine status. Thiamine diphosphate (ThDP) is the biologically active form of the vitamin (4), and concentrations can be assessed in whole blood or erythrocytes using high performance liquid chromatography with a fluorescence detector (27). Unfortunately there is currently no consensus on the most appropriate ThDP cut-offs for thiamine deficiency (28). The other assessment method is a functional marker of thiamine status, transketolase activity coefficient. ThDP is a cofactor with transketolase in the pentose phosphate pathway; *in vitro* measurement of the activity of transketolase before and after the addition of excess ThDP reveals the original level of ThDP saturation, indicating thiamine adequacy (1,25). An erythrocyte transketolase activity coefficient >1.25 is commonly used as a cut-off for thiamine deficiency (1).

3.4 Efforts to improve perinatal thiamine intake

Low maternal thiamine intake reduces the thiamine concentration of human milk (18). Infantile thiamine deficiency and beriberi are not normally seen in countries where maternal thiamine intake is adequate (29). Both supplementation and fortification interventions have been employed in an attempt to improve maternal thiamine status as a means of preventing infantile thiamine deficiency. Between 1987 and 1990 there was an outbreak of infantile beriberi in the Mae La refugee camp on the Thai-Burmese border that accounted for 40% of infant mortality (15,16). As a preventative measure, lactating women in this camp were provided with a weekly 10 mg thiamine supplement until 9 months postpartum (15). However, supplementation has several challenges, including high cost, issues of distribution and a sustainability, and compliance.

3.5 Thiamine fortification

Fortification is a potentially more suitable alternative to supplementation, as it is a sustainable, cost-effective, and passive intervention (30–33). Fortification is a highly successful public health intervention; for example, two thirds of the world's population has access to iodized salt (34), and folic acid fortification in Canada and elsewhere has reduced the incidence of neural tube defects (35). Wheat flour is fortified in many countries (36), however this is not appropriate in Cambodia and other countries where wheat flour is not a food staple. We recently conducted a randomized controlled efficacy trial of thiamine-fortified fish sauce, and found that consumption over 6 months improved blood and human milk thiamine concentrations of mothers, their breastfed infants < 6 months, and children < 5 years (10,11). However, centrally produced fish sauce may not reach the poorest communities who make their own fish sauce, and fish sauce consumption is not universal in all regions where we find thiamine deficiency (37,38).

3.6 Salt as a fortification vehicle

Salt is a common condiment in most regions of the world, and has proven to be a successful global fortification vehicle for iodine (39). Current estimates suggest usual per capita salt intake in Cambodia is 15 g/day day (40). However, we do not know how much is consumed at the individual level. In addition, the WHO has been actively promoting salt reduction programs globally with a goal to decrease salt intake to 5 g/day (41), therefore new measures of salt intake are required.

3.7 Summary of rationale

Suboptimal maternal thiamine intake puts exclusively breastfed infants at risk of low thiamine status, impaired cognitive development, and infantile beriberi, which can be fatal. Thiamine fortification of salt is a potentially low-cost and sustainable means of combating suboptimal thiamine status, however knowledge gaps must be filled before thiamine fortification can proceed. There is limited data available on the dose of thiamine required by lactating women to optimize the thiamine concentrations in their milk. Further, usual salt intake among lactating women is unknown. Finally, although there is emerging evidence that low thiamine intake in early life impacts cognitive development, this has yet to be assessed in a controlled research environment. The overall aim of this study is to obtain the information necessary to formulate a thiamine-fortified salt for future use in Cambodia (ideal dose, usual salt intake), and to explore the impact of various doses of maternal thiamine on makers of infant cognitive development.

4. Study Objectives

Primary Outcome and Analysis Objective:
To estimate the dose on the dose response curve where additional maternal intake of thiamine (oral dose) no longer meaningfully increases human milk total thiamine concentration at 24 weeks postpartum.

Secondary Outcomes and Analysis Objectives:

1. To estimate the dose on the dose response curve where additional maternal intake of thiamine (oral dose) no longer meaningfully increases infant thiamine diphosphate concentrations (ThDP) 24 weeks postnatally, and assess whether this depends on the presence/absence of a genetic hemoglobin disorder.
2. To estimate the dose on the dose response curve where additional maternal intake of thiamine (oral dose) no longer meaningfully increases human milk total thiamine concentration at 4 and 12 weeks postpartum.
3. To estimate the dose on the dose response curve where additional maternal intake of thiamine (oral dose) no longer meaningfully increases infant transketolase activity at 24 weeks postnatally, and assess whether this depends on the presence/absence of a genetic hemoglobin disorder.
4. To estimate the dose on the dose response curve where additional maternal intake of thiamine (oral dose) no longer meaningfully increases maternal ThDP at 24 weeks postpartum, and assess whether this depends on the presence/absence of a genetic hemoglobin disorder.
5. To estimate the dose on the dose response curve where additional maternal intake of thiamine (oral dose) no longer meaningfully increases maternal ETKac at 24 weeks postpartum, and assess whether this depends on the presence/absence of a genetic hemoglobin disorder.
6. To test for differences between the 4 randomised groups on human milk total thiamine at 4, 12, and 24 weeks postpartum.
7. To test for differences between the 4 randomised groups on maternal ThDP at 24 weeks postpartum, and assess whether this depends on the presence/absence of genetic hemoglobin disorder.
8. To test for differences between the 4 randomised groups on maternal ETKac at 24 weeks postpartum and assess whether this depends on the presence/absence of a genetic hemoglobin disorder.
9. To estimate usual household salt intake from mean fortnightly salt disappearance (weight lost, in g).
10. To estimate salt intake among a subset of 100 lactating women, their male partners (if applicable), and their children 24-59 months (if applicable) using observed weighed salt intake records.
11. To estimate sodium intake using 24 hr urinary sodium concentrations among a subset of 100 lactating women.
12. To test for differences between the 0 & 10 mg randomised groups on Composite Mullen and the 5 subscales of the Mullen at 24 and 52 weeks postnatally.
13. To test for differences between the 0 & 10 mg randomised groups on Visual Paired Comparison Novelty Score and the attention and processing speed subscales at 24 and 52 weeks postnatally.

14. To test for differences between the 0 & 10 mg randomised groups on the Language Preference Task Score at 24 and 52 weeks postnatally.
15. To test for differences between 0 & 10 mg randomised groups on oculomotor scores at 24 and 52 weeks postnatally.
16. To determine the effect of inflammation, as measured by C-reactive protein (CRP) and α-1-acid-glycoprotein (AGP) on maternal ThDP at 2 and 24 weeks postpartum, and infant ThDP at 24 weeks postnatal.

5. Study Design

This will be a double-blind, four-parallel arm randomized controlled trial, in which 320 women and their newborn infants will be enrolled. Each dyad will participate in the study between 2 and 24 weeks postnatal, a 22-week intervention period. The primary outcome measure is human milk thiamine concentrations at 24 weeks postpartum (see **Figure 1**). There are additional cognitive assessments at 12 months (52 weeks) postnatal.

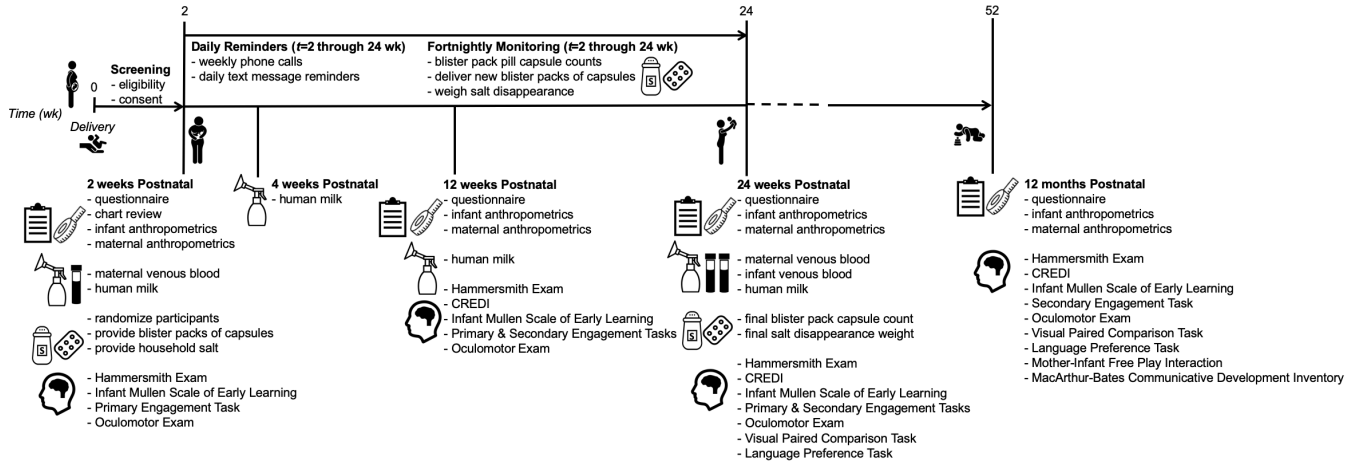

Figure 1: A pictorial description of the study design and data collection scheme.

