

Mount Saint Vincent University
Department of Applied Human Nutrition

**Assessing household and maternal salt intake to model salt as a potential fortification
vehicle for thiamine in Cambodia**

By
Kathleen Chan

A thesis submitted in partial fulfillment of the requirements for the degree of
Master of Science in Applied Human Nutrition

August 2020
Halifax, Nova Scotia
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Acknowledgements

The past two years have been an incredible privilege and would not have been possible without seemingly endless support from so many.

I would first like to thank all the participants of this study for being so open, and generous with their time. These families welcomed us into their homes over a 6-month period and remained committed to the protocols of the study – a huge commitment for new parents. Many friendships were formed throughout this year, and I appreciated the patience of many families who found ways to connect and communicate despite my limited Khmer. I would also like to thank all the staff at Helen Keller International Cambodia for their dedication to this project and for creating such a positive work environment. I hope the findings from this study will contribute towards the efforts of eradicating thiamine deficiency in Cambodia.

I would also like to thank my supervisor, Dr. Kyly Whitfield. I am so grateful for the guidance and encouragement you have provided me ever since my first undergraduate course with you. Your enthusiasm for research is contagious and you continually inspire me. Thank you for the opportunity to be a part of this project, and for the energy you put into your role as a supervisor. I have learned so much from you and grown both professionally and personally. It has truly been a pleasure to work with you.

Many thanks to my committee members, Dr. Frank Wieringa and Dr. Tim Green. Thank you for your guidance and feedback throughout this process, and for your flexibility with coordinating meetings between three very different time zones. In particular, I would like to thank Dr. Green and the Child Nutrition Research Centre at SAHRMI for hosting me for a two-month training period in 2019. It was an invaluable experience, thank you for your time, expertise, and support during my time in Adelaide.

I would like to acknowledge the support of my funders; the Canadian Institutes of Health Research (Frederick Banting and Charles Best Canada Graduate Scholarship-Master's and Michael Smith Foreign Study Supplement) and Research Nova Scotia (Scotia Scholar's Award-Master's Level). As well as the funders of the Trial of Thiamine Supplementation in Cambodia; the Bill & Melinda Gates Foundation and the New York Academy of Sciences.

Finally, my sincere gratitude to my husband, Carlos, thank you for your love and support, as well as your adventurous spirit. To my family, thank you so much for making the effort to visit Cambodia and for always staying connected. I would also like to thank Jelisa Gallant for her companionship throughout this program, it has been so lovely to work, learn, and travel with you. And thank you to the students in the MAMA Lab, you are an exceptionally thoughtful, kind and accomplished group, I am so grateful to know you all.

List of Abbreviations

AI	Adequate intake
ASEAN	Association of Southeast Asian Nations
ATP	Adenosine triphosphate
CDRR	Chronic disease risk reduction intake
CI	Confidence interval
DRI	Dietary reference intake
EAR	Estimated average requirement
eThDP	Erythrocyte thiamine diphosphate concentrations
FAO	Food and Agriculture Organization of the United Nations
FADH ₂	Flavin adenine dinucleotide
FBS	Food Balance Sheets
IFA	Iron and folic acid
IGN	Iodine Global Network
ISU	Iowa State University Method
LMIC	Low- and middle-income countries
mo	Month
MSG	Monosodium glutamate
MSM	Multiple Source Method
NADH	Nicotinamide adenine dinucleotide + hydrogen
NCI	National Cancer Institute Method
RDA	Recommended dietary allowance
SD	Standard deviation
SPADE	Statistical Program to Assess Dietary Exposure
TDD	Thiamine deficiency disorders
ThDP	Thiamine diphosphate
ThMP	Thiamine monophosphate
ThTP	Thiamine triphosphate
THTR	Thiamine transporter
UL	Tolerable upper intake level
UNICEF	United Nations International Children's Emergency Fund
WHO	World Health Organization
y	Year

Abstract

Background: Thiamine (vitamin B₁) is an essential vitamin critical for energy metabolism, cognitive function, and infant development. Thiamine deficiency is a persistent public health issue in Cambodia due to a traditionally thiamine-poor diet. When maternal thiamine intake is low, exclusively breastfed infants are disproportionately at risk of developing thiamine deficiency disorders. Salt has been proposed as a thiamine fortification vehicle in Cambodia, because it is consumed in consistent amounts by the target population, regardless of socioeconomic status. However, accurate salt consumption data among the target group, lactating women, are required to model and assess the feasibility of a mass fortification program.

Objectives: To assess salt intake among rural Cambodian lactating women during the exclusive breastfeeding period, and to model usual salt intake to simulate a thiamine salt fortification scenario.

Methods: This study was part of a larger trial (NCT03616288). Salt intakes were assessed using three methods via two studies. The household salt disappearance study was a longitudinal, exploratory study, where household salt disappearance (in g) was measured fortnightly from 2 through 24 weeks postpartum for all women and their households in the larger trial ($n=331$). The maternal salt and sodium study was a cross-sectional, exploratory study, using repeat 12-hr observed weighed salt and condiment records and repeat 24-hr urine samples collected between 8 and 22 weeks postpartum among a subsample of women from the larger trial ($n=104$). Urinary sodium concentrations were analyzed via ion-selective potentiometry. Demographic information was recorded at the beginning of the study, and descriptive salt use questions were asked during fortnightly home visits. Usual daily salt intake distribution was estimated using the National Cancer Institute (NCI) method, and a thiamine fortification scenario was modelled using a modified EAR-cut point method, with salt as the fortification vehicle and the optimal dose of thiamine from the larger trial (1.2 mg/day) as the cut-off for inadequacy.

Results: Unadjusted mean salt intake from repeat observed weighed intake records was 9.3 (8.3, 10.3) g/day, and not different from unadjusted estimated salt intake from 24-hr urinary sodium concentrations (9.0 (8.4, 9.7) g/day ($p>0.05$)). Mean salt use via household salt disappearance was 11.3 (10.7, 11.9) g/person/day. Adjusted salt intake from all sources was 7.7 (7.4, 8.0) g/day. A fortification dose of 275 mg thiamine/kg salt was found to meet population requirements. At this level of fortification, mean adjusted thiamine intake from fortified salt would be 2.1 (2.0, 2.2) mg/day, ranging from 1.2 to 4.3 mg/day.

Conclusion: Salt appears to be an appropriate fortification vehicle for thiamine, with usual intakes among lactating women of 7.7 (7.4, 8.0) g/day. If salt is fortified at 275 mg thiamine/kg salt, there is a very low risk of both under- and over-consumption of thiamine. These salt consumption findings and fortification models can be used to inform a potential mass fortification program in Cambodia and inform thiamine-fortified salt initiatives in other thiamine deficiency-endemic countries.

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1.0 Introduction

The life-long benefits of adequate nutrition in the first 1000 days, from conception to two years of age, are well established, and there are ongoing global efforts to improve maternal and infant nutrition status. Of particular relevance for this thesis, ensuring adequate maternal nutrition status during the first 6 months of life is vital as infants are reliant on human milk to meet all their nutrition requirements. Thiamine (vitamin B₁) is an essential, water soluble vitamin that must be regularly consumed to maintain adequate status. During the exclusive breastfeeding period, human milk is the sole source of thiamine for infants, and thiamine is one of the few micronutrients where maternal intake/status directly impacts the content of this vitamin in milk. Thiamine is critical for energy metabolism, and plays a role in normal central nervous system function and neurological development in infants. Thiamine deficiency, which can manifest with a variety of cardiovascular and neurological symptoms and is potentially fatal for infants (infantile beriberi), is generally not present in high-income countries where populations have access to a diverse and high quality diet and thiamine fortification programs, except in populations at risk of reduced intake or absorption such as alcohol-dependent people. However, thiamine deficiency remains a public health concern in several low- and middle-income countries (LMICs) that lack fortification programs, and presents a substantial risk for populations that rely heavily on a monotonous diet of thiamine-poor foods, such as polished white rice and cassava. The estimated prevalence of thiamine deficiency is highest in Southeast Asia. Although national data are not routinely collected in Cambodia, cases of infantile beriberi and suboptimal thiamine status are still regularly reported in research publications, prompting interest from both local government and international non-governmental entities to address this preventable, and potentially fatal, deficiency disease.

The overall aim of this study was to measure salt intake among lactating mothers to assess the feasibility of salt as a fortification vehicle for thiamine in Cambodia, with the ultimate goal of preventing thiamine deficiency among the most vulnerable segment of the population, exclusively breastfed infants. This study is part of a larger thiamine dose-response trial; a double blind, four parallel arm, randomized controlled trial among 331 lactating women, conducted in Kampong Thom, Cambodia. The primary objective of the trial was to determine the maternal thiamine dose at which additional thiamine no longer meaningfully increased human milk thiamine concentrations. Results from the larger trial have informed the current study, as this dose has been used to simulate fortification scenarios at which the majority of lactating women would receive adequate thiamine via consumption of a fortifiable food to optimize their human milk thiamine concentrations. The objectives of the current study were:

- 1) To estimate salt and sodium intakes among lactating women ($n=104$) using repeat 12-hour (sunrise to sunset), observed weighed condiment intake records of major sources, table salt, soy sauce, fish sauce, monosodium glutamate (MSG), and fermented fish paste (*prahok*).
- 2) To estimate salt and sodium intakes among lactating women ($n=104$) from repeat sodium excretions using 24-hr urinary sodium concentrations.
- 3) To estimate household ($n=331$) salt intake from fortnightly salt disappearance (weight lost, in g) over 22 weeks during the exclusive breastfeeding period.
- 4) To simulate various thiamine fortification scenarios, with salt as a fortification vehicle (from all major sources: table salt, fish sauce, soy sauce, and *prahok*) using the National Cancer Institute (NCI) usual dietary intake modeling technique and a modified Estimated Average Requirement (EAR) cut-point method.

2.0 Literature Review

2.1 Cambodia

The Kingdom of Cambodia is a largely agrarian nation located in Southeast Asia, bordered by Thailand, Vietnam, Laos, and the Gulf of Thailand (see **Figure 2-1.**) (1). The King figurehead holds mainly a symbolic role, and Cambodia is governed by a national government which oversees provincial and local governments. Cambodia experienced a period of social, political and economic turmoil throughout the 1970s and 1980s, however, the country stabilized with significant intervention from the United Nations in the early 1990s (2). Today, Cambodia has a population of 16 million people, 81% of whom reside in rural areas, and has been undergoing rapid economic transition in recent decades, with an average GDP growth of 7% per year and increased migration from rural to urban areas (2).

In a relatively short time, Cambodia has experienced major changes; poverty rates have fallen from 48% in 2007 to 14% in 2014, and the country was recently reclassified from “low-income” to “low-middle income” by the World Bank (3). However, despite the continued improvement in quality of life in Cambodia, approximately 4.5 million people are still classified as “near-poor” and remain highly vulnerable to economic trends. National health indicators reveal significant challenges persist; the maternal mortality rate is 1.7 per 1,000 live births and there is a high infant (under 1 year) mortality rate of 28 per 1,000 live births (1).



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

2.1.1 Diet in Cambodia

Cambodia has a tropical climate, with warm temperatures year-round and two distinct seasons (a dry northeastern monsoon from December to April, and wet southwestern monsoon from May to October), which drastically changes the landscape of the country and affects food availability and agriculture-related employment throughout the year (1). The Cambodian diet is highly linked to the immense freshwater ecosystems of the Tonle Sap lake, Mekong River, and surrounding flood plains as a direct source of food and for crop irrigation. The traditional Cambodian diet consists mainly of rice, fish and fish products, and vegetables, however domestic availability of these products decreases in the dry season (4,5).

Rice is central to Cambodian life, culture and economy; 75% of agricultural land is dedicated to rice production, and the verb “to eat” in Khmer translates literally to “eat rice” (6). Polished, white rice comprises approximately 60% of total energy intake and is often consumed at least

twice a day, with an estimated average intake range of 302-823 g/person/day (5,7). Fish and fish products, such as fish sauce and raw fermented fish paste (*prahok*), are consumed almost daily at an estimated intake of 120 g/person/day, providing approximately 37% and 28% of total individual protein and fat intake respectively, and is considered to be the main source of micronutrients in the diet (8). Fish constitutes 76% of all animal-based food intake, followed by meat (20%, mainly beef, pork and organ meats), and poultry (4%) (4). While meat and poultry are popular foods, there are often economic barriers to including these foods more regularly, and the lack of a reliable cold chain in most of the country can increase food safety risks with these foods (4).

2.2 Malnutrition in Cambodia

Global malnutrition is a complex and evolving problem that is connected to economic, political, social, and environmental factors (9). Malnutrition can occur through undernutrition or overnutrition, and affects all countries and population subgroups, increasingly creating a “double-burden” of disease in economically developing countries (10). This study will exclusively explore malnutrition in the form of chronic undernutrition and micronutrient deficiencies, as overnutrition is not prevalent in Cambodia.

2.2.1 Infant and young child malnutrition in Cambodia

Infants and children under 5 years in Cambodia are at particular risk of the long-term effects of malnutrition. The first 1,000 days, from conception to two years, is a critical time of growth and development that must be supported with adequate nutrition to avoid the potentially irreversible

adverse effects of stunting, which affects approximately 32% of Cambodian children under 5 years (11). In addition, 10% of Cambodian children under 5 years are wasted.

One area of maternal and child nutrition that has been particularly successful in Cambodia is the promotion and support of breastfeeding in line with global recommendations (12). Ninety-six percent of infants receive some breastmilk in Cambodia, 63% within one hour of birth, and although there have been some declines in breastfeeding rate recently, 65% of infants are exclusively breastfed to 6 months and 37% continue breastfeeding to the recommended 2 years (1). However, micronutrient deficiencies during this period continue to present serious barriers in improving malnutrition rates (2).

2.2.2. Micronutrient deficiency malnutrition

Micronutrient malnutrition programs globally and in Cambodia have mainly been focused on a few, select micronutrients that are commonly deficient and can have detrimental effects for developing children: iron, vitamin A, folate, and iodine (13,14). The most recent Cambodia Demographic and Health Survey (2014) revealed that the prevalence of iron deficiency is actually quite low (3% for mothers and children), however anemia from other (non-nutritional) causes remains a significant problem (1,15). Suboptimal vitamin A status (RBP<1.05 $\mu\text{mol/l}$) affects approximately 9% of mothers and almost 40% of children, and folate deficiency affects 19% of mothers and 29% of children (1). Seventy-eight percent and 66% of mothers and children had insufficient urinary iodine concentrations, respectively, however iodine deficiency prevalence is variable throughout the country (1,16).

One micronutrient deficiency that remains a public health issue in Cambodia, despite its eradication in most of the world, is thiamine deficiency. This deficiency can have disproportionate and detrimental effects for developing infants. Recent and ongoing research into the prevalence and prevention of thiamine deficiency has been supported by both governmental and non-governmental entities and is an important part of addressing maternal, infant and young child malnutrition in Cambodia.

2.3 Thiamine

Thiamine is an essential, water-soluble vitamin also known as vitamin B₁. Thiamine contains a thiazole ring and pyrimidine ring linked through a methylene bridge (17) and exists in four main forms in the human body; free thiamin (non-phosphorylated, normally ionized under physiological conditions), or phosphorylated through an alcohol side-chain as thiamine monophosphate (ThMP), thiamine diphosphate (ThDP, also referred to as thiamine pyrophosphate), or thiamine triphosphate (ThTP) (see **Figure 2-2.**)

In healthy adults, approximately 25 to 30 mg of total thiamine is maintained for active use or distribution, mostly present in the biologically active form of thiamine, ThDP (18,19). Free thiamine, ThMP, and ThTP are all found in low concentrations in the body. Free thiamine plays a role in central nervous system function, such as neuronal signal transmission, and may act as a shuttle molecule across cell membranes (19–21). ThMP is the main form of thiamine found in human milk, and functions as an inactive form of thiamine that can be converted into active forms of thiamine as required (19–21). ThTP is potentially involved in nervous system function and nerve impulse transmission, as the highest concentrations are in the brain and skeletal

muscles, and it may act as a phosphate donor for proteins in the neuromuscular junction, however, additional roles are not well established (19,21,22).

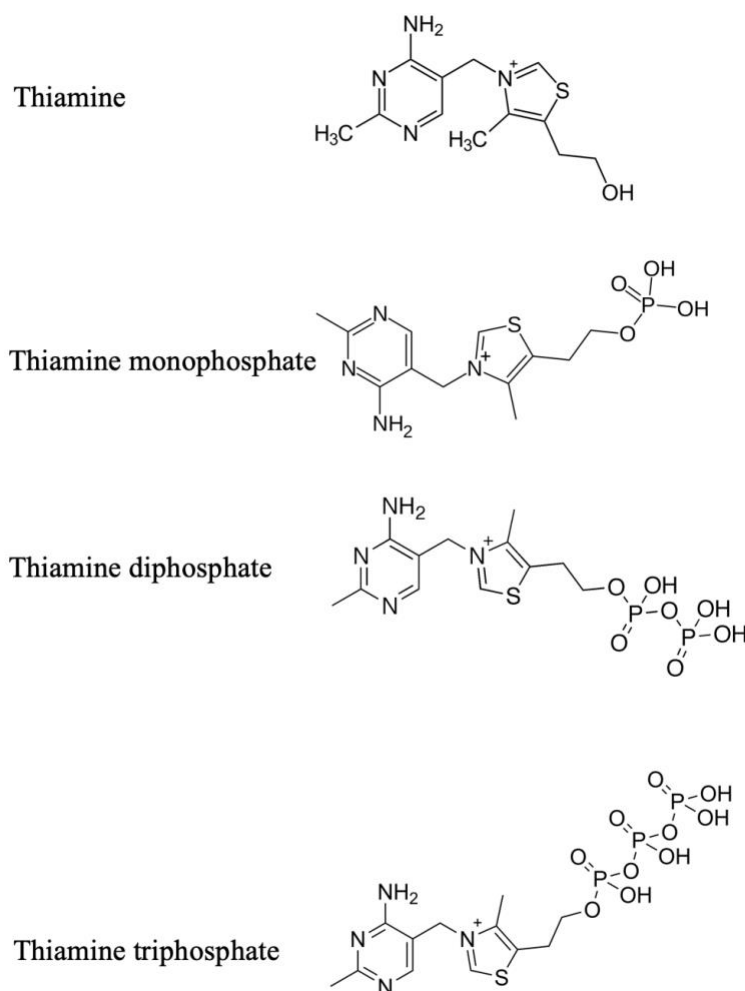


Figure 2- 2. The free thiamine molecule and its phosphorylated esters: thiamine monophosphate (ThMP), thiamine diphosphate (ThDP), and thiamine triphosphate (ThTP) (*public domain*).

