Happy Fish: A Novel Supplementation Technique to Prevent Iron Deficiency Anemia in Women in Rural Cambodia

by

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ABSTRACT

HAPPY FISH: A NOVEL IRON SUPPLEMENTATION TECHNIQUE TO PREVENT
IRON DEFICIENCY ANEMIA IN WOMEN IN RURAL CAMBODIA

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Maternal and child undernutrition are a significant problem in the developing world, with serious consequences for human health and socio-economic development. In Cambodia, 55% of children, 43% of women of reproductive age, and 50% of pregnant women are anemic. Current prevention and control practices rely on supplementation with iron pills or large-scale food fortification, neither of which are affordable or feasible in rural Cambodia. In the study areas, 97% of women did not meet their daily iron requirements.

The current research focuses on the design and evaluation of an innovative iron supplementation technique. A culturally acceptable, inexpensive and lightweight iron ingot was designed to resemble a fish species considered lucky in Khmer culture. The ingot, referred to as ‘try sabay’ or ‘happy fish’, was designed to supply iron at a slow, steady rate. Iron leaching was observed in water and soup samples prepared with the iron fish when used concurrently with an acidifier. More than 75% of daily iron requirements can be met with regular use. Its use in the common pot of soup or boiled water provides supplementation to the entire family.
The effectiveness of the iron fish was investigated in a randomized community trial involving 310 women in rural Cambodia. Blood samples were taken at baseline and every three months thereafter, over a 12-month trial period. Significant increases in hemoglobin concentrations were observed in women allocated an iron fish when compared to controls throughout the study, with an endline difference of 11.6 g/L. Significant improvements in serum ferritin concentration were observed at 9 months (6.9 ng/mL) and endline (30.8 ng/mL) in women who used an iron fish regularly when compared to the control group. Overall, use of the iron fish led to a two-fold reduction in the prevalence of anemia. The supplement was used daily by 94% of the households at the end of the trial.

The study highlights the acceptability and effectiveness of a fish-shaped iron ingot as a means of improving dietary iron content. It offers a promising, simple solution to iron deficiency anemia if the project can be scaled-up for use throughout the country.
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LIST OF ABBREVIATIONS

CDHS: Cambodian Demographic and Health Surveys
CRH: Corticotropin releasing hormone
CRP: C-reactive protein
DALYs: Disability-Adjusted Life Years
Dcytb: Duodenal ferric reductase
DMT1: Divalent metal transporter 1
DRI: Dietary reference intakes
EPO: Erythropoietin
FPN1: Ferroportin
FU: Follow-up group
Hb: Hemoglobin
HCP1: Heme carrier protein 1
ICP-OES: Inductively coupled plasma optical emission spectroscopy
ID: Iron deficiency
IDA: Iron deficiency anemia
IDD: Iodine deficiency disorders
MDS: Multidimensional proximity scaling
NFU: Non-follow-up group
NPNL: Non-pregnant and non-lactating
PK1: Preak Khmeng Village 1
PK2: Preak Khmeng Village 2
PWLW: Pregnant and/or lactating
RDIC: Resource Development International Cambodia
SF: Serum ferritin
SFe: Serum iron
SES: Socioeconomic status
TfR: Transferrin receptor 1
TT: Tuol Trea Village
USD: United States Dollars
VAD: Vitamin A deficiency
WHO: World Health Organization
CHAPTER 1: INTRODUCTION

Despite the focus on poverty reduction spearheaded by the United Nations and numerous civil society organizations, malnutrition, hunger, and food insecurity are still affecting large portions of the global population, with serious consequences for human and economic health and development. Women of reproductive age and children are particularly burdened, and malnutrition during pregnancy and childhood can interfere with physical and mental development of children, and can increase the risk of maternal mortality and morbidity. Undernutrition, including micronutrient deficiency, underweight, stunting and wasting, is the underlying cause of “3.5 million deaths, 35% of the disease burden in children younger than 5 years, and 11% of the total global DALY’s (Disability-Adjusted Life Years)”.

In 2008 a consortium of development economists gathered in Copenhagen to assess the most significant threats to global socioeconomic development. Using cost:benefit analysis and other econometric methodology, the Consensus prioritized those problems which should be quickly, feasibly, and effectively addressed to end suffering on a grand scale. The elimination of micronutrient deficiencies, including iron deficiency and anemia, was identified as one of the most important interventions in order to advance global welfare today.

Prevention and control of iron deficiency and related iron deficiency anemia has been largely reliant on two techniques: widespread distribution of oral iron supplements and/or iron fortification of food staples. In many parts of South East Asia, the latter has yet to show success, and iron pills remain the only way of confronting the challenge. Iron pills have a
number of undesirable side effects, are costly, not easily administered to infants and children, and represent an unsustainable way of supplementing iron. Innovative, cost-effective, accessible and long-lasting treatment and prevention options are needed. Anemia is a persistent problem worldwide and it is not going away – not even at a slow rate like many nutritional problems – and it is time to put effort and money into exploring novel ways to confront the challenge. With all of the rhetoric over the Millennium Development Goals and the priority identified by the Copenhagen Consensus that iron deficiency should be dealt with, it is time for innovative and evidence-based action.

1.1 Research motivation

The Kingdom of Cambodia is a nation that has seen many years of conflict, political and economic instability, and food insecurity. Beginning in 1970, following a civil war that culminated in the rise to power of the Khmer Rouge, the country entered a decade that would see the starvation, torture, and systematic murder of millions of its own citizens. By the end of decade, the Vietnamese had gained control of the impoverished state leading to a period of low intensity conflict and seclusion on the global stage.

Finally, in 1991 the United Nations, for the first time in the institution’s history, entered the country and took over governance and economic provision, a period that would last until 1998 when the first election of modern Cambodia was held. Since this time, the country has taken steps in the long and difficult process of rebuilding the physical, social and human capital that was destroyed by decades of instability. Much progress remains to be made, however, it is threatened by the rule of a kleptocracy that has not been accountable to its people. Yet, in
Cambodia economic growth is visible, there is a renewal of relationships across the region and beyond, and a slow but steady normalization of life is eminent, all which may permit the development of effective public health nutrition policies.\(^5\,^7\)

In 2009, Cambodia developed the first-ever National Nutrition Strategy that is meant to guide nutrition policy until 2015.\(^8\) The Strategy aims to provide a clear focus and long term direction for the addressing maternal and child under nutrition, and recognizes the need for a concerted effort between government, civil society, and multilateral assistance agencies. Specifically, the strategy highlights the need for a reduction of key micronutrient deficiencies for young children and women.\(^8\) The strategy targets a reduction in anemia for children under 5 years of age from 62% in 2005 to 52% in 2010, and finally to 42% in 2015. Anemia in women of reproductive age is to decrease from 44% in 2005 to 32% in 2010, and finally to 19% in 2015.\(^8\) Similarly anemia during pregnancy is targeted for a reduction from 57% in 2005 to 39% in 2010 and finally to 33% in 2015.\(^8\) The results of the most recent Cambodian Demographic and Health Survey indicate that not a single target was met for 2010, and if effective nutritional interventions are not established it would seem unlikely that the 2015 goals will be reached.\(^9\)

Currently, numerous nongovernmental organizations, together with the government and the United Nations, work to improve access and adherence to iron nutritional interventions for high-risk groups in Cambodia. With a focus on oral iron supplements and with only limited success in the fortification of culturally relevant food vehicles, the situation remains grim. Cambodia has one of the highest burdens of anemia in the region, and when compared to worldwide estimates it is clear that anemia is a critical public health problem.\(^3\) There is a real
and sustained need for a novel supplementation technique that is affordable, acceptable, effective and that can be widely distributed immediately.

In the mid-1990s, research into the use of adventitious iron sources to supplement an otherwise deficient diet began. Randomised controlled trials in Ethiopia, Brazil, and Malawi demonstrate that the use of a cast-iron pot for cooking leads to improved blood parameters and decreased prevalence of iron deficiency anemia in women and children.\textsuperscript{10-12} Though iron pots do leach iron into food, the acceptability of this supplementation technique appears limited. Geerligs and colleagues reported that a number of problems exist with iron cooking pots, including: aesthetic and health concerns regarding rusting; increased weight and decreased quality when compared to alternative pots; ease with which the pot can be cleaned, with pots made from different metals perceived as being easier to clean; and a perceived change in taste and appearance of food when left in the pot overnight.\textsuperscript{13} Aluminum cooking pots are widely used throughout Cambodia so research into the development of an alternate method of providing a source of adventitious iron was therefore needed.

In keeping with the iron pot research, I worked with a local non-governmental organization, Resource Development International Cambodia (RDI), to design and manufacture an iron ingot which families could put in their cooking pot to enhance the nutritional content of the food. The research and development stage would span nearly four months, but ultimately I persevered and the result is a safe and efficacious iron source, designed to resemble a local species of fish which is readily recognizable and is considered to be somewhat ‘lucky’ in Khmer folklore—an important component of the design, which would ultimately improve compliance
to the regime and allow for an enhanced acceptance at the household-level. Following this time spent largely in the laboratory, I began the evaluation phase of the ‘iron fish’ project in several communities throughout rural Cambodia. The findings of this project, together with a review of global burden of anemia and an anthropological look at nutritional knowledge and practices in Cambodia, are presented in this thesis.