6. Study Treatments

6.1 Description of study arms

The four study arms are described in **Table 1**, below.

Table 1: Treatment arms for the trial.

Treatment Arm	Rationale	Thiamine Dose
Negative Control	Negative Control (placebo)	0 mg/d
EAR	1 x thiamine EAR for lactating women (1)	1.2 mg/d
Double EAR	2 x thiamine EAR for lactating women (1)	2.4 mg/d
Positive Control	Positive Control (dose currently given in supplemental form in Myanmar)	10 mg/d

Supplements will be opaque capsules containing varying amounts of thiamine hydrochloride and cellulose filler, as indicated in **Table 1**. All thiamine is delivered as thiamine hydrochloride, calculated using a 1.271 correction factor (ratio of molecular weights of thiamine hydrochloride and

thiamine). Capsules will be formulated and compounded by pharmacist Anastasia Hanias at the Quinpool Wellness Centre in Halifax, Nova Scotia, Canada.

6.2 Treatment arm codes and packaging of study capsules

Statisticians at the South Australian Health and Medical Research Institute (SAHMRI) will create a total of 8 alpha-numeric codes consisting of 3 letters followed by 8 numbers to label study supplements, two sets of codes per treatment arm.

Study capsules will be packaged in 14-capsule blister packs and delivered to the participants every two weeks. These blister packs will contain the following labeling in both English and Khmer:

- "For research purposes only"
- "Could contain up to 10 mg thiamine (vitamin B1)"
- "Take one capsule each morning"
- best before date
- alpha-numeric treatment code
- days of the week on each blister slot
- study ID and barcode (individualized sticker)

6.3 Safety of thiamine doses

The thiamine hydrochloride safety data sheet can be found in **Appendix A**. The thiamine EAR is 1.2 mg/day (1), however there is no concern for regular consumption of higher doses because thiamine supplements above these doses are commonly available over-the-counter in Canada and the United States. For example in Canada, Jamieson Natural Sources has Health Canada approval to sell B Complex vitamin supplements containing up to 100 mg each of thiamine. In addition, as noted in *3.2 Dietary thiamin requirements*, there is no UL for thiamine because there have been no reports of adverse effects of excess thiamine intake (1,2,25).

6.3 Product accountability

Initial Assessment
All capsules will be formulated and packed in two batches at the Quinpool Wellness Centre in Halifax, Nova Scotia, Canada, in July and September 2018 using codes assigned by an independent scientist not involved in this study. Before data collection, an independent lab will assess a sample of capsules from each batch to confirm thiamine concentrations. The independent scientist will courier 10 capsules from each of the 8 treatment codes to the independent lab for analysis. The lab report will be shared with an independent scientist, who will cross-check values with the treatment arm codes to ensure the supplements meet the study standards. The supplements will be deemed acceptable if the average thiamine concentration for each code (n=10 samples) falls within 15% of the values shown in **Table 1**.

Throughout the study
A set of supplement blister packs will be stored at the village chief's home (in a manner similar to how women are storing their blister packs) in each of the eight health centre catchment areas in which we will work. Field staff will collect one blister pack from each of the village chief's homes every second month for assessment. We will collect 1 blister pack x 8 treatment codes x 8 village

chief's homes = 64 blister packs will be couriered to the independent lab. Here, the lab technician will select 5 capsules from each blister pack for analysis; supplements will be deemed acceptable if the average thiamine concentration for each code (n=40 samples) falls within 15% of the values shown in **Table 1**.

6.4 Compliance

Compliance will be assessed fortnightly: field staff will visit the participant's home to pick up the old blister pack and complete a capsule count, and to deliver a new blister pack. A woman will be considered compliant if she consumes ≥80% capsules over the 22-week intervention.

In an attempt to ensure high compliance, participants will interact with the same field staff person throughout the entire study in hopes of building rapport. This field staff person will complete in-person check-ins fortnightly throughout the study, and will also make weekly phone calls to encourage women to continue consuming their study capsules daily. All participants will be provided with a mobile phone as part of this study.

We are also conducting a small compliance pilot study in advance of this randomized controlled trial to determine if additional reminders in the form of daily voice messages (e.g. Verboice) or text SMS, in addition to the weekly phone calls and fortnightly visits, are warranted.

7. Sample Size

To detect a clinically meaningful difference of 40 μg/L in human milk total thiamine concentration between any two treatment groups with 90% power, assuming a SD of 43 μg/L (estimated SD of control group in a Cambodian thiamine-fortified fish sauce trial (9)), 48 women are required per treatment group, or a total of 192 women. This sample size allows for 20% attrition and uses a two-sided alpha of 0.0083 for each of the 6 pairwise comparisons between the four treatment groups in order to control the familywise error rate at the 0.05 level using a Bonferroni adjustment for multiple comparisons. Recruitment of 320 participants (80 per group) was planned to allow for some uncertainty in the assumed values, particularly the SD which may be larger than anticipated.

Simulations of 500 dose-response curves for human milk total thiamine concentration were conducted to estimate the precision that this sample size would provide for addressing the primary study objective (i.e. for estimating the dose required to achieve 90% of the average maximum human milk total thiamine concentration). Data were simulated based on an average minimum and maximum concentration of 136 μg/L (9) and 210 μg/L (1), respectively, with a SD of 43 μg/L (9), and reaching 50% and 90% of the maximum average concentration at dose 1.2 mg/d (EAR group) and 2.4 mg/d (double EAR group) respectively. Assuming an Emax dose-response curve, the precision of the estimated dose is ±1.29 mg/d (i.e. the 90% confidence interval for the estimated dose will be within ±1.29 mg/d of the point estimate).

8. Enrolment and Randomization

8.1 Recruitment

We plan to recruit and enroll the majority of participants through antenatal care (ANC) visits, as well as through consultation with local village chiefs, elders, and health centre staff. Pregnant women will be advised of the study during ANC programs within the local health centres in Kampong Thom province in which we will work. The following health centres were selected in consultation with the National Nutrition Programme (Cambodian Ministry of Health) because of their high birth rates: Tboung Kapoeur, Kampong Svay, Sankor, Chey, Salavisai, Prey Kuy, Prey Pros, and Srayov Health Centres. Local midwifes and nurses will provide a general overview of the research study, and will share names and contact information of interested women with the research team. The research team will follow up with women after delivery, screen for eligibility, obtain consent, and enroll women. Recruitment will continue on a rolling basis until 320 women are enrolled (we anticipate rolling recruitment over five months). A record of all women screened but not enrolled will be maintained.

8.2 Eligibility criteria

All women must provide written informed consent to participate. Participants must meet the following criteria:

- mothers of a newborn
- aged 18-45 years
- most recent pregnancy was normal (i.e. no known chronic conditions, preeclampsia, gestational diabetes etc.), and the singleton infant was born without complications (e.g. low birth weight (<2.5 kg), tongue tie, cleft palate)
- intends to exclusively breastfeed for six months
- resides in Kampong Thom province, Cambodia, and is not planning to move in the next six months
- is not currently taking, and hasn't taken any thiamine-containing supplements over the previous 4 months
- is not currently participating in any nutrition programs beyond normal care
- is willing to consume one capsule daily from 2 weeks through 24 weeks postpartum
- is willing for her entire household consume only salt provided by the study team
- is willing for the following biological samples to be collected: a maternal venous blood sample and human milk sample at 2 weeks postpartum, a human milk sample at 4 and 12 weeks postpartum, and maternal and infant blood samples and a human milk sample at 24 weeks postpartum

8.3 Randomization procedures and blinding

Women will be randomly assigned to one of the four treatment groups as described in **Table 1**. Participants, field staff, all study investigators, and data analysts will be blinded to the randomized groups. The randomization will be stratified by study centre and use randomly permuted blocks of size 8 within strata to assign participants to one of eight treatment codes in the ratio 1:1:1:1:1:1:1:1 (2 treatment codes per treatment group). An individual who is independent of the trial will determine

which treatment codes correspond to which treatment group. A computer-generated randomization schedule will be prepared by the study statisticians using ralloc.ado in Stata.

When a participant is enrolled in the study they will be assigned a unique study ID. The field staff will open an envelope labeled with the study ID and containing the study ID and accompanying treatment code. This code will be recorded on the participant's 2 weeks postnatal questionnaire, and the participant will receive their first blister pack of capsules. At every data collection point the participant's study ID will be typed into the data collection tablet and the treatment code will be displayed in order to ensure that the participant is receiving the correct treatment throughout the study.