ThDP is the main, active form of thiamine, and makes up approximately 80% of total thiamine in the body, the majority of which is found in erythrocytes (19,21). ThDP can be synthesized in the body through phosphorylation of free thiamine or ThMP by cytosolic thiamine diphosphokinase, or dephosphorylation of ThTP by 25-kDa TTPase (23,24). ThDP plays various critical roles in

carbohydrate and branched-chain amino acid metabolism as well as nucleotide synthesis. In the cytosol, ThDP plays an important role in the pentose phosphate pathway where it acts as a cofactor for transketolase, which diverts excess sugars from the pentose phosphate pathway to the glycolytic pathway to be converted to pyruvate (23). ThDP also mediates reactions to produce nicotinamide adenine dinucleotide (NADH; an electron carrier in the electron transport chain) as well as precursors for nucleotide synthesis. In the mitochondria, ThDP is necessary for several reactions in the citric acid cycle; ThDP acts as a cofactor for pyruvate dehydrogenase to convert pyruvate to acetyl-coenzyme A, for alpha-ketoglutarate dehydrogenase to convert alpha-ketoglutarate to succinate, and for branched-chain-alpha-keto acid dehydrogenase in the conversion of branched-chain-alpha-keto acids into acyl coenzyme A (20). Ultimately, ThDP is necessary for the production of electron carrying compounds (NADH and FADH₂) in the citric acid cycle for eventual production of ATP in the electron transport chain as well as the direct production of ATP from the cycle (25).

2.3.1 Sources of thiamine

Thiamine has a short half-life (1-12h) (26,27) and no storage depot in the body, therefore humans are dependent on regular dietary intake of thiamine to maintain adequate thiamine status. Some bacteria in the gastrointestinal tract may synthesize small amounts of thiamine, however it is not absorbed (28).

Thiamine-rich food sources include whole grains, yeasts, meats, legumes, and nuts, which mainly provide thiamine in the form of ThDP (see **Table 2-1**). Several species of bacteria, yeast and plants are able to synthesize the pyrimidine and thiazole moieties separately, and then

assemble them to form ThMP by thiamin-phosphate synthase (23). In addition, there is evidence that some plants and microorganisms may be able to re-synthesize degraded thiamine through a salvage pathway (29), and thiamine can also be produced synthetically, as thiamine hydrochloride and thiamine mononitrate (30).

Cambodians are at particular risk of thiamine deficiency due to high intake of thiamine-poor staple foods such as polished, white rice, and fish. Most of the thiamine in rice is located in the bran and germ, which is removed during processing, while thiamine-rich animal sources such as pork are usually unaffordable, and legumes are not part of the traditional diet (21).

Unfortunately, recent outbreaks of African Swine Fever virus in Southeast Asia may also result in reduced pork intake due to pig culling and lack of consumer trust (31). In 2019, the Cambodian Pig Raiser Association stated that up to 20% fewer pigs were being brought to local slaughterhouses (32). In addition, people with high-carbohydrate diets may require higher thiamine intakes for carbohydrate metabolism, potentially exacerbating the problem of deficiency (33). Thiamine is also one of the few micronutrients where maternal intake is directly related to human milk content, which is problematic given that exclusively breastfed infants are completely reliant on maternal thiamine intake to maintain adequate thiamine status (34). When lactating mothers consume a thiamine-poor diet, their infants are at a high risk of thiamine deficiency (35).

Table 2- 1. Thiamine content of common dietary sources. Adapted from (18).

Food	Thiamine content, mg/100g
Wheat flour (whole meal)	0.4-0.5
Rice	
<i>Whole rice</i>	0.50
<i>Polished rice</i>	0.03
<i>Rice bran</i>	2.30
Vegetables	
<i>Peas</i>	0.36
<i>Other legumes</i>	0.4-0.6
<i>Potatoes</i>	0.1
Cow's milk	0.04
Meats	
<i>Beef</i>	0.3
<i>Lamb</i>	≤1.0
<i>Poultry</i>	0.1
<i>Pork</i>	1.30
Refined sugars	Nil

2.3.2 Thiamine antagonists and thiaminases

Another potential risk for thiamine deficiency in Cambodia is the regular consumption of thiamine-degrading enzymes (thiaminases I and II) and thiamine antagonists that reduce thiamine bioavailability or absorption (18). Thiaminases I and II have been isolated in raw and fermented freshwater fish and shellfish, as well as some bacteria and yeast, but is denatured with heat, while thiamine antagonists are heat-stable molecules (often polyphenolic compounds) that are found in coffee, tea, and betel nut (18). Raw *prahok* is a household staple in Cambodia, consumed on average 2.2 days/week/household both as a major meal component or as a condiment (4). Tea and coffee are common beverages in Cambodia, and betel nut chewing is widespread among women; approximately 50% of women over 48 years chew betel nut habitually (36). Betel nut chewing is used as a traditional treatment for morning sickness during pregnancy, however it is unclear if this practice is still common among young Cambodian

mothers (36). Thiaminases and thiamine antagonists may further decrease thiamine availability from an already thiamine-poor diet, however there is still some controversy as to the effect of these compounds on the thiamine status of Cambodians. Coats *et al.* did not find an association between intake of foods rich in thiaminases or thiamine antagonists and low maternal thiamine status ($n=54$) in Prey Veng, Cambodia, and were not able to detect thiaminase activity in food samples from local markets (37). However, it is important to note that the majority of *prahok* is made in the home, and a larger sample may be required to better understand the effect of thiaminases and thiamine antagonists on thiamine status in Cambodia (4).

2.3.3 Dietary requirements of thiamine

Dietary Reference Intakes (DRIs) for thiamine were last published in 1998 by the Institute of Medicine (now National Academy of Medicine) (see **Table 2-2.**) (38). The Estimated Average Requirement (EAR) is the level of nutrient intake at which half of a specific population group's requirements are met (39). For thiamine, the EAR was established through various depletion-repletion studies, although it is notable that most were conducted over 40 years ago, with small sample sizes (38). The EAR is then used to mathematically derive a Recommended Dietary Allowance (RDA; $\text{EAR} + 2\text{SD}$), which is the nutrient intake level that is estimated to meet the nutrient needs of nearly all (97.5%) healthy members of a particular sex-age group (38). An Adequate Intake (AI) is established with there is insufficient evidence available to set an EAR for a particular group. For thiamine, an AI was set for infants 0-12 months using an estimated milk intake of 780 mL/d (0-6 months) or 600 mL/d (7-12 months), and the average thiamine concentration of milk from healthy, well-nourished mothers (38). The tolerable upper intake level (UL) is the maximum daily intake level of a nutrient at which no risk of adverse health

effects can be expected, however thiamine has no defined UL as there have been no adverse effects reported for high doses of thiamine (38)

Specific to this study, the EAR and RDA of lactating women is 1.2 and 1.4 mg/day, respectively. The EAR for lactating females is 0.3 mg higher than the EAR for non-pregnant, non-lactating women to account for thiamine transferred to milk each day and the energy costs associated with milk production(38).

Table 2- 2. Dietary reference intakes for thiamine (adapted from (38)).

Life Stage Group	EAR ^a (mg/day)		RDA ^b (mg/day)		AI ^c (mg/day)
	Male	Female	Male	Female	
0-6 months	-	-	-	-	0.2
7-12 months	-	-	-	-	0.3
1-3 years	0.4	0.4	0.5	0.5	-
4-8 years	0.5	0.5	0.6	0.6	-
9-13 years	0.7	0.7	0.9	0.9	-
14-18 years	1.0	0.9	1.2	1.0	-
19-50 years	1.0	0.9	1.2	1.1	-
51-70 years	1.0	0.9	1.2	1.1	-
>70 years	1.0	0.9	1.2	1.1	-
Pregnancy (14-50 years)	-	1.2	-	1.4	-
Lactation (14-50 years)	-	1.2	-	1.4	-

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

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

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

2.3.4 Thiamine digestion and absorption

Phosphorylated forms of dietary thiamine are dephosphorylated by phosphatases into free thiamine before absorption from the small intestine to the blood stream (20,24). Free thiamine

cations are absorbed both actively and passively at this site in a concentration-dependent manner, independent of pH, temperature, and sodium concentration. At high concentrations ($>2\mu\text{M}$), thiamine is transported via passive diffusion through a proton channel; when thiamine concentrations are low ($<2\mu\text{M}$), thiamine is actively transported across the intestinal membrane via organic cation transporters (20). The rate of absorption slows at higher concentrations of thiamine, and plateaus at very high doses (1500mg) (26,40). There is no difference in absorption of naturally occurring versus synthetic thiamine (41).

Once free thiamine cations have entered the intestinal mucosal cells, thiamine is phosphorylated, then dephosphorylated for transport to serosal cells and eventually the blood stream (42). Free thiamine can circulate in the plasma, or be converted to ThDP and bound within erythrocytes (23). In the body, thiamine is mainly transported across membranes via two types of solute carrier proteins; thiamine transporter 1 (THTR-1) in skeletal muscle and systemic tissues and thiamine transporter 2 (THTR-2) in the intestines (20).

2.4 Thiamine deficiency

2.4.1 Historical context

Thiamine was originally discovered as a cure for beriberi, a thiamine deficiency disease that is characterized by neurological and cardiovascular symptoms (43). The first known recorded incidences of beriberi date back to 10th century China, and in accordance with disease theory at the time, the disease was thought to be caused by miasmas (or poisonous vapours) (44). In the late 19th century, Dutch physician and researcher Christian Eijkmann performed various experiments on chickens to characterize beriberi, building on earlier work by Cornelis

Pekelharing and Cornelis Winkler who believed beriberi was potentially caused by bacterial infection (44). Eijkmann attempted to infect chickens with blood of humans who had been diagnosed with beriberi, however neurological symptoms developed in both the control and experimental groups, and disappeared when the chickens' diets changed from polished rice to whole grain brown rice. This finding revealed that beriberi was most likely not a communicable disease, but rather related to malnutrition (44). Around this same period, Takaki Kanehiro, a Japanese naval physician, found that cases of beriberi could be prevented on long voyages when sailors' diets were changed from largely white, polished rice to meat and vegetables and no white rice (43). Later, physician Gerrit Grijns continued Eijkman's work and conducted research that confirmed that beriberi was caused by a deficiency in some unknown nutrient, that would later be classified as a *vitamin* (44). From this research, Barend Jansen and Willem Donath worked to isolate thiamine and determine its chemical formula by 1933, followed by synthesis and final resolution of structure in 1936 by Robert R. Williams (44).

2.4.2 *Thiamine deficiency disorders*

Thiamine deficiency from low dietary thiamine intake or reduced thiamine absorption can cause a range of symptoms, primarily neurological and cardiovascular. Historically, thiamine deficiency has been classified as Wernicke encephalopathy or different forms of 'beriberi', however overlapping presentations of thiamine deficiency have been problematic and led to confusion around these terms (45). The Thiamine Technical Consultation Group recently proposed the more broad terminology of *thiamine deficiency disorders* (TDD) to describe the range of illnesses that can arise from thiamine deficiency (21). With the classical naming system, Wernicke encephalopathy is an acute form of thiamine deficiency that is mainly associated with

alcohol dependency and malnutrition, and is the most common TDD seen in economically developed countries (46). The main forms of beriberi are dry beriberi, wet beriberi, and infantile beriberi (43). Dry beriberi manifests as a neurological disorder and main symptoms include peripheral neuropathy, while wet beriberi manifests primarily as a cardiovascular disorder with symptoms such as edema, tachycardia, cardiomegaly, and congestive heart failure, as well as neurological symptoms (18). Infantile beriberi can present with a range of cardiovascular, respiratory, and neurological symptoms, making correct diagnosis particularly difficult and with potentially detrimental consequences (21). Infantile beriberi can quickly become fatal if it is not recognized and treated immediately with the administration of thiamine (21). Infants born to thiamine-replete mothers are born with high levels of thiamine, which begin to diminish around 2 months of age, potentially due to changes in neurological development and metabolic needs (47,48). Thiamine must be continually replenished through diet, therefore infantile beriberi often presents between 2-6 months of age, during the exclusive breastfeeding period, if maternal thiamine intakes are insufficient (34).

2.4.3 Prevalence of thiamine deficiency

Whitfield *et al.* and Johnson *et al.* have reviewed major outbreaks of TDDs in recent decades, and have noted significant events in Southeast Asia, South Asia, and West Africa (21,49). Global thiamine deficiency rates have been decreasing over the past 50 years, however a review of national food balance sheets, population data, and food composition tables has revealed that Southeast Asia has consistently had the highest estimated prevalence of thiamine inadequacy from 1961-2011, with estimated rates still around 30-40% (50). Cases of infantile beriberi are still regularly reported in Cambodia, Myanmar, and Laos (21).

The impact of infantile beriberi in Southeast Asia was highlighted in the early 1990s among the Karen refugee population in refugee camps at the Thai border, when it was determined that 40% of all infant deaths were caused by thiamine deficiency before a treatment protocol was instated (51). In Cambodia, thiamine deficiency may also play a significant role in infant mortality. Through retrospective verbal autopsies, researchers determined that 45% of infant deaths during the first year of life in Mesang District, Prey Veng (23 of 51 deaths, among 910 live births) between January 2005 and April 2008 could be attributed to beriberi (52). In addition, biochemical data has revealed high levels of thiamine deficiency among both symptomatic (beriberi) and asymptomatic breastfed infants and their mothers (37,53,54). The high prevalence of thiamine deficiency in Cambodia is thought to be a major public health issue (35). There are several population-based interventions available that could prevent this deficiency and its related disorders.

2.5 Interventions to address thiamine deficiency

There are three main policy and program approaches to addressing micronutrient deficiencies at the population level; increasing dietary diversity, mass food fortification, and supplementation (13). All three must be supported with nutrition education, public health, and food safety measures (13). Whitfield *et al.* and Johnson *et al.* have recently reviewed these traditional interventions to address thiamine deficiency in LMICs (21,49).

2.5.1 Improving dietary diversity

Increasing dietary diversity to include a greater quantity and range of micronutrient-rich foods is considered the optimal approach to addressing micronutrient deficiencies, however there are

often significant barriers to implementing this intervention (13). Increased access to micronutrient-rich foods requires increased resources for purchasing and producing these foods, as well as consumer behaviour change, and education (13). Since the poorest populations are at risk of micronutrient deficiency, this intervention may not be feasible without considerable multi-sectoral coordination and support (political, economic, agricultural) (55). Thiamine deficiency in Cambodia is associated with a thiamine-poor diet based heavily on polished, white rice, therefore an increase in dietary diversity would likely increase thiamine intake, however no trials have been published on this type of intervention on thiamine status (49). Given that the thiamine-rich foods listed in *Section 2.3.1*, such as pork and brown rice, are either expensive or not culturally appropriate, it is unlikely that increasing dietary diversity can be employed to immediately address thiamine deficiency in Cambodia.

2.5.2 Supplementation

Supplementation is the delivery of nutrients outside of traditional dietary sources, often as a pill, capsule, or syrup (13). This allows for high doses of specific nutrients to be provided to targeted population groups of interest in a highly absorbable form. The most common supplementation programs in Cambodia provide iron and folic acid (IFA) during the perinatal period, and vitamin A to children. In 2014, the majority (76%) of pregnant women in Cambodia had received IFA supplements for at least 90 days during their pregnancy, while 49% received IFA postpartum, and 70% of children (6-59 months) received a vitamin A supplement (1). More recently, a thiamine supplementation program has been implemented in Myanmar (10 mg/day for pregnant and lactating women up to 3 months) to address thiamine deficiency among breastfed infants (21).

There are no large-scale thiamine supplementation programs in Cambodia, however several thiamine supplementation studies have been conducted among populations of interest globally and in Southeast Asia (21,49). In Cambodia, Coats *et al.* conducted a pharmacokinetics study to better understand lactating women's response to thiamine supplementation. These researchers provided a daily 100 mg dose of thiamine hydrochloride to 16 lactating women in Prey Veng province (suspected deficient population) for 6 days and measured maternal and infant whole blood ThDP, and maternal milk thiamine at baseline and 24 hours after the final dose (56). A control group of 16 non-supplemented lactating mothers from Minnesota also provided blood and milk samples at one time point for comparison to a sufficient population. After 6 days, the Cambodian mothers' median (range) milk total thiamine increased from 179.5 (85-359) nmol/L to 502.7 (360-808) nmol/L. At the end of the study, total milk thiamine was not significantly different between Cambodian and American mothers ($p=0.395$) (56). Similarly, long-term thiamine supplementation trials have been shown to significantly increase human milk thiamine concentrations in Gambia and India (57,58). Thiamine supplementation is an effective approach to improve thiamine concentrations in human milk, even in a short supplementation regime, however this approach is not necessarily an efficient solution to address population-based thiamine deficiency in Cambodia. The routine supply of dietary thiamine required to maintain adequate thiamine status would make preventative thiamine supplementation a costly, resource-intensive intervention, and require consumer behaviour change (13).