1.2 Contextual framework

In the spring of 2008, I moved to Cambodia with the intention of staying for three months. Three and half years later, here I remain with close ties and an ever-lasting love for the culture, language, food, and people. When I began my fieldwork in community health and nutrition in rural areas, looking broadly at the prevalence of micronutrient deficiencies and parasitic infection, I had no idea that I would stumble upon a project that would present the opportunity to actually affect change for the better. After observing incredibly high rates of anemia in women of reproductive age throughout rural Kandal Province—in some villages nearly 90% of the women displayed signs and symptoms of anemia together with abnormal circulating blood iron—I began to question the effectiveness of existing control practices and started to investigate the use of adventitious iron sources.

1.3 Research objectives

The goal of the current research was to design, develop, and evaluate a novel supplementation technique – an iron fish – as a means of adding iron to an otherwise deficient diet. To my knowledge, this research is the first of its kind in Cambodia to examine the use of adventitious iron as a means of ameliorating and preventing iron deficiency and associated
anemia. The research also represents the first study of its kind to use iron ingots as a means of improving iron content in the longer term.

The key objectives of this research were as follows:

1. Design a culturally acceptable, inexpensive, safe, sustainable, and effective iron ingot for use in non-iron cooking pots as a way to improve nutritional content of the food/water.

2. Investigate the effectiveness of an iron ingot at maintaining both stored iron (as measured by serum ferritin levels) and circulating iron (as measured by hemoglobin levels) and hence reducing anemia over the longer-term in a rural setting through both a small-scale pilot study and larger randomized community trial.

3. Determine whether or not educational follow-up visits to study participants in the follow-up intervention group during the first few weeks of the intervention have an effect on compliance to the supplementation regime compared to those that receive a fish only, without nutritional education.

4. Investigate both the total iron content and the theoretical bioavailability of the iron that is leached from the ingot in a laboratory setting, using authentic food samples.

5. Understand the women’s nutritional status and food-related knowledge, attitudes and practices in rural Cambodia.
6. Better understand the burden of anemia and multifaceted nature of the disease both globally and within Cambodia.

1.4 Thesis organization

This thesis follows a manuscript style format including published papers. The copyright agreements for these paper and permissions to include these articles in this thesis are found in Appendix I. The thesis consists of nine chapters, including the current introduction:

Chapter 2. Iron Deficiency Anemia: A Public Health Problem of Global Proportions

This chapter provides a literature review on the problem of anemia in the global context. Included in the chapter is a biomedical description of the physiological importance of iron in the blood and the production and functioning of hemoglobin, together with a discussion of the functional consequences of iron deficiency and anemia, and finally an examination into the epidemiological and etiological aspects of iron deficiency anemia.14

Chapter 3. Anemia in Cambodia: Prevalence, Etiology and Research Needs

This chapter expands and focuses the discussion of iron deficiency and anemia, and includes a country-specific investigation of the epidemiology and etiology of the related conditions in Cambodia.15
Chapter 4. An Investigation of Women’s Nutritional Status and Food-Related Knowledge in Rural Cambodia

This chapter presents the findings of field research conducted in rural Cambodia using a mixed-methods approach to better understand how Khmer women make foods choices and their understanding of ‘good’ and ‘bad’ nutrition.

Chapter 5. Iron Deficiency Anemia in Rural Cambodia: Community Trial of a Novel Supplementation Technique

This chapter presents the findings of a pilot study that was conducted to assess the cultural acceptability and effectiveness of the iron fish at improving hemoglobin and serum iron status in a rural village in Cambodia.\(^{16}\)

Chapter 6. Iron Content of Cambodian Foods When Prepared In Cooking Pots Containing An Iron Ingot

This chapter details a laboratory study of the total iron content of various food and water preparations using the iron fish. Analytical food science measures were used to determine the extent of iron leaching, as well as to determine the most appropriate food/water vehicle. The findings of this analysis were then used to determine the theoretical bioavailability of the iron that is leached from the iron fish, and the ability of the ingot to supply daily recommended dietary intakes of iron was investigated.\(^{17}\)
Chapter 7. Anemia in Cambodia: A Cross-Sectional Study of Anemia, Socioeconomic Status and Other Associated Risk Factors in Rural Women

Chapter 7 presents the findings of a baseline nutritional and health assessment in one commune in Kandal Province prior to beginning a randomized community field trial of the iron fish. The study examines meat consumption, wealth and education in relation to iron deficiency and anemia.

Chapter 8. “Try Sa’Bay”: Iron Fish for the Amelioration of Iron Deficiency and Anemia in Rural Cambodia, a Randomized Community Trial

This chapter presents the findings of a 12-month long randomized controlled trial in several communities in rural Cambodia. The study investigated the long-term effectiveness of the iron fish as a means of improving nutritional status among women who were allocated an ingot compared to the women in the control group. The effect of educational follow-up visits to enhance compliance was also investigated.

Chapter. Conclusions and Recommendations

The final chapter brings together the findings of the thesis research and provides recommendations regarding the use of the iron ingot throughout rural South East Asia. Future research needs are also discussed.
1.5 References


high malaria prevalence: a randomized trial. Tropical Medicine and International Health 8: 310-315.


CHAPTER 2: IRON DEFICIENCY ANEMIA: A PUBLIC HEALTH PROBLEM OF GLOBAL PROPORTIONS

2.1 Introduction

Iron deficiency anemia (IDA) is the most common micronutrient disorder in the world, negatively affecting the health and socio-economic wellbeing of millions of men, women, and children.\(^1\) According to the World Health Organization (WHO), IDA constitutes a significant public health problem requiring immediate attention from governments, researchers and healthcare practitioners.\(^2\) Iron deficiency (ID) is inherently associated with poverty, and is thus particularly prevalent in the developing world where the problem is often exacerbated by limited access to appropriate healthcare and treatment.\(^3\)

Iron deficiency and IDA result from a long-term negative iron balance, culminating in diminished or exhausted iron stores.\(^4\)-\(^6\) Iron is an element found in every cell, and is involved in numerous biochemical reactions in the body, and is associated with oxygen transport and storage, energy production, DNA synthesis, and electron transport.\(^7\),\(^8\)

Although the etiology of IDA is multifaceted, it generally results when iron absorption cannot meet iron requirements for any number of reasons, including insufficient intake of iron due to poor quantity and/or quality of diet, impaired absorption or transport of iron, or chronic blood loss due to secondary disease.\(^2\)

Consequences of IDA are devastating: inhibited growth, impaired cognitive development, poor mental and motor performance, reduced work capacity, and an overall decreased quality of life.\(^9\)-\(^14\)
Prevention and control is typically achieved through iron fortification of food staples like flour, rice, and pasta, and/or through provision of iron supplements most often in iron pill or, more recently powder/sprinkle form.\textsuperscript{1,6,15,16} Although iron supplements are widely available and fortified foods constitute a major component of the diet in the developed world, access is limited in the developing world and cost if often prohibitive.

National, regional and global efforts to combat the problem of iron deficiency and IDA have garnered momentum in recent years, but the prevalence does not appear to be decreasing considerably and the disorder remains a severe global public health problem. The current review will provide a general summary of the problem, touching upon physiological aspects related to iron and hemoglobin, the etiology and epidemiology of IDA, and current prevention and control measures.

\textbf{2.2 Defining iron nutritional status}

Iron deficiency exists when there are no mobilizable iron stores, resulting from a long-term negative iron balance and leading to a compromised supply of iron to the tissues.\textsuperscript{17} Iron status can be considered as a continuum: the ideal stage is normal status with fluctuating amounts of stored iron within defined ranges; this is followed by iron deficiency, characterized by the absence of measurable iron stores; next, iron-deficient erythropoiesis shows evidence of a restricted iron supply in the absence of anemia; finally, the most significant negative consequence of ID is anemia.\textsuperscript{18}

Anemia in general is characterized by a decrease in number of red blood cells or less than the normal quantity of hemoglobin. Iron deficiency anemia is determined by the expected
normal range of hemoglobin in a population, and is defined as existing in an individual whose hemoglobin concentration (Hb) has fallen below a threshold lying at two standard deviations below the mean for a healthy population of the same demographic characteristics, including age, sex and pregnancy status. 

Anemic conditions can result from a myriad of causes that can be isolated, but more often than not co-exist. These causes include hemolysis with malaria and other infectious diseases, enzyme deficiencies, a variety of hemoglobinopathies, and other micronutrient deficiencies. That said, the most significant contributor to the onset of anemia worldwide is iron deficiency, and thus the terms ID, IDA, and anemia are often falsely used interchangeably. IDA represents the most severe form of iron deficiency, resulting in corresponding alterations in iron indices and recognizable signs and symptoms. Currently, the World Health Organization accepts that generally a little less than 50% of all anemias can be attributed to iron deficiency.