8.4 Breaking of the study blind

Breaking blinding during the study
The master ID list will not be unblinded unless in the unlikely event of an adverse event in the trial. Note that the likelihood of any adverse events is extremely unlikely since there is no UL for thiamine because there have been no reports of adverse effects of excess thiamine intake (1,2,25). If, however, there is a medical emergency and unblinding of a participant is required, our blinding mechanism would allow for only one of the two codes per treatment arm to be revealed (as each treatment arm will have two codes). We would then document and explain to the funding agency and all ethics committees why any premature unblinding occurred.

Breaking the blinding after the study
Once data collection and cleaning is complete, the database will be locked and unblinded treatment codes will be included in the database because a blinded analysis is not possible for estimating dose response curves.

Due to the time-consuming nature of coding video-recorded cognitive assessments, data for the following assessments will not be included in the database before it is locked and unblinded: primary engagement task, secondary engagement task, Visual Paired Comparison Task, and Language Preference Task. Note that the research assistants coding the videos for these assessments will remain blinded.

8.5 Participant withdrawal

Participants are free to withdraw from the study at any time. The reasons for withdrawal will be recorded and reported in a CONSORT-style flow and follow up diagram. Participants who discontinue treatment or are withdrawn from the study will not be replaced. Any data collected on that participant up to the time of withdrawal will be retained for analysis.

9. Study Procedures

Data and biological samples will be collected at enrolment (between t=0 to 2 weeks postpartum), at 2, 4, 12, 24, and 52 weeks postnatal, as well as during fortnightly monitoring visits. A pictorial summary of data collection can be found in **Figure 1.**

9.1 Chart review

Upon enrolment, a primary healthcare worker in the Health Centre (receiving an incentive payment from the study) will record data from the dyad's chart such as any interventions/events at birth, maternal age, number of antenatal care visits, number of iron and folic acid tablets consumed, time and date of birth, sex of infant, and infant anthropometrics.

9.2 Fortnightly monitoring

Every two weeks between 2-24 weeks, a member of the research team will visit the participant in her home to distribute new blister packs and collect used blister packs (compliance measure). At this time the field staff will also ask a small number of questions about capsule compliance, selling or sharing of salt, and about infant and young child feeding (IYCF) practices (was the infant exclusively breastfed over the previous two weeks). The field staff will weigh the household salt container to assess salt disappearance, and will provide more salt to the household.

9.3 Salt disappearance

At the initial home visit after randomization, field staff will check that the household has removed all salt from the home, and will distribute table salt in specialized study containers. Using calibrated scales (sensitive to 1 g), field staff will log the initial weight of the salt container(s). Participants will be asked to consume only salt provided by the study, and since Cambodian families eat from a common pot, they will instructed for all family meals to be prepared using this salt. At each fortnightly monitoring visit, field staff will weigh the salt container(s) to determine household salt consumption from salt disappearance. In addition, field staff will ask women to confirm the household members eating from the common pot.

The fortnightly salt disappearance study will yield household salt consumption, but does not allow us to make strong estimates of intra-household salt consumption, nor the consumption of salt more holistically (including from packaged foods, salt-containing condiments). This information will be important moving forward in order to calculate dosages for thiamine-fortified salt. Also, gathering information not only from lactating women, but also presumably high consumers (husbands) and very low consumers at risk of deficiency (children after the window of micronutrient powder consumption, so 24-59 months) is also vital for future fortification modelling. Therefore, we will collect supplemental information in a randomly selected subset of households (n=100) using observed weighed intakes and maternal urinary sodium concentrations (randomly selected from larger study, and a second day repeat in 75 households on non-consecutive days). The participant (mother), the husband/man in household aged 18-50 years, and a child between 24-59 months (husband/man and child will not be applicable in all households) will be enrolled in this sub-study. For the observed weighed intake sub-study, field staff will sit in the participants home from dawn through dusk, recording, at the individual level, intake for the 1-3 individuals for all table salt and salt-containing condiments (e.g. fish and soy sauces) consumed throughout the day. For the mothers only, we will assess 24-hour urinary sodium concentrations (25). The mother will be instructed to awake, void her urine, record the time, and then collect all urine over the next 24 hours into a provided container; the collection will end when the woman will attempt to void urine at the exact specified time, 24 hours after initiation of collection. The field staff will visit her home to weigh the full container and then take an aliquot for testing (the remaining urine can be discarded). The urine

sample will be placed in the 4°C fridge in Kampong Thom for <1 week before being transported the Pasteur Institute in Phnom Penh for storage at -20°C and subsequent batch analysis via potentiometry using ion selective electrode on an automated ABX PENTRA C400. We will model thiamine fortification of salt using the University of Iowa's Intake Monitoring, Assessment and Planning Program software (http://www.side.stat.iastate.edu/imapp.php).

9.4 Questionnaire

Using an interviewer-administered questionnaire, we will collect demographic and socioeconomic information, health information including questions about sleep (adapted from (42)) and postpartum depression (43), limited dietary intake data (e.g. perceptions of salt intake, postpartum food taboos (44)), and IYCF knowledge and behaviors at 2, 12, 24, and 52 weeks postnatal.

9.5 Anthropometrics

Infants: length, weight, and head circumference; mothers: height and weight. Initial measurements will be taken in the Health Centre at delivery, and all other measurements will be collected in participant's homes (2, 12, 24, and 52 weeks postnatal) using calibrated instruments and standard protocols as per (45).

9.6 Venous blood samples

Trained, Khmer-speaking phlebotomists will meet mothers and infants at their home or a central village location (health centre, or village chief's home) to collect maternal and infant blood samples into EDTA-coated tubes. This is a very low risk procedure; however, participants will be advised that 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 participants will immediately be given a bandage to cover the spot where the blood was taken. Maternal blood samples will be collected at 2 and 24 weeks postnatal; infant samples will be collected only at 24 weeks postnatal. Time of day and time since last meal will be recorded.

9.7 Human milk samples

Human milk samples will be collected using a battery-powered single breast pump (Swing Breast pump, Medela) at 2, 4, 12, and 24 weeks postnatal. One full breast expression (single breast) will be collected from the breast women self-identify as being more 'full' (the breast not most recently emptied). Since time of day has little effect on milk thiamin concentrations (46), samples can be collected at any time of day, however, time of day, time since last meal, and breast side will be recorded.

9.8 Blood and human milk processing and analysis

Biological samples will be collected in the village (woman's home, or central village location such as the village chief's home), placed on ice, and transported to the field lab in Kampong Thom within 5 hours of collection. Phlebotomists will collect 9 mL venous blood from mothers and 5 mL from infants (in EDTA-coated Vaccutainers). Venous blood samples will be processed as per **Figure 2**. All samples will be stored at -20°C for <1 week before being transported the National Institute for

Public Health Laboratory in Phnom Penh for storage at -80°C. Samples will be batch shipped on dry ice after the 24 weeks postnatal data collection is completed.