2.5.3 Fortification

Food fortification is the addition of nutrients to processed foods, and has proven to be a safe, effective, and efficient approach to prevent micronutrient deficiencies around the world (13). Food fortification can occur at three levels; mass fortification (either mandatory or voluntary), targeted, and market driven (55). Mass food fortification is the addition of micronutrients to a food vehicle that is consumed by the majority of the population as: a staple food (e.g. milled grain, salt, or oil), basic food (e.g. dairy product or bread), or value-added food (e.g. condiment) (59). Targeted fortification is an approach used for at-risk populations, such as processed complementary foods for infants and young children (6-24 months), while market driven fortification involves the fortification of food as a business initiative to add value to food products (such as beverages or nutrition bars with added nutrients) (55). Finally, another form of fortification is biofortification, the genetic modification through plant breeding or gene editing to create new variants of staple foods that produce higher concentrations of nutrients of interest (13). There have been recent efforts to develop a rice cultivar with a higher thiamine content, however the majority of the thiamine remained in the outer layers (which are removed during processing) and not the endosperm (60).

Mass food fortification is the most cost-effective approach to preventing micronutrient deficiencies at the population level (13,55). A major advantage of fortification is that it is a passive intervention that does not require consumer behaviour change. This approach is also the fastest and most accessible intervention to implement when coordinated with support of the food industry to use existing manufacturing technology and distribution systems (13). Sustainability of fortification programs depends on the selection of an appropriate fortification vehicle (food)

and fortificant (nutrient), and are most effective at preventing micronutrient deficiencies when legislated as mandatory for industry and integrated within a more comprehensive strategy to address underlying causes of malnutrition (55). Mass food fortification programs were first implemented in the early 20th century to target iodine deficiency with iodized salt, and in high-income countries such as Canada and the United States there are robust mandatory fortification programs for vitamin D, B-vitamins (thiamine, niacin, folic acid), and iron (61).

2.5.3.1 Selection of a fortification vehicle for mass fortification

Selecting an appropriate food as a fortification vehicle is critical to the success of a mass fortification program, and careful considerations must be taken regarding consumption, technical feasibility and processing of the food (62). It is important that the food vehicle is consumed by a large number of the population at risk of deficiency, regardless of age and socioeconomic status, and is consumed regularly throughout the year in consistent quantities. Addition of the micronutrient should not affect the stability or acceptability of the food, and the food should be centrally processed so that fortification is more cost-effective, and quality controls are easier to monitor (13,62). The appropriateness of a fortification vehicle varies by country and context, as it depends on local dietary patterns. For example, wheat, sugar and bouillon cube are consumed daily in large areas of sub-Saharan Africa (63,64), while dairy products are a major part of traditional diets in western Europe (65), yet these foods would not be appropriate vehicles in Cambodia. Polished rice is a staple food that could potentially be fortified in Cambodia, however rice production is highly decentralized in the country and significant losses through washing and cooking have been noted (45).

Globally, the most successful fortification program has been the universal iodization of salt to prevent iodine deficiency disorders. Salt is essentially consumed by everyone in all countries throughout the year, and iodization technology is widely available and inexpensive (66). While there are still barriers to reaching high-risk populations, over 70% of the world's population is covered by iodized salt and iodine status has been continually improving over recent decades (67).

2.5.3.2 Selection of a fortificant

It is equally important to select an appropriate fortificant for effective food fortification. The chemical fortificant compound should: be cost-effective and not affect the affordability of the food, have adequate bioavailability, not reduce the sensory acceptability of the product or separate from the food matrix, be safe for a range of consumption levels, and not interact with other micronutrients or foods (13,62). The World Health Organization (WHO) reviewed acceptable fortificants for mass food fortification in 2006, and the two recommended thiamine fortificants are the two synthetic thiamine-salts: thiamine hydrochloride and thiamine mononitrate (13). Both forms are nutritionally equivalent and cost-effective, and both are sensitive to heat, alkaline pH, oxygen and radiation (30). Thiamine hydrochloride is more soluble, while thiamine mononitrate has more stability in low-moisture foods (41). Thiamine hydrochloride has a greater range of stability, however when degraded, its byproducts have a strong odor and colour change compared to thiamine mononitrate, which can negatively affect sensory acceptability (41).

2.6 Thiamine fortification programs

Recent, comprehensive reviews have recommended that thiamine fortification of culturally appropriate staple foods in LMICs is the most appropriate and effective intervention to prevent and address thiamine deficiency, particularly among women and children (21,49).

Mass fortification with thiamine first began in the United States in 1941, after the American Medical Association determined that refined wheat milling processes were removing over 90% of the thiamine content compared to whole grain wheat flour, drastically reducing thiamine intake in the traditional American diet (68). In Canada, thiamine fortification began in Newfoundland in 1944 with the mandatory enrichment of white wheat flour with thiamine after a population health survey revealed several indicators and risk factors of thiamine deficiency, such as low excretion of thiamine in urine ($<50 \mu\text{g/g}$ of creatinine) among 44% of participants, high intake of processed white flour, and sporadic cases of neurological symptoms associated with thiamine deficiency (69). After four years of fortification, prevalence of low excretion of thiamine in urine decreased significantly to only 1% of participants (69). Today, there are limited thiamine fortification programs in LMICs, and in Southeast Asia, only two countries have mandatory thiamine fortification programs (wheat flour in Indonesia and vitaminized rice in Thailand). However, voluntary thiamine fortification programs exist in The Philippines, Vietnam (wheat flour), and Malaysia (wheat flour) (70). There are no thiamine fortification programs in Cambodia.

2.6.1 Fish sauce fortification with thiamine

Recent research on thiamine-fortified fish sauce in Cambodia has shown that food fortification may be an efficacious approach to improve thiamine status among lactating and non-lactating

women and their children in rural Cambodia, albeit not necessarily with fish sauce as the vehicle (71,72).

Whitfield *et al.* conducted a double-blind, randomized controlled efficacy trial in Prey Veng, Cambodia wherein 276 non-pregnant, non-lactating women and their families consumed study-provided fortified fish sauce (control; low: 2g/L; or high: 8g/L) *ad libitum* over 6 months (71). After 6 months, the baseline-adjusted erythrocyte ThDP concentrations (eThDP) (as mean [95% CI]) were significantly lower among women in the control group (184 [169-198] nmol/L) compared to the high concentration group (257 [237-276] nmol/L; $p < 0.001$). This study also assessed fish sauce intake of participants through an observed, weighed fish sauce intake assessment. Authors reported large variation in fish sauce intakes that translated to mean (SD) thiamine intakes well above the RDAs of 1.1 mg/d: women in the low and high concentration groups consumed 36 (42) mg/day and 127 (153) mg/day, respectively (71).

In a parallel trial, Whitfield *et al.* provided 90 pregnant women in Prey Veng with study-provided fish sauce in the 6 months around the perinatal period (72). Average (95% CI) milk total thiamine concentrations were higher among the low and high concentration groups (20.7 [18.6-22.7] $\mu\text{g/dL}$) and (17.7 [15.6-19.9] $\mu\text{g/dL}$) respectively) compared to the control group (14.4, 12.3-16.5 $\mu\text{g/dL}$) ($p < 0.05$) (72).

Both studies highlight the potential for a thiamine fortification of a commonly consumed food to improve thiamine status among vulnerable populations in rural Cambodia, however, Whitfield *et al.* identified some challenges to using fish sauce as a fortification vehicle (73). While fish sauce

is a commonly consumed condiment in Cambodia (approximately 90% coverage), as a value-added commercial food product, fortified fish sauce may not reach the poorest segments of the population who make their own fish sauce. Also, fish sauce is not centrally produced, but rather manufactured by several smaller producers throughout the country which would complicate implementation, monitoring and quality control, and the highly variable quality of fish sauce across the country may affect the stability of the fortificant (73,74). In addition, fish sauce is not consumed universally and would not be an appropriate fortification vehicle for many thiamine deficient populations around the world (21). Salt may be an optimal alternative vehicle, given that it is consumed universally and could align with existing salt iodization policies and infrastructure (73).

2.7 Salt as a fortification vehicle in Cambodia

Salt (NaCl) is a universally consumed staple food that has been used effectively as a vehicle for iodine fortification since the early 20th century. More recently, it has become technically possible to fortify salt with a range of nutrients, such as iron and vitamin B12 (75). Salt could be a successful thiamine fortification vehicle in Cambodia because it is consumed by the entire population throughout the year (both lean and peak seasons), and domestic salt production is centralized in two adjacent coastal provinces, Kampot and Kep (4,76). In addition, salt fortification legislation (Sub-Decree No. 69 on Management of Iodized Salt Exploitation) has been in place since 2003, which could provide the political framework to legislate thiamine fortification (77).

However, monitoring and enforcement of legislation is critical to the success of the fortification program. Salt fortification has several challenges in Cambodia; issues were initially identified when rates of compliance decreased substantially with the end of external support from the United Nations International Children's Emergency Fund (UNICEF) in 2010 (78). A nationally representative repeat survey of school children's households in 2008 ($n=566$), 2011 ($n=1275$) and 2014 ($n=1862$) found no significant difference in iodine content during periods where iodization was supported by UNICEF; the median (25th/75th percentile) iodine content of salt in 2008 and 2011 was 18.0 (2.0/39.0) mg/kg and 22.0 (2.0/37.0) mg/kg, respectively ($p>0.05$). After external support ended, median iodine content dropped significantly to 0.0 (0.0/8.9) mg/kg in 2014 ($p<0.001$) (78). However, recent implementation of the Cambodia Iodized Salt Project (led by the National Sub-Committee for Control of Iodine Deficiency Disorders, the Ministry of Planning, and UNICEF) has renewed efforts to monitor and improve salt iodization practices with some success. A 2018 survey conducted by this group has shown a decrease in non-iodized fine salt from 29% in 2016 to 10% in 2018 (76).

2.7.1 Salt production in Cambodia

All salt in Cambodia is produced through solar salt evaporation ponds, followed by various purification and refinement processes (76). Salt is produced during the dry season (January - April) and productivity is weather-dependent. Salt is categorized as coarse salt, the initial product from sea water evaporation, or fine salt, which has been boiled in reservoirs to a finer grain (78). Cambodia produces approximately 100,000 tonnes of salt annually, and requires approximately 57,000 tons annually for household consumption, while remaining stock is used both for consumption through industrial food processing or for non-food related industrial

purposes (water softening, tanning hides, farming etc.) (76). Approximately 85% of salt consumed in Cambodia is produced domestically, and an estimated 10,000 tonnes of salt is imported annually from Vietnam and Thailand, which both have mandatory salt iodization legislation and are similar in cost to domestic salt (70,76,78).

2.7.2 Technical aspects of thiamine-fortified salt

Salt has been used as a fortification vehicle for other micronutrients in other countries with success. The majority of double-fortified salt efforts have been focused on iron, and have been ongoing since the 1960s (79). Salt has been shown to be an effective fortification vehicle for iron in several trials (80,81) and there have been recent efforts to scale up the distribution of dual-fortified (iron and iodine) salt within the public food distribution system in India (82). Fortifying salt with iron is technically challenging as iron can change the acceptability of salt and can react with iodine, resulting in reduced availability of iodine, however these barriers can be overcome with microencapsulation of iron (75). This microencapsulation process can also be used for multiple micronutrients when iron is included in the formulation (81). By contrast, fortifying salt with only B vitamins, such as folic acid and vitamin B12, has far fewer challenges (83). Salt can be fortified with B-vitamins through a standard, cost-effective spray-mixing process (similar to iodine fortification), they do not affect the availability of iodine in the mix, and have minimal effects on product stability or acceptability (81,83).

2.7.3 Salt in the postpartum diet

Traditional beliefs around diet are considered an integral part of postpartum recovery and successful breastfeeding in Southeast Asia, and certain foods are considered taboo and are thus

excluded in the perinatal period (84). In Laos, Barennes *et al.* conducted survey of 300 mothers and found that up to 93% of women restricted their diet in the postpartum period (85). Mothers commonly excluded fruits, vegetables, and meats, relying mainly on a diet of glutinous rice in the first months after giving birth. In this survey, 16% of mothers reported eating only glutinous rice with salt within the first 2 weeks after giving birth. In addition, analysis of 24-hr diet recalls revealed that 97% of women had insufficient thiamine intake (mean (SD) intakes of 0.8 (0.5) mg/day) (85). The combination of a high carbohydrate diet and low dietary thiamine intakes could exacerbate thiamine deficiency in this population, where thiamine needs are increased due to lactation and high carbohydrate intakes. Thiamine-fortified salt would likely be an appropriate intervention to address thiamine deficiency among lactating women in Cambodia other beriberi endemic countries, such as Laos, because salt is considered an acceptable food in the postpartum taboo diet.

2.7.4 Sodium in the body

Sodium is an essential mineral that acts as a major electrolyte (Na^+) and has several critical roles in the body, including maintenance of extracellular osmolarity, acid-base balance, transmission of nerve impulses, and general cell function (18). Sodium in the body is highly regulated, as normal metabolic activities in the body must occur within a narrow range of osmolarity (plasma sodium concentrations are maintained between 138-142 mmol/L) (86). Therefore, dietary sodium intake has little effect on the sodium concentration of extracellular fluids, however sodium intake does directly affect extracellular fluid volume (both intravascular and extravascular), as increased sodium intake can trigger thirst to increase body water content to maintain serum sodium concentration, thereby increasing blood pressure (18).

The kidneys play a major role in maintaining sodium concentrations and regulating fluid balance in the body. The renin-angiotensin-aldosterone system is the primary function to correct changes in blood pressure, through vasoconstriction or dilation as well as sodium re-absorption or excretion (87).

2.7.5 Dietary sodium requirements

Sodium requirements have been recently updated by the National Academy of Medicine, as well as a comprehensive review of the risks associated with both low and excessive sodium intake (88). Physiological minimum requirements for sodium to maintain metabolic functions and replace losses are actually quite low (230-460 mg/day) however intakes are generally far above physiological requirements because of the wide use of sodium as a preservative as well as human preference for the taste of salt (89). The updated AI for sodium can be found in **Table 2-3**. The AI for males and females above 14 years is 1500 mg/day, with a Chronic Disease Risk Reduction Intake (CDRR) of 2300 mg/day, however, average sodium intakes are often above this level. A systematic review and meta-analysis of dietary sodium and 24 hr urinary sodium from 66 countries estimated mean (95% CI) global sodium intake in 2010 at 3950 (3890-4010) mg/day (90). There is limited data available on sodium intake in Cambodia, however this review estimated a mean (95% CI) intake of 4410 (3730-5180) mg/day (approximately 11 g salt/day) (90), and other estimates have ranged from 12.5 to 15g salt /day (78,91). The main sources of sodium in the Cambodian diet are from table salt, fish sauce, MSG, and *prahok* (4,91).

Table 2- 3. Dietary reference intakes for sodium (adapted from (88)).

Life Stage Group	AI ^a (mg/day)
0-6 mo	110
7-12 mo	370
1-3 y	800
4-8 y	1000
9-13 y	1200
14-18 y	1500
19-50 y	1500
51-70 y	1500
>70 y	1500
Pregnancy (14-50 y)	1500
Lactation (14-50 y)	1500

^aAdequate Intake (AI), the average intake of a nutrient that appears to meet nutrient intake requirements of a group, used in absence of sufficient evidence to derive an EAR. AIs for sodium do not differ by sex.

2.7.6 Assessing salt and sodium intake

It is critical to have accurate and precise methods to assess population salt and sodium intake, because of the potential risk for chronic disease, and because of its widespread use as a fortification vehicle. The main methods for assessing sodium intake in individuals and in populations include dietary assessment and urinary assessment, however there are several challenges to obtaining accurate and precise estimates (92). Sodium intakes have high inter- and intra-individual variability due to differences in day-to-day dietary patterns. Intra-individual bias can be minimized by collecting repeat data among at least a sub-sample of the study sample, and inter-individual variability can be addressed by ensuring an adequate sample size (92–94). The focus of this thesis is solely on estimating population-level sodium intake.

The most common dietary assessment methods are dietary recalls and weighed diet records. Both methods are widely used, however both methods have several challenges: both are labour intensive for participants and researchers, and participants often under-report food intake

retrospectively (recall) as well as prospectively (weighed record) (92). Because sodium intake is highly related to energy intake, this can result in under-reporting of sodium intake, particularly for participants with higher body mass index (BMI) in populations from high- and middle-income countries (95). It can also be difficult to quantify discretionary salt use in the home, and assess the accuracy of sodium content of recipes and processed foods from food composition databases (92). In resource-poor settings, where literacy rates may be low, an observed, weighed food record by a trained research assistant is the recommended dietary assessment method for nutrient intake (96).

