A cross-sectional evaluation of sodium consumption by people in Cambodia

by

Lauren Calder Ramsay

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ABSTRACT

A CROSS-SECTIONAL EVALUATION OF SODIUM CONSUMPTION BY PEOPLE IN CAMBODIA

Lauren C. Ramsay
University of Guelph, 2014

Advisor:
Alastair J. S. Summerlee

The cross-sectional study took place in four provinces in Cambodia: Kampot, Kandal, Kampong Chhnang and Phnom Penh. This study involved the collection of 24-hour urine samples to assess sodium and potassium excretion. Demographic, anthropometric, blood pressure, physical activity level, and socio-economic data were collected in face-to-face interviews. Mean urinary sodium excretion found in the 24-hour urinary sample of the entire sample population (M=5615.93mg,) is nearly three times higher than the World Health Organization recommended intake of 2000mg of sodium, $p<0.001$. The mean potassium excretion in 24-hours in the sample population is 3455.79mg/day. Sodium excretion has a positive linear relationship with potassium excretion, $p=<0.001$. There was no relationship between sodium excretion and blood pressure. The major contributors to the high-sodium diet were added seasonings daily during cooking including: salt, fish sauce, monosodium glutamate, and prahok (a locally made fish paste).

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Declaration of Work Performed

I declare that with the exception of the items indicated below, all work reported in the body of this thesis was performed by me:

Chapter 2 contains contributions from Christopher Charles. This chapter is forthcoming in the following publication:

Ramsay, L. C., Charles, C. V. Review of Iron Supplementation and Fortification in Public Health, Prof. David Claborn (Ed.). ISBN 978-953-51-4103-7. InTech; forthcoming.

The research in Chapter 4 and 5 was done in collaboration with Christopher Charles. NutriFood Cambodia conducted surveys and collected data in Cambodia and translated all results.

Biomedical Laboratory in Phnom Penh, Cambodia analyzed urine samples included in Chapter 4.

Food samples included in Chapter 5 were analyzed at the University of Guelph Agriculture and Food Laboratory.

Table of Contents

List of Tables

List of Figures

List of Symbols

CDHS: Cambodian Demographic and Health Survey

IDA: Iron deficiency anemia

ID: Iron deficiency

K: Potassium

METs: Metabolic equivalents

MNP: Micronutrient powder

MSG: Monosodium glutamate

Na: Sodium

NaFeEDTA: Sodium iron ethylenediaminetetraacetic acid

NGO: Non-governmental Organization

NFC: Nutrifood Cambodia

PSU: Primary sampling unit

SSU: Secondary sampling unit

STEPS: STEPwise approach to Surveillance

SES: Socioeconomic status

WHO: World Health Organization

Chapter 1: Introduction

I began this program after spending time living and working in Cambodia. I was living in the capital city, Phnom Penh working in the office of a local non-governmental organization (NGO) and had the opportunity to travel around the country visiting villages and meeting Cambodian people. I developed a passion for the country, the people, the politics, and the future of Cambodia. In addition to developing a passion of the people and the country, I became profoundly concerned about the apparent lack of adequate nutrition and wanted to make a contribution to nutritional studies in the country.

I was motivated to work on a project involving the clinical effects of a palliative for iron deficiency (ID) known as the *Lucky Iron Fish*™. The principal objective of my proposed area of study was an in-depth clinical trial of the *Lucky Iron Fish*™ to determine its affects on circulating and stored iron in women in rural villages. I developed the research methodology and protocols for the clinical trial and obtained ethics approval from the Research Ethics Board at Guelph (Appendix 1). Although I had anticipated that obtaining ethical approval for the proposed research in Cambodia would take time because all bureaucratic processes take a long time in the country, I did not expect the approval to be so protracted or delayed. After three semesters when there was still no sign of forthcoming approval, I decided to switch topics of my research to study sodium intake among the general population. Whilst at first glance, these topics might seem disconnected, there is a direct link in terms of nutritional concerns in Cambodia.

Anemia, in all of its different forms, affects 1.62 billion people globally (Balarajan et al., 2011; Benoist et al., 2008); and iron deficiency anemia (IDA) is considered to be the most prevalent micronutrient condition globally, with 50% of anemia cases being caused by iron deficiency according to the World Health Organization (WHO) (Benoist et al., 2008). The Cambodia Demographic and Health Survey in 2010 found that 43.4% of non-pregnant and non-lactating women are anemic and 49.9% of pregnant and lactating women are anemic (CDHS, 2010). Since ID and IDA are significant issues in Cambodia I became interested in other factors that influenced the prevalence and treatment of the condition. Part of this involved considering what are all of the ways that ID and IDA can be treated and how well they work. Among the proposed solutions for alleviating ID, there are currently programs looking into the feasibility of fortifying fish sauce and/or soy sauce, which is an important component of the normal diet in Cambodia. Since these condiments have high sodium content, this sparked the question of how sodium intake could impact health in Cambodia. Asia is notorious for its high sodium delicacies using great amounts of salt, monosodium glutamate, soy sauce, and fish sauce. Could encouraging the use of high sodium products promote negative health consequences associated with a high sodium diet? And so my education took a new course and now focused on collecting baseline data on sodium intake in Cambodia.

Rationale

The WHO is committed to working on sodium reduction strategies with the target sodium intake being less than 2000mg of sodium per person per day by

2025. To develop effective strategies by country it is essential to have baseline studies on sodium intake. There are virtually no data for Cambodia bar one report of one community in Phnom Penh, which found average sodium intake to be 7000mg/day (Van Maaren, 2013). The WHO and Government of Canada developed guidelines for the monitoring of sodium intake around the world. These guidelines highlight the importance of monitoring sodium intake, the main sources of dietary sodium, perceptions of sodium and health, and monitoring the effects of sodium reduction strategies (WHO & Government of Canada, 2010). Therefore the purpose of the current study is to develop baseline knowledge of sodium intake in Cambodia. This study aims to develop a stronger understanding of perceptions of sodium, mean intakes, and consumption habits in Cambodia. Additionally, gaining an understanding of the relationship between blood pressure and sodium intake in Cambodia will shed light on the possible adverse health outcomes of sodium intake in the sample population.

Organization of Thesis

The thesis is set out in six chapters. Chapter 2 is an evaluation of common iron fortification and supplementation methods used around the world that was modified from a chapter that was published in a textbook called *Public Health* edited by David Claborn (Ramsay & Charles, forthcoming). The following chapter provides background information on the role of sodium in the human body, sodium intakes around the world today, and the relation between sodium and cardiovascular health. Chapter 4 reports the results of a cross-sectional study that was conducted in Cambodia in August 2013. The study used 24-hour urine samples to assess the sodium intake and excretion in four provinces in Cambodia and compared these results to the WHO recommended sodium intake. A subset of the sample population provided 24-hr food samples that had their composition analyzed, and Chapter 5 outlines the results from these samples. Using meal duplicates it is possible to analyze samples for food composition and develop an understanding of the typical Cambodian diet in the study sites. The final chapter provides discussion and recommendations based on the results of the two studies on sodium intake and excretion.

Chapter 2: Review of Iron Supplementation and Fortification

This chapter is a modified version of the chapter that is forthcoming in the textbook, *Public Health*.

Ramsay, L. C., Charles, C. V. Review of Iron Supplementation and Fortification in Public Health, Prof. David Claborn (Ed.). ISBN 978-953-51-4103-7. InTech; forthcoming.

Introduction

Iron deficiency anemia (IDA) is the most prevalent micronutrient condition globally, with nearly 50% of anemia cases being caused by iron deficiency according to the WHO (de Benoist et al., 2008). While it is a condition that does not discriminate between the developed and developing world, the prevalence is still higher in developing countries. In South-East Asia, the WHO reported that 65.5% of preschool-age children suffer from anemia, and 48.2% and 45.7% of pregnant and non-pregnant women, respectively, also suffer from anemia, which represents the highest prevalence in the world (de Benoist et al., 2008).

As the most prevalent micronutrient clinical problem in the world, it is critical that there is a strong understanding of how to improve iron intake. In many cases this may involve taking iron supplement pills; however, this is by no means the only approach that is currently being used. This chapter seeks to provide background information on ID and IDA, and review a number of current strategies currently being used to address these conditions around the world. This review is by no means exhaustive, but aims to cover a number of studies in each of the major iron deficiency intervention strategies.

Iron Deficiency and Iron Deficiency Anemia

The word anemia is derived from the Greek word □ναιμία *anaimia*, meaning “without blood” (Clark, 2008). Anemia is a deficiency of red blood cells and/or hemoglobin resulting in a reduction of the oxygen-carrying capacity of blood (Loney et al., 2000). An individual with a circulating hemoglobin concentration less than 120g/L is considered to be anemic, but this varies across age and sex based on iron requirement and hemoglobin thresholds, which can be seen in Table 1 (Balarajan et al., 2011; de Benoist et al., 2008).

Table 1 Hemoglobin concentration thresholds (g/L)below which anemia is present

Hemoglobin thresholds used to define anemia	
Age or Gender	**Hemoglobin Threshold (g/L)**
Children (0.5-4.99 yrs)	110
Children (5-11.99 yrs)	115
Children (12-14.99 yrs)	120
Non-pregnant Women (≥15.00 yrs)	120
Pregnant Women (≥15.00 yrs)	110
Men (≥15.00 yrs)	130

Anemia is caused by a long-term iron imbalance resulting in the depletion of bodily iron stores over time (Clark, 2008; Johnson-Wimbley et al., 2011). Iron is a necessary component in the production of red blood cells, and so the absence of sufficient supplies results in decreased hemoglobin production subsequently red blood cell production (Clark, 2008; Johnson-Wimbley et al., 2011; Means, 2013). The focus of this chapter is on IDA, and this is typically a result of inadequate dietary intake, but anemia could also be a result of excessive blood loss from trauma or post-partum hemorrhage, or other diseases such as parasitic infections, malaria, and inherited hemoglobin disorders (Johnson-Wimbley et al., 2011; Means, 2013).

There are several risk factors of IDA, which have resulted in uneven distribution of prevalence around the world, with the highest concentration in developing countries in Africa and Asia (Balarajan et al., 2011). The most commonly reported risk factors of IDA include: poverty (Balarajan et al., 2011; Charles, 2012); local dietary staples with low bio-availability of iron such as rice (Lynch, 2011); genetic hemoglobinopathies (George et al., 2012); consumption of untreated water that may result in a helminth infection (Cotta et al., 2011); sex where typically women are more likely to experience IDA than men (Tengco et al., 2008); low parental educational attainment (Cotta et al., 2011; Tengco et al., 2008; Choi et al., 2011); maternal anemia (Pasricha et al., 2010); and food insecurity (Pasricha et al., 2010). Although the etiology of the condition is complex and multi-layered, the overriding theme throughout all the studies is that the condition is exacerbated by poverty.

Signs and Symptoms of Iron Deficiency Anemia

The signs and symptoms vary among individuals but there are always adverse effects of IDA. Pregnant and postpartum women and young children are most susceptible to ID and IDA because of the high demands for iron during pregnancy and growth (Stoltzfus et al., 2004). These groups also experience some of the most severe symptoms (Stoltzfus et al., 2004).

Symptoms of IDA may include diminished work capacity, immune system dysfunction, neurocognitive impairment, dizziness, fatigue, and pallor (Charles, 2012; Clark, 2008; Means, 2013). Severe anemia can significantly reduce a woman's ability to survive giving birth due to bleeding during and after childbirth (Stoltzfus et al., 2004; WHO, 2014). Additionally, pregnant women are at higher risk of preterm delivery when anemic (Lynch, 2011). For these reasons, anemia is considered to be a major contributor to maternal mortality in the developing world. The WHO reports that anemia is a factor in 20% of maternal deaths (WHO "Micronutrient Deficiencies").

Children are also highly susceptible to adverse effects of IDA and experience additional signs and symptoms if left untreated. Anemic children may experience height and weight disturbances, and interruptions in physical or mental growth (Choi et al., 2011). IDA can affect neurocognitive development, resulting in reduced psychomotor and cognitive abilities in children (Clark, 2008; Horton & Ross, 2003). Neurocognitive development is most often measured on standardized measures of mental development, cognitive function tests, psychomotor scales, and educational achievement tests (Baltussen et al., 2004). Impaired cognitive and psychomotor development may have a causal relationship to future earning potential in children affected by ID or IDA, however studies have not yet been long enough to quantify this (Horton & Ross, 2003).

Iron in the Body

Iron is a critical component in many metabolic functions, particularly in delivering oxygen throughout the body as an essential component of red blood cells. The majority of human iron stores are found in hemoglobin (McLaren et al., 1983). Iron is a component of every human cell and plays a critical role in many biochemical reactions in the body. It is involved in oxygen transportation, energy production, cellular respiration, DNA synthesis and the production of dopamine and serotonin, which are essential to neurotransmission (Charles, 2012; Stoltzfus et al., 2004; Singh, 2004; Theil, 2004). The storage and transportation of iron are highly dependent on red blood cells morphology and the presence of sufficient amounts of hemoglobin. Iron is toxic when too much is stored in the body and may cause tissue damage when the iron capacity has been exceeded (Johnson-Wimbley et al., 2011; McLaren et al., 1983). This problem can be compounded by the very slow rate at which humans naturally lose iron (Conrad & Umbreit, 2000).

Dietary Iron

Dietary iron intake comes in two forms: heme and non-heme. Heme iron, which is more easily absorbed, comes from meat, poultry, and fish. This source of iron is easily absorbed because it is delivered as the stable porphyrin complex and is unaffected by other food components (Theil, 2004). Contrary to this, non-heme iron is more difficult to absorb. It comes from cereals, legumes and some vegetables (Theil, 2004) and comes in two different chemical forms: ferrous and ferric. The chemical form of iron affects the body's ability to recognize and absorb the iron. For example, the chemistry of ferric (Fe^{3+}) is much more dependent on pH levels in the stomach relative to ferrous (Fe^{2+}) iron; ferric iron requires a more acidic environment to be absorbed (Theil, 2004). Thus, consuming vitamin C with dietary iron or iron supplementation can be a critical step to absorbing the maximum amount of elemental iron possible (Johnson-Wimbley et al., 2011; Sobieraj, 2010). Absorption of non-heme iron is further complicated in the presence of polyphenols (e.g. in tea and coffee), phytate (e.g. in whole grains), albumin (e.g. in egg whites), and soy protein, as these are known to inhibit the absorption of non-heme iron (Cook et al., 1981; Hurrell & Egli, 2010; Johnson-Wimbley et al., 2011; Theil, 2004).

Implications of Parasitic Infections and Malaria

Helminths

Infection with soil-transmitted intestinal helminths is a common problem among populations living primarily in rural areas of low-income countries. These parasites are a large, polyphyletic grouping of multicellular organisms that can generally be seen with the naked eye in their mature stages. Helminths typically include roundworm (*Ascaris lumbricoides*), whipworm (*Trichuris trichiura*), and the hookworms (*Necator americanus* and *Ancylostoma duodenale*) (Chandler, 1936). Some estimates suggest that more than two billion people on the planet are infected with one, or more types of helminths, with the highest prevalence occurring where sanitation is poor and water supplies are compromised by being untreated or using contaminated open water ways (WHO, 2012).

Soil-transmitted helminths live in the intestine of an infected person, and are easily spread when a person comes into contact with the feces of an infected individual. This contact might result from the use of gardens, bushes or fields as an open latrine, or could result from the use of human feces as fertilizer that is intentionally sprayed onto crops and deposited into the soil. A new host is established when an uninfected person then unknowingly ingests the eggs of these parasites (Beaver, 1961).