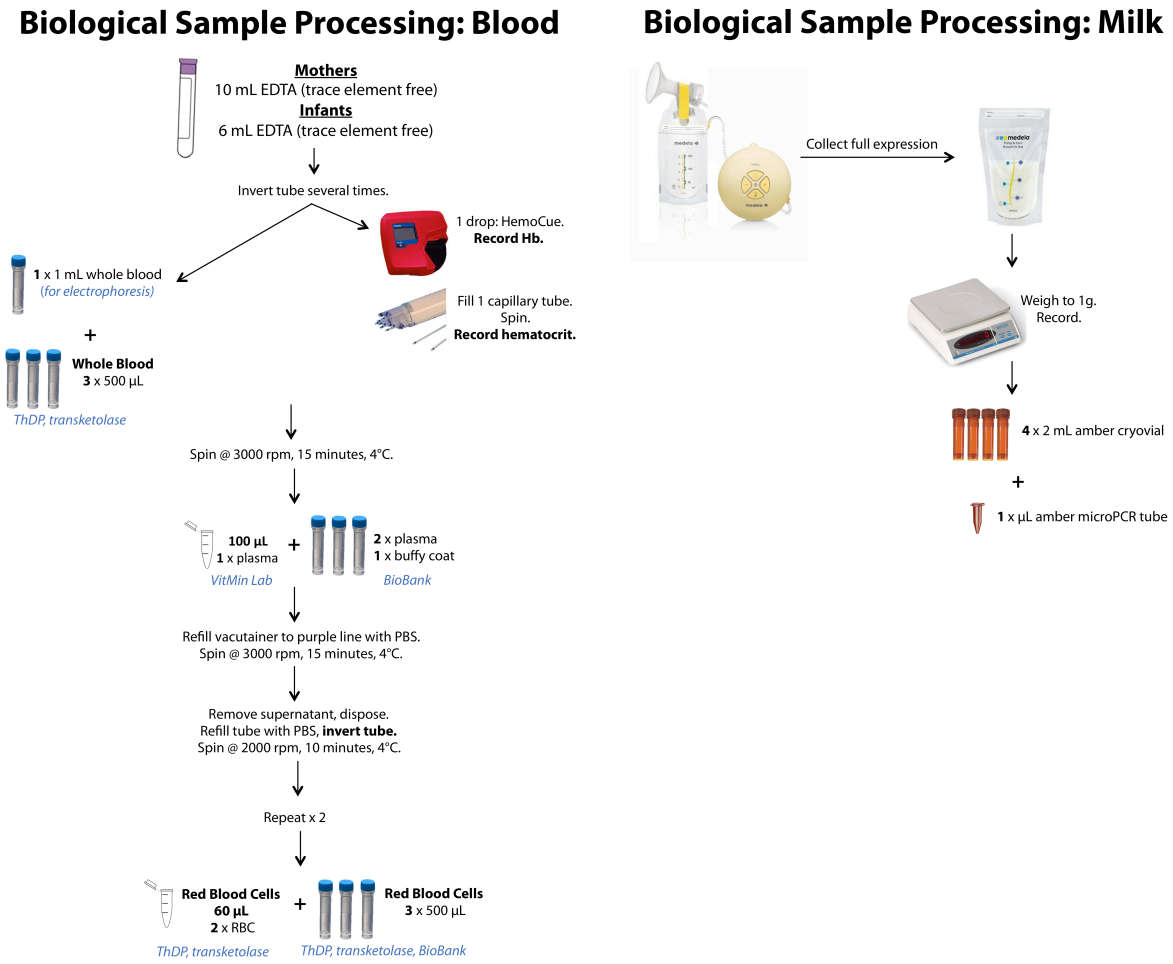

Figure 2: Processing protocol for venous blood and breast milk samples.

Venous blood samples will undergo analysis for both transketolase activity and thiamine diphosphate concentrations, as described in *3.3 Biomarkers of thiamine status*, by Albert Koulman at the NIHR BRC Nutritional Biomarker Laboratory at the University of Cambridge in the United Kingdom. At 2 weeks postnatal, maternal whole blood ThDP and erythrocyte transketolase activity will be measured. At 24 weeks postnatal, ThDP and transketolase activity will be measured in both whole blood and erythrocytes, and infant samples will be assayed for whole blood ThDP and erythrocyte transketolase activity. When ThDP is assessed in whole blood, concentrations must be normalized to hemoglobin concentrations and/or hematocrit (47). Therefore, hemoglobin concentration and hematocrit will be measured on all whole blood samples via a HemoCue 201 portable hemoglobinometer and capillary hematocrit tubes, respectively.

Evidence from the 2014 Cambodian National Micronutrient survey indicates that eThDP is affected by the presence/absence of genetic hemoglobin disorders. With this, it is vital to assess the presence/absence of genetic hemoglobin disorders, as this could influence participant's response to thiamine, it may influence transketolase, and it could change thiamine dosage requirements in other

countries. Therefore, at one timepoint only (2 weeks postnatal for maternal, and 24 weeks postnatal for infant samples), an aliquot of 1 mL whole blood will undergo hemoglobin capillary electrophoresis analysis to identify structural hemoglobin variants (48) at the Pasteur Institute in Phnom Penh. Buffy coat samples will be stored in the BioBank in the Department of Applied Human Nutrition at Mount Saint Vincent University in Halifax, Nova Scotia, Canada, for potential later assessment of genetic hemoglobin disorders.

Biomakers may be influenced by inflammation, which is common in this population (49,50). Plasma samples will be sent to Dr. Jurgen Erhardt at the VitMin Lab in Germany for analysis of C-reactive protein (CRP) and α-1-acid-glycoprotein (AGP) using an immunosorbent assay (51). Note that this assay will also measure retinol binding protein (RBP), ferritin, and soluble transferrin receptor (sTfR). Other plasma samples will be stored in the BioBank for potential future use.

At 2, 4, 12, and 24 weeks postnatal, a full human milk expression will be collected (approx. 35 g) and approximate milk volume (weight of milk to 1 g) will be recorded. Samples will be mixed, and 2 mL aliquots obtained in amber cryovials (see **Figure 2**). Human milk thiamine concentrations will be measured by Drs. Daniela Hampel and Lindsay Allen at the USDA/ARS Western Human Nutrition Research Center, University of California, Davis (52).

9.9 Cognitive assessments

We will conduct cognitive assessments at 2, 12, 24, and 52 weeks postnatal; video-recordings of data collection will be collected to aid in analysis. We will conduct the Hammersmith Infant Neurological Exam (10 minutes), an oculomotor exam (2 minutes), Infant Mullen Scales of Early Learning (10 minutes at 2 and 12 weeks postnatal, 15 minutes at 24 and 52 weeks postnatal), Caregiver Reported Early Development Instruments (CREDI; 15 minutes), a Primary Engagement Task (3 minutes; 2, 12, and 24 weeks only), a Secondary Engagement Task (2.5 minutes), the Visual Paired Comparison Task (7 minutes), and the Language Preference Task (6 minutes), in the women's home. See Figure 1 for the timepoints at which these tasks are conducted. At 52 weeks postnatal, we will also incorporate the Mother-Infant Free Play Interaction (5 minutes), and the MacArthur-Bates Communicative Development Inventory (8 minutes).

The Hammersmith Infant Neurological Exam is a standard tool for providing basic information about infants' neurological status through use of gentle touch and gentle social interaction in order to examine infants' sensory and motor responses; it has previously shown excellent interobserver reliability even among less experienced staff (53). An oculomotor exam was included because oculomotor disturbances are not uncommon among thiamine deficient adults (54). The Infant Mullen Scales of Early Learning is an individually administered, multi-domain measure of early development, with scales measuring development in visual reception, fine motor, gross motor, receptive language, and expressive language (55). The CREDI is a caregiver-reported measure of child development that has been shown to have right validity and reliability in low-resource settings (56). The Primary Engagement Task measures individual variation in infant, caregiver, and dyad-level developmental change in the ability to engage contingently with the partner in direct positive mutual engagement interactions. The Secondary Engagement Task measures individual variation in caregiver and infant ability to engage mutually in relation to an external object. The Visual Paired Comparison task (VPC; 5 minutes) and the Language Preference Task (5 minutes). The VPC probes infants' recognition memory and attention, and has been used to predict subsequent verbal IQ in

middle childhood (57,58), and produce measures of attention predictive of developmental maturity and better cognitive function (59,60). The Language Preference Task is designed to measure individual variation in infants' level of interest in the kind of language that caregivers typically direct toward infants (aka infant-directed talk or 'motherese') relative to a) the kind of language that is typically directed toward adults (aka adult-directed talk), and b) sounds that are matched in complexity but are non-linguistic (non-linguistic analog). Over a series of trials, infants hear these three different kinds of sound samples through an audio speaker (infant-directed talk, adult-directed talk, non-linguistic analog), and the duration that they look toward a visual display during each sound sample is subsequently measured from the videotaped record collected during the session.

The Mother-Infant Free Play Interaction aims to provide behavioral samples of caregiver-infant/child interaction dynamics, and have been used extensively by developmental scientists to detect and measure typical and atypical developmental trajectories starting at birth (61,62). At 52 weeks, we will use a free-play task designed to elicit positive affect that we have used successfully in Laos with undernourished and healthy caregiver-infant dyads (63). As with our Primary Engagement Task at the three earlier study time points, coding of the interactions will target maternal, infant and dyadic behaviors, and will be based on Walker and Thompson's Mother-Infant Play Interaction Scale (64).

The MacArthur-Bates Communicative Development Inventory (CDI) is a parent report inventory developed to assess language and communication development in children aged 8 to 30 months. We propose to use the "short form" of the CDI, which typically takes less than 10 minutes to complete. The CDI has been used extensively to study the language development of typical and atypical populations (65), and has proven capable of discriminating between groups infants/toddlers who differ in terms of birthweight complications and early neurological adversity (66) and suboptimal early caregiver environments (67). The CDI has been used successfully in over 20 different languages. Presently, a Khmer version does not exist, thus our team will utilize the MacArthur-Bates CDI's "adaptation guidelines" to create a Khmer version.

9.10 Participant remuneration

All participants will receive a mobile phone and mobile phone credits. In addition, modest, study-appropriate remuneration such as a sarong, canned fish, or soap will be provided at biological sample collection/cognitive assessment points (2, 4, 12, 24, and 52 weeks postnatal).

10. Adverse Event Reporting

As described in *2.6 Serious Adverse Events Committee / Data Safety Monitoring Board,* there will be no Serious Adverse Events Committee or Data Safety Monitoring Board assembled for this study, because no adverse events have ever been reported for thiamine. There is no UL for thiamine because there has never been an adverse event reported from high thiamine intake (1,2), even though over-the-counter supplements in Canada and the United States commonly contain 50 mg thiamine or more. With this, we are confident that there is a low risk of serious adverse events in this study. There are also no clinical outcomes for which to monitor: the primary outcome of the study is a biochemical measure of thiamine status, but results will only be known after study completion. Also, there will be no mid-study data available to examine because all biological samples

will be batch analyzed to decrease assessment variability (in addition to logistic considerations for batch storing and shipping samples).