2.3. Biochemical and physiological importance of iron in the blood

2.3.1 Human iron metabolism

Iron is important in the formation of a number of essential compounds in the body, including but not limited to hemoglobin, myoglobin, and other metalloproteins. Most well nourished adults in industrialized countries contain approximately 3 to 5 grams of iron, of which about 65% is in the form of hemoglobin. The remaining iron in the body is in the form of myoglobin, other heme compounds that promote intracellular oxidization, or is stored as ferritin in the reticuloendothelial system and cells of liver hepatocytes and bone marrow.
Typically men have more stored iron than women, as women are often required to use iron stores to compensate for iron loss through menstruation, pregnancy, and lactation.\textsuperscript{20}

2.3.2 Dietary iron sources

In food, two basic forms of iron exist: non-heme (inorganic) and heme (organic).\textsuperscript{20,21} In an average diet, 90\% of total dietary iron content is non-heme in nature, while heme iron constitutes the remaining 10\%.\textsuperscript{23} Heme iron is highly bioavailable, and present in meat, fish, and poultry. In contrast, non-heme iron is not as readily bioavailable as absorption is greatly influenced by diet composition.\textsuperscript{24} Enhancers, such as ascorbic acid, and inhibitors, such as phytates and polyphenols, significantly affect inorganic iron absorption.\textsuperscript{25,26} Although total iron content in a meal is an important consideration, it is crucial to appreciate the overall composition of the meal, taking into account these known enhancers and inhibitors of absorption.

2.3.3 Dietary iron absorption

Dietary iron, digested from food and/or supplements is absorbed by the mature villus enterocytes of the duodenum and proximal jejunum.\textsuperscript{27} Non-heme and heme iron are absorbed via different pathways, though currently there is a limited understanding of the pathway(s) used by heme iron.

Transport of non-heme iron (in ferrous form) involves crossing the apical membrane of enterocytes, a process that is mediated by a non-specific divalent metal transporter (DMT1).\textsuperscript{28,29} Because much of the iron that enters the gastrointestinal tract is in the oxidized or
ferric form, reduction of the dietary iron prior to uptake involves a duodenal ferric reductase (Dcytb) that is centralized in the apical membrane of enterocytes.\textsuperscript{30}

In contrast, regarding the understanding of heme iron absorption, it was long thought that heme molecules bind to an apical membrane protein and the combined product is absorbed intact. Upon detection of heme carrier protein 1 (HCP1), our understanding has improved.\textsuperscript{31} HCP1 is a polypeptide belonging to a superfamily of transporter proteins, and has nine transmembrane domains by which heme iron is taken up. Though the mechanism is unclear, research has shown that by altering gene expression in animal models, heme absorption can be enhanced or limited by overexpressing or silencing HCP1 genes, respectively.\textsuperscript{31}

The export of iron into the blood is facilitated by the transmembrane protein ferroportin 1 (FPN1).\textsuperscript{32} The exact mechanism by which FPN1 functions is unclear, though it is thought to be facilitated by the ferroxidase activity of a membrane bound oxidase called hephaestin.\textsuperscript{33,34}

After moving into the plasma, iron binds to transferrin and is transported by the blood to sites of use and storage.\textsuperscript{35} Cellular iron uptake is mediated by transferrin receptor 1 (TfR)-mediated endocytosis.\textsuperscript{33} Once inside the cell, iron is either incorporated into iron proteins (usually as heme) or stored as ferritin.\textsuperscript{36}

**2.4 Regulation of iron homeostasis**

Since uncovering the peptide hormone hepcidin in 2000, our understanding of the way in which iron homeostasis is achieved has evolved.\textsuperscript{37,38} Hepcidin, a hormone that is produced
and predominately expressed in the liver, appears to be the master regulator of iron homeostasis in humans and other mammals.\textsuperscript{39}

When iron levels are high, several regulatory molecules including hemochromatosis gene product, hemojuvelin and transferrin receptor 2, increase hepatic hepcidin expression, in turn stimulating downstream molecular pathways. With up-regulation of hepcidin expression, iron levels are effectively regulated by binding to FPN1, which is found on the surface of iron storage cells. When iron levels are high, hepcidin causes internalization and degradation of FPN1, leading to decreased iron release from iron storage cells and a reduction in intestinal iron uptake.\textsuperscript{40} In addition, hepcidin may also play a role in negatively regulating DMT1 and Dcytb, which are involved in intestinal iron absorption; currently, the mechanism and extent of control is unknown.\textsuperscript{41}

In situations where iron requirements are increased, during periods of increased erythropoietic activity, anemia and hypoxia, the down-regulation of hepcidin expression is observed, though the mechanism is not clear.\textsuperscript{40,42,43}

\textbf{2.4.1 Formation of hemoglobin}

Hemoglobin is an allosteric protein with primary function of binding and transporting of oxygen in the blood to tissues in order to meet metabolic demands.\textsuperscript{44} Synthesis of Hb involves a series of complex steps occurring in the erythrocytes, with production continuing through the early phases of the development and maturation of red blood cells.\textsuperscript{45} The coordinated production of \textit{heme}, the group that mediates reversible oxygenation, and \textit{globin}, which is responsible for protection of the heme group during transport, is required during synthesis.\textsuperscript{46}
Fully functional hemoglobin molecules consist of four globular protein subunits, each made of a protein chain that is tightly associated with a non-protein heme group. The first step in the synthesis of Hb is the binding of succinyl-CoA (formed during the Krebs cycle) with glycine to form a pyrrole molecule. Next, four pyrroles combine to form protoporphyrin IX, which subsequently binds with iron to form the heme molecule. Each heme molecule then combines with a ribosomal-derived long polypeptide chain called a globin, forming a globular subunit of hemoglobin called a hemoglobin chain. Lastly, four Hb chains are loosely bound to produce a whole hemoglobin molecule. The most common form of Hb in adult humans, hemoglobin A, is a combination of two alpha and two beta chains arranged into a set of alpha-helix structural segments connected in a globin fold arrangement.

### 2.4.2 Reversible oxygenation of hemoglobin

Aerobic metabolism is critically dependent on maintaining normal concentrations of Hb, and the protein’s ability to combine with oxygen in a reversible manner is essential for normal physiological functioning. Oxygen binds with Hb in the lungs during respiration and is later released in peripheral tissue capillaries in the form of molecular oxygen where the gaseous tension of the molecule is much lower than in the lungs. This is a cooperative process as the binding affinity of hemoglobin for oxygen is increased by the oxygen saturation of the molecule.

In addition to hemoglobin’s ability to bind oxygen, the protein can also bind with carbon dioxide and carbon monoxide, though not in a cooperative manner. In the presence of carbon monoxide, hemoglobin’s ability to bind with oxygen is hampered as both gases compete
for the same binding site with a much greater binding affinity for carbon monoxide than oxygen.\textsuperscript{55} As a result, small amounts of carbon monoxide can dramatically reduce the oxygen transport in the body and carbon monoxide poisoning can ensue.\textsuperscript{54} On the other hand, hemoglobin’s ability to bind carbon dioxide is a necessary process to allow for removal of carbon dioxide and by-products from the system. Because carbon dioxide occupies a different binding site on the hemoglobin molecule, this type of ligand binding is allosteric in nature.\textsuperscript{53,56}

2.4.3 Physiological control of hemoglobin levels

The primary factor regulating the production of hemoglobin is tissue oxygenation. The peptide hormone erythropoietin (EPO), responding to a feed-back mechanism measuring blood oxygenation, is synthesized in times of decreased tissue oxygenation within 24 hours of the stimulus.\textsuperscript{57} EPO release triggers erythrocyte production in the bone marrow in an effort to achieve homeostasis of tissue oxygenation.\textsuperscript{58} As erythrocyte production increases, transferrin from plasma directly from diet and/or from iron stores enters the erythroblasts of bone marrow and is delivered to the mitochondria where heme synthesis occurs, thus inducing the formation of hemoglobin.\textsuperscript{58}

2.5 Functional consequences of iron deficiency and anemia

2.5.1 Cognitive development

Over the past three decades a large number of studies on the relationship between iron status and cognitive development have been conducted, often with varying results.\textsuperscript{59} While an association between IDA and impaired cognitive development has been reported, research that
takes into consideration the multi-diseased state common among individuals with IDA is needed.\textsuperscript{59}

Experiments employing animal models have demonstrated a key role for iron in brain development and function.\textsuperscript{60-64} Iron-containing enzymes and hemoproteins are necessary for normal human development. Several processes, including neurotransmission, myelination, synaptogenesis, and dendritogenesis, require a sufficient supply of iron. Iron deficiency disrupts these processes, and this disruption may lead to a variety of neurodevelopmental effects that usually do not respond to iron replenishment.\textsuperscript{59}

In humans, the majority of research has focused on developmental and behavioural effects of ID on infancy. Delayed psychomotor development, cognitive performance, and social/emotional functioning have been observed in numerous studies.\textsuperscript{59,65}

Observational research suggests that children who suffered from IDA early in life demonstrate inferior academic performance once they begin formal schooling, when compared to their non-anemic classmates. In Costa Rica, an experiment conducted on school children that were born at full-term and who were free of other health problems (other than moderate iron deficiency) demonstrated the necessity of iron for normal cognitive development. Over time, these children showed delayed motor development, repeated grades more often, and experienced more anxiety and depression, when compared to a non-iron deficient cohort.\textsuperscript{66} Upon entering school, these children attained lower scores on intelligence and other cognitive performance tests, despite controlling for socioeconomic factors that may have acted as confounders.\textsuperscript{67} The long term affects of iron deficiency on intellectual development during
infancy suggest a reduction of 1.73 IQ (intelligence quotient) points per every 10 g/L drop in hemoglobin concentration.\textsuperscript{68}