The association between parasite infection and anemia is well known and has been widely documented (WHO, 2010). Primarily, the organisms cause gastrointestinal bleeding resulting in blood loss and lowered hemoglobin values, resulting in anemia. For example, the two major hookworm species *Ancylostoma duodenale* and *Necator americanus*, produce 5000-10,000 and 10,000-25,000 eggs per day, resulting in 0.03 mL and 0.15-0.23 mL blood loss per day, respectively (Shetty, 2010). In addition, some hookworms release anti clotting factors that ensures continuous blood flow and thereby further leads to poor health outcomes (Shetty, 2010). Further, some organisms disrupt nutrient absorption by damaging the mucosal surface of gut, leading to poor absorption of micronutrients (Rosenberg & Bowman, 1982).

Malaria

Malaria, a well-known cause of morbidity and mortality in the developing world, is initiated by infection by parasites of the genus *Plasmodium*. Estimates of the burden of disease suggest that more than 515 million episodes occur annually, and that the disease represents 18% of all childhood deaths in Sub-Saharan Africa alone (Rowe et al., 2006; Snow et al., 2005). Anemia caused by malaria has a multi-factorial pathophysiology with unknown molecular mechanisms (Haldar & Mohandas, 2009). Research has shown that malaria is implicated in both the destruction of red blood cells through hemolysis of both infected and uninfected erythrocytes, but also through the decreased production of erythrocytes in the bone marrow (Haldar & Mohandas, 2009). Malarial anemia is typically normocytic and normochromic with a distinct absence of reticulocytes.

Severe malarial anemia is of public health concern because of the widespread prevalence of malaria in the developing world where access to appropriate healthcare is limited, and because children and pregnant women are the hardest hit by the condition (Lamikanra et al., 2007). Severe anemia caused by *P falciparum*, one species of malarial infection, is responsible for approximately one-third deaths associated with the disease and the areas hardest hit by malaria are coincident with those also with the highest levels of anemia (Haldar & Mohandas, 2009).

Treating Iron Deficiency Anemia

Treating ID and IDA must continue to be a priority of governments, non-governmental organizations, and international aid agencies alike. The ideal approach to addressing ID is a nutritious and well balanced diet that naturally provides adequate iron intake. However, this is not a reality in much of the developing world where subsistence comes from cereals with low iron bioavailability. This generates the need for a cost-effective, simple and easy to administer solution that may be found in supplementation and fortification. Food fortification is widely considered to be the most cost-effective approach to treating IDA, but this is not without its challenges (Lynch, 2011). While in the developed world fortified products are extensive, and often there are government regulations to ensure fortification of common food items (e.g. milk, breakfast cereals, and salt) this strategy may be more difficult to implement in the developing world. Even the smallest price increase in staple products in developing countries may be a deterrent to purchase them (Lynch, 2011). With escalating food prices, which peaked in the 2008 food crisis, households in developing countries were spending large shares of their incomes on food and commonly had to switch to cheaper but less nutritious foods to combat hunger (Brinkman, 2009).

Along with iron supplements there are several fortification approaches are being implemented. Currently, there are three principal types of fortification (Berry et al., 2012): 1) genetically engineered foods with enhanced nutrition (e.g. genetically engineered cereals); 2) fortified products added during food processing (e.g. wheat flour); 3) fortification added at home during food preparation (e.g. multiple micronutrient powders). In general, iron fortification was found to be successful around the world; in a meta-analysis of randomized controlled trials for ID alleviation, 13 studies on iron fortification for women and 41 for children were assessed (Das et al., 2013). These studies included fortification via sodium iron ethylenediaminetetraacetic acid (NaFeEDTA), ferrous sulfate, fortified candies, fortified curry powder, and ferrous pyrophosphate (Das et al., 2013). In these studies, efficaciousness of iron fortificants all showed significant results in improving hemoglobin concentration and reducing anemia in study populations (Das et al., 2013). A separate meta-analysis of 60 studies showed similar results: iron-fortified foods were efficacious in increasing hemoglobin concentrations and reducing IDA in study populations (Gera et al., 2012).

Iron Supplements

The Lancet Series (2013) on maternal and child nutrition reported that during a trial of regular iron supplements in pregnant women they found a 67% reduction of IDA (Bhutta et al., 2013). Additionally, intermittent use of iron supplements was partially successful in reducing IDA (Bhutta et al., 2013). This review also indicated that daily iron supplementation reduced the incidence of low birth weight by 19% (Bhutta et al., 2013). Despite the high success rates of iron supplementation, distribution, cost, and unpleasant side effects become major concerns and make compliance in ID reduction strategies very difficult to navigate. Iron supplementation, often in the form of a pill or liquid, is an expensive option for treating ID and IDA (Lynch, 2011). Although in most jurisdictions women do not bear the costs of iron supplements, the programs are still costly for governments or aid agencies.

Examining the cost-effectiveness of supplementation versus fortification (Baltussen et al., 2004) showed that in the developed world supplementation was more cost-effective due to well-developed distribution chains already in place (Baltussen et al., 2004). But despite the higher overall impact of iron supplementation on a population’s health, fortification is more cost-effective in rural and developing communities (Baltussen et al., 2004).

Fortified Staples

Certain foods can be fortified after harvesting with iron and some can be genetically modified and bred to include more iron. The universal fortification of foods has been promoted as an important strategy to address micronutrient deficiencies around the world. For example, in Canada and the United States it is a legal requirement to fortify wheat flour with folic acid and iron (Berry et al., 2010; Zimmermann et al., 2005). Universal food fortification is viewed to be an efficacious and cost-effective strategy to address IDA (Hotz et al., 2008). Fortification was reported with flour, rice, and millet, depending on what the main staple in specific populations is. Since 48% of a woman's energy is derived from grains such as rice, and noodles in Kandal province, Cambodia (Wallace et al., 2014), the remainder of the current review will be devoted primarily to rice fortification, but will also include some information on wheat and millet fortification.

Rice contains relatively little iron. In fact, most of the iron is present in the coating, which is commonly removed when rice is polished. There are two principal ways of increasing the iron content of rice grains: first, to leave the coat on (not polish the rice) and second, genetic modification of the grain (Slamet-Loedin, 2011). A number of approaches to genetic modification were suggested: (1) adding a gene from soybeans that adds the protein ferritin to rice, (Slamet-Loedin, 2011); (2) adding an edible coating that is high in iron (Mridula & Pooja, 2014); and (3) use a product known as 'Ultra Rice' – this is a simulated rice grain that is high in iron that can be mixed with regular rice (Hotz et al., 2008; Arcanjo et al., 2012).

Fortification of rice improved the health outcomes and iron status of women in different jurisdictions including: the Philippines (Angeles-Agdeppa et al., 2011); Mexico (Hotz et al., 2008); and Brazil (Arcajo et al., 2012). The degree of effectiveness from fortification is related to the acceptance of the fortified rice (Beinner et al., 2009), and the serving size and frequency of use (Angeles-Agdeppa et al., 2011; Hotz et al., 2008; Arcanjo et al., 2012).

Iron fortified wheat flour and maize flour is also used to address ID in communities where these foods make up a large proportion of the diet (Grimm et al., 2012). The WHO supports the fortification of wheat and maize flour and expects that it will be the most effective in reducing ID when it is mandated at the national level (WHO, 2009). Fortified wheat flour was found to be efficacious in improving iron status in women who consumed fortified flour more regularly (Grimm et al., 2012), and in school children with consistent use over seven months (Muthayya et al., 2012).

Iron biofortified pearl millet also has been tested as an approach to address ID in communities of developing countries. A study in Benin found that consuming iron fortified pearl millet can double the absorption of iron in women and may be a highly effective approach to combating ID in millet-eating communities (Cercamondi et al., 2013).

Despite the improvement in hemoglobin concentration in trial communities, there are challenges and limitations to this approach for addressing ID and IDA. Primarily, the cost to households associated with switching to fortified products proved to be a challenge. For example, in the Philippines when the market was flooded with unfortified rice by the government, the population began purchasing the cheaper, unfortified variety rather than the iron-fortified rice that was slightly more expensive, despite the added benefit (Angeles-Agdeppa et al., 2011). Other studies reviewed did not test the possibility of selling fortified products in markets so it is uncertain how this would work in different countries. It is likely that higher costs of fortified rice could be a barrier to the sale of the fortified rice, and this strategy may be difficult to sustain within a competitive market environment.

Golden rice is genetically modified rice that is fortified with vitamin A. The challenges associated with golden rice provide an example of some of the challenges associated with biofortified foods. One of the main issues that golden rice has encountered is protest from anti-genetically activist groups such as Green Peace (Yang et al., 2014). Some countries, such as Thailand, have chosen to avoid allowing biofortified rice crops in the country (Enserink, 2008). The regulation and approval systems that are in place to monitor biofortified foods are time consuming and onerous which resulted in golden rice taking over ten years to be accepted publicly and still undergoes challenges associated with the regulatory systems (Khush, 2012). From the consumers' standpoint a study found that there was not enough positive information available to increase their willingness to pay for golden rice (Depositario et al., 2009).

Micronutrient Powders (MNPs)

Micronutrient powders include any fortified powders that have at least two micronutrients in their composition. These powders are added to food being prepared in the home and then consumed, providing the benefits of the micronutrients they contain (Zlotkin et al., 2001). The distinct advantage of MNPs is that this strategy has the potential to address multiple deficiencies at once.

Sprinkles™, a brand of MNP, were developed as a fortification method by creating a powder with iron and various micronutrients that can be ‘sprinkled’ on foods such as porridge and rice before being fed to children (Zlotkin et al., 2001; Zlotkin et al., 2005). Various studies have shown that this is an effective method of increasing hemoglobin concentration and treating IDA in children (Zlotkin et al., 2005; Ip et al., 2009; Macharia-Mutie et al., 2012; Troesch et al., 2011; Serdula et al., 2013). In Cambodia two studies were conducted that demonstrated a significant decline in IDA among groups of children who were on a daily regiment of sprinkles versus a control group with no intervention (Giovannanini et al., 2006; Jack et al., 2012). A study in Kenya assessed Sprinkles in a more real world setting by selling the product in local markets and found that in this situation they remained efficacious in reducing ID (Suchdev et al., 2012). In India, where IDA is prevalent, the use of MNPs resulted in significantly reduced levels of IDA in children after 24 weeks of treatment (Varma et al., 2007). In a meta-analysis conducted on 17 studies in November 2012, micronutrient powders reduced the prevalence of anemia by 34%, IDA by 57%, and improved hemoglobin concentration (Salam et al., 2013). These results were from studies that occurred in various countries including Ghana, India, Bangladesh, Kenya, Pakistan and Haiti (Salam et al., 2013).

While the randomized clinical trials that were conducted to test various MNPs have been successful, these are all in highly controlled situations. Studies consistently have shown success in improving biomarkers such as hemoglobin concentration and a reduction in anemia prevalence, however the adherence to such programs in a less-controlled environment may differ. Distribution, cultural cooking practices, and cultural perceptions of MNPs may impact the effectiveness of such programs. Micronutrient powders were accepted in many communities by caregivers around the world because they could be incorporated into the regular feeding practices (Macharia-Mutie et al., 2012; Jack et al., 2012). In a study conducted in Bangladesh on sprinkle adherence it was found to be higher with greater hematological improvements in groups who received more flexible instructions for use of 60 Sprinkles packets, rather than being told to use them daily (Ip et al., 2009). Further research into adherence and acceptability would be useful in order to develop a strong implementation strategy for different regions of the world.

Food Fortification Involving Sodium Ferritin

It is possible to fortify condiments such as fish sauce and soy sauce, which are commonly consumed in Asian countries. A study conducted using iron fortified Thai fish sauce showed that iron absorption from ferric sulfate fortified fish sauce added to a meal of rice and vegetables showed positive results (Walczyk et al., 2005). This finding suggests that fortified fish sauce may be an effective solution to combat ID in countries with high fish sauce use. A similar study conducted in Switzerland assessed NaFeEDTA fortified fish sauce and found that it is a potential fortification method to address ID and IDA (Fidler et al., 2003). In Vietnam a study was conducted and found that women using iron fortified fish sauce for six months had higher hemoglobin concentrations by 8.7g/L, on average, compared with women who used non-fortified fish sauce (Thuy et al., 2003). However, there are still challenges associated with fortifying condiments which include a changing of the colour of the sauce due to the addition of fortificants as well as political challenges to monitoring and rolling out this type of initiative (Walczyk et al., 2005; Theary et al., 2013). Although not extensively tested in Cambodia yet, Theary and colleagues (2013) report that the Government of Cambodia is considering legislation to insist that fish sauce and soy sauce should be fortified with iron.

While the cost-effectiveness ratio of iron fortified soy sauce and fish sauce has been positive, these analyses do not include the costs of potential health implications of high sodium diets (Longfils et al., 2008). Sodium is known to cause adverse health problems including hypertension, increased risk of cardiovascular disease and increased risk of stroke (WHO, 2012). It is possible that interventions that promote additional consumption of sodium might have negative health impacts that may outweigh the benefit of increased iron intake (WHO, 2013). In fact, the WHO recommends that "the use of salt as the vehicle for new fortification initiatives other than iodine and fluoride should be discouraged" (WHO pg. 7, 2013).

Taking Advantage of Adventitious Sources of Dietary Iron

Cast Iron Pots

Early studies demonstrated that cooking food in cast iron pots increased the iron content of certain foods and that this iron is bioavailable (Drover & Maddocks, 1975). Researchers believed that promoting the use of cast iron pots could be efficacious in reducing the incidence of ID and IDA.

A laboratory study was conducted in order to assess the impact on the iron contents of green leafy vegetables when cooked in different pots. This study demonstrated that the material of the pot has a significant role in the bioavailable iron in green leafy vegetables, and those cooked in iron utensils had 9% more available iron than those cooked in non-iron pots (Kumari et al., 2004). There is still some debate whether or not the iron that leaches into the food is bioavailable (Sharieff et al., 2008).

The main limitation of using cast iron pots to improve iron status is that there was low acceptability during randomized controlled trials (Geerligs et al., 2002; Geerligs et al., 2003a; Geerligs et al., 2003b). Cast-iron pots were reported to be heavy, rust easily, and required more attention for cooking due being prone to higher cooking temperatures (Geerligs et al., 2002). In one study it was found that participants were selling the cast iron pots in the market in order to supplement low family incomes (Tripp et al., 2010). On the other hand, cast iron pots required less wood for stoves since cooking times were faster, and the pots were considered to be very durable (Geerligs et al., 2002).

Despite increases in hemoglobin concentrations and a reduction in anemia in the trial communities, the use of cast iron pots is not an effective strategy for addressing ID and IDA in most cases. However, in communities where cast iron pots are commonly being used, promoting the continued or increased use could be part of a larger strategy to treat ID (Kapur et al., 2002).

The Lucky Iron Fish™

The *Lucky Iron Fish™,* an intervention based on the same principles of cooking with a cast iron pot, has been shown in a randomized controlled trial to be effective in increasing hemoglobin concentrations by 11.6g/L, and reducing anemia by half in the study population, compared with the control group after 12 months (Charles, 2012). This method involves boiling water or cooking soup with the *Lucky Iron Fish™* (an iron ingot shaped like a common Cambodian fish) for 10 minutes and adding some form of ascorbic acid (citrus juice) (Charles, 2012; Charles et al., 2011).

While the *Lucky Iron Fish™* has potential to be effective in addressing ID it has only been tested on women of reproductive age, and thus it is not certain that it will provide the adequate amount of iron for children. Like other methods of treating ID, additional challenges associated with this approach are predominantly surrounding acceptability and education, as this intervention requires significant behavior change in home practices. As a promising intervention, additional research regarding the acceptability, adherence and cost-effectiveness of this strategy should take place in order to present the *Lucky Iron Fish™* to be included in a national nutrition strategy.