However, the study will employ a Cambodian medical doctor from the Kampong Thom Operational District to answer medical questions from mothers, and, on an *ad hoc* basis, to investigate adverse events. As described above in *2.6 Serious Adverse Events Committee / Data Safety Monitoring Board,* we do expect 2 – 4 infant death during the study due to events not related to the study.

11. Statistical Analysis

The full statistical analysis plan can be found in **Appendix B**. The primary analyses will be performed on an 'intention to treat' basis, where all participants will be analyzed according to their allocated treatment group regardless of compliance. A secondary 'per-protocol' analysis will be performed on the subset of women who consumed ≥80% capsules over the study period. The parameters of the Emax dose-response curves will be estimated using non-linear least squares models. Separate curves will be fitted for outcomes measured at 4 weeks, 12 weeks, and 24 weeks postpartum. The dose where additional maternal intake of thiamine no longer meaningfully increases milk thiamine concentration, defined as the dose that achieves 90% of the maximum concentration, will be estimated from the fitted curve with standard errors estimated by bootstrapping for calculating 95% confidence intervals.

Comparisons between treatment groups will be performed using linear mixed-effects models to account for repeated measurements and adjusted for randomization strata and levels at 2 weeks postnatal. Interaction tests will be performed to assess whether treatment effects depend on genetic hemoglobin disorder. Missing data will be addressed using multiple imputation to create 100 complete datasets for analysis, with a sensitivity analysis performed on the raw (unimputed) data (68). All analyses will follow a pre-specified statistical analysis plan (see **Appendix B**).

12. Administrative Aspects, and Data Security and Management

12.1 Obtaining informed consent

Field staff will follow up with interested women and screen for eligibility in their homes. If women are eligible and wish to participate, the field staff will obtain consent by reading them the consent form and obtaining written informed consent via a thumbprint stamp. Signatures are not common in Cambodia; instead participants will provide a thumbprint stamp, as is common practice in research studies in Cambodia. During this initial meeting, women will be told that they can withdraw from the study at any time without penalty. Women will be reminded of the consent process at 2, 4, 12, 24, and 52 weeks postnatal, before data and biological samples are collected.

If women are not interested or are unwilling to provide consent, the field staff will thank them for their time and leave their homes.

As part of the consent process participants will be also be asked if they would provide permission for the repurposing of their biological samples after this study to be 'biobanked' for use in future nutrition-related research (re-consent will not be obtained). Participants will be informed that any future use would involve only de-identified data, and would be for nutrition-related research only.

Participants can chose whether or not to provide this consent for future use of samples: the consent form is clear that this is secondary use of the biological samples, and is in no way required for participation in this study.

12.2 Ethics

We will obtain ethics approvals from the following Research Ethics Boards:
- National Ethics Committee for Health Research (Cambodia)
- Mount Saint Vincent University Research Ethics Committee (Canada)
- University of Oregon Institutional Review Board (USA)

This research will also be registered at clinicaltrials.gov.

12.3 Confidentiality and data security

Participants will be given a unique alpha-numeric study ID code. This unique identifier will not be derived from personal identifiers. A key linking the subject code to participant information will be kept on a password protected computer in a secure area in the Helen Keller International (HKI) office in Phnom Penh, Cambodia. There is no need for co-investigators elsewhere to have information linking participant names with their unique identifier.

All electronic data files will be stored on password protected computers and/or secure servers accessible only to members of the research team. Archived electronic data files and any hard copies of data (consent forms) will be stored in locked filing cabinets in locked research rooms at HKI Cambodia.

Once all data is collected, all information will be de-identified. Only the study statistician and Principal Investigator will have access to the master list once data collection is complete; otherwise, all data will be completely de-identified.

Biological samples will be transported to MSVU on dry ice, and stored in -80°C freezers in a locked freezer room. After analysis, all biological samples will be stored for 5 years in -80°C freezers at MSVU, then destroyed following biological safety protocols.

All paper copies of consent forms and questionnaires will be retained for at least 5 years after publication of results. As per granting agency rules, all data will be made available on a public repository, but we will also store data on an external hard-drive, in a locked drawer, in the Principal Investigator's locked office for a minimum of 25 years. After this time, all paper copies of consent forms will be shredded, and the hard drive deleted and physically destroyed.

12.4 Modifications to the protocol

This study will be conducted as per the current version of the protocol. Any change to the protocol document or related study tools that affects the scientific intent, study design, participant safety, or that may affect a participants willingness to continue participation in the study is considered an amendment, and therefore will be written and filed as such.

12.5 Protocol deviations

Protocol deviations will be documented and reported to the Principal Investigator if data collection does not fall within the following windows:

- chart review: anytime between 0 – 16 days postnatal
- 2 weeks postnatal: 2 weeks postnatal ± 2 days
- 4 weeks postnatal: 4 weeks postnatal ± 2 days
- 12 weeks postnatal: 12 weeks postnatal ± 4 days
- 24 weeks postnatal: 24 weeks postnatal ± 4 days
- 52 weeks postnatal: 52 weeks postnatal ± 7 days
- fortnightly monitoring: ± 2 days

12.6 Trial closure

The Principal Investigator (or designee) may terminate the study prematurely if data become available which raise concern about the safety of the study treatment(s) that have potential to cause unacceptable risks to subjects. If premature study closure occurs, the Principal Investigator (or designee) must contact all participants within two weeks, and written notification must be sent to the Ethics Committees.

13. Use of Data, and Dissemination

13.1 Layperson dissemination

We will create various lay outputs from this study that can be used by NGOs and government agencies working with families, or can be accessed directly by families. These will include short video clips, infographics, and 'hot tips,' which we will make available online (website maintained through Mount Saint Vincent University; HKI will provide Khmer-translated materials) and in print for distribution at local agencies.

We will host a Dissemination Workshop in Phnom Penh open to relevant stakeholders (NGOs, government, researchers, media, clinicians, public health, all sectors) to share the main outcomes of the study, and officially 'launch' resources online. Relevant parties from Myanmar and Laos PDR (e.g. Ministry of Health representatives) will be invited to this workshop; we will also engage with these stakeholders to explore take-up and/or scaling in these countries.

Updates on the study will be shared at the monthly Cambodian Nutrition Working Group meeting, hosted by the National Nutrition Programme, Ministry of Health, and attended by all researchers and NGOs engaging in nutrition research and programming in Cambodia. Scaling Up Nutrition (SUN) and other relevant groups will also be updated regularly, and we will share dissemination materials, and invite these groups to the dissemination workshop.

We will return to each community in Kampong Thom to conduct a village-wide meeting relaying study results.

13.2 Academic dissemination

Results of this research will be presented at academic nutrition, public health, and psychology conferences (i.e. Micronutrient Forum, Canadian Nutrition Society and American Nutrition Society Annual Meetings, International Society for Research in Human Milk and Lactation Conference, Society for Research in Child Development, etc.), and in peer-reviewed, journals that offer open access (e.g. Nutrients, American Journal of Clinical Nutrition, Pediatrics, etc.).

We plan to make data available on a public repository by August 31, 2020.

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Appendices

Appendix A: Thiamine hydrochloride safety data sheet.

Appendix B: Statistical Analysis Plan for *Trial of thiamine supplementation in Cambodia.*

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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	

HMIS 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	$C_{12}H_{17}ClN_4OS.HCl$
CAS NUMBER	67-03-8

ALTERNATE CAS NUMBER	Not applicable
MOLECULAR WEIGHT	337.3

COMPOSITION

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-metalic 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

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
POSSIBLITY 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 Proposition 65	This product does not contain any chemicals known to the State of California to cause cancer, birth defects, or any other reproductive harm.
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.	

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	**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.

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

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 355 Appendix A	EPA 40 CFR Part 355 Appendix B	Right-to-know Pennsylvania	Right-to-know New Jersey	Right-to-know Massachusetts	California Prop 65
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

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Trial of Thiamine Supplementation in Cambodia

STATISTICAL ANALYSIS PLAN

SAP Authors: Shalem Leemaqz (Statistician)
Lisa Yelland (Senior Statistician)

SAP Version: 1.7

SAP Date: 14 August 2019

Approved by:

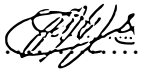
	Signature	*Date*
Kyly Whitfield (Principal Investigator)		August 14, 2019
Hou Kroeun (Co-Investigator)		14 August 2019

TABLE OF CONTENTS

1 PREFACE

This statistical analysis plan (SAP) describes the planned analyses for the trial:

Trial of thiamine supplementation in Cambodia.