The detrimental effects of iron deficiency have been ameliorated with iron supplementation. Randomized controlled trials of various iron supplements show improvements in cognitive development, including motor,\textsuperscript{69} social-emotional,\textsuperscript{70} and language outcomes.\textsuperscript{71,59}

\subsection*{2.5.2 Resistance to infection}

The role that iron deficiency plays in decreased immune response has been reported in both animal and human studies.\textsuperscript{72} Leukocytes (neutrophils, in particular) appear to have a reduced capacity to ingest and neutralize microorganisms,\textsuperscript{9,73,74} while mitogen-stimulated lymphocytes exhibit a decreased ability to replicate.\textsuperscript{75} Additionally, depressed T-cell responses have been widely documented, with the depression proportional to the severity of iron deficiency.\textsuperscript{73,74,76,77} Observations of reduced morbidity from infectious disease following treatment regimens such as iron supplementation and food fortification programs have been reported, further implicating a role for iron in immune response.\textsuperscript{78}

\subsection*{2.5.3 Working capacity}

Anemia has long been known to impair work performance, endurance, and productivity.\textsuperscript{79} Studies in developing countries in South America,\textsuperscript{79,80} East Africa,\textsuperscript{81,82} and Sri Lanka\textsuperscript{83} report a near linear relationship between ID and work capacity. Piecework labourers, including rubber tappers and tea pickers, are often examined as experimental models to
demonstrate productivity gains following iron supplementation. In Indonesia and Sri Lanka, substantial gains in productivity were observed following treatment of IDA.\textsuperscript{83,84} A separate investigation conducted in China revealed that production efficiency could be increased by 14% with every 10 g/L increase in hemoglobin concentration in response to iron supplementation to treat IDA.\textsuperscript{85}

A meta-analysis of 29 studies demonstrated a strong causal effect of severe and moderate IDA on aerobic work capacity in animals and humans.\textsuperscript{86} The presumed mechanism for this effect is reduced oxygen transport and reduced cellular oxidative capacity due to tissue iron deficiency.\textsuperscript{86,87} In laboratory and field trials, iron deficiency and IDA at all levels of severity also appears to affect energetic efficiency\textsuperscript{14,85} and endurance capacity.\textsuperscript{88,89} Conversely, iron supplementation has been shown to improve endurance and aerobic work capacity in iron-depleted humans.\textsuperscript{90-92}

### 2.5.4 Maternal mortality

Two meta-analyses drawing upon the same published studies reported a relationship between ID and maternal mortality. In a 2001 paper, Brabin et al. suggested that there is an association between a higher risk of maternal mortality with severe anemia.\textsuperscript{93} Stoltzfus and colleagues, using a methodologically different analysis, corroborated these findings, suggesting that the risk of maternal mortality increased with decreasing hemoglobin levels, though not in a linear manner. Causal evidence for the role that mild or moderate anemia may play in maternal mortality is lacking.\textsuperscript{68}
In spite of these findings, a causal link between iron deficiency and mortality related to pregnancy and childbirth (i.e., maternal mortality) remains unclear due to methodological concerns. To date there have been no large scale, placebo-controlled, prospective interventions to test the effect of iron supplementation on maternal mortality as large sample sizes would be required and it is considered unethical to withhold treatment from pregnant, anemic women. In addition, research in this field often does not take into consideration other possible causes of anemia and maternal mortality, such as concurrent micronutrient deficiencies, infectious disease, and other related conditions. For this reason, better observational data that controls for confounders are required.

2.5.5 Preterm delivery and growth

A negative correlation between maternal IDA with length of gestation is well established. There are currently two widely accepted biological mechanisms that explain this phenomenon. One theory suggests that anemia (leading to hypoxia) and iron deficiency (which increases serum nor-epinephrine concentrations) induces maternal and fetal stress, ultimately leading to stimulation of the production of corticotropin-releasing hormone (CRH). Elevated CRH is a major risk factor for preterm labour, pregnancy-induced hypertension, eclampsia, premature rupture of the membranes, maternal infection (leading to yet more CRH synthesis), and increased fetal cortisol production (inhibiting longitudinal growth of the fetus). A second theory suggests that iron deficiency may increase oxidative damage to erythrocytes and the fetal-placental unit.
Maternal iron deficiency with and without anemia is also strongly associated with low birth weight and impeded growth.\textsuperscript{68} While infants born at full-term normally have sufficient iron stores, infants have high iron requirements and the diets offered to infants in the developing world are frequently inadequate in terms of satisfying the iron requirements for growth. Although iron in breast milk is highly bioavailable, maternal iron reserves are depleted after 4-6 months of feeding, thus infants commonly develop iron deficiency and IDA if the diet is not altered to include a readily absorbable source of iron.\textsuperscript{103}

Iron supplementation of infants appears to ameliorate the problem of impaired growth. A number of studies conducted in Indonesia,\textsuperscript{104} Kenya,\textsuperscript{105} Bangladesh,\textsuperscript{106} and the United Kingdom\textsuperscript{107} provide evidence that iron supplementation of iron deficient children leads to improved growth.

\textbf{2.5.6 Heavy-metal absorption}

Iron deficiency may also result in an enhanced ability for heavy-metal uptake, leading to heavy-metal poisoning. Iron deficiency is strongly associated with an increased absorption capacity, resulting in the uptake of divalent heavy-metals like lead, cadmium, mercury and arsenic from the environment.\textsuperscript{108} Heavy metal poisoning is a particular concern in children, as impaired cognitive development and irreversible physical and mental disability can result.\textsuperscript{109,110} For this reason, prevention of iron deficiency is important, predominantly in areas where exposure to heavy metals is a high risk.
2.6 Prevalence and epidemiology

2.6.1 Prevalence of iron deficiency and iron deficiency anemia

Globally, nearly two billion people are classified as anemic. The majority of those affected live in developing countries where the problem is exacerbated by limited access to inadequate resources and appropriate treatment. Currently there are no figures specifically for IDA, but it is widely accepted that approximately 50% of all cases of anemia are caused by iron deficiency. While the extent to which IDA is a problem in women and children has been widely documented, data on the prevalence of anemia in adolescents, men, and the elderly are scarce.

The level of hemoglobin concentration in the blood is used as an indicator to estimate the prevalence of anemia. Hemoglobin values that indicate the threshold for IDA have been published by the WHO and are widely acknowledged (Table 2.11.1).

Worldwide, the prevalence of anemia is highest in non-industrialized nations where prevalence is three to four times higher than developed countries (Table 2.11.2). Africa, Eastern Europe and the Western Pacific have a large burden of disease, with over 1 billion people in these three regions estimated to be anemic. That said, anemia in South-East Asia is more prevalent than any other region in the world, with nearly 800 million affected. While the prevalence of IDA among women and children in the developed world is lower when compared to the developing world, a high prevalence is still reported in high-risk groups, including preschool-aged children and pregnant women.
Women and children are hardest hit by this nutritional disorder due to increased iron requirements during periods of peak growth as well as during menstruation and pregnancy. Nearly 40% of preschool children and women (aged 15-59 years) and more than 50% of all pregnant women in developing countries estimated to be anemic.2

2.6.2 Etiology of iron deficiency and iron deficiency anemia

The prevalence of ID and IDA varies greatly from population to population according to a variety of host and environmental factors. The etiology of anemia is multifaceted and often several factors are at play in an anemic individual. Nutritional anemia, resulting from iron deficiency, is the most common cause of anemia worldwide, with approximately 50% of all cases attributed to a lack of iron in the diet. A number of host and environmental factors are associated with iron deficiency, and in more severe forms contribute to IDA as well. These include:

1. Inadequate dietary iron intake: Diets low in iron or diets low in acceptable quantities of bioavailable iron are a major cause of IDA, particularly in non-industrialized countries. Typically, high levels of IDA are also observed in old age when dietary quality and quantity deteriorates in both quality and quantity.5,111

2. Menstruation and pregnancy: Blood losses associated with menstruation and pregnancy are common causes of ID and IDA. Typically non-menstruating women lose about 1 mg Fe per day, while menstruating women lose a further 10 mg Fe per day during menses. Pregnancy is associated with an iron loss of approximately 1000 mg in a 55kg woman.20
3. Infectious disease: In the developing world common infections which may be both chronic and recurrent are associated with blood loss leading to iron deficiency, and ultimately to IDA. Hemolytic malaria and parasitic infections such as hookworm, trichuriasis, amoebiasis, and schistosomiasis are particularly common diseases that contribute to the depletion of iron stores and often result in IDA.\textsuperscript{112}

4. Interactions with medication: Several pharmacological agents can interfere with iron uptake and/or transport leading to iron loss or defective absorption, including non-steroidal anti-inflammatory drugs, H2 blockers, proton pump inhibitors, and aspirin.\textsuperscript{113}

5. Gastrointestinal conditions: Both acute and chronic gastrointestinal illness is associated with IDA and is an important consideration in clinical diagnosis of the condition. Duodenal or gastric ulcers, carcinoma, polyps, irritable bowel disease, erosive gastritis, celiac disease, altered hepatic function for any number of reasons, and/or compromised protein status may lead to IDA.\textsuperscript{5}

6. Periods of growth: Iron deficiency and IDA are particularly prevalent during peak periods of growth. Though infants born at full-term normally have adequate iron stores, if complementary foods containing iron are not introduced to the diet after six months of age then an infant is at risk of developing ID, and ultimately IDA. Requirements for iron on a body weight basis are proportional to the speed of growth, thus iron deficiency and IDA are common in preschool years and during puberty.\textsuperscript{2,114,115}

7. Socioeconomic status: Iron deficiency and IDA are most prevalent among groups of low socioeconomic status for a number of reasons, including but not limited to: malnutrition, poor education regarding health and hygiene, and greater presence of
concomitant disease when compared to populations of higher socioeconomic status.\textsuperscript{116,117}

2.7 Prevention and control

Interventions to control iron deficiency and its anemia are available, affordable, and sustainable.\textsuperscript{2,118} Food-based strategies are the most sustainable method for prevention of IDA, with dietary improvement representing the most cost-effective and sustainable option. Advancements in the fortification of food staples and condiments have also shown promise. In addition, supplementation using multivitamins and vitamin complexes containing high levels of iron are accessible, though often at a higher cost than other preventive and treatment methods.