Treating ID and IDA in Cambodia

Giovannanini and colleagues (2006) reported that 63% of infants and children in Cambodia suffer from anemia. Current projects from non-governmental organizations, social enterprises, and aid agencies for addressing ID in Cambodia include: *Sprinkles*™; weekly iron folic acid supplements for women of reproductive age; helminth control; fortified foods such as rice and fish sauce; and the *Lucky Iron Fish*™ (HKI, 2001; Charles et al., 2012). However, the government does not support these programs. The official government stance, as advised by the WHO, is for a supplementation program for pregnant and post-partum women, and thus no official fortification program is currently in place or widely accepted by the numerous government and non-government groups working to address nutrition issues in Cambodia (Cambodia Ministry of Health, 2007). Recent research assessing the possibility of iron-fortified condiments has put fortification on the government's radar and is speculated to result in a change in government legislation (Theary et al., 2013). ID and its associated anemia remains a salient health issue in Cambodia and looking ahead a more multi-faceted approach may be necessary to successfully combat this challenge.

Government Supplementation Program

Table 2 outlines the guidelines that are followed by Health Centres in Cambodia (Cambodia Ministry of Health, 2007).

Table 2 Cambodian government guidelines on iron supplementation for pregnant women over 15 years

Dose	Timing
60mg iron and 400μg folic acid daily	At first contact give 60 tablets
60mg iron and 400μg folic acid daily	At second contact give 30 tablets
60mg iron and 400μg folic acid daily	During postpartum period give 42 tablets at first postpartum contact

Daily iron requirements for women range from 8mg/day for non-menstruating women to 27mg/day for pregnant women (Health Canada, 2005). It is difficult for women to meet iron requirements during pregnancy through diet alone even when their diet is rich in bioavailable iron (WHO, 1998), which is why iron supplementation programs during pregnancy are important for maternal and child health. Pregnant women need 350-500mg of additional iron to maintain iron balance during pregnancy; however, iron stores are typically not high enough to meet the demands of pregnancy, and in developing countries iron stores are severely low (WHO, 2001). High-dose iron supplementation can ensure that women are not iron deficient during pregnancy and improve pregnancy and birthing outcomes (Stoltzfus & Dreyfuss, 1998; WHO, 2001).

Iron supplementation is known to be effective due to the high bioavailability of iron (Kanal et al., 2005; Allen, 2002; Beard, 2000). This is especially important to during pregnancy when iron demands of women are greater, and the risks can be more severe (de Benoist et al., 2008; Beard, 2000). Daily iron supplementation is the most effective strategy at protecting iron stores in women during pregnancy but large doses of iron are also associated with negative side effects (Beard, 2000) such as nausea and constipation. The negative side effects may cause problems with compliance, thus damaging the effectiveness of these programs (Allen, 2002). Currently an urgent need for evaluation on the government’s supplementation program exists. The strategy included National Nutrition Guidelines is not adequate in addressing ID and IDA and should be revised to include multiple approaches to improve the overall reach of the program.

Two informal focus groups were held in Preah Vihear province in February 2014 as a precursor to the proposed formal focus groups that were being developed for the clinical trial of the *Lucky Iron Fish*™. Two villages were visited (Koh Ker and Ker) and approximately 30 women participated in each village. In both villages fewer than 5% of participants visited a health centre during their pregnancy (LC Ramsay, personal observation, 2014). Collectively, the women reported that they were aware that they would have received free iron supplements for during pregnancy and after delivering their child at their health centre. Despite this, women chose (by personal preference or circumstances dictated this) to deliver in their homes with a local midwife from their village. The health centres can be expensive to travel to and are far away from their villages, in the case of the two villages that were visited. The roads used to travel are barely passable, which only becomes more difficult during the rainy season (June – November) (LC Ramsay, personal observation, 2014). The difficult travel distances and conditions suggests that in some parts of Cambodia the government initiatives may not be reaching marginalized populations, the population that demonstrates the greatest need and a more multi-faceted approach should be considered to reach these populations.

Chapter 3: Sodium

Introduction to Sodium Intake

The recommended daily intake of sodium is no less than 230-460mg with an upper limit of 2000mg of sodium per day (Elliot & Brown, 2006; He & MacGregor, 2010). High sodium intake is a common problem around the world, particularly in South-East Asia, where many foods use high volumes of high sodium sauces (e.g. soy sauce and fish sauce), dried salted foods, pickled foods and fermented foods (Yu et al., 1999; Elliot & Brown, 2006). Sodium plays a critical role in the human body, but when consumed in excess has the potential to cause negative health outcomes.

Sodium causes a number of adverse health problems including hypertension, increased risk of cardiovascular disease, and increased risk of stroke (WHO Guidelines, 2012). A reduction in sodium intake reduces systolic and diastolic blood pressure in both adults and children, which may have a significant effect on the prevalence of non-communicable disease (WHO Guidelines, 2012). The WHO recommends that sodium intake should not exceed 2000mg per day, which is equivalent to 5000mg of salt (WHO Guidelines, 2012). The WHO reports that long-term modest sodium reduction may reduce the incidence of stroke by 14% and coronary deaths by 9% for hypertensive people (Salt as Vehicle for Fortification, 2007).

Sodium in the Body

Sodium is involved in a number of critical events in the body (Guyton & Hall, 2010). Fundamentally, sodium is involved in the maintenance of membrane potential and integrity. Membrane potential is the electrical potential difference between the intracellular and extracellular fluid and is measured in millivolts (mV). Sodium's charge and osmotic potential work in tandem with potassium and chloride ions determine intra and extracellular fluid balance and maintain membrane potential (Hodgkin, 1951). The amount of sodium and other cations in the extracellular fluid influences the size of the extracellular space, and higher levels of sodium may result in greater cell volume which can translate to higher blood pressure in some individuals (Preuss et al., 2010). The resulting higher blood pressure often presents complications for cardiovascular health (Karppanen & Mervaala, 2006; Strazzullo et al., 2009; Brown et al., 2009; Bibbins-Domingo et al., 2010; Stamler, 1998; INTERSALT, 1988).

Regulation of Sodium Balance

The human body maintains its plasma sodium concentration within a small optimal range: 138mmol/L – 142mmol/L (Schrier, 2008). The kidney plays an essential role in maintaining the body's sodium balance, and thus regulating the fluid volume (Schrier, 2008). The serum sodium concentration is affected primarily by water intake (induced by feelings of thirst or personal habit of drinking water), but also by insensible loss of fluid (e.g. sweating and breathing) and urination (Reynolds et al., 2006). There is a panoply of factors that monitor and control fluid balance including the critical regulation of sodium (Anderson, 1977; Hall, 1986; Rossier et al., 2002).

Blood pressure and volume and the volume of the extracellular fluid are linked to sodium ions in the blood plasma (Suckling et al., 2012). Moreover, blood sodium concentrations are exquisitely sensitive to dietary sodium (He et al., 2005; Suckling et al., 2012). An increase of sodium in blood plasma can directly raise blood pressure, as well as lead to the increase of extracellular fluid, which can also raise blood pressure (He et al., 2005; Suckling et al., 2012; de Wardener et al., 2004).

Sodium and Cardiovascular Health

High sodium intake is associated with increased rates of stroke and overall cardiovascular disease (Strazzullo et al., 2009; Brown et al., 2009; WHO Guidelines, 2012; Bibbins-Domingo et al., 2010). Conversely, reductions in sodium intake have been associated with significant decreases in the incidence of high blood pressure and related cardiovascular disease (Karppanen & Mervaala, 2006; Bibbins-Domingo et al., 2010; He & MacGregor, 2002). However, the data are not entirely consistent. For example, O'Donnell et al. (2012) argue that local context must be considered, rather than assuming the direct relationship between dietary sodium and high blood pressure. For example, dietary habits differ across populations, regions, and countries. Although the relationship between high salt intake and blood pressure is well documented in North America, people in this part of the world readily have access to fast food and fast food *per se* has been shown to be associated with poor cardiovascular health. In contrast, access to fast food in developing countries is more restricted (Alter & Eny, 2005; Esposito & Giugliano, 2006).

There is also a body of evidence that suggests that there is inconsistency in the data and that under some conditions there is no significant association between sodium intake and increased blood pressure (O-Donnell et al., 2012; Preuss et al., 2010; Cohen et al., 2008). First, overall dietary habits may differ significantly in different populations across countries or regions (O'Donnell et al., 2012). The complexity of the diet and overall nutrition may have an impact on blood pressure, rather than only considering sodium intake (O'Donnell et al., 2012). In North America, where high blood pressure is a documented problem, there is a high-sodium diet, along with affordable access to fast foods and packaged processed foods that are high in sodium. In many less developed regions of the world fast food, which is known to be associated with poor cardiovascular health (Alter & Eny, 2005; Esposito & Giugliano, 2006), is much less accessible. Second, salt sensitivity can be impacted by obesity, ethnicity and hypertensive status, which will impact the relationship between sodium intake and blood pressure (O'Donnell et al., 2012; Luft & Weinberger, 1991; Sanada et al., 2011). A linear relationship between sodium intake and blood pressure may be too simplistic, and in reality it likely is related to overall health, genetics, and other compounding factors such as obesity and smoking, more so than a direct relationship with sodium intake.

A recent study suggests that the current recommendations on sodium intake are controversial and that the optimal range is between 3g and 6g of sodium intake, and below 3g and over 6g is a greater risk for cardiovascular disease (O'Donnell et al., 2014). This study also suggested that the relationship between sodium intake and blood pressure is non-linear, and that the studies that determined the current intake recommendations were too short-term to be conclusive (O'Donnell et al., 2014). Additionally, another study found that lower levels of sodium intake were associated with higher cardiovascular mortality (Stolarz-Skrzypek et al., 2011).

Sodium in the World Today

Salt was first recorded to be produced in China approximately 800 B.C. and since then has been the focus of war, trade, preservation, and cooking (Kurlansky, 2002). Some of the earliest uses of salt included making soy sauce and preserving food such as meats and olives (Kurlansky, 2002). Today, we may have less of a need for salt in preservation for long journeys or cold winters, but it is still used frequently in cooking and processed foods (He & MacGregor, 2010).

An adequately balanced diet that consists of a variety of foods provides approximately 230-460mg of sodium daily which is sufficient for daily needs but with the discovery of salt as a food preservative sodium intake increased greatly (He & MacGregor, 2010). Today, sodium intake exceeds the physiological requirements in most of the world, and in many countries the average daily sodium intake exceeds the upper tolerable limit by more than 2 times (Elliot & Brown, 2006). Some of the highest sodium intake in the world can be found in Akita Prefecture, Japan where on average 10626mg sodium is consumed daily, and the lowest intake is found among the Yanomamo Indians of Brazil who essentially consume no sodium, with a mean intake of 23mg Na/day (Elliot & Brown, 2006; Brown et al., 2009).

A meta-analysis that included 142 research studies helped to demonstrate regional and national differences in sodium intake around the world. This study showed that Central Asia, Asia Pacific and East Asia had the highest sodium intakes for all age groups in the world (Powles et al., 2013). Some of the regions with the lowest sodium intake are East Sub-Saharan Africa, Central Sub-Saharan Africa and Oceana (Powles et al., 2013).

Despite geographic differences in sodium intake, few studies have found any differences in sodium intake across different demographic indicators in the developing world. However, in the developed world studies have found that socioeconomic status and education level are related to sodium intake (Grimes et al., 2013; Ji et al., 2013; Murakami et al., 2009; Tian et al., 1996). In Australia those with lower socio-economic status consume more sodium than those who are wealthier (Grimes et al., 2013). In Britain participants with lower educational attainment consumed significantly more sodium, and participants who work manual jobs who were of lower socioeconomic status also consumed more sodium (Ji et al., 2013). A study conducted in Japan found that women living in lower class neighbourhoods had a higher ratio of 24-hour sodium to potassium, which is consistent with high sodium intake and low potassium intake (Murakami et al., 2009).

In hot climates where people sweat more than in cooler climates, people become acclimatized and only small amounts of sodium are lost in sweat (Fukumoto et al., 1988; Sawka & Montain, 2000). Even in cases where there is high physical activity in hot climates and more sodium is lost through sweat, only small amounts of sodium may be necessary to make up for sodium loss (Coyle, 2007).

Potassium

Along with sodium, potassium is a critical ion in the human body and works with sodium to control the body's electrolyte balance. Potassium and sodium have a critical inter-relationship in the control of cellular and intracellular fluid balance (O'Donnell et al., 2014; Mente et al., 2014; D'Elia et al., 2011).

The INTERSALT study, one of the largest and earliest studies on hypertension, concluded that high sodium intake in conjunction with low potassium intake is a risk factor for increased blood pressure (INTERSALT, 1988). Potassium intake greater than 1.5g is associated with lower risk of stroke and hypertension (O'Donnell et al., 2011). Conversely, a study focused on serum potassium levels found that low serum potassium levels was associated with higher incidence of stroke among older individuals (Green et al., 2002). There is also research that suggests that increasing potassium intake is an important part of the blood pressure reduction strategy, rather than focusing on decreasing sodium intake alone, due to its negative linear relationship with blood pressure and stroke (Khaw & Barrett-Conner, 1987; Tian et al., 1996; O'Donnell et al., 2011; D'Elia et al., 2011).

Chapter 4: Sodium Intake in Cambodia Determined by 24-hour Urine Samples

Introduction

High sodium intake has a number of adverse health effects including hypertension, increased risk of cardiovascular disease, and increased risk of stroke (WHO, 2012). A reduction in sodium intake reduces systolic and diastolic blood pressure in both adults and children, which may have a significant effect on the prevalence of non-communicable disease, including hypertension and coronary heart disease (WHO, 2012). The WHO recommends that salt intake is less than 2g of sodium per day (equivalent to 5g of salt) since even a modest reduction of sodium intake could reduce stroke deaths by 14% (He et al., 2002).

Potassium excretion likely also plays a critical role in determining the presence or severity of adverse health outcomes of a high sodium diet. Potassium, the main ion in intracellular fluid, works in conjunction with sodium to control cellular and intracellular fluid balance, and has been shown to reduce the risk of high blood pressure even when sodium intake is high (O'Donnell et al., 2014; Mente et al., 2014). Consumption of potassium rich foods may also prevent stroke and reduce the incidence of cardiovascular disease and could be promoted as a strategy to preventing these adverse health problems (D'Elia et al., 2011). Foods that are high in potassium include nuts, legumes, fruits, and vegetables (Marinangeli & Jones, 2014).

Demographic indicators can play a role in sodium intake. It has been established that individuals of lower socio-economic status tend to have a higher sodium intake than those of higher socio-economic status in some countries (Grimes et al., 2013; Ji et al., 2013; Murakami et al., 2009; Tian et al, 1996). Tian et al. (1996) showed that among Chinese people there was an inverse relationship between education and sodium intake: as education levels rise, sodium intake decreases. This relationship also was true for the relationship between blood pressure and education (Tian et al., 1996). Studies in Japan and Britain showed that high sodium intake was associated with lower socio-economic status (Ji et al., 2013; Murakami et al., 2009). An analysis of the INTERSALT study showed that sodium intake is particularly high in Asian countries where mean daily excretion is over 4600mg/day (Brown, 2009).

A pilot analysis of a salt intake survey in 2013 in just one community in Phnom Penh, Cambodia found that 70% of adults consume more than 5g of salt per day, which is equivalent to 2g of sodium (Van Maaren, 2013). The mean intake was 7g of salt per day (Van Maaren, 2013). High salt intake, therefore, may represent a very real threat to the health of the nation if these findings are representative of the greater Cambodian population. It is therefore important to understand sodium intake in Cambodia to better understand the association between sodium intake and health.

The principle objective of this study was to establish baseline sodium intake data in a large study population in Cambodia and determine what, if any, factors are associated with sodium intake. This observational, cross-sectional study was completed to collect urine to determine sodium and potassium excretion whilst collecting a description of the typical daily foods that might have high sodium content. A secondary purpose was to determine any associations between sodium excretion and lifestyle including socioeconomic status, physical activity, and blood pressure.