Repeat twenty-four hour urine collection is considered the gold standard for assessing population sodium intake, and is often used to validate other sodium intake methods (92). This method requires that participants collect all urine voided over a 24 hour period. Urinary sodium is most commonly determined using the well-established potentiometric method, where the measured electric potential across a membrane (an ion-selective electrode) can determine various electrolyte concentrations in an aqueous solution (97). A recent meta-analysis of 35 studies on sodium excretion found that an estimated 93% of dietary sodium is excreted in the urine (98), and the remaining losses occur through sweat and feces. While it is commonly assumed that additional sodium is lost through sweat in hot climates, the total amount is often negligible (approximately 0.6-4.1 g/L of sweat), with heat-acclimated populations, such as Cambodian residents, experiencing losses at lower levels (99). Challenges associated with this method of assessment include significant participant burden, which can result in high attrition, and inaccurate, often under-collection of urine (92). This is usually addressed by assessing completeness of the urine sample, through para-aminobenzoic acid (PABA) tablets, creatinine

excretion, and urine volume (92). PABA is completely excreted at a constant rate (a cut-off of 85% is assumed to be a complete 24-hr urine sample), however PABA tablets place an additional burden on participants, as they must be taken at three, evenly distributed times throughout the collection day (100). In resource-poor settings, PABA tablets are not recommended for population assessments of sodium intake as this places undue burden on participants and adherence may be low (101). Thus, 24-hour creatinine excretion and urine volume are more commonly used. Creatinine excretion is related to body weight and lean body mass, age, sex, and protein intake (102). Urinary creatinine is determined via the Jaffe reaction, a colorimetric reaction that occurs when creatinine reacts with picric acid in an alkaline medium and concentration is quantified at 520 nm (103). There are limitations with using urinary creatinine as a proxy for sample completeness, as prediction models for 24-hr creatinine have been shown to have low sensitivity (102), however, several studies use standard cut-offs of 4 mmol/day for females and 6 mmol/day for males (104,105). It is important to note that these cut offs have been developed using healthy populations, and are often applied in high income settings (105). It is unclear if these cut-offs are appropriate low and low and middle income countries where populations may have low protein intakes, or live with chronic malnutrition. Various cut-offs for 24-hr urine volume have also been used, most commonly $\leq 500\text{mL}$, $\leq 400\text{mL}$ and even as low as $\leq 250\text{mL}$ to assess completeness of sample (106). Additionally, self-reporting of spilled urine or incomplete samples can be used. Single spot-urines have also been widely used in to estimate mean 24-hr sodium population intake (107,108), however there is still no consensus on a validated, unbiased formula that can be used for epidemiological trends (88,92).

2.7.7 Setting fortification targets

Several factors must be carefully considered before mass food fortification policies and programs are designed and implemented, as these policies will have a blanket effect on the entire population and could potentially pose new risks to population subgroups. Clear guidelines have been set out by the Food and Agricultural Organization of the United Nations (FAO) and the WHO to help minimize the risk of micronutrient deficiency or excess (13). Mass food fortification must first be justified by a demonstrated risk of micronutrient deficiency through biochemical data on nutrition status of different population groups, data on usual dietary patterns, and specific dietary intakes of the micronutrient of interest to determine the prevalence of inadequate intake (13).

One of the most important issues related to estimating nutrient intakes in regards to fortification programs, is accounting for variability associated with dietary intake (109). Because individuals' dietary intake changes each day, as well as by season, it can be difficult to understand dietary habits from short-term assessment, however long-term dietary assessment is generally not logistically feasible. There is a high risk of introducing bias if raw data is used to estimate population intake, therefore statistical models can be used to reduce high intra-individual variability in daily dietary intake to better understand the *usual* daily intake of population subgroups, which has been described as a “long-run daily average [over the period of] a year” (109). Adjusting data for intra-individual, day-to-day, and seasonal variance reduces the effect of outlying data and random variability to produce a more realistic intake distribution (see **Figure 2-3**). Four main statistical methods have been developed to account for this intra-individual variability and model estimated usual dietary intake distributions; the Iowa State University

(ISU), the National Cancer Institute (NCI), the Multiple Source Method (MSM), and the Statistical Program to Assess Dietary Exposure (SPADE) methods (110). All four methods can be used for both foods or nutrients that are consumed episodically or daily, and transform the data (ISU with grafted polynomial function to fit a normal probability plot; NCI, MSM and SPADE with Box-Cox transformations), model the transformed data to a usual intake distribution, and back-transform data to the original scale (110,111). Laureano *et al.* conducted a simulation study, running 1000 replicates of 12 different daily nutrient intake scenarios of varying sample sizes and intra- and inter-individual variance, and compared the accuracy and precision of each method (110). These authors found all four methods to be acceptable, however all methods tended to shrink the distributions (underestimate 10th and 90th percentiles). The NCI method had less accuracy at smaller sample sizes ($n=150$) and greater variances compared to the other three methods (these differences disappeared at larger sample sizes of $n=300$ and $n=500$) (110). It was determined that any of the four methods could be used at the discretion of the researcher, depending on preference and practicality, however cases with small sample sizes or large variances should also use a method other than the NCI method to confirm results (110). Each method also has different data requirements that should be considered before use. The MSM method requires a minimum of two repeat observations for each participant and was developed to incorporate data from multiple sources (e.g. 24-hr recalls and food frequency questionnaires), although it is also possible to use only one source of data (111). The SPADE method is more appropriate for diverse samples that require age-specific nutrient requirement analysis (111). The ISU and NCI methods have similar data requirements (repeat observations are only required among a sub-sample of the population); however they are computed differently (111).

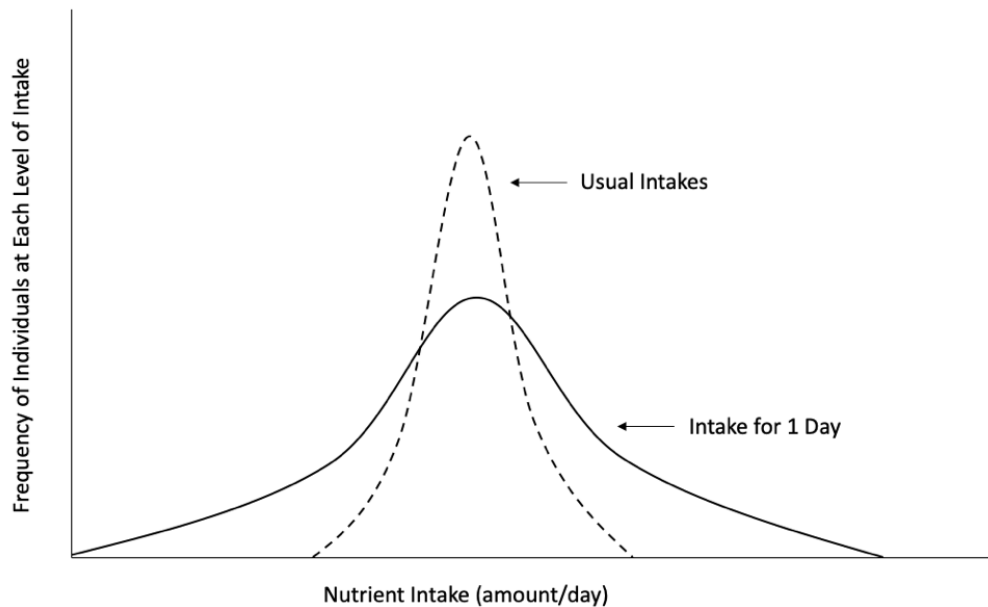


Figure 2- 3. Concept of estimated usual nutrient intake distribution from 1 day of intake data, and from adjusted intake data from repeat intake data. This figure illustrates the potential to over-estimate the prevalence of inadequacy with only 1 day of intake data, and regression toward the mean with adjusted repeat data. Adapted from (112).

After observational data on usual micronutrient intake of different population groups has been obtained, population subgroups with the highest and lowest prevalence of inadequate intakes must be identified. Then usual intake of the potential food fortification vehicle consumed by these groups must be determined, followed by fortification scenario simulations at levels to optimize nutrient intakes for the majority of the population, while minimizing risk of excessive intakes (13).

There are two main methods to determine optimal levels of fortification; the full probability approach or EAR cut-point method (13,39). When nutrient requirements are not normally distributed, the full probability approach must be used, which involves determining the risk of inadequate usual intake among all individuals in the population subgroup, then averaging the

probabilities of the individuals to calculate a population risk of inadequate intake. This is time-consuming and resource-intensive; however this approach is not necessary for thiamine fortification (38,39). The EAR cut-point method is more commonly employed, given three assumptions are met: that intake and requirement distributions are independent from one another, that the variance of intakes is larger than of requirements, and that the requirements of the group are normally distributed(13). For this approach, usual intakes below the EAR are considered inadequate, and the percentage of the population group intake below the EAR is assumed to equal the prevalence of inadequacy. The recommended strategy for fortification programs is to shift the usual intake distribution up to a level at which only 2-3% of the target population group has an intake below the EAR (see **Figure 2-4**) (13). However, as discussed in *Section 2.5.3.2*, feasibility of this target is dependent on several factors such as cost, technical limitations, and risk of excessive intakes.

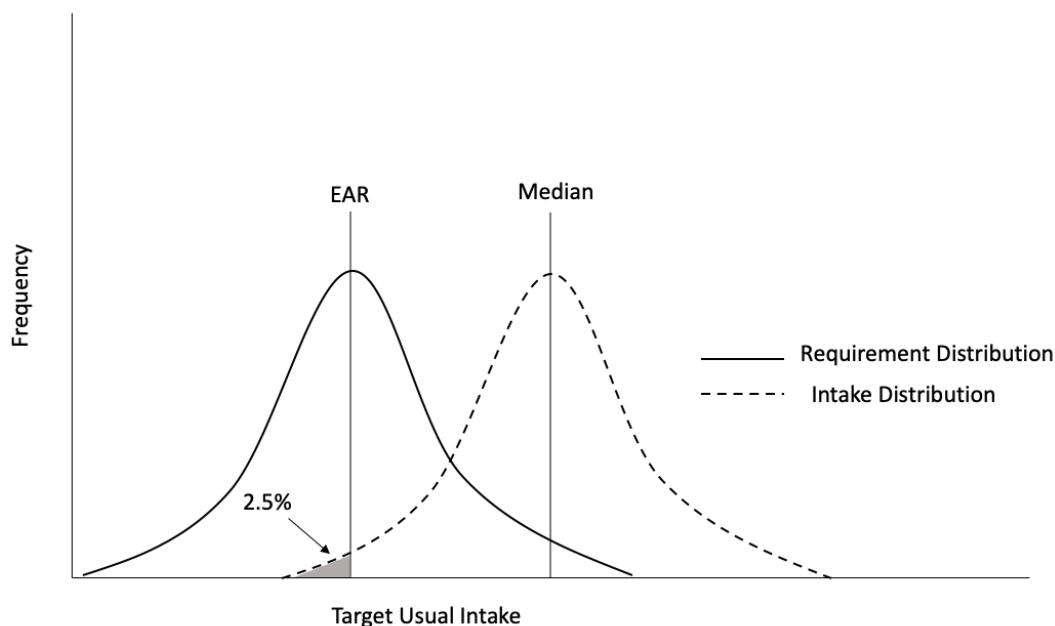


Figure 2- 4. Concept of nutrient requirement distribution and usual intake distribution, with only 2-3% of inadequate intakes. Adapted from (39).

2.8 Conclusions and research rationale

Thiamine is an essential vitamin that is critical for normal functioning and development in infancy (18,113). Thiamine deficiency is a persistent public health issue in Cambodia due to a traditional thiamine-poor diet based largely on polished white rice, however there are currently no programs to prevent thiamine deficiency at the population level (21,70). Human milk thiamine concentrations are directly related to maternal dietary thiamine intake (34), therefore exclusively breastfed infants are at a disproportionate risk of developing infantile beriberi if the maternal diet is low in thiamine. Infantile beriberi is a potentially fatal thiamine deficiency disease that likely contributes to Cambodia's high infant mortality rate (1,50,52).

Recent reviews of thiamine deficiency in LMICs have concluded that mass food fortification is an ideal intervention to prevent thiamine deficiency, as it is passive, cost-effective, and can have a broad impact on a population (21,49). Before a mass fortification program can be implemented, part of the feasibility assessment must include sufficient data on the required dose of thiamine, as well as usual intake of a potential fortification vehicle among the target populations (13,62).

Salt is a universally consumed condiment and has proven to be a successful global fortification vehicle for iodine. Current estimates suggest that usual per capita salt intake in Cambodia is between 11-15g/day, however this average may not be representative of salt consumption among lactating women (78,90,91). Furthermore, the WHO has been promoting messages to decrease salt intake to ≤ 5 g/day, therefore new measures of salt intake are required (114).

This research aims to estimate the usual salt intake of lactating women in rural Cambodia to address this gap.

3.0 Rationale and Objectives

This study is part of a randomized control dose-response supplementation trial designed to determine the optimal daily dose of thiamine required by lactating Cambodian women to optimize milk thiamine content for the ultimate prevention of infantile beriberi (clinicaltrials.gov entry NCT03616288) (115). The larger randomized controlled trial will inform the dose of thiamine required to optimize human milk thiamine concentrations. The current study aims to quantify salt intake among lactating women in rural Cambodia and their households to assess the potential use of salt as a fortification vehicle for thiamine. This will be examined through the assessment of salt intake and sodium status among lactating mothers, as well as household salt disappearance, and modeling various fortification schemes with the thiamine dose obtained from the larger trial.

3.1 Study Objectives

The study objectives are:

- 1) To estimate salt and sodium intakes among lactating women ($n=104$) using repeat 12-hour (sunrise to sunset), observed weighed condiment intake records of major sources, table salt, soy sauce, fish sauce, monosodium glutamate (MSG), and fermented fish paste (*prahok*).
- 2) To estimate salt and sodium intakes among lactating women ($n=104$) from repeat sodium excretions using 24-hr urinary sodium concentrations.
- 3) To estimate household ($n=331$) salt intake from fortnightly salt disappearance (weight lost, in g) over 22 weeks during the exclusive breastfeeding period.

- 4) To simulate various thiamine fortification scenarios, with salt as a fortification vehicle (from all major sources: table salt, fish sauce, soy sauce, and *prahok*) using the National Cancer Institute (NCI) usual dietary intake modeling technique and a modified Estimated Average Requirement (EAR) cut-point method.

4.0 Methods

4.1 Study Design

Individual salt and sodium intakes were determined via a cross-sectional, exploratory study that used dietary and urine assessments, hereinafter referred to as the maternal salt and sodium study. Repeat 12 hr (sunrise to sunset) observed, weighed salt and condiment (table salt, fish sauce, soy sauce, MSG, and *prahok*) records, and 24-hr urinary sodium concentrations, were collected among a sub-set of 100 study participants (lactating women) on two non-consecutive days within a 7-day window.

Household salt disappearance was determined via a longitudinal, exploratory study that assessed fortnightly measurements of salt disappearance (weight lost in g) of study-provided salt from all households in the larger trial ($n=320$) recorded for the 22-week duration of the trial, from 2 through 24 weeks postpartum. This is referred to as the household salt disappearance study throughout this thesis.

These three methods were used to assess salt intake to ensure accurate estimates, with the goal of using repeat observed weighed salt and condiment records to model adjusted salt intake and develop thiamine fortification scenarios. The 24-hr urinary sodium concentrations were used to corroborate the observed weighed intake records, while household salt disappearance was measured to assess broader salt use trends in rural Cambodia.

4.2 Sample

4.2.1 Participants

The study sample included healthy lactating mothers residing within the catchment area of 8 provincial health centres in Kampong Thom, a centrally-located Cambodian province with a population of 700,000 people (116). All participants took part in the household salt disappearance study, and a sub-sample of 104 women were randomly selected to take part in the maternal salt and sodium study.

4.2.2 Eligibility criteria

Participants of the household salt disappearance study met the following criteria:

- mothers of a newborn;
- aged 18-45 years;
- most recent pregnancy was normal and the singleton infant was born without complications;
- intended to exclusively breastfeed for 6 months;
- resided in Kampong Thom province, Cambodia, and was not planning to move in the proceeding 6 months;
- was not currently taking, and had not taken any thiamine-containing supplements over the previous 4 months;
- was not currently participating in any nutrition programs beyond normal care;
- was willing to consume one capsule daily from 2 weeks through 24 weeks postpartum;
- was willing for her entire household to consume only salt provided by the study team;
- was willing to have biological samples collected from herself and her infant to assess nutrition status;
- and provided written informed consent to participate.

To participate in the maternal salt and sodium study, mothers met the following criteria:

- were already participants of the household salt disappearance study;
- were willing to have field officers in their home to conduct observed weighed intake records on two days;
- and were willing to collect two 24-hr urine samples.

4.2.3 Ethical considerations

Approval for this study was obtained from the National Ethics Committee for Health Research in Cambodia (#112NECHR), and the Mount Saint Vincent University Research Ethics Board in Canada (MSVU UREB #2014-141). Participants received two bags of laundry soap (one for each day of data collection) for participation in the maternal salt and sodium intake study, and informed, written consent was obtained from all participants (see **Appendix A**).

4.2.4 Sample size

Sample size calculations for the larger trial can be found in Whitfield *et al.* (115). For the maternal salt and sodium study, a minimum sample size of 100 participants (with repeat observations) was needed based on software requirements for estimating usual dietary intake (117), and protocol recommendations on population sodium intake assessment in similar low-resource settings (101).

4.3 Data collection procedures and data management

4.3.1 Screening, recruitment and sampling procedure

Participants for the larger trial were screened and recruited via rolling recruitment (details outlined in Whitfield *et al.* (115)). The subsample for the maternal salt and sodium study were randomly selected from the larger study. Each participant's unique alphanumeric identifier was assigned a random number using a random number generator (Microsoft Excel 2016; IBM Corp, Armonk, New York), then sorted in ascending order. At 6 weeks postpartum, participants were provided with information about the sub-study and invited to participate. The first 100 participants who agreed to participate and provided written, informed consent, were included. If a participant declined participation, they were thanked for their time. Data collection for the

maternal salt and sodium study took place between 8 through 22 weeks postpartum to ensure a breadth of data was collected through the exclusive breastfeeding period, and because postpartum physiological changes occur in three main stages: the first 7 days post-delivery, then from 7 days to 6 weeks postpartum, and from 6 weeks to 6 months postpartum (118). We wanted to ensure 24-hr urine samples were collected during this more physiologically stable period of 6 to 24 weeks postpartum.