2.7.1 Dietary improvement

Efforts towards the promotion, access to and availability of iron-rich foods are a key prevention technique. Foods containing high levels of iron include: animal-source proteins (including organ meat), as well as non-animal foods such as legumes/pulses and green leafy vegetables.\textsuperscript{119}

As overall meal composition is as important as total iron content of a meal, it is important to promote the consumption of food that enhances iron absorption and while limiting the consumption of foods that act as inhibitors.\textsuperscript{120-124} Foods that enhance absorption of iron typically contain high levels of vitamins A, vitamin C, and folic acid; this includes various fruits, vegetables, and tubers. Conversely, phytates, found in cereal grains, tannins and other
polyphenols found primarily in tea and coffee, and calcium from milk and milk products should be avoided where possible to limit the inhibition of iron absorption.

Typically, diets of individuals in the developing world do not provide adequate iron. In a typical South-East Asian diet consisting of rice, vegetables and spices, iron absorption was reported to be inadequate.\textsuperscript{125,126} Even with the addition of fruit, meat and fish to these simple meals, iron absorption remained lower than the estimated requirement. These findings would suggest that individuals consuming such a diet could only maintain their iron balance in a state of iron deficiency, and would therefore greatly benefit from ID and IDA treatment and prevention programs.\textsuperscript{125}

**2.7.2 Iron fortification**

Iron fortification involves the addition of iron to an appropriate food vehicle that is distributed widely to the general population. Fortified flour and other cereals have historically been the most commonly used.\textsuperscript{1,6} Research into self-fortification through plant breeding is also gaining momentum and in the future may have a great impact on improving nutritional status.\textsuperscript{127}

**2.7.2.1 Fortification in the developed world**

In the developed world, iron fortification has played an central role in the decline in the prevalence of ID since the 1960’s.\textsuperscript{6,128} The addition of elemental iron powder to flour and other cereals has since been commonplace, with approximately 30 to 60 μg/g enrichment
concentration depending on the food product.\textsuperscript{1} In Canada, for example, it has been mandatory to enrich all white flours, and enriched pastas and precooked rice since 1976.\textsuperscript{129}

As the demand for processed foods has increased over the past 50 years, vitamins and minerals have slowly been added to an increasing variety of foods. Ready-to-eat cereals in particular play an important role in daily iron intake in the Western world. In Germany, approximately 78\% of total iron intake in children aged 2 to 13 years may be attributed to ready-to-eat cereals.\textsuperscript{129}

The fortification of infant formulas and foods provides particularly convincing evidence for the benefits of food fortification.\textsuperscript{130} Following the introduction of fortification guidelines in the U.S. in the late 1960’s a clear reduction in IDA among infants and young children was noted.\textsuperscript{131}

\textbf{2.7.2.2 Fortification in the developing world}

In developing countries a much lower consumption of food from animal sources is observed and typically the overall nutritional value of the diet is lower when compared to developed nations.\textsuperscript{6} In addition, both a high relative cost and a decreased availability of fortified products like cereal flours, ready-to-eat cereals, and infant formula leads to an overall decreased use of industry-prepared food that would otherwise benefit the population.\textsuperscript{6} Thus, the relative absence of fortified food products from diets in developing nations could at least partly explain the high prevalence of iron deficiency and IDA and why current fortification practices have not ameliorated the situation. Research into fortifiable products that are culturally acceptable and desirable in developing nations should be conducted.
2.7.3 Iron supplementation

Iron supplementation, using oral iron supplements, is the most common and cost-effective strategy used to control ID and IDA in the developing world and is used as both a preventive measure and a treatment option. World Health Organization guidelines suggest that iron supplementation should include administration of 60 mg of iron daily with a dose of 400 μg of folic acid for women of reproductive age, 30 mg of iron and 250 μg of folic acid for school-aged children, and approximately 2 mg/kg body weight per day for preschool-aged children. Weekly iron supplementation also exists, though is considered to be a less effective treatment option and requires additional research and evaluation.

The majority of supplementation studies to date have examined a variety of treatments in women of reproductive age, as infants, preschool and school-aged children. It is becoming increasingly clear that a main target group for iron supplementation in the developing world should be all women of reproductive age, regardless of pregnancy status at the time, thus ensuring adequate iron reserves for both the mother and fetus during pregnancy and breast-feeding infants. Of concern is the relative cost of iron supplements in developing nations, coupled with issues surrounding delivery to infants and children exist. Other problems with iron supplementation include: undesirable side effects (including gastrointestinal irritation, black stools, and constipation); poor adherence to treatment guidelines; poor awareness and motivation, often due to inadequate health and nutrition education; quality and packaging of iron supplements; and risk of iron overload if supplementation guidelines are not followed correctly.
2.7.4 The use of adventitious iron sources

Research conducted in the latter half of the 20th century has reported on the use of iron pots for cooking as an innovative way to reduce IDA, with the first study conducted in 1986.\textsuperscript{137} Wistar rats fed a basal diet low in iron though cooked in an iron pot demonstrated comparable hemoglobin, hematocrit, protoporphyrin, serum iron, and transferrin saturation levels to those rats fed a complete diet, thus implicating the iron pot as an adventitious source of iron.

Since this time several studies have examined this supplementation technique in humans with similar findings. Experiments conducted on Ethiopian children aged 2-5 years and pre-term infants (between months 4 and 12) from Brazil reported that cooking food in iron pots led to lower rates of anemia than children whose food was cooked in non-iron pots.\textsuperscript{138,139} Significantly improved hematologic values between iron pot and non-iron pot groups were noted, including increased hemoglobin, hematocrit, mean corpuscular volume, free erythrocyte protoporphyrin, and serum ferritin. In addition, the Ethiopian study indicated moderate height and weight gains in children assigned to treatment groups.\textsuperscript{138} A more recent study conducted in Malawi verifies this research, noting a reduction in iron deficiency among children and increased hemoglobin levels in adults living under malarial endemic conditions.\textsuperscript{140,141}

Research into the beneficial aspects of contaminant iron and adventitious iron sources, should be conducted. This supplementation technique has the possibility of providing a low-cost and sustainable way of improving dietary iron content, and may be particularly effective in the developing world where resources are limited.
2.8 Conclusion

Anemia is a global public health problem with serious consequences for both the human and socio-economic health. Despite high-level spending over the past 50 years and a concerted effort to ameliorate the worldwide prevalence, the problem of iron deficiency and anemia persists. In 2004, the Copenhagen Consensus brought together a panel of world-renowned development economists to consider and confront the ten most pressing challenges to “global welfare” that we face today.\(^{142}\) Micronutrient interventions, including iron fortification, ranked at the top of the list and offered the highest benefit: cost ratio of any development intervention. These findings were confirmed in 2008, at the most recent Consensus meeting, where iron and zinc fortification were placed within the top three global challenges.\(^{143}\) This prioritization of iron and other micronutrient interventions emphasizes the need for well-designed, sustainable and effective programming efforts to combat iron deficiency anemia.

The adverse effects of anemia on mortality, morbidity and development are abundantly clear. Anemia affects how individuals participate in all areas of life, including work, school and social activities, and this limits the ability to generate income and afford iron-rich sources of food, medical treatment, and school fees. In turn, this leads to constrained social and economic development, ultimately contributing to a viscous cycle of poverty that is difficult to overcome.

The widespread prevalence of anemia, both in the developed and developing worlds, is great cause for concern. The current review highlights some of the most promising research on the etiology, prevention and control of the disorder. From this, it should be clear that although we have made strides, there is still much that we do not understand about iron deficiency and
anemia, especially in relation to treatment and prevention. A renewed effort to find effective ways to combat this problem is needed, as anemia is unique and complex public health crisis that is of global proportions.
2.10 References


placental but not hypothalamic CRH. Journal of Clinical Endocrinology and Metabolism 70: 1574-1580.