Materials and Methods

Study Site and Population Sample Selection

The cross-sectional study took place in four provinces in August 2013: Kampot, Kandal, Kampong Chhnang, and Phnom Penh. These provinces represent four of the main geographic regions of Cambodia: the coastal region, the Tonle Sap River, the plain region, and urban communities, respectively. Using the WHO Stepwise Approval of Surveillance (STEPS) random sampling tool, communes were selected as Primary Sampling Units (PSU): 23.2% urban and 76.8% rural, which makes up the distribution of urban and rural population as reported in the 2008 Cambodian census. In each PSU two villages, known as Secondary Sampling Units, were randomly selected by probability proportionate to size. In total 46 villages were selected for inclusion in the study and information on sampling in each village can be seen in Appendix 2. Two villages were reselected using the random sampling tool in Kandal province due to roads leading to the villages being flooded during the study time. In each village the village chief provided a list of participants within the age range of the study and participants were chosen randomly from these names by pulling names from a hat. Village members were divided into age blocks and sex prior to random selection to ensure equal sampling from each age bracket and sex. Potential participants were excluded from participating if they knew they were hypertensive in order to have more generalizable results.

The sample size was calculated with a 95% confidence interval, a margin of error of 5% and with an estimation of baseline level of indicators of 50%. Given a non-response rate of 20%, approximately 660 households were approached to determine willingness to participate. The population sample included 552 participants: 275 women and 277 men aged 18-60 years. The population was divided into 6 strata based on age and gender: Women 18-30 (n = 91); Men 18-30 (n = 91); Women 31-44 (n = 91); Men 31-44 (n = 92); Women 45-60 (n = 93); Men 45-60 (n = 94). Twenty-six people recruited to participate in the trial failed to collect urine samples properly and were deleted from the data. This means that the sample size for statistical comparisons was 526 participants. This loss to follow up was anticipated in the original sample size calculations. Previous work has shown that it is not appropriate to include data from urine samples if the complete 24-h urine was not collected (Elliot & Brown, 2007). Therefore, to mitigate the possibility of sample loss, samples with ≤ 500mL were excluded from the analysis, similar to the exclusion criteria used by other researchers (Charlton et al., 2008; Espeland et al., 2001). In the end 44 samples were excluded reducing the overall sample pool to 482 urine samples. The final sample distribution across strata can be seen in Table 3.

Table 3 Sample Information, Age and Province

Name of Province		Age Bracket			Total
		18-30	31-44	45-60	
Kampong Chnnang	Male	10	13	11	34
	Female	10	12	11	33

	Total				**67**
Kampot	Male	18	20	21	59
	Female	19	20	23	62
	Total				**121**
Kandal	Male	34	35	35	104
	Female	34	35	39	108
	Total				**212**
Phnom Penh	Male	13	14	15	42
	Female	11	14	15	40
	Total				**82**
Total	Male	75	82	82	239
	Female	74	81	88	243
	Total	**149**	**163**	**170**	**482**

Data Collection

Demographic, anthropometric, blood pressure, physical activity level, and socio-economic data were collected in a face-to-face interview, which can be seen in Appendix 3. The socioeconomic information was collected using an asset-based approach (Morris et al., 2000). Physical activity was established using the Global Physical Activity Questionnaire (GPAQ) that seeks to collect information about work, traveling to and from places, and recreational activities (WHO GPAQ). An Omron HEM-907XL automated sphygmomanometer (OMRON Corporation, The Netherlands) was used to measure blood pressure by taking 3 readings and taking the average of the systolic and diastolic readings at the time of recruitment. Weight and height were determined using a digital electronic balance and a Harpenden digital stadiometer, respectively. Percentage of body fat was measured using electrical bioimpedance using an OMRON BF306 Body Fat Monitor (OMRON Corporation, The Netherlands) and the mean of three readings was used for each participant.

The current study involved the collection of 24-hour urine samples, as well as a spot urine sample collected on the final morning of data collection. Urine was collected in two 1.5L containers and stored in a cooler with ice in a dark location during the collection period. The spot urine sample was read within 2 hours of urination using Chemstrip 10 pH, density, and abnormal substances test strip. The 24-hour urine sample was tested for volume, sodium, potassium, and creatinine excretion.

All data were collected by trained local enumerators hired by NutriFood Cambodia. The data collectors were trained in interview techniques, body measurement methods, and how to use the equipment for blood pressure and body fat monitor. All potential participants were provided information about the study and what would be required, and had the opportunity to decline participation. Participants who agreed to participate signed an informed consent form, and were also made aware that they had the right to leave the study at any time, and to refuse to answer any questions. Each participant received financial compensation for participation. Results were collected in Khmer, the local language in Cambodia, and translated by NutriFood Cambodia.

Ethics approval was obtained from the University of Guelph Research Ethics Board, Canada, and the Cambodian Research Ethics Board (Appendix 4).

Statistics

All data were analyzed using SPSS for Macintosh version 21 (IBM Corporation, New York). Descriptive statistics were determined for demographic information, consumption habits, and urine analysis results. A Student's *t*-test was performed to analyze differences in 24-hour sodium excretion and potassium excretion from recommended levels. One-way analyses of variance were used to determine mean differences in sodium excretion, potassium excretion and blood pressure between geographic area by province, sex, age category, and diastolic and systolic blood pressure readings. Differences were considered significant at $p<0.05$. Linear regression analysis was used to determine significant predictors of blood pressure and sodium intake.

As described in Morris et al. (2000), the asset-based approach was used to assign a score associated with an individual's socioeconomic status. A questionnaire (Appendix 3) was conducted to determine if the household owned 23 different assets. The asset score was determined by calculating the weight equal to the reciprocal of the proportion of the study households who owned that item. The weighted score for each asset was summed for each household to provide an overall household asset score. This method assigns a higher weight for more rare or luxury items, that not many households have, and lower weights for common assets (Cordova, 2009).

Physical activity levels were assessed using the WHO Global Physical Activity Questionnaire. Time spent doing different intensities of physical activity during work, leisure, and travel were used to calculate metabolic equivalents (METs) for that time.

Results

Urine samples were collected from 526 participants and after removing samples that were not complete, 482 urine samples were included in the final analysis. Information on sample by province, sex, and age can be seen in Table 3.

Sodium Excretion

The mean urinary sodium excretion found in the 24-hour urinary sample of the entire sample population was M=5615.93mg, 95% CI [3377.13, 3854.72]. This mean sodium excretion is significantly greater than the WHO recommended intake of 2000mg of sodium, $t(481)=29.75$, $p<0.001$. Only eight participants had a sodium intake equal to or less than 2000mg. Data divided into 1g increments of sodium intake can be seen in Table 4.

Table 4 Sample Characteristics Based on 1g Increments of Sodium Intake

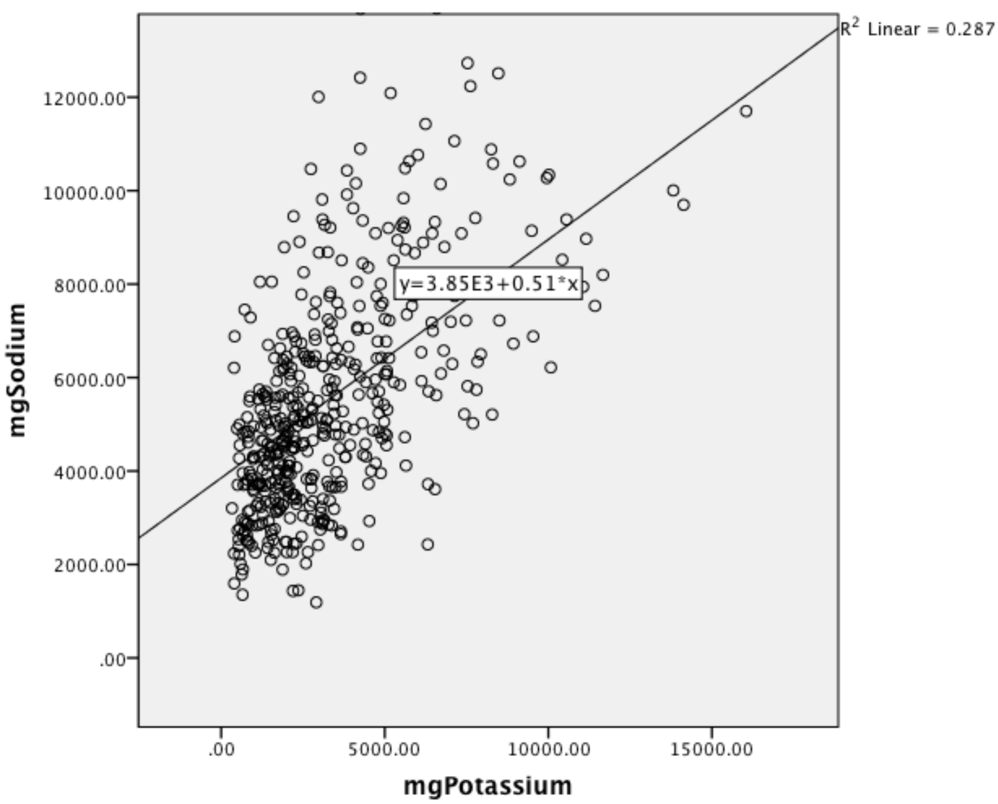
Na Excretion	**<2g**	**2-3g**	**3-4g**	**4-5g**	**5-6g**	**>6g**
n	8	57	80	89	76	172
% men	50%*	52.6%	47.5%	50.6%	50%	48.8%
Avg systolic BP	119.08	120.47	114.18	114.14	116.48	115.3
Avg diastolic BP	81.42	81.04	74.49	76.14	77.8	76.76
% high BP	12.5%	10.5%	7.5%	6.8%	10.5%	7%
% pre-hypertensive	37.5%	21.1%	17.5%	20.5%	25%	23.8%
% High Physical Activity	75%	54.4%	58.8%	59.6%	60.5%	58.7%
% land ownership	62.5%	63.2%	61.3%	65.2%	55.3%	50%
% trying to reduce salt intake	12.5%	38.6%	33.8%	53.9%	44.7%	50%

*Percentages indicate the percentage of participants within the sodium excretion level

Sodium excretion had a positive linear relationship with potassium excretion (Figure 1): as potassium excretion increases by 1 mg, sodium excretion increases by 0.51 mg, p=<0.001.

mg Sodium excretion = 3852.02 + 0.51(mg Potassium excretion)

Figure 1 Regression Analysis for Sodium and Potassium



Demographic Assessment of Sodium Excretion

Sodium intake was not associated with province, sex, age, household size, or educational attainment. Detailed data is not presented.

Consumption of High-Sodium Foods

Salt, MSG, fish sauce, and prahok (Cambodian fish paste) were used by the majority of participants in all groups during cooking; with reported daily use by 99.8%, 99.6%, 94.6% and 93.8% of the sample population, respectively. Not only were these used during cooking, but also 45.3% of participants added additional high-sodium products to their food at the table. On average, the sample population reported that they purchased 2.55kg of salt each month for their household. There were no data on the composition of table salt in Cambodia but in North America table salt, sodium chloride (NaCl), is composed of 40% sodium and 60% chloride (NCCDP, 2014). This suggests that on average households were buying just over 1kg of sodium each month. The average household size in the sample population was 5.02 individuals. High-sodium foods also had an important place in the Cambodian diet; 72.0% and 72.2% of participants reported that they consumed salted fish and salted meat weekly, respectively.

Perceptions of Sodium Intake

There was little understanding of the role, benefits and risks of sodium in the diets in the sample population. When asked what health problems were caused by eating too much salt (and high sodium foods), only 25.1% thought that a high-sodium diet led to high blood pressure, and 3.3% thought that it may result in stroke. Despite a limited knowledge of the health implications of a high-sodium diet, 59.1% of participants said that reducing sodium intake was somewhat important to them, and 7.3% reported that it is very important; however, there were no significant differences in sodium excretion between these groups. In addition, almost half (45.2%) of participants reported to make a conscious effort to reduce their sodium intake. Of this subset of participants, 93.1% tried to use less salt and sauces when cooking, 50% (n=109) did not add salt at the table, and 21.1% (n=46) bought low sodium products. The participants who actively tried to reduce sodium intake did not have significantly different excretion levels as those who do not.

Blood Pressure

Blood pressure results were categorized based on the generally accepted interpretations of blood pressure (<120/<80, normal; 120-139/>80, pre-hypertensive; >140/90 hypertensive). A one-way analysis of variance indicated no significant differences in sodium intake between the three blood pressure groups, $p=0.88$.

The mean systolic blood pressure between men and women were significantly different, $F(1, 479)=15.206$, $p<0.001$. Men, on average, had higher systolic blood pressure (M=119.27, SD=21.47) than women (M=112.33, SD=17.35). There was no significant difference between diastolic blood pressure of men and women: men M=77.5, SD=19.85, and women M=76.55, SD=12.61.

Systolic blood pressure was positively associated with BMI, age, and was lower in females than males. This model accounts for 19.7% of the variation in systolic blood pressure, $p<0.001$. However, sodium excretion was not significantly associated with blood pressure.

Systolic Blood Pressure = 67.89 + 1.78(BMI) + 0.331(age) -7.97(if female)

Socioeconomic Status

The SES weighted scores ranged from 0.00 to 103.56 with a mean score of M=23.18 (SD=15.31) and median score of 19.76. These data were not normally distributed, as assessed by Shapiro-Wilk's test ($p<0.05$).

There was no relationship found between the SES weighted score and sodium intake. However, participants who own land (M=5382.38mg, 95% CI [5072.21, 5692.54]) consumed significantly less sodium than those who did not owned land (M=5928.84mg, 95% CI [5556.67, 6301.02]), p=0.026. Participants who owned land (M=24.77) were significantly wealthier than those who do not own land (M=21.05, p=0.008). A one-way ANOVA and Tukey HSD post-hoc analysis found that participants with no education (M=16.58) were significantly less wealthy than those with secondary education (M=24.58, p=0.038) and than those who had attended college or university (M= 30.61), p=0.027.

Obesity

Based on body mass index (BMI) calculations this study showed that 19.09% of participants were overweight based on a BMI of 25. BMI was not a significant predictor of sodium excretion. On average, both men and women had body fat percentage within the normal range for their sex; men M=23.44% body fat, and women M=26.86% body fat.

Physical Activity

The average METs in this sample was M=7790.45 and the median score was 3640 METs. Using the classification guidelines provided by the WHO it was determined that 58.9% of participants were in the high physical activity group, 23.2% were moderately physically active and 17.8% had low levels of physical activity.

Potassium Excretion

The mean potassium excretion in 24-hours in the sample population was 88.61mmol/day (SD=71.78), which is equivalent to an intake of 3455.79mg/day. A Student's *t*-test confirmed that this was significantly lower than the recommended daily intake of 4700mg/day, $p<0.001$. No relationship was found between potassium excretion and diastolic or systolic blood pressure. Similarly, no significant relationship existed in our data between the sodium potassium ratio and diastolic or systolic blood pressure.

Discussion

In the sample population sodium intake in Cambodian adults was significantly higher than the WHO recommended daily intake of 2g sodium per day. The average sodium excretion in Cambodia exceeded the WHO recommendations by 165%, or 3062g of sodium. More than 98% of participants in the study consumed ≥2g of sodium and 35.5% of participants consumed ≥6g. The major contributors to the high-sodium diet were added seasonings including: salt (2.37g sodium per teaspoon), fish sauce (1.73g sodium per teaspoon), MSG

(1.17g sodium per teaspoon) and prahok (a locally made salty fermented fish paste, about 1g sodium per teaspoon). Cambodia follows the trend of high-sodium diet in the current study as found in other Asian countries (Powles et al., 2013). There were no differences across age bracket for sodium intake, which may be explained by cultural eating habits in Cambodia. Typically, multiple generations of Cambodians live together in a household, and consume food from the same cooking pot. Similarly, when eating at restaurants, food tends to be ordered and shared communally, resulting in people of all ages consuming the same foods, and thus having similar sodium intake.