4.3.2 Sociodemographic information

All household characteristics and sociodemographic data were collected on the first home visit of the larger trial (2 weeks postpartum), using a tablet-based questionnaire (Open Data Kit Collect Version 1.22.4 software on a Samsung Galaxy tablet) (115). For the purposes of this thesis, data on education, daily per capita income, national wealth equity index, household size, and maternal age are presented (see **Appendix B**). Household education attainment is described here as a categorical measurement of the highest education level attained by either the mother or father in the household. Daily per capita income was calculated from monthly income and household size, then categorized based on the World Bank Lower Middle Income Class Poverty Line of US\$3.20 (119). Households were categorized into a National Wealth Equity Index quintile using the EquityTool survey, a standardized tool based on data from the 2014 Cambodian Demographic and Health Survey that assesses various household characteristics (i.e. building materials of the home, availability of electricity, ownership of various assets etc.) (120). Household size included all members ≥ 2 years living and regularly eating meals in the home. Maternal age was measured as the woman's age (in years) at two weeks postpartum.

4.3.3 Observed, weighed salt and condiment intake procedure

Data were collected via in-person household visits by trained field officers during two non-consecutive weekdays within a 7-day window, avoiding all major holidays. Dietary assessment and urine collection occurred on the same day. On each data collection day, trained field officers remained in the participant's household from sunrise until sunset (approximately 6am to 6pm). Field officers observed all meals and snacks prepared in the home for the duration of the day. Using calibrated scales (sensitive to 0.1 g; Amir Technology Co. Ltd.) field officers weighed and recorded all salt and condiment use (table salt, fish sauce, soy sauce, MSG, and *prahok*) during food preparation, discretionary consumption of these foods at meal time, and also weighed the intake of all meals and snacks via indirect weights of the dishes before and after consumption. Prospective weighed intake records are considered the most precise method of intake assessment, and observed-weighed intake records by a trained data collector is the recommended nutrient assessment method in low-resource settings where literacy rates may be low (96). This method was also chosen in consultation with staff from Helen Keller International Cambodia, as it was determined that it was socially acceptable to be in the women's homes and to observe meals without participating in them. In addition, the link between food choices and social desirability does not seem to be pronounced in rural Cambodia as it is in many high-income settings, particularly around salt and sodium intake (121), possibly due to the monotonous quality of the traditional diet.

Condiment and meal weights were computed by recording the total weight of all empty pots being used to prepare the meal or snack as well as the initial weight of the study-provided salt container, and household fish sauce, soy sauce, MSG, and *prahok* containers before food

preparation. After meal preparation, the final weights of all full pots as well as condiment containers were recorded once again.

Cambodians often eat from a common pot, however study participants were asked to eat separately for individual intake records. Plain, steamed rice is consumed with most meals, but is not prepared with condiments in Cambodia, therefore participants were asked to use a separate bowl for steamed rice to allow for a more accurate estimate of salt and sodium intake from the meal. All data were recorded in a field notebook and paper data collection sheets (see **Appendix C**), and later entered into a database (Microsoft Excel 2016; IBM Corp, Armonk, New York). This procedure was repeated on the second day of data collection.

For foods that were consumed, but not prepared, during the observation period (i.e. leftovers, foods purchased from the market, previously salted fish, etc.), data collectors still recorded the net weight of the meal consumed. Meal duplicates were recorded at the end of the study and salt and condiment estimates were applied to these meals.

4.3.4 24-hr urine collection procedure

Field officers visited the participant's household to explain the urine collection protocol and to provide the participant with urine collection equipment (5L container, urine pot, and funnel) one day before data collection. Participants were instructed to void their bladder the following morning (first day of data collection), discard as usual, and record the time using the clock on their study-provided mobile phone. Women were instructed that all urine passed after this time was to be collected and stored in the 5L study-provided container up to and including the first

urine of the next morning (participant recorded time again). Field officers picked up the urine sample within 24 hours of collection, (48 hours if the sample was collected on a Friday), recorded the final weight of the urine sample in study-provided 5L container (using calibrated scales, to 0.1 g; Amir Technology Co. Ltd.), and time of first urine on both days on a paper questionnaire (see **Appendix D**). The samples were then mixed thoroughly, and 6 mL of urine (3 x 2 mL aliquots) was collected for analysis. Samples were transported in coolers at 4°C to the field lab in Kampong Thom town, and stored at -20°C for ≤ 2 weeks before transportation to the National Institute of Public Health Laboratory in Phnom Penh for analysis.

4.3.4.1 Urine sample analysis

Biochemical urine analysis was completed at the National Institute of Public Health Laboratory in Phnom Penh. Urine aliquots were assessed for 24-hr urinary sodium concentrations (mmol/24 hrs) using the EasyLyte Na/K/Cl Analyser (Medica, Dusseldorf, Germany) via ion-selective potentiometry.

4.3.5 Household salt disappearance procedure

All salt was removed from the home during the initial home visit (2 weeks postpartum). This was replaced with study-provided salt in a standardized container; the initial weight of the container was recorded (calibrated scales, to 1 g; Tanita Corporation). Participants were asked to only consume study-provided salt for the 22-week duration of the study, for their household only. At fortnightly visits, trained field officers weighed the salt containers, re-filled and re-weighed containers, and confirmed the number of people in the household residing and eating in the home. Participants were also asked four questions about salt use in the previous 14 days (see

questionnaire in **Appendix E**). All data were collected electronically on a tablet-based questionnaire (Open Data Kit Collect Version 1.22.4 software on a Samsung Galaxy tablet).

4.3.6 Data storage

All participants were given unique alpha-numeric identifiers, which were used to identify all paper questionnaires and urine samples. The observed, weighed salt and condiment intake records, urine collection records, and written consent forms were kept in locked filing cabinets in locked offices of Helen Keller International Cambodia during data collection, then securely transported from Phnom Penh, Cambodia to MSVU in two locked, expanding files (consent forms kept separate) in hand luggage. All files are currently stored at MSVU in locked filing cabinets in locked research rooms (consent forms kept in separate locked filing cabinet) and access to the documents is limited to the master's student, principal investigator of the larger trial, and research assistants. All electronic data files are stored on password-protected computers and/or secure servers accessible only to members of the research team.

All paper and archived electronic data will be stored for at least 5 years following publication of research findings. After this time, they will be physically destroyed (e.g., paper copies will be shredded), and electronic files will be permanently deleted.

4.4 Statistical analysis

All descriptive statistics were computed using IBM SPSS Software (Version 25; IBM Corp, Armonk, New York). Adjustments for usual salt intake distributions were completed using RStudio (Version 1.2.1335). Throughout this thesis, raw intake data will be referred to as

unadjusted intake, usual intake (i.e. the estimated long-run average intake that has been adjusted for intra-individual variance) will be referred to as adjusted intake.

4.4.1 Statistical analysis for sociodemographic information

Sociodemographic characteristics are presented as n (%) for categorical data, and mean (95% CI) for continuous data. Comparisons in characteristics between participants in the maternal salt and sodium study and the remaining participants from the larger trial were assessed using χ^2 tests and Mann Whitney U tests, with a significance level of $p < 0.05$.

4.4.2 Statistical analysis for the maternal salt and sodium study

4.4.2.1 Statistical analysis for repeat observed weighed salt and condiment intake

Descriptive statistics (mean (95% CI) and median (IQR)) were computed for overall daily unadjusted intakes of table salt, fish sauce, soy sauce, MSG, and *prahok*. Sodium intakes from soy sauce, fish sauce and *prahok* were calculated using the Association of Southeast Asian Nations (ASEAN) Food Composition Database Electronic Version 1 (Institute of Nutrition, Mahidol University, February 2014, Thailand), while sodium intakes from table salt and MSG were estimated using chemical composition calculations. Salt (NaCl) was assumed to be the main source of sodium in soy sauce, fish sauce and *prahok*, therefore salt content of each was calculated by dividing sodium: 393.4 mg sodium/1 g salt. Total estimated salt from all sources was computed by adding salt intake from table salt, soy sauce, fish sauce, and *prahok*. Coverage of use in this study is described as consumption of the food item (i.e. table salt, soy sauce, etc.) at least once during the observation day (reported as n (%)). In addition, to gain perspective around

the volume of condiment intakes, mean (95% CI) and median (IQR) of unadjusted intakes for each condiment were computed using consumption days only (all null values removed).

4.4.2.2 Statistical analysis for repeat 24-hr urinary sodium concentrations

Descriptive statistics (mean (95% CI) and median (IQR)) were computed for urine sample volumes and unadjusted 24-hr urinary sodium concentrations. A cut-off of <400 mL was used to exclude 24-hr urine samples as incomplete (106). Urinary sodium concentrations were used to estimate salt intake by assuming 100% excretion of sodium from intake, and assuming salt as the source of all sodium excreted. Urinary sodium concentrations (mmol/24 hrs) were converted to grams of sodium (dividing by 1000, then multiplying by 22.989 g/mol), then to grams of salt (multiplying by 2.542).

4.4.3 Statistical analysis for the household salt disappearance study

Fortnightly salt disappearance (in g) was divided by 14 days, and by the number of people residing in the household (≥ 2 years of age) to estimate salt disappearance/person/day. Mean (95% CI) and median (IQR) salt disappearance/person/day over the 22-week period was computed. Households that withdrew from the larger trial before 6 weeks postpartum were excluded from analysis. Exploratory comparisons in salt intake were made by household size, household National Wealth Equity Index quintile, and by agricultural season (lean agricultural season defined as April-May and peak agricultural season defined as November-December). Normality of data were assessed using the Shapiro Wilks test ($p > 0.05$ considered normally distributed). Independent t-tests and One-Way Analysis of Variance (ANOVA; if data were normally distributed), or Mann-Whitney-U tests and Kruskal-Wallis tests (if data were not

normally distributed) were employed, with statistical significance level of $p < 0.05$. Dunn-Bonferonni post-hoc analysis was used to assess differences in salt disappearance by household National Wealth Equity Index quintile.

4.4.4 Statistical analysis for adjusted salt intake and thiamine fortification scenario

4.4.4.1 Adjusted salt intake

Adjusted daily salt intake from all major sources (table salt, fish sauce, soy sauce, and *prahok*) was estimated using data from the repeat observed weighed salt and condiment intake records using the NCI method (refer to *Section 2.7.7*). Adjusted daily salt intake from table salt only was also computed, however adjusted table salt from all major sources was used for the fortification scenario. The NCI method was selected after consultation with the statistician (Dr. Shalem Leemaqz) from the larger trial. Despite the limitations of this method outlined in *Section 2.7.7*, the NCI method was determined to be the most appropriate approach for this study. As discussed previously, the MSM method requires a minimum of two repeat observations for each participant so although we planned to collect repeat data from every participant, we expected to have missing data given the labour-intensive nature of observed weighed intake records and the population group (new mothers in a resource-poor setting). The SPADE method is more appropriate for diverse samples with age-specific nutrient requirements, however our sample is homogeneous (all lactating women of reproductive age) so this method was also determined to be inappropriate. The ISU and NCI methods only require repeat observations among a sub-sample of participants; however, the ISU method fits the data to a normal distribution, which we anticipated may not be appropriate for our data, which was likely to be skewed. Therefore the NCI method was deemed more appropriate for analysis.

The NCI method involves three main steps:

1. Observed intakes are transformed by one-parameter (λ) Box-Cox transformation and residuals are modelled to improve and assess normality, where;

$$y(\lambda) = \frac{(y^\lambda - 1)}{\lambda} \text{ if } \lambda \neq 0$$

$$y(\lambda) = \log(\lambda) \text{ if } \lambda = 0$$

2. Linear mixed effects model on transformed data is fit to estimate adjusted individual intakes. Best Linear Unbiased Predictor (BLUPs) are calculated for each individual.

$$\text{Log}(x) = \beta + \left(\frac{1}{k}\right), \text{ when } \lambda = 0$$

Where;

- i. x = observed salt intake for each individual
 - ii. β = fixed effect of inter-individual intake (overall mean of salt intake)
 - iii. k = random effect of intra-individual intake
3. BLUPs are back-transformed to the original scale (from log scale) to predict adjusted salt intake for each individual.

Descriptive statistics (mean (95% CI) and range) were computed for adjusted daily salt intake from all major sources. Adjusted individual intakes were also used to create a distribution of adjusted salt intake among this population.

4.4.4.2 Fortification scenarios with modified EAR cut-point method

An EAR-cut point method (see *Section 2.7.7*) was used to simulate various thiamine fortification scenarios using salt as the fortification vehicle. We proposed a modified method because instead of using the EAR as the cut-off for inadequacy from all dietary sources, we planned to model using the optimal supplementation dose of thiamine determined from the larger trial. The optimal supplementation dose of thiamine was determined to be 1.2 mg/day, which also happens to be the thiamine EAR for lactating women (38). Iterative models were run with different fortification concentrations (mg thiamine/kg salt) in an attempt to minimize the proportion of the population with intakes below the optimal dose through salt intake.

As noted in *Section 2.3.3*, thiamine has no defined UL (38), and no adverse effects have been recorded, even at very high, chronic thiamine intakes. For this study, we attempted to minimize intakes above 10 mg/day through fortified salt, as 10 mg thiamine is a known safe dose used for long-term supplementation among lactating women in Myanmar (21). Ideally, only 2-3% of the population group will have thiamine intakes through fortified salt below the optimal dose, and only 2-3% above our imposed upper limit (10 mg), however these fortification targets are highly dependent on the adjusted daily salt intake distribution and the optimal dose, and technical aspects of fortification. As such, the fortification simulations were exploratory in nature, as opposed to having defined guidelines *a priori*.

4.5 Dissemination of study findings

Results of this research will be presented at academic nutrition or public health conferences (i.e. Micronutrient Forum, Canadian Nutrition Society etc.) and in peer-reviewed, journals that offer open access (e.g. *Annals of the New York Academy of Sciences*, etc.)

Results will also be disseminated in collaboration with study co-investigators with the National Sub-Committee for Food Fortification in the Cambodian Ministry of Planning. 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.

5.0 Results

5.1 Participant Characteristics

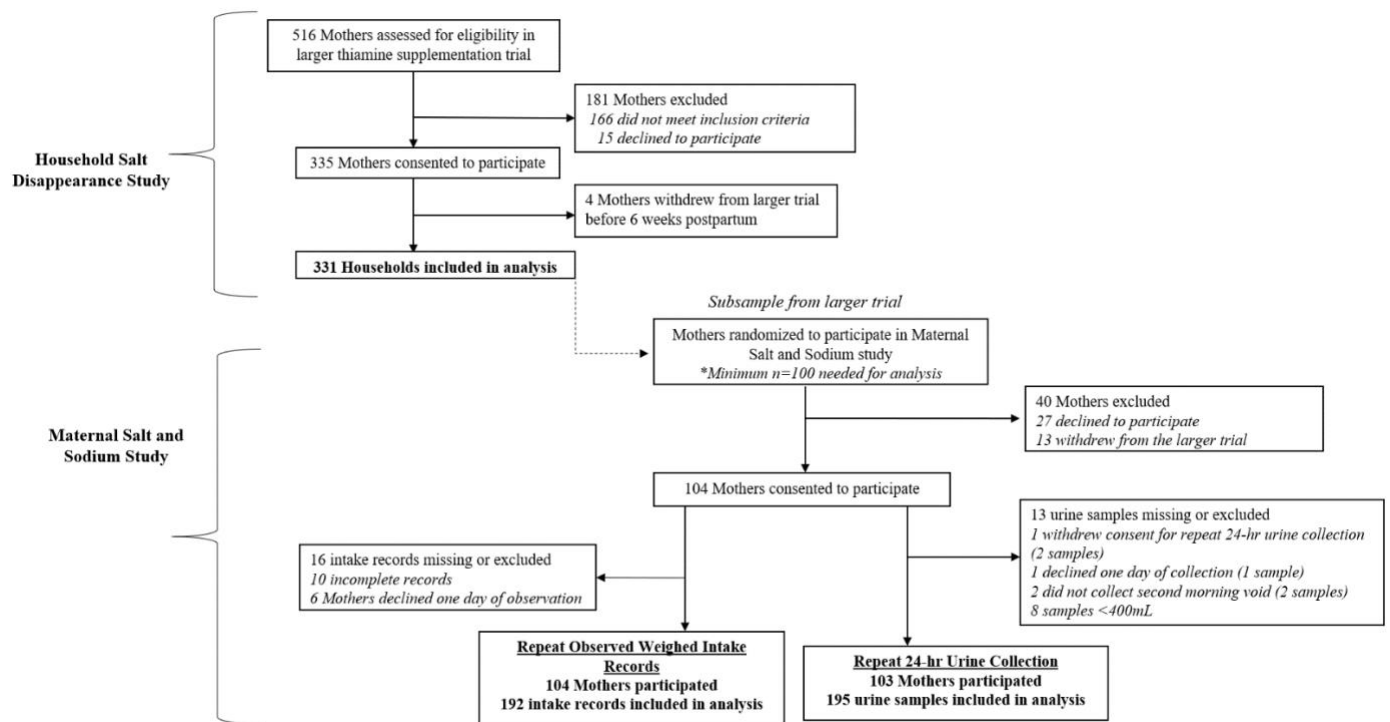


Figure 5- 1. Participant flow chart for the household salt disappearance study and maternal salt and sodium study.

The participant flowchart is presented in **Figure 5-1**. All households from the larger trial participated in the household salt disappearance study: 335 mothers and their households consented to participate, and 331 households were included in the final analysis. Households were excluded from analysis if they withdrew from the larger trial before 6 weeks postpartum ($n=4$). For the maternal salt and sodium intake study, 144 mothers were invited to participate, and 104 provided consent and participated. Repeat observed weighed salt and sodium intake records were completed for 104 participants ($n=192$ complete intake records included in

analysis), and repeat 24-hr urine samples were collected from 103 participants ($n=195$ samples included in analysis).