2.11 Tables

Table 2.11.1 Hemoglobin levels below which anemia is present in a population.²

<table>
<thead>
<tr>
<th>Age or gender group</th>
<th>Hemoglobin (g/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>6 – 59 months</td>
<td>110</td>
</tr>
<tr>
<td>5 – 11 years</td>
<td>115</td>
</tr>
<tr>
<td>12 – 14 years</td>
<td>120</td>
</tr>
<tr>
<td>Non-pregnant women (&gt;15 years)</td>
<td>120</td>
</tr>
<tr>
<td>Pregnant women</td>
<td>110</td>
</tr>
<tr>
<td>Males (&gt;15 years)</td>
<td>130</td>
</tr>
</tbody>
</table>
Table 2.11.2 Anemia prevalence and number of individuals affected in pre-school aged children, pregnant women, and non-pregnant women by WHO region.²

<table>
<thead>
<tr>
<th>WHO Region</th>
<th>Preschool-age Children</th>
<th>Pregnant Women</th>
<th>Non-pregnant Women</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Prevalence (%)</td>
<td># Affected (millions)</td>
<td>Prevalence (%)</td>
</tr>
<tr>
<td>Africa</td>
<td>67.6 (64.3-71.0)</td>
<td>83.5 (79.4-87.6)</td>
<td>57.1 (52.8-61.3)</td>
</tr>
<tr>
<td>Americas</td>
<td>29.3 (26.8-31.9)</td>
<td>23.1 (21.1-25.1)</td>
<td>24.1 (17.3-30.8)</td>
</tr>
<tr>
<td>South-East Asia</td>
<td>65.5 (61.0-70.0)</td>
<td>115.3 (107.3-123.2)</td>
<td>48.2 (43.9-52.5)</td>
</tr>
<tr>
<td>Europe</td>
<td>21.7 (15.4-28.0)</td>
<td>11.1 (7.9-14.4)</td>
<td>25.1 (18.6-31.6)</td>
</tr>
<tr>
<td>Eastern Mediterranean</td>
<td>46.7 (42.2-51.2)</td>
<td>0.8 (0.4-1.1)</td>
<td>44.2 (38.2-50.3)</td>
</tr>
<tr>
<td>Western Pacific</td>
<td>23.1 (21.9-24.4)</td>
<td>27.4 (25.9-28.9)</td>
<td>30.7 (28.8-32.7)</td>
</tr>
<tr>
<td>Global</td>
<td>47.4 (45.7-49.1)</td>
<td>293.1 (282.8-303.5)</td>
<td>41.8 (39.9-43.8)</td>
</tr>
</tbody>
</table>
CHAPTER 3: ANEMIA IN CAMBODIA: PREVALENCE, ETIOLOGY AND RESEARCH NEEDS

3.1 Introduction

Anemia is a global public health problem that is common in both the developed and developing worlds, with major consequences for human health and socio-economic development. The condition is characterized by a decrease in the normal number of erythrocytes and/or decreased quantities of circulating hemoglobin in the blood.\(^1\) Specific cut-off values of hemoglobin for various demographic groups for determining the presence of anemia were established by the World Health Organization (Table 3.7.1).\(^2\)

The etiology of anemia is multifaceted and often several factors are at play in an anemic individual. Nutritional anemia, resulting from iron deficiency, is the most common cause of anemia worldwide: approximately 50% of all cases of anemia are attributed to a lack of iron in the diet.\(^1,2\) Additionally, other micronutrient deficiencies, some infectious diseases, genetically determined hemoglobinopathies, and chronic or acute blood loss are also known to cause anemia.\(^3\)

The consequences of anemia can be severe and are often irreversible, affecting both human and socio-economic health of individuals and families. Mild to moderate anemia leads to weakened immunity, reduced work capacity, reduced cognitive ability and an overall decreased quality of life.\(^1,4\) Severe anemia (Hb < 70g/L) reduces a woman’s ability to survive bleeding during and after childbirth and is considered a major cause of maternal morbidity and mortality, particularly in the non-industrialized world.\(^5\) Anemia during pregnancy is also
associated with increased risks of premature delivery, low birth-weight, and perinatal mortality.\textsuperscript{5} Delayed cognitive development and limitation in intellectual development is also a major concern in children and adolescents.\textsuperscript{4}

Globally, more than 1.62 billion people are anemic.\textsuperscript{2} Based on the prevalence of anemia in a country, the WHO classifies the public health significance of anemia in a population. These estimates are derived from measurement of hemoglobin concentrations, and encompass four categories: severe (>40.0%), moderate (20.0-39.9%), mild (5.0-19.9%) and normal (<5.0%).\textsuperscript{2} In Cambodia, anemia represents a severe public health problem, and requires immediate attention.

Anemia is inherently associated with poverty, and is therefore particularly prevalent in the developing world where the problem is often exacerbated by limited access to appropriate healthcare and treatment options.\textsuperscript{6} Regional estimates show that the highest prevalence of anemia is in Africa (with 47.5-67.6% of the population affected), but largest number of affected individuals reside in South-East Asia, where more than 315 million people are anemic.\textsuperscript{2} Anemia occurs at all stages of life, but is particularly prevalent in women of reproductive age and children. Globally, the highest prevalence of anemia is in preschool-aged children, while the lowest prevalence is in men. Non-pregnant women, however, make up the population group with the greatest number of individuals affected is, with more than 464 million worldwide estimated to be anemic.\textsuperscript{2}

The paper presents a situation analysis of the burden of anemia in Cambodia. Drawing upon available information, prevalence estimates will be examined by demographic
subpopulations and trends will be briefly discussed. The data presented come from reports, publications and reviews that are readily available, and are therefore not based exclusively on representative national sampling. The second half of the paper will discuss both general and country-specific etiologies of anemia, including nutritional and non-nutritional factors.

3.2 Situation Assessment and Analysis

To date, a national anemia survey or a comprehensive national nutrition survey has not been conducted in Cambodia. Data on the prevalence of anemia, however, can be drawn from two internationally sponsored and facilitated Cambodian Demographic and Health Surveys (CDHS) conducted in 2000 and 2005. These surveys are considered to be nationally representative and involved more than 15,300 and 16,800 women aged 15 – 49 in 2000 and 2005, respectively. To add context to these survey results, the prevalence of anemia by age group is compared over time using chi-square tests.

Anemia prevalence data can also be taken from individual studies that have been conducted in Cambodia to investigate various treatments for anemia and/or to study the prevalence of anemia in specific population groups. These studies are not nationally representative and much of the data comes from rural Cambodia. Regardless, this information is valuable in demonstrating the extent of the problem. Data has been presented according to urban/rural status, the study setting, age, sample size of the specific study, and method of diagnosis, where possible; sampling methodology varied by study.
3.2.1 Infants and Preschool Children

By far, the largest body of data on the prevalence of anemia in Cambodia has been collected on this high-risk demographic. The two Cambodian Demographic Health Surveys and several additional studies provide prevalence data, with anemia present at hemoglobin levels < 110 g/L (Table 3.2.2). The CDHS conducted in 2000 indicated a prevalence of 57.3% and 64.4% of anemia in children aged 0.50 – 4.99 years in urban and rural Cambodia, respectively.\(^7\) Using chi-square analysis, these estimates were not found to differ significantly in 2005: 59.7% of children from urban populations \((P = 0.12)\) and 62.2% of children from rural areas \((P = 0.15)\) were found to be anemic.\(^8\) In addition to these national surveys, prevalence estimates from various studies suggest that anemia affects between 54.0 – 82.0% of infants and preschool children in the country.\(^3,9\)-\(^15\)

3.2.2 School-aged children and Adolescents

Unfortunately, high quality prevalence data in school-aged children in Cambodia is limited (Table 3.7.3). Longfils and colleagues estimated that the prevalence of anemia was 64.1% among grade-one students at two primary schools in rural Kampot province, using a sample size of 450.\(^16\) Interestingly, the ages of these students ranged from approximately 6 to 15 years of age, thus this study provides important data on the prevalence of anemia across a wide age-range.

McLean reported 21.2% prevalence of anemia in 231 girls enrolled in secondary school, ranging from 9 – 21 years of age.\(^17\) This prevalence estimate was lower than anticipated, and these results must be interpreted cautiously. In Cambodia, only about 25% of girls attend
secondary school, and those that do are typically of higher socioeconomic status and are therefore more likely have a diet that is higher in heme iron when compared to those who are less affluent. Future surveying efforts should focus on collection of data from both adolescents who are and who are not attending secondary school, representing the full range of socioeconomic strata in a weighted representative sample.

One additional intervention study included baseline measurement of anemia caused specifically by iron deficiency. In Kampot province, 805 students from a secondary school, and 699 students from a primary school were assessed for hemoglobin and iron status. Prevalence of iron deficiency anemia was approximately 7% and 13% in the secondary and primary schools, respectively. The primary school selected for this study was, prior to the trial, part of a school-feeding program run by the World Food Programme and this may partly account for the low prevalence of iron deficiency anemia reported.

### 3.2.3 Adult Women

The reproductive status of women is an important factor when considering the prevalence of anemia. Pregnant and lactating women have a higher demand for nutritional sources of iron in order to maintain normal hemoglobin levels because they require an adequate iron supply for the growth of the foetus and/or the production of milk. For this reason, it is beneficial to categorize women as non-pregnant and non-lactating (NPNL) or pregnant and/or lactating (PWLW) (Table 3.7.4).