The Canadian and United States governments recommend a daily intake of 4700mg of potassium per day, and specify no upper tolerable limit due to a lack of suitable data. Though the average intake in the sample population was less than the recommended levels, a positive relationship was found between potassium excretion and sodium excretion. This may suggest that because participants who had the highest sodium intake tended also to have the highest potassium intake, the cardioprotective qualities of potassium had a moderating effect on the negative impacts of high sodium intake.

No relationship was found between sodium intake and blood pressure in the sample population; however, BMI, age, and sex were all significant predictors of blood pressure. The majority (69.5%) of the sample population had blood pressure that fell within the normal range, 22.4% were prehypertensive, whereas 8.1% had hypertension (blood pressure >140/90). While many studies suggest a significant positive relationship between sodium intake and blood pressure, the current results are in line with many other studies in which a significant relationship was not found (Cohen et al., 2008; O'Donnell et al., 2012; O'Donnell et al., 2011; Thomas et al., 2011). The results of the current study support the evidence that sodium intake does not necessarily impact blood pressure, and that there may be no serious clinical implications of the high sodium diet in the study population.

One explanation of the inconsistencies in the relationship between sodium intake and blood pressure is that the overall dietary habits of the population may differ significantly among countries or regions (O'Donnell et al., 2013). In North America, people eat a high-sodium diet, and have access to affordable fast foods and packaged processed foods that are high in sodium. In Cambodia fast food is much less accessible. Such an observation might suggest that the assumed link between sodium and high blood pressure is not as simple as predicted: the presence of fast food diets appears to be correlated more strongly with cardiovascular disease and whilst fast food does contain large amounts of sodium, the assumption that it is sodium alone that predisposes an individual to cardiovascular disease may be too facile (Alter & Eny, 2005; Esposito & Giugliano, 2006). Furthermore, salt sensitivity can be impacted by obesity, ethnicity and hypertensive status, which will impact the relationship between sodium intake and blood pressure (O'Donnell et al., 2013; Luft et al., 1991; Sonada et al., 2011). For instance, in the sample population in the current study only 19.09% were overweight based on their BMI, compared to Canada where 60% of adults are overweight (Stats Canada, 2013). Being overweight or obese are contributing factors to high blood pressure and cardiovascular disease (Mertens & Gaal, 2000; Paugova, 2006), and so it may be the case that differences in average BMI across countries may result in variance in the relationship between sodium intake and blood pressure. Overall, physical activity was high in Cambodia and may contribute to good health: 59% of participants had high physical activity and an additional 23% had moderate physical activity. Moderate levels of physical activity have been shown to reduce blood pressure (Collier et al., 2008; Whelton et al., 2001; Whelton et al., 2002). In the United States and Canada less than 5% of adults adhere to the recommendation of participating in 30 minutes of physical activity per day (Troiano et al., 2008; StatsCan, 2014) and 68% of Canadian adults' waking hours are sedentary (StatsCan, 2014). The low levels of physical activity in North America might contribute to high blood pressure.

Overall, sodium intake exceeded the recommended daily intake in the sample population; however, there was no relationship between sodium intake and blood pressure. On average, the sample population had normal blood pressure, high levels physical activity, and low levels of obesity. These findings suggest that sodium reduction strategies may be of questionable necessity in the regions included in the current study.

Chapter 5: Evaluation of 24-Hour Food Samples in Three Provinces in Cambodia

Introduction

In 2002 the WHO reported that 61% of all deaths in Cambodia resulted from non-communicable diseases (excluding including cardiovascular disease), maternal and perinatal, and nutritional deficiencies (WHO, 2002). It is critical that health researchers seek to understand nutrition and dietary habits in Cambodia in order to address the public health concern that poor nutrition presents. Detailed information on the Cambodian diet is important in understanding the prevalence of nutrition related diseases such as IDA, as well as promoting a healthy, well-balanced diet.

Dietary recall is a common methodology to estimate nutrient intake, including that of potassium and sodium. However, there are challenges with this methodology due to the tendency for participants to under-report or over report intake (Novotny et al., 2003; Livingstone et al., 2004). Sodium consumption is sometimes underreported by 30-50% (Leiba et al., 2004). When results collected from dietary recall are compared with the ‘gold standard’ 24 hour urine sample, the sodium intakes often do not match suggesting that dietary recall is less accurate of a methodology to estimate dietary intakes (Espeland, 2001). Additionally, participants over-estimated potassium intake (Espeland, 2001). The current study chose to mitigate the challenges with dietary recall by employing a different methodology that may be more accurate.

Materials and Methods

Study Site and Population Sample Selection

The study took place in three Cambodian provinces: Kampong Chhnang, Kandal and Kampot. These provinces were selected to represent three of the main geographical regions in Cambodia, which are the plain region, the Tonle Sap River, and the coastal region. Using the WHO STEPS random sampling tool communes and villages were selected as PSUs and SSUs, respectively. Within each village participants were randomly selected from a list of eligible village members that was provided by the village chief.

The sample population included 86 participants: 44 men and 42 women, selected randomly from the three provinces. Table 4 details the sample population by sex and province.

Table 5 Sample Information for Food Sample Analysis

	Kampong Chnnang	Kampot	Kandal	Total
Men	13	7	24	44
Women	11	9	22	42
Total	24	16	46	86

Data Collection

A face-to-face interview was conducted with each participant (Appendix 3) to collect demographic information, dietary habits, anthropometric information, blood pressure, and physical activity information. The questionnaire was written in English then translated into Khmer, the local language, by the research coordinator from NutriFood Cambodia, and reviewed with the translator to ensure the questions remained the same. Experienced local enumerators that were trained by NutriFood Cambodia and researchers from the University of Guelph conducted data collection. The data collectors were trained in interview techniques, body measurement collection, and in how to operate the automated sphygmomanometer and body fat monitor. Upon being visited in their home, participants were given an explanation of the project and asked to sign a consent form if they wished to participate in the study. All participants had the right to refuse to participate, remove themselves from the study at any time, and refuse to answer any questions during the survey. Participants were provided financial compensation for participating, as well as money to account for the additional food that was purchased for the meal duplicates. Answers were recorded in the local language by the data collectors and later translated by staff at NutriFood Cambodia. A member of the research team from NutriFood Cambodia supervised the data collection process.

An Omron HEM-907XL automated sphygmomanometer (OMRON Corporation, The Netherlands) was used to measure blood pressure at the time of recruitment. Weight and height were determined using a digital electronic balance and a Harpenden digital stadiometer, respectively. Percentage of body fat was measured using electrical bioimpedance using an OMRON BF306 Body Fat Monitor (OMRON Corporation, The Netherlands) and the mean of three readings was used for each participant. The Global Physical Activity Questionnaire was used to assess physical activity level based on time spent doing physical activity while working, traveling, and recreating.

Meal duplicate samples were collected from each participant for 24-hours in a plastic container stored in a cooler with ice. Participants were instructed to collect an identical serving of all food and beverages that were consumed within 24-hours. Samples were collected at the end of the 24-hour period and transported to Phnom Penh to be homogenized, frozen at -30°C, and an aliquot of 100g was shipped to the University of Guelph for analysis. The University of Guelph Agriculture and Food Laboratory analyzed the concentration of calcium, magnesium, phosphorous, potassium, sodium, sulpher and iron per one gram of sample using the macronutrients elements panel. The method detection limit of iron was 1.8ug/g.

Statistics

All data were analyzed using SPSS for Macintosh version 21 (IBM Corporation, New York). Descriptive statistics were examined for demographic information, consumption habits, physical activity, blood pressure, and amount of micronutrients in one gram of sample. One-way ANOVA and linear regression were used to determine whether blood pressure changed by levels of minerals. Blood pressure results were categorized based on the generally accepted interpretations of blood pressure (<120/<80, normal; 120-139/>80, pre-hypertensive; >140/90 hypertensive) to be included in an ANOVA. Physical activity was assessed using the analysis guidelines for the Global Physical Activity Questionnaire.

An asset-based approach was used to assign the SES weighted score for each participant. The asset score was determined by calculating the weight equal to the reciprocal of the proportion of the study households who owned that item. The weighted score for each asset was summed for each household to provide an overall household asset score (Morris et al., 2001).

Results

Demographic Information

Table 6 shows data from the demographic survey conducted with each participant. In total 86 samples were included in the analysis; the samples included 52.2% men and 48.8% women. The participants' ages ranged from 18 to 60 years, and the average age of participants was 39.27 years.
Sixty-six percent of participants had high levels of physical activity, and an additional 23.3% had moderate levels of physical activity. The average body fat percentage of men was 19.92% and of women was 25.1%.

The average blood pressure was 116.78/74.33mmHg for men and 112.40/76.29mmHg for women. Most participants had normal blood pressure levels; however, 5.81% of participants had high blood pressure. A one-way ANOVA determined that blood pressure classifications (normal, prehypertensive and hypertensive) were not associated with the concentration of any of the micronutrients.

In an assessment of dietary habits salt, MSG, prahok (Cambodian fish paste), and fish sauce were used daily during cooking: 100%, 98.8%, 95.3% and 90.7% of participants reported using these condiments respectively.

Table 6 Participant Demographic Information

Participant Information	Men	Women	Total

Sex (n)	44	42	86
Mean Age (years)	37.39	41.24	39.27
Some Primary Education (%)	34.09	50	41.9
Mean SES Score	28.16	22.99	25.64
Mean Systolic Blood Pressure (mmHg)	116.78	112.40	114.64
Mean Diastolic Blood Pressure (mmHg)	74.33	76.29	75.29
High Blood Pressure (%)	4.55	7.14	5.81
High Physical Activity (%)	81.82	50	66.28
Moderate Physical Activity (%)	13.64	33.33	23.26
Low Physical Activity (%)	4.55	16.67	10.47
Mean Body Fat (%)	19.92	25.1	22.45

Micronutrient Concentrations

Despite knowing the micronutrient concentrations in the meal samples, it was not possible to translate this into an estimation of daily intake of particular nutrients. An error in collecting the data was made. The volume of each meal from which the samples were taken for analysis was not recorded. It is not possible to know how large the meals were. However, it was possible to determine the elemental components of the diets. Table 7 shows the ug/g of the micronutrients included in the food analysis.

Table 7 Micronutrient Concentrations Descriptive Statistics, ug/g

	N	Minimum	Maximum	Mean	Std. Deviation
Calcium	86	18	2600	177.00	383.92
Magnesium	85	21	420	66.62	51.17
Phosphorous	86	51	1400	237.73	219.36
Potassium	86	40	2600	379.74	321.84
Sodium	86	52	5800	1287.00	884.09
Sulpher	86	48	540	199.55	113.55
Iron	49*	2	28	4.47	4.12

**Only 49 samples had iron levels that were detectable in the analysis, which is of significance and will be discussed in a separate paragraph*

Iron

The method detection limit of the lab where the analysis took place for iron was 1.8ug/g. 43% of participants had iron levels in their food samples that were below the method detection limit. No demographic indicators were associated with iron concentration, $p>0.05$.

Discussion

The current study set out to assess the concentration of different micronutrients in the typical diet in three provinces in Cambodia. Twenty-four hour food samples were collected from 86 participants and analyzed for the concentration (ug/g) of calcium, magnesium, phosphorous, potassium, sodium, sulpher, and iron.

The mean age of participants in the current study was 39.27 years; however, according to the CDHS (2010) 45% of Cambodians are under the age of 20 years. However, the current study only sampled participants aged 18-60 years and is representative of the adult population in Cambodia but not the entire country population. The education profile of participants is similar to that of the CDHS (2010); 41.9% of participants completed some primary education, which is similar to the national proportion. The socio-economic status of the sample population based on assets owned compared with the CDHS (2010) is low. In 2010 22% of Cambodians owned a car or truck, and in the sample population just 4.7% do, which suggests that the participants sampled may own fewer assets than the average level in Cambodia.

The mean concentration of sodium was 1287.00ug/g, which is equivalent to 1.29mg/g of sodium. White rice has approximately 0.1mg/g of sodium. Pringles original potato chips (common in Cambodia) contain 5.36mg/g of sodium. Since rice makes up a major component of the Cambodian diet (Wallace et al., 2014), the high sodium concentration is likely from condiments added during cooking and eating some processed foods.

The analysis found that 43% of the samples had iron levels that were below the method detection limit, and the mean iron concentration of the remaining 57% was 0.0045mg/g. Spinach contains 0.027mg/g of iron. Chicken contains 0.012mg/g of iron. Steamed white rice contains 0.004mg/g of iron. The mean iron concentration in the sample population was very similar to the concentration of iron in 1g of steamed rice, which is consistent with the typical Cambodian diet that is primarily rice.

Cambodia has a high prevalence of ID and IDA and in order to address this problem it is important to have a good understanding of the causes and types of anemia that are present. It is concerning that 43% of samples had iron levels below the method detection limit. If the Cambodian diet is low in iron, this could lead to IDA. There are a number of coexisting factors that increase the likelihood of anemia in Cambodia including concomitant disease (such as malaria and hookworm), high prevalence of hemoglobinopathies, which may exacerbate the danger of low or even absent iron in the diet. Lack of dietary iron in conjunction with other factors that contribute to IDA is a real threat to health in

Cambodia, particularly during life stages that have high iron demands such as pregnancy, pre-menopausal women, and childhood.

Chapter 6: Overall Discussion

Although the original intent of my MSc research project was to assess the efficacy of the *Lucky Iron Fish*™, circumstances beyond my control necessitated that in order to complete my thesis work within the allocated period of time I shift the focus of my project to carry out a baseline study on sodium intake in Cambodia. The principal objectives were: (1) to evaluate sodium intake through measuring 24 hour urine collection and estimating intake based on the food study; and (2) to relate the observed and calculated sodium intake to a variety of factors that might be associated with sodium intake including blood pressure, physical activity, and socioeconomic status.

In total, 482 samples were included in the analysis of 24-hour urine samples. Twenty-four hour urine samples are considered to be the gold-standard for estimating sodium intake because intake is closely linked to urinary sodium excretion (Elliot & Brown, 2006). Sodium intake in Cambodia was found to be significantly higher than recommended daily intake of 2g/day; the mean sodium intake in the sample population is estimated to be 5.6g/day. There were no differences in mean intake across any demographic indicators including sex, province, education, age, and socio-economic status. Landowners consumed significantly less sodium than those who do not own land. Landowners are also wealthier and more educated than participants who do not own land. Similar studies in Britain, Australia, and Japan found similar results; wealthier participants consumed less sodium on average than less wealthy participants (Grimes et al., 2013; Ji et al., 2013; Murakami et al., 2009; Tian et al., 1996).

Though the sodium concentration in the food samples cannot be translated to an estimated daily intake, the comparison of sodium concentration in 1g of white rice and potato chips implies that sodium intake is high. The high sodium diet in the sample population was likely because of the use of high-sodium products in the diet that are added during cooking or consumed often. The results from the qualitative survey confirm this: 99.8%, 99.6%, 94.6% of participants reported the daily use of salt, MSG and fish sauce respectively. Fish sauce is a high sodium product, with 78.51mg of sodium per gram of fish sauce.

The WHO is promoting worldwide sodium reduction strategies based on data that are available that shows a relationship between poor cardiovascular health and high sodium intake (WHO, 2006). However, there are conflicting opinions on the relationship between sodium intake and cardiovascular disease, specifically on the role and negative impact that sodium intake has on blood pressure (O-Donnell et al., 2012; Preuss et al., 2010; Cohen et al., 2008).