Table 5- 1. Household and maternal sociodemographic characteristics of study participants.

	<i>Household Salt Disappearance Study</i>	<i>Maternal Salt and Sodium Study</i>		
Characteristic	All Households ($n= 331$)	Mothers Included^a ($n=104$)	Mothers Not Included ($n=227$)	p^b
Household size ^c				0.771
≤ 2 persons	27 (8%)	10 (10%)	17 (8%)	
3-6 persons	242 (73%)	74 (71%)	168 (74)	
≥ 7 persons	62 (19%)	20 (19%)	42 (19%)	
Daily per capita income ^d				0.389
$\leq$ US\$3.20	284 (86%)	91 (88%)	193 (85%)	
$>$ US\$3.20	47 (14%)	13 (12%)	34 (15%)	
Household education level ^e				0.774
None	10 (3%)	2 (2%)	8 (4%)	
Primary School	125 (38%)	37 (36%)	88 (39%)	
Lower Secondary School	122 (37%)	38 (37%)	84 (37%)	
Upper Secondary School	56 (17%)	21 (20%)	35 (15%)	
Higher Education	18 (5%)	6 (6%)	12 (5%)	
National Wealth Equity Index Quintile ^f				0.759
Lowest	80 (24%)	23 (22%)	57 (25%)	
2 nd lowest	69 (21%)	21 (20%)	48 (21%)	
Middle	107 (32%)	35 (34%)	72 (32%)	
2 nd highest	53 (16%)	15 (14%)	38 (17%)	
Highest	22 (7%)	10 (10%)	12 (5%)	
Mothers age (years)	28 (27, 29)	28 (27, 29)	28 (27, 29)	0.893

Data are presented as n (%) or mean (95% CI).

^a Participants of the maternal salt and sodium study were randomly selected from the household salt disappearance study.

^b χ^2 test or Mann-Whitney U test, comparing subsample of mothers ($n=104$) to remaining larger sample ($n=227$), $p<0.05$

^c Includes household members ≥ 2 years.

^d Daily per capita income categorized as below or above the World Bank Lower Middle Poverty Line (US\$3.20/person/day)

^e Household education level is defined as the highest education level attained within the household (either mother or father).

^f National Wealth Equity Index score is calculated using EquityTool, quintiles standardized to 2014 Cambodian Demographic and Health Survey (<https://www.equitytool.org/cambodia/>).

As shown in **Table 5-1** the majority of households were made up of 3-6 persons (73%), had daily per capita incomes below the Cambodian poverty line (86%), and the highest level of education attained in most households was lower secondary school or less. The subsample of mothers who participated in the maternal salt and sodium intake study lived in households with the same socioeconomic characteristics as the remaining larger sample ($p>0.05$).

5.2 Maternal salt and sodium study

Maternal salt and sodium intakes were assessed using repeat observed weighed intake records and 24-hr urinary sodium concentrations. The intakes and 24-hr urinary sodium concentrations were not normally distributed (Shapiro Wilks $p<0.05$), and tended to be positively skewed. Unadjusted observed weighed intakes of salt and major sodium-containing condiments (soy sauce, fish sauce, MSG, and *prahok*) can be found in **Table 5-2**. Table salt was the largest contributor to mean (95% CI) daily sodium intake, 2.51 (2.21, 2.81) g/d. Estimated total daily salt intake from all major sources (table salt, soy sauce, fish sauce, and *prahok*) was 9.3 (8.3, 10.3) g/d. MSG was also a relatively large source of non-salt-based sodium in the diet, 0.44 (0.36, 0.53) g/ day. Coverage of each condiment was variable: table salt and MSG were consumed daily or almost daily by all participants (99% and 100% of observations, respectively), while soy sauce was only consumed in only 6% of visits (**Table 5-3**).

Table 5- 2. Unadjusted repeat, salt and sodium intakes from 12hr observed weighed intake records ($n=104$ participants).

	n (observations_{b,c})	Observed Weighed Intake (g/day)		Sodium Intake_a (g/day)	
		Mean (95% CI)	Median (IQR)	Mean (95% CI)	Median (IQR)
Table Salt	192	6.4 (5.6, 7.1)	5.1 (3.2-7.9)	2.51 (2.21, 2.81)	2.01 (1.27-3.11)
Soy Sauce	192	0.2 (0.0, 0.4)	0.0 (0.0-0.0)	0.01 (0, 0.02)	0 (0-0)
Fish Sauce	192	9.9 (7.4, 12.3)	5.5 (1.1-12.0)	0.92 (0.69, 1.14)	0.51 (0.10-1.12)
MSG	144	3.6 (2.9, 4.2)	2.8 (1.7-4.1)	0.44 (0.36, 0.53)	0.34 (0.20- 0.50)
<i>Prahok</i>	115	5.6 (3.3, 7.8)	1.5 (0.0-8.1)	0.35 (0.21, 0.49)	0.09 (0.00-0.51)
Total salt_d and sodium intakes	192	9.3 (8.3, 10.3)	7.8 (5.1-11.7)	3.61 (3.18, 4.04)	3.15 (2.18-4.73)

^a Sodium contents of soy sauce, fish sauce and *prahok* were calculated using the ASEAN Food Composition Database Electronic Version 1 (Institute of Nutrition, Mahidol University, February 2014, Thailand). Sodium content of table salt and MSG were computed based on chemical composition.

^b Observation is defined as one 12-hr period (sunrise to sunset) of observed weighed salt and condiment intakes; missing $n=16$ observations due to: incomplete records ($n=10$) and declined participation for one day of observation ($n=6$).

^c n differs due to changes in questionnaire (addition of MSG and *prahok*) to reflect field observations, no selection bias in missing data.

^d Total salt includes table salt, soy sauce, fish sauce and *prahok* only (calculation assumes sodium content from soy sauce, fish sauce and *prahok* is from added salt during processing).

Table 5- 3. Coverage of salt and condiment use and unadjusted intakes on days consumed only.

	n (observations_{a,b})	Observed Weighed Intake on Days when condiment actually consumed (g/day)		
		Coverage_c n (%)	Mean (95% CI)	Median (IQR)
Table Salt	192	191 (99%)	6.4 (5.6, 7.1)	5.1 (3.3-7.9)
Soy Sauce	192	11 (6%)	3.9 (0, 8.0)	0.2 (0.1-6.2)
Fish Sauce	192	155 (81%)	12.3 (9.3,15.1)	7.6 (3.3-13.3)
MSG	144	144 (100%)	3.6 (2.9, 4.2)	2.8 (1.7-4.1)
<i>Prahok</i>	115	62 (54%)	10.4 (6.5, 14.2)	7.2 (3.3-11.1)

^a Observation is defined as one 12-hr period (sunrise to sunset) of observed weighed salt and condiment intakes; missing $n=16$ observations due to: incomplete records ($n=10$) and declined participation for one day of observation ($n=6$).

^b n differs due to changes in questionnaire (addition of MSG and *prahok*) to reflect field observations, no selection bias in missing data.

^c Coverage is considered use of the condiment at least once during an observation day.

Repeat 24-hr urinary sodium concentrations can be seen in **Table 5-4**. Mean (95% CI) estimated daily salt intake, based on sodium concentrations, was 9.0 (8.4, 9.7) g. Mean estimated total daily salt intake from observed weighed intake records and 24-hr urinary sodium concentrations were not significantly different (Mann-Whitney U test, $p=0.3$, data not shown).

Table 5- 4. Unadjusted repeat 24-hr urinary sodium concentrations and estimated salt intake ($n=103$ participants)

	n (samples^{a,b})	Mean (95% CI)	Median (IQR)
Urine Volume (L)	195	1.1 (1.0, 1.2)	1.0 (0.7-1.4)
Urinary sodium excretion (g/24 hr)	195	3.56 (3.30, 3.82)	3.19 (2.19-4.52)
Estimated salt intake ^c (g)	195	9.0 (8.4, 9.7)	8.2 (5.6-11.4)

^a Sample is defined as one 24-hr urine collection.

^b Missing $n=13$ samples due to: incomplete collection ($<400\text{mL}$, $n=8$), declined participation ($n=3$), or participant did not collect first void of the second morning ($n=2$).

^c Assumes all sodium excretion from salt (NaCl) intake.

5.3 Household salt disappearance study

As seen in **Table 5-5**, mean (95% CI) salt use for the households throughout the entire 22-week study period was 11.3 (10.7, 11.9) g/person/day. Mean salt use was lower among households within the highest National Wealth Equity quintile, as compared to the three lowest quintiles ($p=0.022$), and salt use was higher during fortnightly visits in the lean season (April – May) compared to the peak season (November – December; $p<0.01$).

Table 5- 5. Fortnightly salt disappearance among lactating women’s households in rural Cambodia between 2 through 24 weeks postpartum.

	n (households)	Mean (95% CI) g	Median (IQR) g	p_a
Daily salt use/person/day	331	11.3 (10.7, 11.9)	9.6 (7.2-13.5)	-
Household size ^b				0.054
≤ 2 persons	27	11.0 (8.3, 13.7)	10.1 (6.6- 13.8)	
3-6 persons	242	11.6 (10.9, 12.3)	10.2 (7.4- 13.8)	
≥ 7 persons	62	10.2 (8.7, 11.7)	8.9 (6.3- 11.6)	
National Wealth Equity Index quintile ^c				0.022
Lowest	81	11.6 (10.3, 12.9)	10.1 (7.3- 15.0) †	
2 nd lowest	69	11.4 (10.2, 12.6)	10.2 (7.5- 13.4) †	
Middle	106	12.1 (10.8, 13.3)	10.7 (7.9- 14.0) †	
2 nd highest	53	10.5 (9.1, 11.9)	9.0 (6.9-13.5)* †	
Highest	22	8.0 (6.6, 9.5)	7.3 (5.7-10.6)*	
Agricultural season	n (observations^d)			<0.01
Lean season (April-May)	396	12.4 (11.6,13.4)	10.2 (6.3-16.9)	
Peak season (November-December)	1030	10.5 (10.0, 10.9)	8.4 (5.5-12.6)	

^a Assessment for differences in salt disappearance by household size and National Wealth Equity Index quintile were evaluated using Kruskal Wallis test (with Dunn-Bonferroni post-hoc test, absence of superscript symbol indicates no significant difference); differences by agricultural season assessed using Mann Whitney U test.

^b Includes household members ≥2 years.

^c National Wealth Equity Index score is calculated using EquityTool, quintiles standardized to 2014 Cambodian Demographic and Health Survey (<https://www.equitytool.org/cambodia/>).

^d Observation is one fortnightly home visit.

Households rarely reported selling, sharing or using the salt for business purposes, and used study-provided salt almost exclusively (**Table 5-6**). However, salt was used for non-consumption purposes, specifically for cleaning fish and/or vegetables, in 27% (n=666) of all fortnightly questionnaires, with 181 unique households reporting using salt in this way (data not shown).

Table 5- 6. Descriptive fortnightly salt use practices among lactating women’s households in rural Cambodia between 2 through 24 weeks postpartum.

	n (observations^a)	n (%)
Sold or shared salt	3425	15 (0.4%)
Used any salt for business use	3425	0 (0%)
Used any rock salt	3425	56 (1.6%)
Used any salt for cleaning fish or vegetables ^b	2514	666 (26.5%)

^a Observation is defined as one fortnightly home visit.

^b *n* differs due to changes in questionnaire to reflect field observations, no selection bias in missing data.

5.4 Adjusted maternal salt intake and fortification scenario

5.4.1 Modelling adjusted salt intake

After adjusting for intra-individual variance using the NCI method, mean (95% CI) adjusted salt intake from all sources was 7.7 (7.4, 8.0) g/day, and adjusted table salt intake 7.0 (6.6, 7.2) g/day (Table 5-7). The frequency distribution of salt intakes from all sources before and after NCI method adjustment can be seen in Figure 5-2.

Table 5- 7. Adjusted salt intake from all sources and from table salt only among lactating women in rural Cambodia.

	n (participants)	Mean (95% CI) g/day	Range g/day
Adjusted ^a daily table salt intake (g)	104	7.0 (6.9, 7.2)	5.3 – 11.3
Adjusted daily salt intake from all sources (g) ^b	104	7.7 (7.4, 8.0)	4.4 – 15.7

^a Adjusted intakes modelled using National Cancer Institute method to control for intra-individual variance.

^b Salt from all sources includes: table salt, soy sauce, fish sauce and *prahok*.

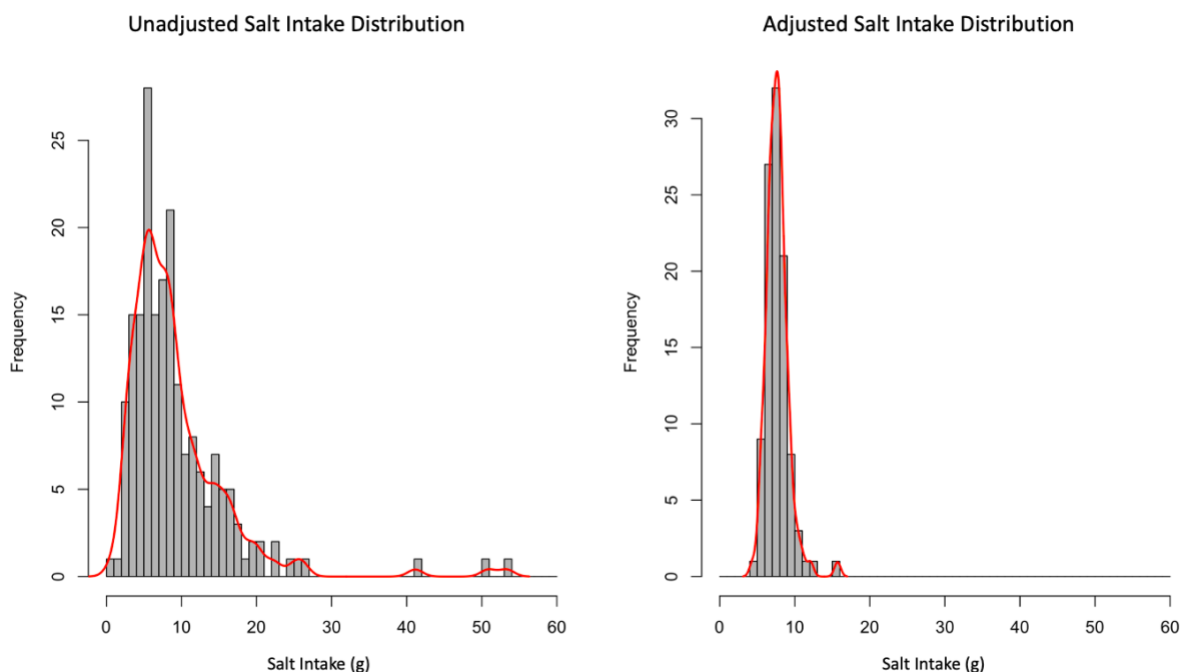


Figure 5- 2. Salt intake distributions (from table salt, soy sauce, fish sauce and *prahok*) for lactating women in rural Cambodian before and after adjusting for intra-individual variance with the National Cancer Institute Usual Dietary Intake method.

5.4.2 Thiamine fortification scenario

The thiamine fortified salt scenario was modelled using adjusted salt intake from table salt, soy sauce, fish sauce and *prahok*, and the optimal dose of 1.2 mg thiamine above baseline dietary thiamine intake. Various models of potential thiamine fortification were run by multiplying thiamine doses by the adjusted salt intake distribution, however a thiamine fortification level of 275 mg thiamine/kg salt was determined to meet all scenario parameters outlined in *Section 4.4.4.2*. In this model, mean thiamine intakes from fortified salt would be 2.1 (2.0, 2.2) mg/day, ranging from 1.2 to 4.3 mg/day. At this level of fortification, 100% of intakes would be above 1.2 mg and below 10 mg per day. There is limited data available on current levels of dietary thiamine intake in Cambodia. Gibson *et al.* estimated daily per capita thiamine intake of 0.58 mg

based on national Food Balance Sheets (FBS) (122). This hypothetical baseline intake and distribution of adjusted thiamine intakes from fortified salt can be seen in **Figure 5-3**.

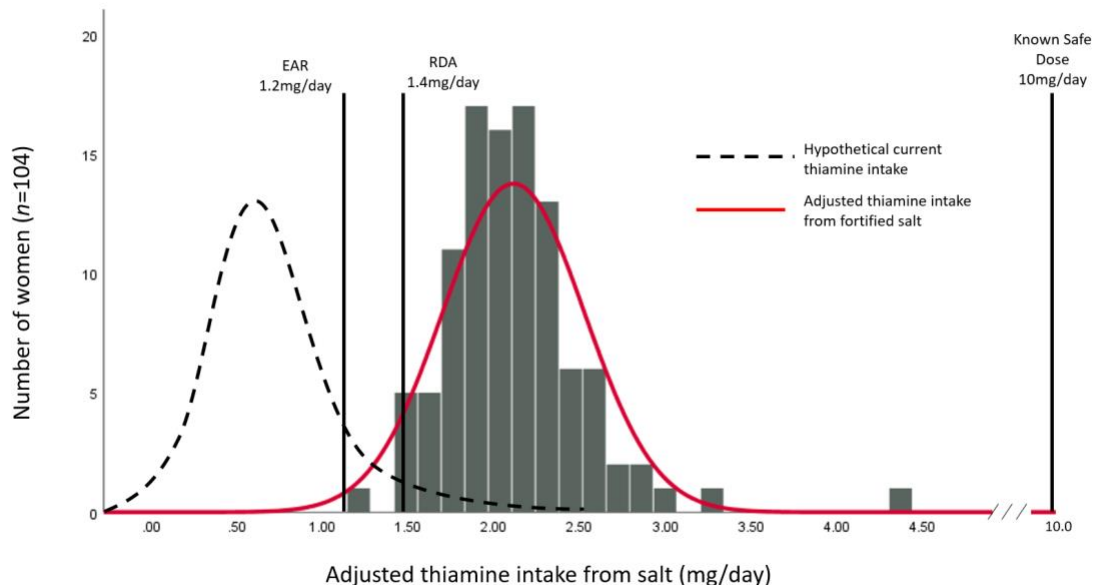


Figure 5- 3. Hypothetical distributions of current dietary thiamine intakes (approximately 0.58 mg/day)(122), and adjusted thiamine intake from thiamine-fortified salt (at 275mg thiamine/kg salt).