The following documents were reviewed in preparation of this SAP:

- Trial of thiamine supplementation in Cambodia: Protocol version 2.1 (dated 24 July 2018)
- Questionnaires and consent forms (dated 6 April 2018 and 19 July 2018)

2 STUDY OBJECTIVES

2.1 Primary Objective

The primary objective is to estimate the dose of maternal intake of thiamine where the dose-response curve reaches 90% of the maximum average human milk total thiamine concentration at 24 weeks postpartum.

2.2 Secondary Objectives

1. To estimate the dose on the dose-response curve where 90% of the maximum average is reached in the following outcomes:
    a. Infant thiamine diphosphate concentrations (ThDP) at 24 weeks postnatal
    b. Human milk total thiamine concentration at 4 and 12 weeks postpartum
    c. Infant transketolase activity coefficient (TKac) at 24 weeks postnatal
    d. Maternal ThDP at 24 weeks postpartum
    e. Maternal TKac at 24 weeks postpartum

    and assess whether this depends on the presence/absence of genetic hemoglobin disorder for the following outcomes:
    a. Infant ThDP at 24 weeks postnatal
    b. Infant TKac at 24 weeks postnatal
    c. Maternal ThDP at 24 weeks postpartum
    d. Maternal TKac at 24 weeks postpartum
2. To test for differences between the 4 randomised groups on:
    a. Human milk total thiamine at 4, 12, and 24 weeks postpartum
    b. Maternal ThDP at 24 weeks postpartum
    c. Maternal TKac at 24 weeks postpartum

    and assess whether this depends on the presence/absence of genetic hemoglobin disorder for the following outcomes:

   a. Maternal ThDP at 24 weeks postpartum
   b. Maternal TKac at 24 weeks postpartum

3. To estimate the usual household salt intake from mean fortnightly salt disappearance (weight lost, in g).
4. In a subset of 100 lactating women, to estimate:
   a. Salt intake of lactating women, their male partners (if applicable), and their children 24-59 months (if applicable) using observed weight salt intake records
   b. Sodium intake using 24 hr urinary sodium concentrations in lactating women
5. To test for differences between the 0 mg and 10 mg treatment groups on the following outcomes at 24 and 52 weeks postpartum:
   a. Composite and 5 subscales of Mullen
   b. VPC Novelty score and the attention and processing speed subscales
   c. Language Preference Task scores
   d. Oculomotor scores
6. To determine the effect of inflammation, as measured by C-reactive protein (CRP) and α-1-acid glycoprotein (AGP) on the following outcomes:
   a. Maternal ThDP at 2 and 24 weeks postpartum
   b. Infant ThDP at 24 weeks postnatal

3 STUDY METHODS

3.1 Overall Study Design

Randomised placebo controlled, double-blinded, four-parallel arm, multicentre trial.

3.2 Selection of study population

3.2.1 Inclusion criteria

Mothers of a newborn who:

1. are aged 18 – 45 years
2. had a recent normal pregnancy (i.e. no known chronic conditions, no preeclampsia, gestational diabetes etc), and the singleton infant was born without complications (e.g. low birth weight (<2.5 kg), tongue tie, cleft palate)
3. are intending to exclusively breastfeed for six months
4. reside in Kampong Thom province, and are not planning to move in the next six months

5. are willing to consume one capsule daily from 2 weeks through to 24 weeks postpartum
6. are willing for her entire household consume only salt provided by the study team
7. are willing to have study samples collected at 2, 4, 12 and 24 weeks postpartum

3.2.2 Exclusion criteria

1. Currently taking or has taken thiamine-containing supplements over the past 4 months
2. Currently participating in nutrition programs beyond normal care

3.3 Treatment groups

Participants are randomised to receive supplements containing one of the following:

1. 0 mg thiamine (placebo, negative control group)
2. 1.2 mg thiamine as thiamine hydrochloride (EAR group)
3. 2.4 mg thiamine as thiamine hydrochloride (double EAR group)
4. 10 mg thiamine as thiamine hydrochloride (positive control group)

The supplements will be packaged in 14-capsule blister packs delivered to participants every two weeks. Participants will be asked to consume one capsule per day from 2 weeks to 24 weeks postpartum.

3.4 Method of treatment group assignment and randomisation

Two product codes for each intervention group (total of 8 product codes), in the form of 3 letters followed by 8 numbers, were used to assist with blinding. The randomisation schedule was produced by the study statistician using ralloc.ado version 3.7.6 in Stata version 15.1. Health centre (listed below) was used to define the strata and randomly permuted blocks of size 8 were used within strata to assign participants to one of the 8 product codes in the ratio 1:1:1:1:1:1:1:1. The assignment of product codes to intervention groups was performed by an independent individual and kept separate from the randomisation schedule. The randomisation was performed using sealed envelopes containing a unique participant ID and the assigned product code that were distributed to each health centre.

Stratification Variable	**Categories**
Health centre	Tboung Kapoeur Health Centre Kampong Svay Health Centre Sankor Health Centre Chey Health Centre Salavisai Health Centre Prey Kuy Health Centre Prey Pros Health Centre Srayov Health Centre

3.5 Blinding

Participants, study staff and study investigators are blinded to treatment group allocation. Data analysts will necessarily be unblinded while performing the analysis.

3.6 Sample size

To detect a clinically meaningful difference of 40 μg/L in human milk total thiamine concentration between any two treatment groups with 90% power, assuming a SD of 43 μg/L (estimated SD of control group in a Cambodian thiamine-fortified fish sauce trial[1]) 48 women are required per treatment group, or a total of 192 women. This sample size allows for 20% attrition and is based on a two-sided alpha of 0.0083 for each of the 6 pairwise comparisons between 4 treatment groups to maintain an overall alpha of 0.05. Recruitment of 320 participants (80 per group) was planned to allow for some uncertainty in the assumed values, particularly the SD which may be larger than anticipated.

Simulations of 500 dose-response curves for human milk total thiamine concentration were conducted to estimate the precision that this sample size would provide for addressing the primary study objective (i.e. for estimating the dose required to achieve 90% of the average maximum human milk total thiamine concentration). Data were simulated based on an average minimum and maximum concentration of 136 μg/L[1] and 210 μg/L[2] respectively, with a SD of 43 μg/L[1], and reaching 50% and 90% of the maximum average concentration at dose 1.2 mg/d (EAR group) and 2.4 mg/d (double EAR group) respectively. Assuming a dose-response Emax curve, the precision of the estimated dose is ±1.29 mg/d (i.e. the 95% confidence interval for the estimated dose will be within ±1.29 mg/d of the point estimate).

4 SEQUENCE OF PLANNED ANALYSIS

4.1 Interim analysis

There are no planned interim analyses for this study.

4.2 Final analysis and reporting

Once data collection and cleaning is complete, the database will be locked and unblinded treatment codes will be included in the database; a blinded analysis is not possible for estimating dose response curves.

Due to the time-consuming nature of coding video-recorded cognitive assessments, data for the following assessments will not be included in the database before it is locked and unblinded: primary engagement task, secondary engagement task, Visual Paired Comparison Task, and Language Preference Task. Note that the research assistants coding the videos for these assessments will remain blinded.

Analysis of all primary and secondary outcomes will be performed as described in this SAP and the results of the statistical analyses will be made available to the Principal Investigator.

Any post-hoc, exploratory analyses which were not specified in this SAP will be clearly identified in the final report.

5 GENERAL ISSUES FOR STATISTICAL ANALYSIS

5.1 Analysis software

All analyses will be performed using R version 3.4.4 or later.

5.2 Analysis approach

The primary analyses will be performed using an intention-to-treat (ITT) approach, where analyses will be conducted according to the randomised treatment group regardless of participants' compliance with the protocol. Secondary 'per-protocol' analyses will be performed including only women who consumed ≥80% capsules over the 22-week intervention period.

5.3 Methods for withdrawals, missing data and outliers

All available data collected up until time of withdrawal will be included in the statistical analysis.

Multiple imputation using chained equations will be performed separately by randomised treatment group to address missing data under a missing at random assumption. A total of 100 imputations will be performed to ensure <1% loss of power compared to full information maximum likelihood[3]. Covariates pre-specified for adjustment in the analysis will be included in the imputation model, along with other auxiliary variables useful for improving the prediction of missing values. Conclusions will be based on the results obtained using imputed data, with sensitivity analyses performed on unimputed data.

Outliers will not be excluded from the primary analysis unless a data entry error is confirmed.

5.4 Protocol violations and deviations

No participants will be excluded from the primary intention-to-treat analyses due to protocol deviations.

For the secondary 'per-protocol' analyses, only women who consumed ≥80% capsules over the 22-week intervention period will be included.

5.5 Data transformations

No data transformations are planned. The statistical analyses detailed in Section 7 are based on assumptions about the distribution of the outcomes. If these assumptions turn out to be invalid, data transformations may be required. Log transformation is expected to be required for analysis of Oculomotor score.