The CDHS conducted in 2000 estimated a 55.1% and 66.3% prevalence of anemia in NPNL (Hb < 120 g/L) and PWLW (Hb < 110 g/L), respectively. These estimates decreased
significantly \((P < 0.001)\) five years later with anemic NPNL women representing 44.3% of the population, and 55.4% of PWLW estimated to be anemic.\(^8\)

Additional, non-nationally representative studies report a wide range in the reported prevalence of anemia. Estimates suggest that 61.0% - 69.0% of non-pregnant women and 72.0% - 77.0% pregnant women are anemic.\(^9,10,14\) Two studies with much smaller sample sizes did not separate according to reproductive status, and estimate 25.0% - 33.0% prevalence of anemia in all women surveyed.\(^17,19\)

### 3.2.4 Summary of the Situation Analysis

Anemia is assessed by hemoglobin levels and requires a blood sample, either venous or capillary. In Cambodia, many people are fearful of having a blood sample taken and the extent of representative survey data therefore tends to be limited with highly variable results, depending on who is included in the study population and how individuals are selected.

The lack of data presented herein highlights the need for a comprehensive national nutrition survey among all ages, involving individuals from both genders. There is a relative abundance of data on infants and preschool-aged children, and this is an important group to monitor, as effects of anemia can be most severe during these important developmental years. Iron requirements are increased during periods of marked growth, including puberty; therefore the lack of data on the prevalence of anemia in school-aged children and adolescents is a concern. Quite obvious is the complete absence of data on the prevalence of anemia in men; anemia is often considered a disease of women and children, but men are also at risk and nationally representative data for this group should be collected. Similarly, data on the
prevalence of anemia in the elderly is absent, with the majority of surveys setting an upper age limit for inclusion of 50 years. Given that the quantity and quality of diet typically decreases with age, there is likely to be a high prevalence of anemia in older individuals that is not captured by the current research.

Overall, the two nationally representative health surveys show that the prevalence of anemia remained unchanged in children aged 0.50-4.99 years. In contrast, in women of reproductive age, anemia prevalence dropped approximately 11 percentage points for both NPNL and PWLW between 2000 and 2005.

3.3 Etiology of Anemia

Anemia may result from a wide variety of causes, both in isolation and concurrently. Most commonly, anemia results from nutritional factors with dietary iron deficiency responsible for the majority of all cases of anemia worldwide. Non-nutritional causes of anemia are numerous and diverse. In the developing world, common infections, which may be both chronic and recurrent, are associated with blood loss that can result in iron deficiency and ultimately anemia. In addition, inherited abnormal hemoglobin traits, acute haemorrhage and various chronic diseases are also contributing factors.

3.3.1 Iron deficiency anemia

An individual’s iron status can be described as a continuum. If an individual has too much iron, they are considered to be in a state of iron overload. If iron homeostasis is achieved, normal iron status is observed. Finally, if an individual is not meeting iron requirements, they
may be classified as having iron deficiency, or more severely, iron deficiency anemia.\textsuperscript{2} Widely accepted estimates suggest that approximately 50% of all anemias can be attributed to iron deficiency.\textsuperscript{2}

Iron deficiency anemia may result from a single, or any combination of four main factors: i) insufficient daily iron intake and/or low bioavailability of consumed iron; ii) increased iron requirements at various life stages, including pregnancy, childhood, or puberty; iii) chronic iron depletion due to menstruation, stomach ulcers or parasitic infection; and, iv) compromised iron utilization following absorption as a result of repeated infection and/or concomitant micronutrient deficiencies.\textsuperscript{1}

In Cambodia, data are scant on the prevalence of IDA and inference from surveys on the prevalence of general anemia must be used. To determine the prevalence of iron deficiency, hemoglobin concentration plus an indicator of iron status, most commonly serum ferritin, must be examined.\textsuperscript{1,21} Hemoglobin is easily measured with a capillary blood sample using a portable hemoglobin monitor, such as the HemoCue® system (HemoCue AB, Ängelholm, Sweden) but serum ferritin analysis requires a venous blood sample that is analyzed in a laboratory setting at a significantly higher cost. Further, serum ferritin is an acute-phase protein that is raised during periods of infection or inflammation, and this must be taken into account when conducting an analysis of this data.\textsuperscript{1} For this reason, the majority of research on the prevalence of anemia in the developing world, including Cambodia, provides only hemoglobin data and the prevalence of IDA can therefore only be estimated.
A community study of children of Cambodian refugees living in Dunedin, New Zealand can provide interesting proxy data. Within the community, 19 infants between 3 and 19 months of age were identified, and 18 of those submitted to blood sampling. Iron deficiency (serum ferritin less than 10 micrograms/L) was identified in 65% (11/17) of the children, while iron deficiency with anemia was observed in 37% of those tested. Although this prevalence estimate is disconcertingly high, this study is not a measure of iron status of children living in Cambodia and therefore must be interpreted with caution.

Trend analysis from the 2000 and 2005 CDHS show that hemoglobin levels decreased in children aged 6 to 24 months, but that these levels eventually improve after 2 years of age. These markedly decreased hemoglobin levels coincide with a child’s peak period of growth when iron requirements would be elevated; thereby suggesting that iron deficiency is a significant cause of anemia in this age group.

Three relatively recent studies did include markers of iron deficiency and the prevalence of iron deficiency and/or IDA can therefore be calculated. A 2006 intervention study involving 204 children selected from a single district in Kompong Chhnang province showed that iron deficiency was prevalent in 13.2% of children aged 6 months. However, by 18 months, more than 50% of children in the placebo group (n=62) were reported to be iron deficient, even when accounting for inflammation. Similarly, iron deficiency was estimated to be present at baseline in 44.0% of children aged 6-24 months in a separate population based study involving 250 children, also accounting for high rates of inflammation. In order to determine the effectiveness of a supplementation program involving 60,000 school girls in five target
provinces and 33,000 women of reproductive age in one operational district, McLean surveyed a subsample of the school girls (n=231) and women of reproductive age (n=231) for indicators of iron status.\textsuperscript{17} Iron deficiency was found to be present at baseline in 18.2% of school girls, with IDA affecting 10.4% of participants. In women of reproductive age, iron deficiency and IDA were estimated to affect 19.9% and 12.6% of survey participants, respectively. Following intervention with oral iron supplements, iron deficiency decreased significantly among anemic and non-anemic women at baseline but the proportion of anemia was unchanged. These findings suggest that iron deficiency was only partially responsible for cases of anemia in the study group, and that various other etiological factors may be at play.\textsuperscript{17} Future surveying efforts should assess iron status of the population in order to determine the contribution of iron deficiency to the prevalence of anemia that is currently reported.

3.3.2 Other micronutrient deficiencies

Deficiencies of several key micronutrients may also lead indirectly to anemia, typically by influencing hemoglobin metabolism. These include vitamin A, folate, vitamin B12, vitamin C, protein, and copper.\textsuperscript{23} Vitamin A deficiency, in particular, is a well-established contributor to anemia and is also common in the developing world where dietary diversity is limited.\textsuperscript{21} Vitamin A is essential for hematopoiesis, and it is thought that the mineral is required for the mobilization of iron for hemoglobin synthesis.\textsuperscript{24,25}

Data describing the prevalence of vitamin A deficiency in Cambodia is limited. In 2000, 22% of rural children aged 6-59 months had severe vitamin A deficiency as assessed by serum retinol levels.\textsuperscript{7} Despite this, surveys in 2000 and 2005 observed that only
one in three children received a vitamin A supplement in the 6 months before the survey.\textsuperscript{7,8} The most recent nation-wide survey collected data on the consumption of vitamin A-rich foods during the 24-hour period prior to surveying.\textsuperscript{8} Foods rich in vitamin A were consumed by 87.3% of last-born children in the 24-hour period before the survey. Among women aged 15-49 with a child under 3 years living with them, 98.5% consumed vitamin A-rich foods. Data was not collected on the total daily vitamin A intake, so it is not possible to determine whether or not daily vitamin A requirements are being met.

### 3.3.3 Helminths

Helminths are a division of eukaryotic parasites that live inside their host, commonly in the intestinal tract. The organisms can disrupt nutrient absorption leading to weakness and disease and may contribute to anemia by causing blood loss and depletion of iron stores and/or by inhibiting vitamin A absorption; diarrhoea and chronic inflammation are also common symptoms.\textsuperscript{26} Helminth infections are distributed throughout the world with particularly high prevalence in the tropics. In Cambodia, helminths constitute a significant health problem.\textsuperscript{27} A survey of intestinal parasite infection in 251 primary school children in Kompong Cham Province was conducted in 2002 and reported an infection prevalence of 57% in males and 51% in females.\textsuperscript{28} Another survey of 623 schoolchildren in Battambang province reported an overall infection rate of 25.7%.\textsuperscript{29} Hookworm (*N. americanus, A. duodenale*), whipworm (*T. trichuria*), roundworm (*A. lumbricodes*), *Opisthorchis sp.* and *Echinostoma sp.* were identified, and multiple infections were noted in a subsample of the cases.\textsuperscript{3,28,29} Data on the presence of
helminth infection in Cambodia is limited and out-of-date and there is a great need for current, nationally representative data on the presence and geographical distribution.