In Cambodia it was reported in 2010 that 12.3% of Cambodians were hypertensive, and in the sample population in Chapter 4 just 8.1% were hypertensive. The WHO reported that 14% of all deaths in Cambodia in 2002 were caused by cardiovascular disease. This is approximately half what was reported in Canada in 2010, despite sodium intake being greater in Cambodia than Canada. Statistics Canada reported that in 2008 29% of all deaths in Canada were caused by cardiovascular disease. It was also reported that 19% of Canadians had high blood pressure (Wilkins et al., 2010). Statistics Canada estimates that on average Canadians consume 3.4g sodium per day. The results of the study in Chapter 4 align well with the lower incidence of cardiovascular disease. The results of the current thesis suggest that the high sodium intake in the sample population was not related to blood pressure. In fact, the level of hypertensiveness in the sample population was less than what was anticipated based on national estimates that 11.2% of Cambodians have high blood pressure (Van Maaren, 2013).

Fifty-nine percent of participants had high physical activity, and an additional 23.2% had moderate physical activity. The WHO GPAQ characterizes high physical activity by calculating the metabolic equivalents for time spent doing different intensity levels of physical activity. The WHO recommends that adults reach 600 metabolic equivalents to promote good health, and in the sample population just 14.32% of participants do not reach that threshold. The livelihoods of many Cambodians are physically demanding, such as farming rice fields and taking care of a home. Physical activity levels impact blood pressure: moderate physical activity is proven to lower blood pressure (Collier et al., 2008; Whelton et al., 2001; Whelton et al., 2002). Since physical activity levels in the study sample were relatively high compared to Canada where the majority of adults waking hours are sedentary (Statistics Canada, 2009), physical activity may be mitigating the effects of sodium on blood pressure.

It is likely that the inference that sodium intake results in high blood pressure is too simple and there are other possible explanations and causes of high blood pressure. Possible explanations may shed light on why the results of the current thesis do not represent a positive relationship between sodium intake and blood pressure. Overall dietary differences, such as access to fast food, may explain contradictions in the relationship between sodium and blood pressure. Fast food is high in sodium, but it is also high in fats and low in other healthy nutrients such as potassium (Alter & Eny, 2005; Esposito & Giugliano, 2006), and the overall diet may be more closely linked to blood pressure than sodium alone. Additionally, salt sensitivity is linked to obesity and may change the impact that sodium has on blood pressure (O'Donnell et al., 2013; Luft et al., 1991; Sonada et al., 2011). Being overweight or obese negatively impacts blood pressure (Mertens & Gaal, 2000; Paugova, 2006). In the Cambodian sample from the current thesis, the incidence of obesity and overweight adults were relatively lower than in the Canada. In Cambodia 19.09% of participants are overweight compared to 60% in Canada (Statscan, 2013). Obesity is likely a contributing factor to lower rates of hypertension found in Cambodia.

Another possible explanation of the lack of clinical problems related to high sodium intake exists if potassium intake is considered. In Canada, where sodium intake is related to blood pressure, it was found that mean potassium intake is 2.46g/day (Mente et al., 2014), which is significantly lower than what was found in Cambodia: 3.4g/day. Potassium has a cardioprotective effect when consumed and even in the presence of high sodium intake can control and/or reduce blood pressure (D'Elia et al., 2011; Khaw & Barrett-Conner, 1987; O'Donnell et al., 2011; Tian et al., 1996).

The original intent of understanding sodium intake in Cambodia and the health implications that may exist was to assess the possible outcomes of fortifying fish sauce and soy sauce with iron to treat ID and IDA. The cost-benefit analyses that exist regarding iron fortified fish sauce and soy sauce did not include the possible adverse health outcomes of a high sodium diet (Longfils et al., 2008). My data would suggest that it might not be a concern to fortify fish sauce or soy sauce with iron from the perspective of sodium intake and cardiovascular health. Indeed, almost all of the participants consumed fish sauce daily therefore indicating that fortification of this food will provide a source of iron to most people in Cambodia. In the short term, this may be beneficial; however, as the education level and economic prosperity of Cambodians increase, the fortified fish sauce may not continue to be useful. The current research indicates that participants with land and those with more education are less likely to consume high sodium diets.

The food sample results showed that 47% of the samples had iron levels that were lower than the detectable limit. This suggests that lack of dietary iron is a challenge in the study population in Cambodia.

Overall I believe that the samples used in the current thesis were representative of the adult population in Cambodia. While around 40% of Cambodians are under the age of 20, this study focused only on adults and had evenly distributed participants from the ages of 18-60 years, which is consistent with the demographic information available in the 2010 CDHS. It would be difficult to assert the findings of the current thesis to some provinces that were not included in the study because of great differences in proximity to water, main staples, and wealth distribution. Some provinces in Cambodia have higher proportions of poor people than the provinces that were included in the study (CDHS, 2010). However, for many of the central provinces in Cambodia, and those close to the Tonle Sap or Mekong rivers have similar wealth distributions and the sample population may be representative of these regions.

Limitations

While 24-hour urine samples are considered the gold standard for assessing sodium intake, the collection of the samples was unmonitored and relied on the participants to collect each urination over the collection period. When compared to other methods of assessing sodium intake 24-hour urine samples, despite being difficult to monitor, are more accurate than other methods that rely on participant self-reporting or smaller urine samples (Ji et al., 2012). The limitations of 24-hour urine collection include high participant burden, incomplete samples, and issues with timing 24 hours (WHO, 2007). In Cambodia the use of latrines is not widespread and participants were likely urinating on their farm fields, and around their property making it difficult and burdensome to collect their urine. The study design attempted to control for this with clear instructions on collection, financial incentives for participation in the study, and a follow-up survey to record any problems and report any missing urine so that those samples could be excluded from analysis. Additionally, it is recommended that urine samples that have a volume of under 500mL be excluded from analysis due to the likelihood that they are incomplete (Charlton et al., 2008; Espeland et al., 2001). I am confident that with the measures taken to control for missing urine in samples, as well as the data cleaning to remove any possible incomplete samples that the urine samples included in analysis in Chapter 4 are accurate.

Some of the data collected required participants to recall the frequency and amount of certain foods that they consumed. Dietary recall is known to be a challenging methodology because participants may under or over-estimate the amounts or frequencies of consumption of particular food items (Novotny et al., 2003; Livingstone et al., 2004). Additionally, accurately estimating the specific number of grams or portions consumed is very difficult for most individuals. These data were used only for qualitative purposes to make some estimates of dietary habits in the study population, but not used to estimate the sodium intake.

Finding credible and recent data about cardiovascular health in Cambodia is challenging. The WHO released a report in 2002 about causes of death in Cambodia but has not issued more recent data since then.

In the meal duplicate study discussed in Chapter 5 of the current thesis the volume of the food samples collected was not recorded at the time of homogenization. This was necessary in order to comment on the consumption of sodium and potassium for this study. In the future it may be possible to repeat the food collection in a sample population and calculate an average volume of food consumption in 24 hours. The average volume can then be applied to the food analysis results from the study in Chapter 5.

Future Research

- A longitudinal study to assess cardiovascular heath and sodium intake over a long period of time would be beneficial in the discussion on causality of sodium and cardiovascular disease
- Research sodium concentration in sweat during period of high physical activity to assess sodium demands in hot climates
- Future studies on the efficacy of iron fortified fish sauce should repeat the study from Chapter 4 to provide information on the impact of high sodium intake on cardiovascular health
    - Do cooking habits change in the presence of fortified fish sauce compared to regular use of non-fortified fish sauce? If so, what is the change in sodium intake?
    - How much fish sauce would need to be consumed for the fortification to be considered efficacious, and how much sodium does this correlate to?
- Run similar studies in Vietnam, Thailand and Laos to compare results

Conclusion

The conflicting research on sodium intake by people suggests that the current understanding and popular sentiment of “high sodium contributes significantly to high blood pressure” may be too simple of an explanation. There are multiple factors that should be considered when considering the impact of sodium on health including obesity, physical activity, the effect of living in a hot climate, and the intake of other essential nutrients such as potassium. In the sample population in Cambodia no association was found between high sodium intake and hypertension.

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Appendix 1: *Lucky Iron Fish™* Preliminary Work

<u>Testing the Lucky Iron Fish™ as an iron supplementation intervention in rural Cambodia</u>

Main Protocol

I. Background:

Iron deficiency is more prevalent in the developing world and the largest population affected by anemia (315 million) is found in South-East Asia. Anemia remains a major public health problem in Cambodia, particularly among women and young children. However the causes of anemia in Cambodia have not been thoroughly documented. Some research estimates that approximately 28-50% of the anemia is caused by iron deficiency with hemoglobinopathies also a major factor in the high prevalence. Anemia, a condition of low red blood cells, is primarily caused by deficiency of iron. It's a common condition, but it impedes daily routines by causing exhaustion and lack of focus, or sometimes more extreme lethargy, dizziness, or birth complications. Iron deficiency anemia is impacts negatively on maternal and childhood morbidity and mortality. The consequences of anemia in pregnancy include an increased risk of potentially fatal hemorrhage at and after delivery, premature birth; and low birth weight.

Despite some positive changes in the anemia status of women and children since 2000, anemia prevalence improved little in the five years between the 2005 and 2010. The Cambodia Demographic and Health Survey (CDHS) 2010 found that 55.1% of children under five, 44.4% of women of reproductive age (WRA) and 53% of pregnant women are anemic.

Preventing anemia is a priority for Cambodia and can contribute to achieving Millennium Development Goals 4 and 5. Moreover Cambodia set a target for the reduction in the prevalence of anemia among pregnant women in 2010 at 39% and other groups.

The concept of the *Lucky Iron Fish™* (LIF) emanated from two published studies in Cambodia. The first demonstrated that simply adding a lump of iron to the cooking pot during meal preparation or boiling drinking water boosted levels of iron in the food. However, the results of this first trial were confounded by the source of the water used at different times throughout the study and the initial improvement of iron status seen was not maintained. Based on the results from the first trial, a more extensive study was completed that showed unequivocally that using the fish every day for nine months significantly increases circulating and stored levels of iron in women and reduces anemia by half. Improvement in iron status was maintained for at least 12 months. Adding the fish to one litre of water, slightly acidified with lemon juice, when boiling it to make it safe to drink, provides about 75% of the daily iron requirement of a women of reproductive age. Based on the appearance of the fish, and the self-reported impacts on health, compliance for use of the fish over nine months of the study **increased** to over 90%. The second pilot field trial was carried out in three villages in rural Cambodia. With such a level of demonstrated success, there is an imperative to expand the distribution and use of the fish to as many households across Cambodia as possible.

II. Study Aim:

The aim of this study is to develop a more complex understanding of anemia in Cambodia, as well as to assess the Lucky Iron Fish as a treatment option for iron deficiency anemia and to inform policy and strategy on the prevention and reduction of iron deficiency in Cambodia. With this information this product can be promoted as an inexpensive and effective supplement.

Hypotheses:

H0: There will be no significant changes in anemia prevalence or hemoglobin levels in the presence of use of the Lucky Iron Fish
H1: There will be a significant change over time (0 months, 3 months, 8 months and 12 months) in the group using the LIF
H2: There will be a significant difference between the two treatment groups (those using the LIF and those who are not)

III. Objectives:

- Estimate the prevalence of iron deficiency among women in the study area
- To assess the effectiveness of Lucky Iron Fish^{TM} among women and the level of iron blood level at baseline, and follow up and endline
- To assess the feasibility and acceptability of Lucky Iron Fish^{TM} outside the health system
- To determine other risk factors including smoking and alcohol use on iron deficiency

IV. Methodology:

Study Design
The study has two arms, Control and Treatment. 5 villages will be selected for a control group and another 5 villages will be selected for intervention. Both control and treatment village must have the same socio-economic characteristic.

Location
The study will be conducted in the village under the coverage of Kien Svay Operational District (OD) of Kandal province.

Study population

This study will occur within 10 villages in Kandal Province, Cambodia. The World Health Organization Stepwise Approach to Surveillance will be used to randomly select the primary sampling units (villages) and village chiefs will assist with the random selection of households.

This study aims to include 415 participants, with an anticipated drop out rate of 25%, leaving the study with 332 participants. Sample size was calculated based on information from Dr. Charles study on the changes of hemoglobin levels and anemia prevalence over time. Each randomly selected village will have an even distribution of participants in the treatment group (using the Lucky Iron Fish) and control group. The treatment group will receive a Lucky Iron Fish and clear instructions on product use, as well as asked to cease using any other iron supplements or fortified products. These participants will be encouraged to use the product daily and will receive follow up throughout the year in order to ensure compliance. The control group will not use the fish, or any other iron fortified product.

Sample Selection

a)Selection of village or cluster

The Probability-Proportionate-To- Size (PPS) will be used for the village selection. The list of village from the Cambodian Census 2008 will be used as the base for village selection. 5 villages will be selected for control and another 5 village will be selected for treatment group.

b. Sample size

Using a confident level of 95%, margin error of 0.05, and estimated proportion of 0.3, approximately 415 women will have to be included in the study.

c. Sampling procedures and Interviews

A list of all women age from 15-49 years old in each village will be prepared before the data collection. The list will be used for the random selection of 40 women per village for the interview.

d. Inclusion Criteria:

- Women age from 15-49 years old
- Healthy group
- Participants who permanently live in the target village and who has given their consent to undertake the study.

c. Exclusion Criteria:

- Participants who has been diagnosed or have history of Thalassemia or malaria
- Participants who have been identified with severe anemia(hemoglobin levels less than 70g/)
- Participants who have been regularly using iron fortified products

- Participants who are pregnant or lactating
- Participants who are not able to give the consent

e. Dealing with sampling operational problems

Monitoring non-response:
Non-response is encountered when no respondent is at home or when study subjects refuse to be interviewed. Although some level of non-response is built into calculation of sample size requirements, to the extent that it does occur, it can bias the study results. This is because there are often systematic differences between people who choose to respond and those that do not and these differences may be reflected in the indicators that are being measured. The best way to deal with such possible non-response bias is to minimize non-response to the extent possible. Accordingly, field operational plans should allow sufficient time for follow-up of non-responders during data collection. In case, some of non-respondents still occur after thorough follow-ups by monitoring teams during data collection, it is strongly recommended that they are not replaced.

Procedures for dealing with these problems:
Not at home: When there is no reply from a respondent, inquiries should be made from neighbors as to (1) whether the dwelling unit is inhabited and if so, (2) what time of the residents are usually home. If the dwelling unit is not occupied, no further action is required. If it is, at least one (and better still more) revisit(s) should be made, preferably at the time of day that the neighbor indicated that the residents were usually home.

Refusal: When respondent refuses to be interviewed, at least one revisit, perhaps by another monitoring team member or the team supervisor, should be made. The priority for revisits, however, should be for the not-at-homes.

V. Data Collection

a) *Coordination with Local Authority*.
Project staff will inform Operational Health District and local authority of the study and request permission to conduct data collection within their communities. Project staff will inform local authority exact data and time of the data collection, so that the health centers or village chief could help coordinate and mobilize the participants accordingly. The survey team will work in close cooperation with the health centers and project staff during data collection.

b) Survey and blood collection Teams:
The surveys and anthropometric measurements will be conducted by 11 trained enumerators who each have a number of years' experience working in nutrition research. They will be trained by research directors from NutriFood Cambodia (NFC) and the University of Guelph. NFC will seek collaboration from 3 staff of National Lab of the National Institute of Public Health to assist in blood collection, processing and storage of the bio-specimen.

c) Training of survey teams:
Survey teams will be trained on the survey objectives and methodology, interview techniques, anthropometric and blood collection. After the training the survey teams will conduct a 'field practice to ensure that they understand and know how to ask each question, consistency, observation and feedback before conducting the real data collection". Duration of training will be two-day plus one day for field practice.

d) Data and blood collection:

Data and blood samples will be collected from each participant four times throughout one year as in the following:

- **Baseline data collection:** at 0 months
- **First follow up data collection:** at 6 months after the baseline
- **Second follow up data collection:** at 9 months after the baseline; and
- **Endline data collection:** at 12 months after the baseline.