6.0 Discussion

The overall purpose of this exploratory, cross-sectional study was to assess salt intake among lactating women in rural Cambodia to model salt as a potential fortification vehicle for thiamine. Thiamine deficiency continues to be a public health issue in Cambodia, and exclusively breastfed infants are particularly at risk for developing TDD. Assessing salt intake can be difficult due to various challenges, such as under-reporting or accounting for food waste (92), therefore we employed three methods of assessing salt intake in an attempt to corroborate findings: repeat 12-

hr observed weighed salt and condiment intake records, repeat 24-hr urinary sodium concentrations, and fortnightly household salt disappearance over a 22-week period. Mean (95% CI) adjusted salt intake from all major sources among lactating women was 7.7 (7.4, 8.0) g/day, and an estimated fortification dose of 275 mg thiamine/kg salt was determined to optimize thiamine intake among this population. To our knowledge, this is the first study that has explored salt intake among lactating women in rural Cambodia, and assessed salt as a fortification vehicle for thiamine.

6.1 Salt and condiment intakes in Cambodia

6.1.2 Maternal salt and sodium intakes from observed weighed intake records and 24-hr urinary sodium

There is limited research on salt and sodium intakes in Cambodia, and no research available specifically for lactating women. Previous estimates have relied mainly on indirect assessment methods, such as national FBS. The FAO publishes national FBS annually to capture trends of food supply and utilization in a country using various sources, such as commodity production, and data on imports and exports (123). FBS are an inexpensive and accessible method of intake assessment, however this method is more suitable for assessing trends over time as opposed to actual intakes, since this data cannot be disaggregated by population sub-groups, and does not account for waste at the household and retail level (123). For example, in 2010 Powles *et al.* used FBS and surveys from other countries in the region to model an estimated mean (95% CI) sodium intake of 4.14 (3.73, 5.18) g/day for Cambodian women (approximately 11 g of salt daily) (90). Laillou *et al.* cited an average per capita salt consumption of 15 g/day among the general Cambodian population from national FBS in 2011 (78). Due to the lack of high-quality

data salt and sodium intake data, we felt it was important to assess salt and sodium intakes using household-level methods to triangulate our findings.

In this study, the unadjusted mean (95% CI) sodium intake from repeat observed weighed intakes and 24-hr urinary sodium concentrations were 3.61 (3.18, 4.04) g/day and 3.56 (3.30, 3.82) g/day, respectively, lower than previous estimates. Twenty-four-hour urinary sodium is considered the gold standard for measuring sodium intake (92), and is often used as a proxy to estimate daily salt (NaCl) intake. Researchers will assume a sodium excretion rate of 100%, and all sodium is assumed to come from dietary salt sources (104,105,124). However, we decided to use repeat 12-hr observed weighed salt and condiment intake records as our primary method of assessing salt intakes to model adjusted salt intake and develop a thiamine fortification scenario, because MSG is a known source of sodium in the diet and would not be distinguishable from salt-based sodium in the results. In our findings, MSG was consumed in 100% of observations, and mean daily sodium intake from MSG was 0.44 (0.36, 0.53) g/day. Despite the universal use of MSG among participants, there was no significant difference in estimated daily salt intakes from observed weighed intakes (9.3 (8.3, 10.3)g /day) and 24-hr urinary sodium concentrations (9.0 (8.4, 9.7) g/day; $p>0.05$). This may be due to the higher intake of salt and higher sodium content of table salt, compared to MSG. Regardless, corroboration of the results from these two methods of assessment indicate that both methods are appropriate measures of salt and sodium intake among lactating women in rural Cambodia.

Similarities between the estimated intake findings for each method also shows that virtually all sodium intake was being captured through observed weighed intake records, therefore sodium

intake in the participants' diets was largely coming from discretionary salt and condiment use while cooking. This is in line with previous research (91,108), and contrary to dietary trends in North America, where up to 70% of dietary sodium intake is from commercially processed foods (125). Although not an objective of this study and not presented in this thesis, data collectors anecdotally reported minimal maternal consumption of ultra-processed foods during observations, however consumption of commercial “snack” foods was commonly seen among children in the households. This trend has been documented previously in Cambodia. Pries *et al.* conducted a survey of 222 mother-child dyads in Phnom Penh, Cambodia and found that 81% of children 6-23 months had consumed commercially produced foods and beverages within the week prior to data collection (126). As the Cambodian population ages, it will be important to reassess the sources of salt and sodium intake and changes in dietary patterns to ensure appropriate thiamine-fortification levels are employed.

6.1.3 Table salt and condiment coverage

Table salt was consumed almost daily by all participants (99%) and MSG was consumed in all observations, however, table salt contributed almost 70% of salt and sodium from all sources. Fish sauce was consumed on 81% of the observation days and mean (95% CI) intake was 9.9 (7.4, 12.3) g/day (contributing 2.3 (1.7, 2.9) g of salt per day). *Prahok* use was more variable, being consumed in only 54% of visits, and mean intake was 5.6 (3.3, 7.8) g/day. This is much lower than a previous estimate of 18 g/day among the general population (7), however the method of assessment used to derive this previous estimate was not described and likely relied on indirect methods. This discrepancy may also be related to cultural food restrictions in the postpartum period. Wallace *et al.* conducted focus groups with 67 women in Kandal Province,

Cambodia and found that *prahok* and other salty foods were considered “harmful” for lactating women (127). This is in contrast to Laos where salt appears to be one of the few foods maintained during the postpartum taboo diet: here, 16% of the 300 women surveyed consumed only rice and salt for the first 2 weeks postpartum (85). As outlined in *Section 2.7.3*, postpartum food restrictions are common in several beriberi-endemic countries, so it is critical to accurately assess the coverage and utilization of fortification vehicles among target populations before implementing a fortification program (128).

One unexpected finding in our study was the limited use of soy sauce in rural Cambodian homes. Soy sauce was only used on 6% ($n=11$) observation days, among 9 unique participants. This finding should be explored further as it is unclear if soy sauce is more commonly used in urban settings or retail settings, such as restaurants. This finding is particularly important because there have been substantial efforts to promote soy sauce as a fortification vehicle for iron in Cambodia (129). Soy and fish sauce iron-fortification initiatives in Cambodia have been in place since 2011, and are supported by the Global Alliance for Improved Nutrition (GAIN), the Reproductive and Child Health Alliance (RACHA), the United States Agency for International Development (USAID), and the National Subcommittee on Food Fortification in Cambodia (129). In 2014, Laillou *et al.* reported that almost 25% of total fish and soy sauces produced in Cambodia were fortified (74). However, given our findings, these efforts may have more impact if focused solely on fish sauce. Soy sauce may not be a suitable fortification vehicle for any micronutrient in Cambodia if the goal is to reach vulnerable, rural populations.

6.1.4 Household salt disappearance and practices

Household salt disappearance had several benefits and limitations as a method of assessing salt intake. This method was not as resource-intensive as collecting 24-hr urine samples or completing observed weighed intake records, and was useful in understanding broader trends in salt use. However, it was ultimately not accurate enough to assess the salt intake of lactating women as a means of estimating adjusted salt intake and modelling a fortification scenario. This method over-estimated table salt intake (unadjusted mean salt use by household was 11.3 (10.7, 11.9) g/person/day, compared to only 6.4 (5.6, 7.1) g/person/day using observed weighed intake records), and household salt disappearance does not capture intake of other sources of salt and sodium in the diet (i.e. soy sauce, fish sauce, MSG, and *prahok*). This method of assessment was also unable to capture differences in salt intake by household member, which is particularly important in this study where accurate salt intakes among the target population, lactating mothers, are needed in order to prevent thiamine deficiency among exclusively breastfed infants, and since unequal intrahousehold food distribution is expected (130).

We found that salt was commonly used in the non-consumptive practice of cleaning fish and vegetables: this practice was reported in 26% of fortnightly visits ($n=666$ visits, $n=181$ unique households). The frequency of this practice provides further understanding for the over-estimated salt uses when compared to the other two methods of salt assessment. This finding also highlights why household salt disappearance is not an appropriate proxy for dietary salt intakes among lactating women in rural Cambodia.

Trends in household salt use by various sociodemographic and environmental characteristics provided important information related to the use of salt as a fortification vehicle. Household salt use varied significantly by National Wealth Equity Index quintile, with significantly lower salt disappearance among the highest quintile as compared to the lowest three quintiles ($p < 0.05$). However, this result should be interpreted with caution as the number of households in this quintile was quite low ($n = 22$). This difference could be due to several reasons, such as wealthier households being more likely to purchase pre-made foods from markets or eat out at restaurants, as opposed to cooking at home. Alternatively, these wealthier households may not have been using salt for non-consumption purposes such as cleaning salt or vegetables, opting instead for specific cleaners or tools, or may have been consuming less salt due to health beliefs around sodium intake. Regardless, low-income women are at higher risk of thiamine deficiency (21), therefore relatively uniform intake among the lowest three quintiles indicate that salt would be an appropriate fortification vehicle for thiamine.

Salt use also varied by season, with increased salt use during fortnightly visits in the lean season (12.4 (11.6, 13.4) g/day) compared to the peak season (10.5 (10.0, 10.9) g/day; $p < 0.01$). This difference should be explored further, as it is unclear if this difference is related to non-consumption uses, such as cleaning fish and vegetables, for food preservation purposes in the early lean season, or if salt is being consumed in higher amounts during the lean season. The sample size of the maternal salt intake study was insufficient to assess intake by agricultural season and there is a lack of research on seasonal salt use in Cambodia.

6.2 Adjusted salt intake and thiamine fortification

Mass fortification with thiamine-fortified salt has been proposed as the ideal intervention to address thiamine deficiency in Cambodia (21), because salt is consumed daily by everyone in the population, at relatively consistent amounts throughout the year, is manufactured in a centralized area of Cambodia, and policies are already in place for salt fortification with iodine (77).

Using the NCI method, mean adjusted salt intake from all sources was determined to be 7.7 (7.4, 8.0) g/day, and the optimal daily dose of supplemented thiamine from the larger trial was 1.2 mg/day. As discussed in *Section 4.4.4.2*, we aimed to model various fortification scenarios to maximize thiamine intakes from fortified salt above 1.2 mg/day and minimize intakes above 10 mg/day. This objective was met with a single fortification scenario: a dose of 275 mg thiamine/kg salt would result in mean adjusted thiamine intake of 2.1 (2.0, 2.2) mg/day from salt, with intakes ranging from 1.2 to 4.3 mg/day. It is important to note that the NCI method tends to minimize the 10th and 90th percentiles of intakes (110), therefore there could be a small proportion of adjusted thiamine intakes below and above the modeled intake levels. However, the maximum adjusted intake in this model (4.3 mg thiamine/day at the 99th percentile of salt intake) is still well below the known safe intake of 10 mg/day, therefore this fortification dose is likely still appropriate even if adjusted salt intake is underestimated.

When selecting a fortification vehicle, it is important to consider how this product will be used and distributed commercially, as well as all dietary sources of the vehicle in the target population. Given the current salt fortification legislation in Cambodia, as well as the consumption of locally produced salt and condiments, we modelled adjusted salt intake from all

dietary sources (table salt, soy sauce, fish sauce, *prahok*) as opposed to table salt alone. In Cambodia, *Sub-Decree No 69 on the Management of Iodized Salt Exploitation* indicates: “Individuals, restaurants, factories, enterprises, cottage industries, hospitals and all places that produce and prepare food in the Kingdom of Cambodia must use iodized salt as an ingredient” (77). While almost all countries with salt iodization legislation include its use in processed foods, fortification programs and iodization monitoring tend to focus on table salt at the household level, even though salt intake through condiments is an important source of fortified salt (131). The Iodine Global Network (IGN) has found that the use of fortified salt in condiments is variable in Southeast Asia, however compliance appears to be higher in Cambodia (132). In 2017, the IGN and UNICEF conducted a survey of the 30 largest Cambodian fish sauce and soy sauce producers, as well as several *prahok* producers, to assess past use of iodized salt (132). All producers reported using iodized salt from 2003-2010, and samples from 5 out of 6 *prahok* producers were iodized in 2017 (129). The IGN states “Cambodia appears to be the only country in the region to have fully implemented the requirement to use iodized salt in the production of salty condiments, namely fish and soya sauce and fermented fish paste, to date” (pg. 2). In addition, the majority of salt and dominant salt-containing condiments (fish sauce and *prahok*) consumed in Cambodia are produced domestically: almost all Cambodian consumption needs are met through domestic production (as described in *Section 2.7.1*), and only 10% of fish sauce is imported (129). Fish sauce is also produced at home, particularly in rural areas (133). *Prahok* is produced exclusively in Cambodia, either in the home (using table salt), or commercially (134). If a national salt fortification program were to be implemented, these domestically produced condiments would also contain thiamine-fortified salt. However, it is important to note that table salt intake was the most consistently consumed salt source, while the highly variable condiment

intakes added variance to the model of adjusted salt intake from all sources. The distribution of adjusted table salt intakes was actually within the distribution of adjusted salt intakes from all sources. If the fortification dose of 275 mg thiamine/kg salt is applied to table salt only, this population still meets the parameters of our fortification scenario (100% of adjusted thiamine intake between 1.2 and 10 mg/day).

Salt intakes from this study may also be useful in evaluating current iodization practices in Cambodia. Fortification programs should be continually monitored and evaluated to ensure the program is meeting the needs of the population, particularly target populations that are at risk of deficiency (128). Current iodization policies recommend a fortification dose of 30-60 ppm (i.e. 30-60 mg iodine/kg salt), and iodine dietary reference intakes for lactating women are as follows: an EAR of 209 µg/day, a RDA of 290 µg/day, and a UL of 1100 µg/day (39). If mean fortified levels are 45 mg iodine/kg salt, using our estimated salt intake of 7.7 (7.4, 8.0) g/day, lactating women could be receiving approximately 360 µg of iodine from iodized salt, an appropriate 'dose' as it falls between the EAR and UL (13).

6.2.1 Controversies around salt

While salt iodization has been the most widely implemented fortification program globally, there is some controversy around the use of salt as a fortification vehicle, for fear that addition of micronutrients could cause consumers to eat more of the fortified product in hopes of additional health benefits.

The relationship between chronic sodium intake at levels above the Chronic Disease Risk Reduction Intakes (CDRR) and disease risk has been widely studied, and while there is

consensus that sodium is directly linked to blood pressure, there have been inconsistencies in determining disease risk, as many sodium intake studies are observational, may be too short to observe incidence of disease or mortality, may not account for confounding factors that affect blood pressure, and may have inherent bias in the assessment of sodium intake (88).

It is also important to consider the source of sodium in the diet and potential confounding factors. In North America, the positive relationship between sodium intake and blood pressure is well established, however, the majority of dietary sodium comes from commercially processed foods which are also typically of lower nutritional quality (lower fibre and micronutrient density, high calorie content) (125). In the United States, a 2015-16 nationally representative study reported an estimated mean intake of 9 g salt/day, with significant positive associations between sodium intake and blood pressure, and an age-standardized prevalence of hypertension of 45.4% in the population (135). This may differ from economically developing countries, such as Cambodia, where dietary sodium intake among adults appears to come almost entirely from discretionary salt and condiment use while cooking. For example, in a nationally representative cross-sectional study in Vietnam, participants consumed an average of 10 g salt/day and there was no significant relationship between sodium intake and systolic blood pressure among men or women ($\beta = -0.12$ [95% CI 0.83, 0.58] and $\beta = -0.06$ [95% CI 0.73, 0.61], respectively) or prevalence of hypertension (only 15% among men and 5% among women at the time of the study) (108). The relationship between sodium intake and hypertension is likely much more complex than simply sodium intake (136).

While the WHO does not recommend additional fortification of salt universally (114), the Cambodian context is unique in both its traditional diet and the severity of the risks associated with thiamine deficiency. In Cambodia, salt is the only food that meets the criteria for an optimal fortification vehicle (consumed in consistent amounts by all segments of the population, and is produced in centralized industrial facilities), and thiamine deficiency among exclusively breastfed infants can be fatal or have deleterious developmental consequences. The implementation of a fortification program to eradicate thiamine deficiency in Cambodia is an urgent public health issue that likely outweighs potential cardiovascular disease risk later in life, where prevalence of hypertension remains relatively low in Cambodia (approximately 15%) (137). It is also important to re-iterate that mass fortification is a passive intervention, with program success dependent on accurate fortification vehicle intake assessment and compliance at the manufacturer level (13). Salt fortification programs, whether with iodine or other micronutrients, do not encourage additional salt intake for populations to meet their micronutrient requirements and there is no evidence that implementation of salt fortification programs are related to increased salt intake (114).