5.6 Covariates for adjustment

Both unadjusted and adjusted analyses will be performed for some outcomes. Conclusions for these outcomes will be drawn from the adjusted analyses, with unadjusted analyses performed for completeness and to potentially confirm the results of the adjusted analyses.

All adjusted analyses will be adjusted for health centre, since it was used as a stratification variable in the randomisation process. Adjustment will also be made for additional variables collected at 2 weeks postnatal which are potential confounders for some outcomes and these have been identified a priori (see Section 7).

If convergence is an issue, some potential confounders may be excluded from the adjusted analysis. Any deviation from the planned adjustment covariates will be documented in the final report.

5.7 Planned subgroup analyses

For the secondary outcomes of maternal and infant ThDP and TKac, secondary analyses will be performed to test for evidence of effect modification by presence/absence of genetic hemoglobin disorder.

Analysis of secondary outcomes on Mullen, VPC Novelty Scores, Language Preference Task scores, and oculomotor scores will be performed in a subset of women comparing only those who were assigned to receive 0 mg/d (placebo) to those who were assigned to receive 10 mg/d (positive control) of thiamine.

5.8 Multiple comparisons and multiplicity

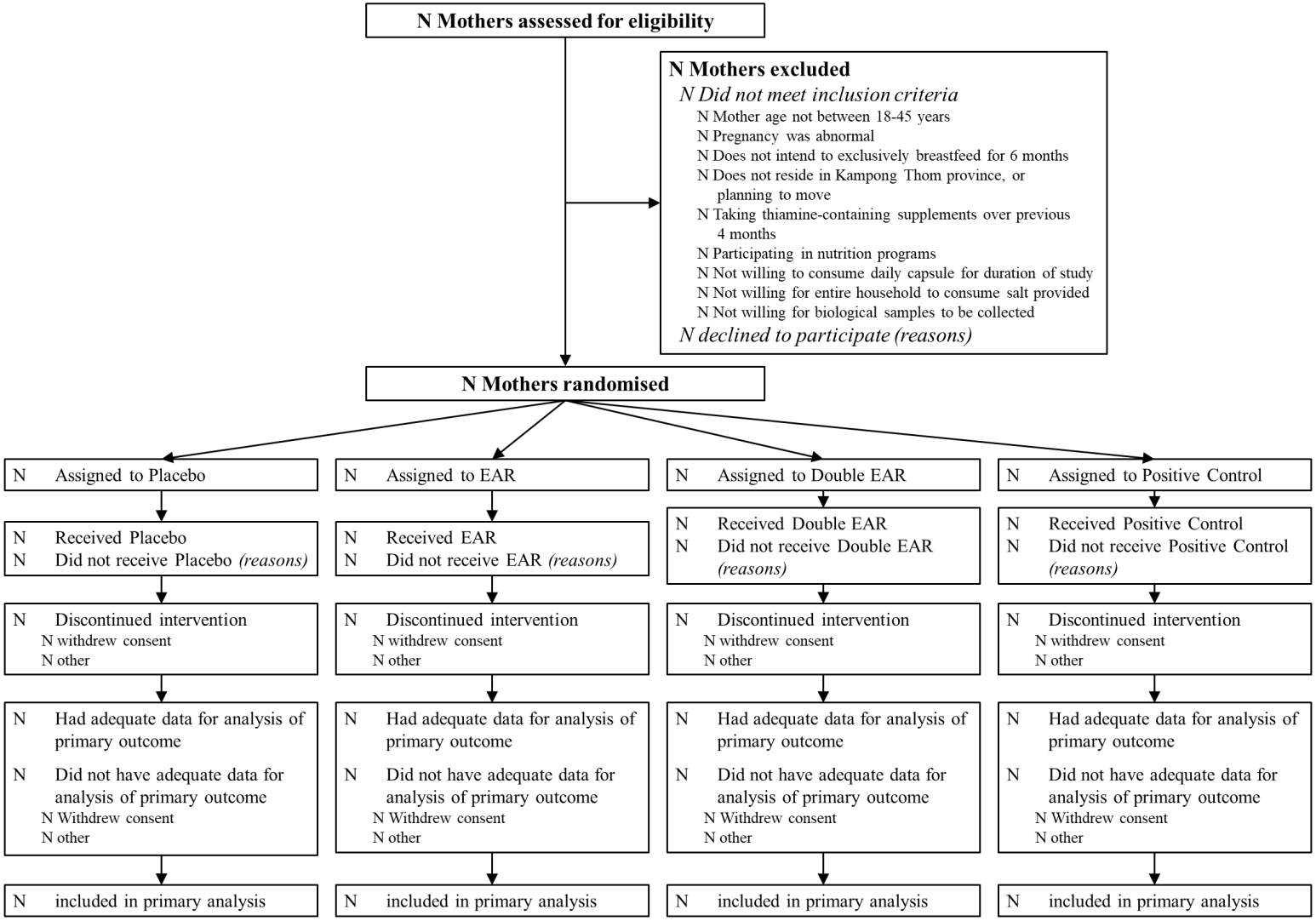
Since there is only one primary outcome for the trial and less importance will be placed on the results of the secondary outcomes and the secondary analyses testing for interactions, no adjustment will be made for multiple outcomes and analyses being performed.

Since conclusions will be drawn based on the adjusted results (where performed) using the imputed data, no adjustment will be made for the fact that both adjusted and unadjusted analyses are being performed (where applicable) on both raw and imputed datasets.

For secondary outcomes where the objective of the analysis is to compare the four treatment groups, multiple comparisons will be addressed by first testing whether there is any evidence of a treatment group effect. Where this test reaches statistical significance ($P<0.05$), post-hoc tests will be performed to test for differences between each pair of treatment groups and Tukey's honestly significance difference (HSD) will be used to control the type I error rate across the 6 pairwise tests.

6 DESCRIPTIVE STATISTICS

6.1 Participant flow



6.2 Baseline characteristics (2 weeks postnatal)

A descriptive comparison of the randomised groups will be conducted on all baseline characteristics in the 2 weeks postnatal questionnaire. Iron biomarkers ferritin and soluble transferrin receptor, vitamin A marker retinol binding protein, and C-reactive protein (CRP) and α-1-acid glycoprotein (AGP) at baseline will also be included. Means and standard deviations, or medians and interquartile ranges will be reported for continuous variables. Frequencies and percentages will be reported for categorical variables.

6.3 Measurement of treatment compliance

Treatment compliance will be assessed descriptively by treatment group based on the number of unused capsules (Module 1.2 from Fortnightly monitoring data collection sheet) counted every fortnight over the 22 week intervention period.

6.4 Missing data

Missing data will be assessed descriptively by treatment group for each outcome variable and potential confounder.

6.5 Randomisation errors

Information listed below will be summarised descriptively by treatment group:

- Number of mothers randomised in the wrong stratum
- Number of mothers given the wrong treatment
- Number of mothers discovered to be ineligible after randomisation

7 STATISTICAL ANALYSES

In this section, the following details are provided for each outcome variable that will be analysed to address the study objectives specified in Section 2:

- **Outcome:** A detailed description of the outcome variable, including the type of variable and how it will be calculated (if applicable).
- **Analysis:** The type of statistical analysis to be performed and parameters to be estimated.
- **Adjustment:** Where relevant, the covariates at 2 weeks postnatal (stratification variables and potential confounders) to adjust for in the adjusted analysis.

For each outcome variable, parameter estimates will be accompanied by 95% two-sided confidence intervals and statistical significance will be assessed at the 0.05 level using a two-sided test, unless otherwise specified.

7.1 Emax curve

Analysis of the primary and several secondary outcomes will be based on the Emax curve. The Emax curve is defined as:

$$Y = E_0 + E_{\max}\left(\frac{\text{dose}^h}{ED_{50}^h + \text{dose}^h}\right)$$

where E_0 is the baseline concentration without supplementation, $E_{\max}$ is the maximum effect of supplementation, ED_{50} is the dose that produces half of the maximum effect of supplementation and corresponds to the inflection point at which the effectiveness of higher doses starts to decrease, and h is the slope or Hill factor.

The nonlinear Least squares model for the primary outcome will be formulated as:

$$Y = E_0 + E_{\max}\frac{\text{dose}^h}{ED_{50}{}^h + \text{dose}^h} + \epsilon$$

For secondary outcomes involving testing for effect modification by presence or absence of genetic hemoglobin disorder (GD), additional coefficients (α, β, γ and δ) will be added to all parameters of the Emax curve. The model will be formulated as:

$$Y = (E_0 + E_0\beta\text{GD}) + (E_{\max} + E_{\max}\gamma\text{GD})\frac{\text{dose}^{(h+h\delta\text{GD})}}{(ED_{50} + ED_{50}\alpha\text{GD})^{(h+h\delta\text{GD})} + \text{dose}^{(h+h\delta\text{GD})}} + \epsilon$$

Evidence of curve shape differences between presence or absence of genetic hemoglobin disorder will be determined by global Likelihood ratio tests. Further testing of the four additional coefficients, in the form of H_0: $\text{coefficient} = 0$, will be conducted following evidence (P<0.05) from the global test.