Two times of blood collection will be conducted at baseline and endline.

Questionnaires Interview: will be used as the tools for face to face interview. Data will be collected from all subjects on their demographic and socio-economic information (household members, educational attainment, housing characteristics and household possessions); consumption habits, smoking and alcohol use. Participants will be asked a series of questions in order to determine their usual physical activity status. This information will be used to calculate estimated energy expenditure. Participants will indicate the length of time spent sleeping, eating, working, etc. during the weekdays and on weekends. Participants will then be categorized as sedentary, low active, active, or very active, according to reference standards from the Institute of Medicine. 10% subjects will be revisited for quality control purpose.

Anthropometric measurement: will be also collected from all subjects. Weight and height will be determined using a digital electronic balance and a Harpenden digital stadiometer, respectively. All data will be collected following WHO norms. Body Mass Index will be calculated from the body weight and height measurements.

Blood sample collection: Total 8 ml of blood will be drawn from each subjects using phlebotomy and the collection will take place in the field, and all samples will be withdrawn by properly trained lab staff. There will be three components to the blood sample collection:

1. Hemocue test: This test requires a finger prick blood sample in order to assess the hemoglobin levels of the participant. If the participant shows severe anemia (hemoglobin levels less than 70g/L) will be sent for immediate medical attention and not be included in the study

2. Blood smear: From the finger that has already been pricked for the hemocue assessment a drop of blood will be placed on a glass sheet in order to collect blood smears from each participant.
3. Venous blood sample, 8mL: Each nurse will use a vein in the participants arm to withdraw an 8mL blood sample. This will be done with proper sterilization and fresh needles for each participant. This blood collection will be combined with a clot-activator (microscopic silica particulate) and placed on ice immediately for transportation.

Storage and transport of blood sample: Blood samples will be transported daily from the field to the National Institute of Public Health (NIPH) on ice. Blood will be stored at the NIPH in Phnom Penh. Samples will be stored at -80 Celsius in order to maintain the integrity of the samples. All specimens will be shipped by air freight and the testing will be outsourced to a lab outside of Cambodia that has the capacity to do all of the aforementioned analyses for laboratory test. Before exporting of bio-specimens, the study will seek authorization from NIPH and Ministry of Health in advance. These specimens will be sent in accordance with international regulations for bio-security of exempt human blood and urine specimens (WHO/HSE/EPR/2008.10) and according to International Air Transport Association (IATA) January 2005 guidelines.

Blood analysis: The following tests will be run on the 8ml sample that is collected from each participant: serum ferritin, hemoglobin, C-reactive protein, serum retinol, serum B-12, alpha-1 acid glycoprotein, as well as a complete blood cell count. Furthermore, in order to assess the status of the red blood cells a blood smear from each participant will be visually analyzed and red blood cells will be counted, measured, and assessed for any abnormal variations. Quality control will be done in order to ensure the validity of the analyses.

VI. Data Entry, data cleaning and storage

A unique identifier will be assigned to each participant. This identifier will not derived from personal identifiers. All electronic data files will encrypted and be stored on password-protected computers and/or secure servers accessible only to members of the research team. Archived electronic data files and any hard copies of data, consent forms, questionnaires or other papers containing data will be stored in locked filing cabinets in locked storage rooms at NutriFood Cambodia. Access to the documents will be limited to the principal investigator and co-investigators.

Data collection and management will be conducted by NFC. The SPSS data builder program will be used to develop the data entry sheet for entering all data collected. After developing the data entry sheet, the data builder program becomes a data entry station for data entry. This program is automatically linked with the SPSS program for analysis. Data analysis will be performed using SPSS 20.00 version.

Quality and consistency of data will be ensured by double entries of data. In order to maintain data quality, the raw data will be reviewed, verified and cleaned before performing data analysis. All irregularities will be crosschecked with the survey teams for clarification and correction, where necessary.

NFC will send de-identified data from Cambodia to the University of Guelph. Data will be sent by email over a password-protected spreadsheet. All co-investigators and research assistants working on the project will have access to the data. Responsibilities concerning privacy and confidentiality will be discussed with the research assistants.

Paper and archived electronic data will be stored in locked filing cabinets in locked research rooms at the University of Guelph 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; CD's will be made unusable).

VII. Reporting and Dissemination of findings

Upon completion of this study, data will be compiled and analyzed. It will be shared with the scientific community at pertinent scientific conferences, and a manuscript of the findings will be prepared for a peer-reviewed nutrition journal. This information will also be shared with the participants in their communities, with the Cambodian National Nutrition Working Group (a committee of Cambodian NGOs and government officials involved in the food security and nutrition sector), as well as the Cambodian Ministry of Health

Comprehensive research reports will be produced and an article will be submitted to a free access peer reviewed journal or to a journal which will be paid to allow for free access. A compilation of the finding will also be provided provincial and national interest group, particular to fine tune program at the community level.

VIII. Significance:

The significance of this research is quite profound in the global health field. Iron deficiency anemia (IDA) is the most common form of anaemia in the world, affecting approximately 3 million people. The LIF has the potential to be a low cost, effective and simple to use product that may alleviate IDA. This product could be useful in treating IDA all over the world, with only a simple change in lifestyle to the things that are often already done daily (i.e. boiling water). In communities where there is low bioavailability of iron in the typical foods this could be a powerful tool for development. The amelioration of IDA has the potential to improve educational attainment, future earning potential and thus the economic status of households and countries.

IX. Ethical consideration:

All respondents will be informed about the purpose and the benefits of this study before conducting the interview or focus group discussion with them. Each respondent is asked to sign a consent form if she agrees to participate in the survey. All information released by respondents is confidentially ensured. Respondents have the right to refuse to participate in the survey or refrain from answering any question they might not want to answer. The monitoring assessment will be conducted only after study approval from the National Ethics Committee and from the individual.

Information Sheet and Informed Consent Form

Introduction

You are being invited to participate in this research study that aims to assess the effectiveness of a health medical device aimed to address iron deficiency anaemia. The Lucky Iron Fish will be given to approximately 215 women, and 200 women without the fish will also be tested. The group that does not receive the Lucky Iron Fish at the beginning of the study will receive one at the end of the study if the results are positive.

We are studying this as a possible treatment method for iron deficiency anaemia. This is considered to be the most common micronutrient deficiency in the world, and is thought to be a serious problem in Cambodia.

The Lucky Iron Fish™ is a product that is currently being produced in Cambodia by local manufacturers and each batch is tested for contaminants at the Royal University of Phnom Penh. A previous study has occurred that has tested the safety of this product as well as the expected health outcomes. This product will not harm you or your family in any way if used as per the instructions provided to you by the research team. It is not possible to overdose on iron in this method of supplementation. The experimental nature of this study comes from examining the extent of the impact rather than the positive effects themselves. It is already known that there are health benefits associated with the use of the Lucky Iron Fish™, however we wish to find more specific information about the level of impact in order to promote this medical device as a strong iron supplement in Cambodia.

Participation

Your participation is entirely voluntary, so it is up to you to decide whether or not to take part in this study. If you wish to take part in the study, you will be asked to provide verbal consent to the trained interviewer and sign this consent form. If you do decide to take part in this study, you are still free to stop at any time and without giving any reasons for your decision. You have the right to skip any question or component of the study that you do not feel comfortable with without consequence. Participation will involve a 20-minute interview, followed by collection of 8mL of blood. Each participant will be visited 3 more times throughout the course of one year and at one more of these visits another 8mL blood sample will be collected by trained nurses.

At any time during this study you are free to withdraw your consent. To do this you may call Mr. Seang Ny (+855 (0)12 700 143) at any time. You may also refuse the follow up visit when a trained interviewer arrives at your household. If you withdraw from the study your data will not be included in the analysis and all records will be destroyed.

Who is conducting the study?

This study is being conducted by researchers from the Department of Biomedical Science at the University of Guelph in Canada, in collaboration with Nutri Food Cambodia.

You may contact Mr. Ny Seang, the Director of Nutrifood Cambodia, at +855 (0)12 700 143 for more information.

The University of Guelph will be the sponsor of this study, and follow the requirements of the Health Canada guidelines: ICH-GCP E6 and TCPS2

Who can take part in the study?
In order to participate in this study, you must:

- be a Cambodian woman
- be aged 18 - 50 years old
- not be pregnant or lactating
- not be taking any iron supplementation or using iron fortified products at the beginning of the study
- be living in Kandal province
- able to give informed consent

What will happen to me if I agree to take part?

You will be contacted by researchers 4 times over the course of 1 year.

Visit 1:

If you agree the researcher will ask you to do the following things:

- If you are in the group that receives the Lucky Iron Fish™ you will be asked to cook soup or boil water with the LIF™ in your pot everyday. You will be provided with clear instructions and information about the safety of the product. Cooking with the LIF™ will not harm your or your family.
- Survey – you will be asked some questions;
    - questions about you, such as your age, and education level;
    - questions about the food you regularly eat; and
    - questions about your activity level, alcohol consumption, and smoking habits
- Measurements – the researcher will take some measurements
    - height – using a tape measure
    - weight - using a scale that the researchers will provide}
    - fat composition – which will be measured using a simple handheld device

- Blood collection: A trained nurse will withdraw 8mL of blood from a vein in your arm. This blood will be stored and sent to a lab to be tested for the following things: serum ferritin, haemoglobin, C-reactive protein, serum retinol, serum B-12, alpha-1 acid glycoprotein, as well as a complete blood cell count.

Risks and Benefits

This survey will take some time away from your work and family, but you will not be asked to do anything that can harm you. There are risks associated with blood withdrawal that include: bleeding, which is common but only a few drops and is not cause for concern; fainting or feeling lightheaded, which is unlikely however the health worker will remain with you until this risk subsides (about 20 minutes); bruising is quite common however will go away in a few days and does not pose any risk to you; and infection which is very unlikely to occur however if it does you should call the number provided and visit your local health centre. There will be no benefits for you. The study is not therapy and researchers cannot diagnose disease. The information learned will help the people who provide health care promote this product to address iron deficiency in Cambodia.

Who will know I took part?

Other people in the village may know that you took part, but only the research team will know the information we collect about you. When we discuss this information, we will do so without using your name.

Your confidentiality will be respected. However, research records and health or other source records identifying you may be inspected in the presence of the Investigator or his or her designate by representatives of Nutri Food Cambodia, Health Canada, and University of Guelph Research Ethics Board for the purpose of monitoring the research. No information or records that name you will be published, nor will any information or records that name you be removed or released without your consent unless required by law.

Will it cost me anything to take part?

The cost to you will be your time, and the small samples of blood that will be withdrawn at two visits.

What will I receive for taking part?

We will provide you with a small gift at each visit during this study; each participant will receive a Lucky Iron Fish (215 at the beginning of the study and 200 at the completion of the study), and $1 will be given at each visit that includes blood withdrawal, and 2000 Cambodian Riel at the other two interview visits. Participation in this study will not tell you about your personal health, will not diagnose disease nor provide treatment. The results of the study will be distributed by the National Nutrition Program, Ministry of Health, Kingdom of Cambodia.

Consent

This study has been explained to you and you have been given the chance to ask questions about taking part in this study. If you have questions you can ask the interviewer, or contact Mr. Ny Seang at +855 12 700 143. You can also contact the Research Ethics Board (Ms. Samnong Phom +855 017 333 459)

I consent to participate in this study.

Printed Name of Subject	Signature/Thumb Print	Date

Printed Name of Person Obtaining Consent	Study Role	Signature	Date

Investigators

Alastair Summerlee, PhD
University of Guelph, Ontario, Canada
a.summerlee@uoguelph.ca
+1(519) 824-4120 x52200

Lauren Ramsay, MSc Student
University of Guelph, Ontario, Canada
lramsay@uoguelph.ca
Cambodia: +855 089 521 429
International: +1 (647) 746-8948

Seang Ny, Director of Nutrifood Cambodia
Phnom Penh, Cambodia
+855 (0)12 700 143

Appendix 2: Chapter 4 Sample Selection by Village

Sample Information by Village

Name of village			Age Bracket			Total
			1	2	3	
Ampel Tuek	Sex of respondent	Male	2	2	2	6
		Female	2	2	2	6
	Total		4	4	4	12
Andoung Preng	Sex of respondent	Male	2	2	2	6
		Female	2	2	2	6
	Total		4	4	4	12
Angk Rumduol	Sex of respondent	Male	2	2	2	6
		Female	1	2	2	5
	Total		3	4	4	11
Angkor Chey Kraom	Sex of respondent	Male	1	2	2	5
		Female	1	2	2	5
	Total		2	4	4	10
Bariveas	Sex of respondent	Male	2	2	2	6
		Female	2	1	2	5
	Total		4	3	4	11
Cheang Toang	Sex of respondent	Male	1	2	1	4
		Female	2	1	1	4
	Total		3	3	2	8
Chey Otdam	Sex of respondent	Male	2	2	1	5
		Female	2	1	2	5
	Total		4	3	3	10
Chheu Teal	Sex of respondent	Male	1	2	2	5
		Female	2	2	1	5
	Total		3	4	3	10
Damnak Trayueng	Sex of respondent	Male	2	1	2	5
		Female	1	2	2	5
	Total		3	3	4	10
Doun Yay	Sex of respondent	Male	2	2	2	6
		Female	2	1	2	5
	Total		4	3	4	11
K'am Samnar Kraom	Sex of respondent	Male	2	2	2	6
		Female	2	1	2	5
	Total		4	3	4	11
Kampong Tnaot	Sex of respondent	Male	1	1	1	3
		Female	2	1	2	5
	Total		3	2	3	8
Kaoh Lot	Sex of respondent	Male	2	3	2	7
		Female	1	2	2	5
	Total		3	5	4	12
Kaoh Thkov	Sex of respondent	Male	0	2	2	4
		Female	1	2	2	5
	Total		1	4	4	9
Kdei Kandal	Sex of respondent	Male	2	2	1	5
		Female	1	2	2	5
	Total		3	4	3	10

Khpob Run	Sex of respondent	Male	2	2	1	5
		Female	1	2	2	5
	Total		3	4	3	10
Koun Satv	Sex of respondent	Male	0	1	2	3
		Female	2	2	2	6
	Total		2	3	4	9
Krasang Mean Chey	Sex of respondent	Male	1	2	2	5
		Female	2	2	2	6
	Total		3	4	4	11
Krol Kor	Sex of respondent	Male	2	1	2	5
		Female	1	2	2	5
	Total		3	3	4	10
La Tuek Trei	Sex of respondent	Male	2	2	2	6
		Female	2	2	2	6
	Total		4	4	4	12
Mlu Meun	Sex of respondent	Male	1	2	2	5
		Female	2	2	2	6
	Total		3	4	4	11
Ou Popol	Sex of respondent	Male	2	1	2	5
		Female	2	2	1	5
	Total		4	3	3	10
Pat Lang Choeung	Sex of respondent	Male	2	2	2	6
		Female	2	2	2	6
	Total		4	4	4	12
Phnom Damrei	Sex of respondent	Male	1	2	1	4
		Female	1	1	2	4
	Total		2	3	3	8
Phum 1	Sex of respondent	Male	4	4	4	12
		Female	1	3	4	8
	Total		5	7	8	20
Phum 5	Sex of respondent	Male	2	2	2	6
		Female	1	2	2	5
	Total		3	4	4	11
Phum 7	Sex of respondent	Male	1	1	2	4
		Female	2	2	2	6
	Total		3	3	4	10
Pong Tuek	Sex of respondent	Male	2	2	2	6
		Female	2	2	2	6
	Total		4	4	4	12
Preaek Kranh	Sex of respondent	Male	2	2	2	6
		Female	2	2	2	6
	Total		4	4	4	12
Preaek Mrinh	Sex of respondent	Male	1	2	2	5
		Female	1	1	2	4
	Total		2	3	4	9
Preaek Samraong	Sex of respondent	Male	1	1	3	5
		Female	2	2	2	6
	Total		3	3	5	11
Preaek Slaeng	Sex of respondent	Male	2	2	2	6
		Female	1	2	2	5
	Total		3	4	4	11

Preaek Ta In	Sex of respondent	Male	2	2	2	6
		Female	2	2	2	6
	Total		4	4	4	12
Preaek Ta Prak	Sex of respondent	Male	2	2	1	5
		Female	1	2	2	5
	Total		3	4	3	10
Reang Chuor	Sex of respondent	Male	2	2	2	6
		Female	2	2	2	6
	Total		4	4	4	12
Sampan Leu	Sex of respondent	Male	2	2	2	6
		Female	2	2	2	6
	Total		4	4	4	12
Sansam Kosal 1	Sex of respondent	Male	2	1	2	5
		Female	2	2	2	6
	Total		4	3	4	11
Srae Ampil	Sex of respondent	Male	0	1	1	2
		Female	1	2	2	5
	Total		1	3	3	7
Stueng	Sex of respondent	Male	2	2	2	6
		Female	2	2	2	6
	Total		4	4	4	12
Ta Chou	Sex of respondent	Male	2	1	2	5
		Female	2	2	2	6
	Total		4	3	4	11
Thmei	Sex of respondent	Male	2	2	2	6
		Female	2	1	3	6
	Total		4	3	5	12
Tnoat Chrum	Sex of respondent	Male	2	2	2	6
		Female	2	2	2	6
	Total		4	4	4	12
Tuol Angkunh	Sex of respondent	Male	2	1	0	3
		Female	2	1	1	4
	Total		4	2	1	7
Tuol Kralanh	Sex of respondent	Male	2	2	1	5
		Female	2	2	1	5
	Total		4	4	2	10
Vimean Troang	Sex of respondent	Male	1	2	2	5
		Female	1	2	2	5
	Total		2	4	4	10
Total	Sex of respondent	Male	75	82	82	239
		Female	74	81	88	243
	Total		149	163	170	482

Appendix 3: Sodium Intake Questionnaire

A SURVEY ON SALT INTAKE IN URBAN, PERI-URBAN AND RURAL CAMBODIA

SURVEY QUESTIONAIRE

CONFIDENTIAL

All information collected in this survey is strictly confidential and will be used for statistical purposes only.