6.3 Strengths and Limitations

This study had several strengths, namely that it was the first study to assess salt intake among lactating women in Cambodia and model salt as a fortification vehicle for thiamine. We employed multiple methods of salt and sodium assessment, including biochemical (repeat 24-hr urinary sodium concentrations), and weighed diet records (repeat observed weighed intake records), as well as household salt disappearance during the exclusive breastfeeding period, from 2 through to 24 weeks postpartum. Corroboration of results from the 24-hr urinary sodium and observed weighed intake records indicate accurate assessment of salt and sodium intake. The

sample size of the household salt disappearance study was large and representative of lactating women in rural Kampong Thom province, and collection of repeat measurements for the maternal salt and sodium study allowed us to control for intra-individual variance in salt intake, and thus model usual salt intake. Another strength was the use of the optimal thiamine dose used in the fortification scenario, which was determined from the large randomized-controlled thiamine supplementation trial using infant and maternal biochemical data (blood and human milk). With the traditional EAR cut-point method, baseline dietary thiamine intakes are assessed (i.e. via food frequency questionnaires, intake records etc.), and food composition databases are used to determine the prevalence of inadequacy in the population. While we do not have baseline dietary thiamine intakes in this study, the larger trial controlled for baseline dietary thiamine intake through the placebo group. Ultimately, this optimal dose, and thus our modified EAR cut-point model, may represent a more accurate level of thiamine inadequacy among lactating women compared to the traditional EAR-cut point method of estimating prevalence of inadequacy using food intake and food composition databases. If the traditional EAR cut-point method were used to determine a fortification dose to meet the needs of the full population (using dietary thiamine and salt intake) it is possible that a lower fortification dose would be appropriate, however, this is not within the scope of the current study.

Limitations for this study include a relatively small sample size for the maternal salt and sodium intake study. While the sample size ($n=104$) was sufficient to adjust for intra-individual variance and model salt fortification, it was not large enough to explore intake trends that were observed in the household salt disappearance study, such as changes by agricultural season. Also, due to the exploratory nature of the study and rolling recruitment, some questions were added to the

observed-weighted intake questionnaire and salt use questionnaire to reflect observations in the field. This led to more missing data for certain questions, such as salt use for cleaning fish and vegetables, however there was no selection bias for these missing data so this should not affect our results. Another limitation is the use of the ASEAN food composition database to estimate sodium content of fish sauce, soy sauce, and *prahok*. While this is standard practice for estimating nutrient content and intake, salt content in fish sauce and soy sauce is known to be quite variable in Cambodia (74). Laillou *et al.* collected 252 samples of fish and soy sauces and found salt content varied from approximately 80 to 340 g/L (74), in the ASEAN food composition database the salt content of fish sauce was 236 g/L (9294 mg of sodium/100 g of fish sauce). Finally, 12-hr observed weighed intake records and 24-hr urinary sodium concentrations may under-estimate actual intakes. Because the observed weighed records were only 12 hours in duration, it is possible that participants consumed salt and sodium-containing foods outside of the observation period, however we attempted to minimize this by regularly communicating with participants and having data collectors travel to the participants' homes the day before observation to drop off urine collection equipment and remind participants about the observed weighed intake process. Given that there was no difference in the calculated sodium intakes between the intake and excretion methods, it is unlikely that large amounts of salt or sodium-containing condiments were consumed outside the dawn-to-dusk observation periods. Similarly, urinary sodium does not account for potential sodium losses through sweat or feces, however, sweat losses are expected to be minimal (108). Regardless, it is important to note that the estimates in this study are conservative, and therefore actual intakes of thiamine via fortified salt may be slightly higher than projected. This should be considered in the development of a thiamine-fortified salt program. However, it should be noted that thiamine fortification vehicles

in other countries may be consumed at highly variable rates, such as refined wheat flour in Canada (138), with no evidence of harm.

6.4 Recommendations for future research

Future research should include testing related to the development of a thiamine-fortified salt formulation, such as stability, organoleptic, and acceptability tests, as well as efficacy trials to inform a national thiamine fortification program. The optimal dose from the larger trial was determined from a single, daily bolus dose of thiamine via a supplement, therefore efficacy trials will be particularly important to understand the effect of intermittently consumed thiamine-fortified salt with food. Similarly, stability testing of thiamine-fortified salt will help determine the retention factor of the salt when used in cooking and manufacturing processes such as fish sauce production. Analysis of the postpartum food avoidance data from the larger trial will also be important in developing the rationale of salt as the ideal fortification vehicle for thiamine in Cambodia, and table salt and condiment intakes among lactating women should also be assessed in urban settings (such as in the capital city of Phnom Penh) to determine if there are regional differences in intake, particularly with soy sauce. Furthermore, an updated survey assessing the salt content of condiments produced in Cambodia and current use of iodized salt practices in food processing would provide insight into future opportunities and barriers to a national thiamine-fortified salt program.

7.0 Conclusions

In this study, we assessed salt and sodium intake, and modelled a thiamine-fortified salt scenario, for lactating women in rural Cambodia. Unadjusted mean salt intake from observed weighed

intake records was 9.3 (8.3, 10.3) g/day, and was not significantly different from unadjusted estimated salt intake from 24-hr urinary sodium concentrations (9.0 (8.4, 9.7) g/day; $p>0.05$). Daily table salt use via household salt disappearance was 11.3 (10.7, 11.9) g/person/day, an over-estimation of lactating women's salt intake that also did not capture salt consumption from other condiments. After adjusting for intra-individual variance, mean salt intake from all sources was 7.7 (7.4, 8.0) g/day. Using a modified EAR-cut point method, a fortification dose of 275 mg thiamine/kg salt was determined to meet the needs of this population, with a modelled mean adjusted thiamine intake of 2.1 (2.0, 2.2) mg/day, ranging from 1.2 to 4.3 mg/day.

We found that repeat 12-hr observed weighed salt and condiment intake records were a valid method of assessing salt and sodium intake among lactating women in rural Cambodia, however after adjusting intake with the NCI method, observed weighed table salt intakes alone resulted in the same recommended fortification dose as salt intake from all sources. As such, assessing only table salt intake via observed weighed intake records may be sufficient in future salt fortification studies for this population.

Trends in both observed weighed intake records and the household salt disappearance study confirm that salt would be an appropriate fortification vehicle for thiamine. Salt was consumed universally, in relatively consistent amounts each day, and there was no significant difference in intake among the three lowest national Wealth Equity Index quintiles. Conversely, coverage and utilization of salt and condiments revealed that soy sauce was not regularly consumed by lactating women in rural Cambodia.

A mass fortification program with thiamine-fortified salt would likely help reduce thiamine deficiency in rural Cambodia and prevent TDDs among the most vulnerable segment of the population, exclusively breastfed infants. Future research should focus on the feasibility of such a program, including studies on the technical and cost-related aspects of thiamine-fortified salt, efficacy trials, and working with stakeholders to plan for implementation.

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Appendix A: Consent form for maternal salt and sodium study

Consent Form

Trial of thiamine supplementation in Cambodia

Individual Salt Consumption and Sodium Status Study: Maternal 24hr Urinary Sodium Concentrations and Observed Weighed Salt & Condiment Intakes



Introduction

We are inviting you to participate in research about your salt and sodium status. We will provide you with information about the study before asking for your authorization to participate. A member of the research team will be available to answer any questions you have. You may decide to not participate or you may withdraw from the study at any time. Participation is entirely voluntary.

Potential Conflict of Interest

The researchers have no conflicts of interest to report.

Purpose of the research

As a participant in the *Trial for Thiamine Supplementation in Cambodia*, you know that we are tracking your household salt intake over 22-weeks to determine if we should use salt as a fortification vehicle for thiamine. However, it is important that we understand how much salt is being consumed by individuals. To accomplish this, we will ask 100 families to allow us to measure 24-hr urinary sodium concentrations (mothers only) and will measure salt, fish sauce and soy sauce intakes of mothers, their husband (if applicable) and one of their children (oldest within 24-59 month range, if applicable).

For this study, you will be asked to provide two 24-hr urine samples and allow a field officer observe and weigh salt, fish sauce and soy sauce consumed by yourself, your husband (if applicable) and one of your children (oldest within 24-59 month range, if applicable) on two full days (sunrise to sunset). We will do this on two non-consecutive weekdays within a 7-day window.

Participation in this study is entirely voluntary and will not cost you anything. As a thank you for your time and participation, you will receive one bag of laundry soap.

Study Procedure

Who can participate?

We will randomly ask 100 families already in the *Trial of Thiamine Supplementation in Cambodia* study to participate, all at about 8 weeks postnatal.

What will participation in this study look like?

If you wish to participate in this study, we will contact you to schedule two data collection days where we will collect a 24-hour urine sample and observe your families salt and condiment intakes.

24-Hour Urine Collection:

On the day before a data collection day, a field officer will visit your home to drop off the urine collection equipment and give more specific instructions. You will collect all of your urine into a 5L study-provided container for 24 hours.

Observed Weighed Salt and Condiment Intake:

A field officer will arrive at your home at sunrise on a data collection day and will stay until sunset. We will record all food prepared and consumed in the home on that day that contains salt, soy sauce, or fish sauce.

The field officer will weigh the meals during cooking, as well as the individual portions consumed by yourself, your husband (if applicable) and one of your children (oldest within 24-59 month range, if applicable). Additional individual use of salt, fish sauce or soy sauce at meal time will also be weighed and recorded.

Confidentiality

Your confidentiality will be respected; your records will be kept in a locked cabinet in the Kampong Thom Operational Health District office. You will be anonymous to the researchers as you will only be referred to by your study-provided ID (i.e. A001) in all analysis. All documents will be kept for a minimum of five years and then securely destroyed.

Risks

We do not believe there are any risks involved with participation in this study.

Benefits

You will not receive direct benefits from participating in this study other than 1 bag of laundry soap.

Consent Form for Participant Participation

PARTICIPANT AUTHORIZATION:

I have read or had read to me this information and authorization form and have had the chance to ask questions which have been answered to my satisfaction before moving forward. I understand the nature of the study and I understand the potential risks and benefits. I understand that I have the right to withdraw from the study at any time without any problems. I have received a copy of this consent form for future reference. I freely agree to participate in this research study.

Name of Participant: _____
fingerprint): _____

Signature (or

Date: _____

STATEMENT BY PERSON PROVIDING INFORMATION ON STUDY AND OBTAINING CONSENT

I have explained the nature and demands of the research study and judge that the participant named above understands the nature and demands of the study. I have explained the nature of the consent

process to the participant and judge that they understand that participation is voluntary and that they may withdraw at any time from participating.

Printed Name of Subject

Signature

Date

Printed Name of Person Obtaining Consent

Signature

Date

Appendix B: Abridged baseline questionnaire

Trial of thiamine supplementation in Cambodia



BASELINE QUESTIONNAIRE

MODULE 2: DEMOGRAPHIC INFORMATION		
2. What is your date of birth? (DD/MM/YYYY)	__ __ / __ __ / __ __ __ __	
8. Please describe the sex and ages of the all people living in your household who eat from the household common pot (excluding yourself).	Sex	Age (circle m/y)
	1. Male	
	2. Female	__ __ months / years
	1. Male	
	2. Female	__ __ months / years
	1. Male	
	2. Female	__ __ months / years
	1. Male	
2. Female	__ __ months / years	
9. Have you attended school?	1. Yes 2. No → code, then proceed to Q11 __ __	
10. What is the highest level of school you attended?	1. Primary school 2. Lower Secondary school 3. Upper Secondary school 4. Higher education __ __	
11. Has your husband/partner attended school?	1. Yes 2. No → code, then proceed to Q13 __ __	
12. What is the highest level of schooling your husband/partner attended?	1. Primary school 2. Lower Secondary school 3. Upper Secondary school 4. Higher education __ __	
14. What was the income for your household last month ?	US\$ __ __ __ __ __	

MODULE 3: EQUITY INDEX	
23. Does your household have electricity?	1. Yes 2. No _____
24. Does your household have a television?	1. Yes 2. No _____
25. Does your household have a refrigerator?	1. Yes 2. No _____
26. Does your household have a CD / DVD player?	1. Yes 2. No _____
27. Does your household have a wardrobe?	1. Yes 2. No _____
28. Does your household have a generator / battery / solar panel?	1. Yes 2. No _____
29. Does any member of your household own a motorcycle / scooter?	1. Yes 2. No _____
30. Does any member of your household own a watch?	1. Yes 2. No _____
31. Does any member of this household have a bank account?	1. Yes 2. No _____
OBSERVATION ONLY: What is the main material of the floor of the living house? 32. RECORD ONLY ONE OBSERVATION.	Natural floor: 1. Earth/sand 2. Dung Rudimentary Floor: 3. Bamboo/palm 4. Wood planks Finished floor: 5. Parquet or polished wood 6. Ceramic tiles 7. Cement 8. Carpet 99. Other – Specify _____
OBSERVATION ONLY: What is the main material of the exterior walls of the living house?	0. No Walls Natural walls: 1. Earth/sand 2. Dung Rudimentary walls: 3. Bamboo/palm with mud

33. RECORD ONLY ONE OBSERVATION	4. Stone with mud 5. Uncovered adobe 6. Plywood 7. Carboard 8. Resued wood Finished Walls: 9. Metal 10. Cement 11. Stone with lime / cement 12. Bricks 13. Cement blocks 14. Covered adobe 15. Wood planks / shingles 99. Other – Specify _____
34. What type of fuel does your household mainly use for cooking?	1. Charcoal 2. Wood 3. Electricity 4. LPG (natural gas) 5. Biogas 6. Straw/shrubs/grass 7. Animal dung 99. Other – Specify _____
35. What is the main source of drinking water during the rainy season for members of your household?	1. Piped into dwelling 2. Open well 3. Covered well 4. Drilled Borehole (with hand pump or other type of pumping system) 5. Surface water (e.g. spring, river/stream, pond/lake/dam) 6. Rainwater 7. Bottled water 99. Other – Specify: _____
What kind of toilet facility do members of your household usually use? 36. ASK TO SEE.	0. No facility—bush, field → NO, then proceed to Module 4, q 38 1. Flush to piped sewer system (not shared with other households) 2. Flush to septic tank (not shared with other households) 3. Flush or pour toilet piped sewer system (shared with other households) 4. Flush or pour toilet to septic tank (shared with other households) 5. Traditional pit latrine 6. Ventilated Improved Pit (VIP) latrine 7. Pit latrine without slab 8. Composting toilet

	9. Bucket 10. No permission to see 99. Other – Specify: _____
37. Do you share this toilet facility with other households?	1. Yes 2. No _____

Appendix C: Observed weighed salt and condiment record

OBSERVED WEIGHED SALT AND CONDIMENT RECORD

Participant ID: _____ **Date:** ____/____/____ (DD/MM/YYYY) **Visit Number** (circle): 1 or 2

Age of Husband (if applicable): _____ years

Age of Child (oldest child within 24-59 months, if applicable): _____ months Sex of Child (circle): F or M

Is today a typical day for meal preparation and consumption? (circle): Yes or No If no, please explain: _____

Enumerator Name:

Record all **salt, fish sauce and soy sauce** used during meal or snack preparation. Do not include plain, steamed rice.

Meal Preparation Part 1			
Code	Type of Meal (short description)	Total Weight of <u>Empty Pots*</u> (g)	Total Weight of <u>Full Pots*</u> (g)

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Meal Preparation Part 2										
Code	Initial Weight: Salt Container (g)	Initial Weight: Soy Sauce Container (g)	Initial Weight: Fish Sauce Container (g)	Initial Weight: MSG container (g)	Initial Weight: Prahok container (g)	Final Weight: Salt Container (g)	Final Weight: Soy Sauce Container (g)	Final Weight: Fish Sauce Container (g)	Final Weight: MSG container (g)	Final Weight: Prahok container (g)

1. Record details on meal consumption and any additional, individual salt, fish sauce and soy sauce use at meal time for the study participant, her husband and one child (oldest within 24-59 month age range).

Ensure plain, steamed rice is eaten from a **SEPARATE bowl and measured separately.

Ensure salted, dried fish is eaten from a **SEPARATE bowl and measured separately

Appendix D: 24-hr Urinary Sodium Concentration Questionnaire

24-Hour Urinary Sodium Concentration Questionnaire

1. Participant ID: ____ ____ ____ ____

2. Visit Number (circle): 1 or 2

3. Enumerator Name: _____

4. Did you eat anything last night after I left the house? YES or NO

a. If yes, what: _____

5. First Morning Void:

Date: ____ ____ / ____ ____ / ____ ____ ____ ____ (DD/MM/YYYY)

Time: ____:____

6. Second Morning Void:

Date: ____ ____ / ____ ____ / ____ ____ ____ ____ (DD/MM/YYYY)

Time: ____:____

7. Final Weight of 24-Hr Urine Sample: ____ ____ ____ ____ (g)

8. Container Code: _____

Appendix E: Abridged fortnightly questionnaire

Trial of thiamine supplementation in Cambodia



FORTNIGHTLY MONITORING DATA COLLECTION SHEET

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

MODULE 1: SUPPLEMENT ADHERENCE

MODULE 2: SALT DISAPPEARANCE

7. Number of people regularly eating from the household's common pot in the last fortnight.	a) 0 - 6 months _____ b) 7 - 23 months _____ c) 2 - 5 years _____ d) 5-17 years _____ e) >=18 years _____
8. Weight of salt in study-provided container. Total of all salt and ensure that all salt in the household is being weighed (e.g. check ID card and ask about other salt storage containers)	_____ g
INSTRUCTION TO INTERVIEWER:	
Top up the family's salt supply, then re-weigh the salt container(s).	
9. New weight of salt in study-provided container (total weight).	_____ g
10. Did the household sell or share salt in the last fortnight?	1. Yes 2. No → skip to Q12 _____

12. Did the household use any salt for business use in the last fortnight? (e.g. salted fish, fish sauce, etc. not for home consumption)	1. Yes 2. No → skip to Q14 _____
14. Did you use any rock salt in the last fortnight?	1. Yes 2. No → skip to Q16 _____
16. Did you use any study-provided salt for cleaning fish/vegetables in the last fortnight?	1. Yes 2. No → skip to Q18 _____