The parameter of interest for this study is the dose required to achieve 90% of the maximum concentration (i.e. 90% of $E_0 + E_{\max}$) and this will be estimated from the fitted Emax curve with variance estimated from non-parametric bootstrapping of 100 resamples. For outcomes where there is evidence of curve difference by genetic hemoglobin disorder status (P<0.05 for likelihood ratio test), separate estimates for the dose required to achieve 90% of the maximum concentration with and without genetic hemoglobin disorder will be reported.

7.2 Primary outcome variable

7.2.1 Human milk total thiamine concentration (24 weeks)

Outcome:	Continuous outcome of total thiamine concentration (μg/L) in maternal human milk at 24 weeks postpartum.
Analysis:	Fit an Emax curve using nonlinear least squares approach. Estimate of the dose that reaches 90% concentration (with 95% CI) will be reported.

7.3 Secondary outcome variables

7.3.1 Infant thiamine diphosphate concentration (24 weeks)

Outcome:	Continuous outcome of infant thiamine diphosphate concentration (ThDP; nmol/L) at 24 weeks postpartum.
Primary analysis:	Fit an Emax curve using nonlinear least squares approach. Estimate of the dose that reaches 90% concentration (with 95% CI) will be reported.
Secondary analysis:	Fit an Emax curve using nonlinear least squares approach allowing curve to vary by presence/absence of genetic hemoglobin disorder. Estimates of the dose that reaches 90% concentration by genetic hemoglobin disorder status will be reported following evidence from the global test. Second, test for a linear relationship using linear regression with CRP at 24 weeks and treatment group as predictors. Estimate of the mean change (with 95% CI) in ThDP for one unit increase in CRP will be reported. Third, test for a linear relationship using linear regression with AGP at 24 weeks and treatment group as predictors. Estimate of the mean change (with 95% CI) in ThDP for one unit increase in AGP will be reported.
Adjustment:	Health centre (linear regressions only)

7.3.2 Human milk total thiamine concentration (4 weeks and 12 weeks)

Outcome:	Continuous outcome of total thiamine concentration (μg/L) in maternal human milk at 4 weeks and 12 weeks postpartum.
Primary analysis:	Fit a separate Emax curve for 4 and 12 weeks using nonlinear least squares model. Estimate of the dose that reaches 90% concentration (with 95% CI) at each timepoint will be reported.
Secondary analysis:	Fit a linear mixed effects model to outcome at 4, 12, and 24 weeks with treatment group, time, and treatment by time interaction as predictors. Treatment effects (difference in mean total concentration) will be estimated separately for each time point if the interaction p-value is <0.05 and for both time points combined otherwise.
Adjustment:	Health centre, human milk total thiamine at 2 weeks postnatal (linear mixed effects model only)

7.3.3 Infant transketolase activity coefficient (24 weeks)

Outcome:	Continuous outcome of infant transketolase coefficient (TKac) at 24 weeks postpartum.
Primary analysis:	Fit an Emax curve using nonlinear least squares approach. Estimate of the dose that reaches 90% concentration (with 95% CI) will be reported.
Secondary analysis:	Fit an Emax curve using nonlinear least squares approach allowing curve to vary by presence/absence of genetic hemoglobin disorder. Estimates of the dose that reaches 90% concentration by genetic hemoglobin disorder status will be reported following evidence from the global test.

7.3.4 Maternal thiamine diphosphate concentration (24 weeks)

Outcome:	Continuous outcome of maternal thiamine diphosphate concentration (ThDP; nmol/L) at 24 weeks postpartum.
Primary analysis:	Fit an Emax curve using nonlinear least squares approach. Estimate of the dose that reaches 90% concentration (with 95% CI) will be reported.
Secondary analyses:	First, fit an Emax curve using nonlinear least squares approach allowing curve to vary by presence/absence of genetic hemoglobin disorder. Estimates of the dose that reaches 90% concentration by genetic hemoglobin disorder status will be reported following evidence from the global test. Second, test for a treatment effect using linear regression and estimate a difference in mean concentration. Third, test for a treatment effect using linear regression with treatment group and treatment by genetic hemoglobin disorder interaction. Treatment effects (difference in mean concentration) will be estimated separately by genetic hemoglobin disorder where the relevant interaction term has a p-value <0.05. Pairwise comparisons will be reported following evidence of a treatment group effect (see Section 5.8). Fourth, test for a linear relationship using linear mixed effects model with CRP at 2 and 24 weeks postnatal, time and treatment group as predictors. Estimate of the mean change (with 95% CI) in ThDP for one unit increase in CRP will be reported. Fifth, test for a linear relationship using linear mixed effects model with AGP at 2 and 24 weeks postpartum, time and treatment group as predictors. Estimate of the mean change (with 95% CI) in ThDP for one unit increase in AGP will be reported.
Adjustment:	Maternal ThDP at 2 weeks postpartum, health centre (linear regressions and mixed effects models only)

7.3.5 Maternal transketolase activity coefficient (24 weeks)

Outcome:	Continuous outcome of maternal TKac at 24 weeks postpartum.
Primary analysis:	Fit an Emax curve using nonlinear least squares approach. Estimate of the dose that reaches 90% concentration (with 95% CI) will be reported.

Secondary analyses:	First, fit an Emax curve using nonlinear least squares approach allowing curve to vary by presence/absence of genetic hemoglobin disorder. Estimates of the dose that reaches 90% concentration by genetic hemoglobin disorder status will be reported following evidence from the global test. Second, test for a treatment effect using linear regression and estimate a difference in mean TKac. Third, test for a treatment effect using linear regression with treatment group and treatment by genetic hemoglobin disorder interaction. Treatment effects (difference in mean TKac) will be estimated separately by genetic hemoglobin disorder where the relevant interaction term has a p-value <0.05.
Adjustment:	Maternal TKac at 2 weeks postpartum, health centre (linear regressions only)

7.3.6 Household salt intake

Outcome:	Continuous outcome of total salt consumption (g) in one day.
Primary analysis:	Descriptive means and standard deviation (overall and by number of individuals in the household) of total salt consumption within a day in a subset of 100 households.
Secondary analysis:	Descriptive means and standard deviation of the difference in salt intake, and intraclass correlation coefficient, between two days in a subset of 75 households.

7.3.7 Individual salt intake

Outcome:	Continuous outcome of individual table salt intake (g) measured throughout one day by field staff.
Primary analysis:	Descriptive means and standard deviation of salt intake for a subset of 100 lactating women, their male partners (if applicable), and their children 24-59 months (if applicable).

7.3.8 Sodium intake

Outcome:	Continuous outcome of urinary sodium concentration (mmol/24 hours) in lactating women.
Primary analysis:	Descriptive means and standard deviation of urinary sodium concentration in a subset of 100 lactating women.

7.3.9 Mullen (composite and 5 subscales)

Outcome:	Continuous outcomes of Composite Mullen and the 5 subscales of the Mullen at 24 and 52 weeks postpartum.

Primary analysis:	Test for a treatment effect using a separate linear regression model for each outcome and estimate a difference in means (positive control minus placebo).
Adjustment:	Mullen scales at 2 and 12 weeks postpartum and health centre

7.3.10 VPC novelty score and attention and processing speed subscales

Outcome:	Continuous outcomes of VPC novelty score and the attention and processing speed subscales at 24 and 52 weeks postpartum.
Primary analysis:	Test for a treatment effect using a separate linear regression model for each outcome and estimate a difference in means (positive control minus placebo).
Adjustment:	Hammersmith at 2 weeks postpartum and health centre

7.3.11 Language Preference Task score

Outcome:	Continuous outcome of Language Preference Task score at 24 weeks postpartum.
Primary analysis:	Test for a treatment effect using a linear regression model and estimate a difference in means (positive control minus placebo).
Adjustment:	Hammersmith at 2 weeks postpartum and health centre

7.3.12 Oculomotor scores

Outcome:	Continuous outcome of Oculomotor scores at 24 weeks postpartum. This outcome is expected to follow a skewed distribution and hence a log or other appropriate transformation will be applied prior to analysis.
Primary analysis:	Test for a treatment effect using a linear regression model and estimate a difference in means (positive control minus placebo).
Adjustment:	Health centre

8 REFERENCES

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3. Graham, J.W., A.E. Olchowski, and T.D. Gilreath, *How many imputations are really needed? Some practical clarifications of multiple imputation theory.* Prev Sci, 2007. **8**(3): p. 206-13.