IDENTIFICATION INFORMATION	
GEOGRAPHIC IDENTIFICATION	INTERVIEW RECORD
PROVINCE :___________________ ☐☐	Interviewer's name:________________________
	Signature :_______________Date:____________
DISTRICT :___________________ ☐☐	Monitor Name: ___________________________
	Signature: __________________ Date: _______
COMMUNE :__________________ ☐☐	
VILLAGE :___________________ ☐☐	
CLUSTER NUMBER :	

☐☐ ID HOUSEHOLD : ☐☐ ID OF RESPONDENT: ☐☐☐ Sex of Respondent: ☐ (1=male, 0=female) **INCLUSION CRITERIA:** Do you have: **Hypertension?** ☐ Yes = 1, No = 0 **Diabetes?** ☐ **Renal disease?** ☐ **Have you been prescribed diuretics?** ☐ **Are you pregnant or lactating?** ☐ *If answer to the previous 5 questions is no, proceed. If answer is yes, the participant in ineligible to participate. STOP NOW!*	**Informed consent given:** ☐ Yes = 1, No = 0

DEMOGRAPHICS	
1. How old are you?	☐☐ years old
2. How many people currently live in your household? (i.e., eat from the same cooking pot) in the last 6 months	☐☐ people
3. What is the highest level of school you attended?	1) None ☐ 2) Primary school 3) Secondary school

	4) High school 5) Higher education (college/university) 6) Other e.g. literacy program, vocational or skills training (exclude formal school)
ECONOMIC STATUS	
OBSERVE AND RECORD 4. What is the main material of the roof of the living house	□ 1 = roof of bamboo, thatch, grass, hay, leaves or other temporary materials 2 = roof of wood/plywood, concrete, brick, stone, galvanized iron/aluminum, other metal sheets and asbestos cement sheets, or other permanent materials
OBSERVE AND RECORD 5. What is the main material of the floor of the living house?	□ 1) Natural floor: earth, sand, clay, dung 2) Rudimentary floor: wood, palm, bamboo 3) Finished floor: Parquet or polished wood, vinyl, asphalt, ceramic tiles, cement tiles, cement
OBSERVE AND RECORD 6. What is the main material of the walls of the house?	□ 1 = walls of bamboo, thatch, grass, reed, earth and salvaged materials, hay, leaves, or other temporary materials 2 = walls of wood, plywood, concrete, brick, stone, galvanized iron/aluminum and other metal sheets and asbestos cement sheets, or other permanent materials

7. Does your household have the following:?	Electricity from power line? ☐ A working well? ☐ A working latrine? ☐ A radio? ☐ A television? ☐ (1= yes, 0=No) A mobile telephone? ☐ A non-mobile telephone? ☐ A refrigerator? ☐ A wardrobe? ☐ A sewing machine or loom? ☐ A CD/DVD player? ☐ A generator/battery/solar panel? ☐ A watch? ☐ A bicycle or cyclo? ☐ A motorcycle? ☐ A motorcycle cart? ☐ An oxcart or horsecart? ☐ A car or truck? ☐ A boat with a motor? ☐ A boat without a motor? ☐ Water buffalo? ☐ Cows/bulls? ☐ Horses/donkeys/mules? ☐ Pigs? ☐
8. Does any member of this household own any agricultural land?	1= yes ☐ 0= No
SALT CONSUMPTION	
9. In the food that you eat at home, which of the following do you usually add in cooking?	a) Fish sauce ☐ (1=yes, 0=no)

(check all applied)	b) Soy sauce ☐ c) Salt ☐ d) MSG ☐ e) Knorr/Magi (stock cubes/packet) ☐ f) Brohoc/baok/mam/kapi ☐ g) Rodi	
10. Do you add additional salt or sauce/paste (including dtuk prahok) to food at the table? (please circle)	1) Never ☐ 2) Rarely 3) Sometimes 4) Often 5) Always☐	
11. How many kilos of salt per month does the family eat? (please circle)	1) Less than half a kg ☐ 2) Between half a kg and 1 kg 3) 2-3kg 4) More than 3kg 5) Don't know	
12. Does your household currently use iodized salt? (please circle)	1) Yes ☐ 2) No 3) Don't know	
13. How often do you eat salted fish, salted meat instant noodle and salted egg? (check appropriate answer)	a) Salted fish ☐ b) Salted meat ☐ c) Instant noodles ☐ d) Salted eggs ☐ e) Pickles ☐ f) Kimchi ☐ g) Prahok ☐	1= Daily 2= 3-4 times / week 3= 1-2 times / week 4= Rarely/never
14. What health problems do you think are caused by eating too much salt? (check all that the participant mentions)	1) High blood pressure ☐ 2) Stroke ☐ 3) Heart attack ☐ 4) Blindness ☐ (1=yes, 0=no)	

	5) Stomach cancer ☐ 6) Diarrhea ☐ 7) Kidney disease ☐ 8) Don't know ☐
15. How important to you is lowering the salt/sodium in your diet (please circle answer)	1) Not important ☐ 2) Somewhat important 3) Very important 4) Don't know
16. Do you try to do to reduce your salt/sodium intake	1) Yes ☐ 2) No ⟶ skip to Q18 3) Don't know
17. If yes, what do you do? (1=yes, 0=no)	a) Use less salt and sauces in cooking ☐ b) Don't add salt at the table ☐ c) Buy low sodium salt ☐ d) Don't eat instant noodles ☐ e) Don't eat salted fish ☐ f) Don't eat salted meat ☐ g) Look at the labels packet foods and chose low sodium/salt ☐ h) Don't eat fast foods (KFC, Lucky Burger, etc.) ☐ i) Don't eat processed foods ☐
18. What is the recommended amount of salt you should be eating to be healthy? (circle)	1) Less than 10g (2 tsp) ☐ 2) Less than 5g (1 tsp) 3) Less than 2g (1/2 tsp) 4) Don't know
SMOKING & ALCOHOL USE	
19. Do you currently smoke cigarettes?	1= yes ☐ 0= no (if no skip to Q21)
20. Yesterday, how many cigarettes did you smoke?	☐☐cigarettes
21. Do you currently smoke or use any other type of tobacco?	1= yes ☐ 0= no
22. Have you ever consumed an alcoholic drink?	1= yes ☐ 0= no

23. Do you currently drink alcohol? (1=yes, 0=no)	1= yes 0= no (if no skip to Q25) ☐
24. How often do you drink alcohol? (circle)	1= Daily 2= 3-4 times / week 3= 1-2 times / week 4= Rarely/never ☐
PHYSICAL ACTIVITY	
Next I am going to ask you about the time you spend doing different types of physical activity in a typical week. Please answer these questions even if you do not consider yourself to be a physically active person. ***Think first about the time you spend doing work. Think of work as the things that you have to do such as paid or unpaid work, study/training, household chores, harvesting food/crops, fishing or hunting for food, seeking employment. [Insert other examples if needed]. In answering the following questions 'vigorous-intensity activities' are activities that require hard physical effort and cause large increases in breathing or heart rate, 'moderate-intensity activities' are activities that require moderate physical effort and cause small increases in breathing or heart rate.***	
25. Does your work involve vigorous-intensity activity that causes large increases in breathing or heart rate like [carrying or lifting heavy loads, digging or construction work] for at least 10 minutes continuously?	1 = Yes 0 = No (if no skip to 28) ☐
26. In a typical week, on how many days do you do vigorous- intensity activities as part of your work?	Number of days: ☐☐
27. How much time do you spend doing vigorous-intensity activities at work on a typical day?	Hours ___________: Mins __________
28. Does your work involve moderate-intensity activity that causes small increases in breathing or heart rate such as brisk walking [or carrying light loads] for at least 10 minutes continuously?	1 = Yes 0 = No (if no skip to 31) ☐
29. In a typical week, on how many days do you do moderate- intensity activities as part of your work?	Number of days: ☐☐
30. How much time do you spend doing moderate-intensity activities at work on a typical day?	Hours _________ : Mins _________
The next questions exclude the physical activities at work that you have already mentioned. Now I would like to ask you about the usual way you travel to and from	

places. For example to work, for shopping, to market, to place of worship.	
31. Do you walk or use a bicycle (pedal cycle) for at least 10 minutes continuously to get to and from places?	1 = Yes ☐ 0 = No (if no skip to 34)
32. In a typical week, on how many days do you walk or bicycle for at least 10 minutes continuously to get to and from places?	Number of days: ☐☐
33. How much time do you spend walking or bicycling for travel on a typical day?	Hours ____________ : Mins ____________
The next questions exclude the work and transport activities that you have already mentioned. Now I would like to ask you about sports, fitness and recreational activities (leisure).	
34. Do you do any vigorous-intensity sports, fitness or recreational (leisure) activities that cause large increases in breathing or heart rate like [running or football,] for at least 10 minutes continuously?	1 = Yes ☐ 0 = No (if no skip to 37)
35. In a typical week, on how many days do you do vigorous-intensity sports, fitness or recreational (leisure) activities?	Number of days: ☐☐
36. How much time do you spend doing vigorous-intensity sports, fitness or recreational (leisure) activities on a typical day?	Hours _________ : Mins _________
37. Do you do any moderate-intensity sports, fitness or recreational (leisure) activities that cause large increases in breathing or heart rate like [running or football,] for at least 10 minutes continuously?	1 = Yes ☐ 0 = No (if no skip to 40)
38. In a typical week, on how many days do you do moderate-intensity sports, fitness or recreational (leisure) activities?	Number of days: ☐☐
39. How much time do you spend doing moderate-intensity sports, fitness or recreational (leisure) activities on a typical day?	Hours _________ : Mins _________
The following question is about sitting or reclining at work, at home, getting to and from places, or with friends including time spent [sitting at a desk, sitting with friends, travelling in car, bus, reading, playing cards or watching television], but do not include time spent sleeping.	
40. How much time do you usually spend sitting or reclining on a typical day?	Hours _____ : Mins _____
PHYSICAL MEASURMENTS	
41. Height and Weight	Height ☐☐☐.☐ cm

*If a difference of 0.5, record a third measurement	Height □□.□□ kg Height □□□.□ cm Weight □□.□□ kg Weight □□□.□ cm Weight □□.□□ kg
BLOOD PRESSURE	
42. Device ID number	□□□
43. B.P. Reading 1	Systolic (mmHg): □□□ Diastolic (mmHg): □□□
44. B.P. Reading 2	Systolic (mmHg) □□□ Diastolic (mmHg) □□□
45. B.P. Reading 3	Systolic (mmHg) □□□ Diastolic (mmHg) □□□
BODY FAT COMPOSITION	
46. Device ID number	□□□
47. B.F. Reading 1	Percentage □□.□
48. B.F. Reading 2	Percentage □□.□
49. B.F. Reading 3	Percentage □□.□

Appendix 4: University of Guelph Research Ethics Approval

UNIVERSITY of GUELPH	**RESEARCH ETHICS BOARD – Natural, Physical, and Engineering Sciences** **REB-NPES** Certification of Ethical Acceptability of Research Involving Human Participants

APPROVAL PERIOD:	**September 26, 2013 to September 26, 2014**
REB NUMBER:	**13JL021**
TYPE OF REVIEW:	**Delegated Type 1**
RESPONSIBLE FACULTY:	**Summerlee, Alastair (a.summerlee@exec.uoguelph.ca)**
DEPARTMENT:	**Biomedical Sciences**
SPONSOR(S):	**University of Guelph, Faculty Discretionary Fund**
TITLE OF PROJECT:	**Salt intake in urban, peri-urban and rural Cambodia**

The members of the University of Guelph Research Ethics Board have examined the protocol which describes the participation of the human subjects in the above-named research project and considers the procedures, as described by the applicant, to conform to the University's ethical standards and the Tri-Council Policy Statement, 2nd Edition.

The REB requires that you adhere to the protocol as last reviewed and approved by the REB. The REB must approve any modifications before they can be implemented. If you wish to modify your research project, please complete the Change Request Form. If there is a change in your source of funding, or a previously unfunded project receives funding, you must report this as a change to the protocol.

Unexpected events or incidental findings must be reported to the REB as soon as possible with an indication of how these events affect, in the view of the Responsible Faculty, the safety of the participants, and the continuation of the protocol.

If research participants are in the care of a health facility, at a school, or other institution or community organization, it is the responsibility of the Principal Investigator to ensure that the ethical guidelines and approvals of those facilities or institutions are obtained and filed with the REB prior to the initiation of any research protocols.

The Tri-council Policy Statement, 2nd Edition, requires that ongoing research be monitored by, at a minimum, a final report and, if the approval period is longer than one year, annual reports. Continued approval is contingent on timely submission of reports.

Membership of the Research Ethics Board: B. Beresford, *Community Member*; F. Caldwell, *Physician;* K. Cooley, *Alt. Health Care;* D. Dyck, *CBS*; D. Emslie, *Physician (alt);* G. Holloway, *CBS (alt);* J. Knapman, *Grad Rep (alt);* S. Logan, *Grad Rep (alt);* A. Niel, *OVC (alt);* B. Nonnecke, *CPES;* A. Papadopoulos, *OVC;* L. Peterson, *Community Member (alt);* B. Power, *Community Member;* R. Regan, *Legal (alt);* L. Spriet, *CBS (alt)*; J. Srbely, *CBS (alt),* D. Stacey, *CPES (alt.);* S. Sutherland, *Legal;* L. Vallis, *CBS (alt);* K. Wendling, *Ethics;* J. Whitfield, *Graduate Rep*

Approved:
per
Chair, REB-NPES

Date: ______________________