3.3.4 Malaria

The world over, malaria is a significant cause of anemia as the parasitic infection ultimately leads to hemolysis of red blood cells and a decrease in hemoglobin production. Since 1991, the Cambodia National Malaria Centre has been responsible for monitoring the prevalence of malaria in the country.\textsuperscript{3} Though malaria was once considered a significant cause of morbidity and mortality in Cambodia,\textsuperscript{30,31} a high level of deforestation has occurred over the past two decades (29% of primary tropical forest between 2000 and 2005) and this has dramatically decreased the size of suitable habitat for malaria-infected mosquitoes.\textsuperscript{32} In turn, the Cambodian Ministry of Health report that the prevalence of malaria has significantly dropped, though data from these groups is not readily accessible.\textsuperscript{3}

In 2009, approximately 64,000 confirmed cases of malaria were reported, with the majority caused by the \textit{Plasmodium falciparum} species of the malaria parasite.\textsuperscript{33} Cases appear to be concentrated in rural areas, with a particular clustering along the western border of the country.\textsuperscript{30,31} Though malaria may be a contributing factor, the relatively low prevalence and marked clustering in isolated areas of the country suggest that malaria is not a significant cause of anemia in Cambodia.
3.4.5 Hemoglobinopathies

Hemoglobinopathies have been widely reported throughout much of Southeast Asia. These conditions are genetically inherited defects that result in abnormal structure of one of the globin chains of the hemoglobin molecule, ultimately leading to anemia. In Cambodia, no nationally representative data is available and future surveying efforts should address this deficiency of data, including the prevalence of co-existent hemoglobinopathies.

Three studies on the prevalence of hemoglobinopathies in Cambodia were identified: a study involving 322 urban children aged 6 to 36 months from a single district in Phnom Penh, a population-based sample of 250 children aged 6 to 24 months, and a hospital study involving 260 patients from the Angkor Children’s Hospital in Siem Reap province. The prevalence of any, non-type specific hemoglobinopathy ranged from 29.0% to 51.5% in children aged 6 months to 16 years of age. Data on the specific type of hemoglobinopathy is yet more limited. Abnormal hemoglobin variant E was found in all three studies, with prevalence of 28.8 – 32.0%; further analysis in one study revealed that heterozygous variant type E was found in 22.0%, while homozygous variant type E accounted for the remaining cases. Data on the prevalence of thalassemia was identified from the hospital study only, with 35.4% and 0.8% prevalence of α-thalassemia and β-thalassemia, respectively. Despite this high prevalence, the majority of hemoglobinopathies were mild and not clinically significant and it is therefore improbable that they pose a major health burden in Cambodia.

One study on the prevalence of abnormal hemoglobin traits in Cambodian refugees was identified. In a hospital-based study, 59 children aged 0 to 18 years were assessed. Prevalence
of hemoglobinopathies is in-line with those reported previously: 36% were found to have abnormal hemoglobin E (19% heterozygous, 17% homozygous), 12% were identified as possessing the α-thalassemia trait, and 3% possessed the β-thalassemia trait. All hemoglobinopathies were shown to be benign conditions that resulted in microcytosis and mild, if any, anemia.\textsuperscript{37} Although this study confirms prevalence estimates and clinical pathology previously reported, it was not conducted on children living in Cambodia and therefore the results must be interpreted with care.

Finally, one study that compared the prevalence of hemoglobinopathies in Cambodian refugees in a rural Thai-Cambodian border province (Prajinburi, Thailand) to a smaller group of subjects from Phnom Penh and suburban areas was identified.\textsuperscript{38} Of those from the rural population (n=264), 21.9% were heterozygous hemoglobin E, 4.2% were homozygous hemoglobin E, and 3.1% had the β-thalassemia minor trait. In the urban population (n=96), 31.4% were heterozygous hemoglobin E, 7.2% were homozygous hemoglobin E, and 3.4 had the β-thalassemia minor trait.

\textbf{3.3.6 Others}

Several other diverse factors can also cause anemia. Chronic blood loss, from peptic ulcers for example, can be a contributing factor to IDA, though prevalence data in Cambodia has not been collected.\textsuperscript{4} Trauma, resulting in acute blood loss, may also induce iron deficiency and IDA dependent upon the severity of haemorrhage.\textsuperscript{4} Finally, complications during pregnancy and/or childbirth can result in anemia. In Cambodia, approximately 78% of deliveries take place
in the home, often without the presence of a skilled birth attendant, thus obstetric
haemorrhage may be a significant cause of anemia, particularly in rural areas.\textsuperscript{8}

\textbf{3.4 Iron Intake in Cambodia}

Given that approximately 50\% of all anemias can be attributed to iron deficiency, it is
imperative to examine iron intake and overall diet composition throughout the various
subpopulations. Unfortunately, data is scant and the only nationally representative data is from
the 2005 CDHS.\textsuperscript{8} This survey provides limited data on the consumption of iron-rich foods by
women and children that were included in the overall survey, and although this data is
relatively useful, we still do not have a clear picture of the nutritional content, nor quantity or
quality of the diet in Cambodia.

The period of birth to two years of age is a critical period for child development, and
adequate nutrition is important for optimal growth and health. The most recent nation-wide
data suggests that 87.3\% of children consume foods rich in iron, with some differences by
background characteristics.\textsuperscript{8} No gender difference was reported, however children living in
rural areas and those still breastfeeding may have a lower intake of iron-rich foods. Adequate
iron intake is also important for pregnant women and women of reproductive age. Women
aged 15-49 with a child under three years living with them were also surveyed for iron intake.
Approximately 98\% of women reported consuming foods rich in iron during the 24-hour period
prior to the survey; these foods include meat (including organ meat), fish, poultry and eggs.\textsuperscript{8}

These values for both women and children only provide data on the consumption of iron
rich foods, not whether or not daily iron requirements are met by the diet. Typical overall meal
composition, including consumption of iron-rich foods, but also consumption of enhancers and/or inhibitors of iron absorption should be investigated. Given that the majority of women in Cambodia lack access to iron-fortified food products and other sources of highly bioavailable iron, this group should be considered a priority for effective intervention programming.

Additionally, there is a need for research on the types of iron-rich foods available in Cambodia, including seasonality, cost, and access across the various geographic regions and demographic groups.

Supplementation with oral iron tablets also contributes to overall iron intake. Cambodia does not have a preventive iron supplementation program for children, despite anemia being documented as a severe public health problem for infants and young children. For this reason, households would need to purchase iron supplements out-of-pocket and preventive supplementation is therefore quite rare. The cost of iron supplements is highly variable depending on the brand, quantity purchased, location of purchase (i.e., small village shops that sell medicines, provincial pharmacies, health centres), type of supplement (i.e., daily or weekly), and a number of other factors. In Kandal province, Charles et al. reported that daily iron supplementation for one month costs approximately $2.50 (USD).\textsuperscript{19} In contrast, Longfils reported that weekly iron oral iron supplements for children are very cost-effective at only $0.05 (USD) per year.\textsuperscript{16} Household purchasing of iron supplements is not currently a priority for the vast majority of families, and this may be attributed to cost, awareness or a combination of the two factors.
The 2005 CHDS collected limited data on the intake of and access to micronutrient supplements. Of those children surveyed, only 1.2% of children aged 6-59 months received iron supplements in the previous 7 days. Among women aged 15-49 with a child under three years living with them, only 17.6% of women took the recommended number (90+ tablets) of iron/folic acid supplements during their most recent pregnancy. Some women (9.0%) took 60-90 tablets, while 30.8% reported taking fewer than 60 tablets. The majority of women (36.8%) did not take iron/folic acid supplements at all.

According to the National Guidelines for Iron and Folic Acid Supplementation established in 2007, pregnant and post-partum women should have access to at least 90 fully-funded iron/folic acid tablets during each pregnancy. Distribution is expected to occur at hospitals, health centres and/or through outreach activities where available. Access to supplements appears to be limited by stock-outs of tablets, problems with outreach staffing, limited community mobilization for attending outreach activities, inadequate interest and/or poor knowledge about anemia on the receiving side. The effectiveness of this program must be investigated through nation-wide surveying in order to better inform on ways to overcome these problems.

3.5 Discussion

This paper reviews the available data on the magnitude and country-specific etiology of anemia in the Cambodian context. Anemia represents a severe public health problem among many women and children in Cambodia, with serious consequences for human health and well-being. Available data on the etiology of anemia in Cambodia points to iron deficiency as a
significant cause of anemia, and this is in-line with global trends.\textsuperscript{2} Other nutritional deficiencies, such as vitamin A deficiency, hemoglobinopathies, helminths, malaria, and other non-nutritional factors likely contribute marginally to the overall prevalence. Additional research on the etiology of anemia in Cambodia is needed to clarify the primary causes of anemia within the population. It is impossible to design effective treatment and prevention programmes without decisive etiological data in-hand.

This review highlights the need for a comprehensive, nationally representative anemia and nutrition survey that elicits data on the nutrition status of all age groups for both men and women. Currently, there is an obvious and detrimental deficiency of data for a large portion of the population; in particular, there is a complete lack of data for men and the elderly of both genders, and only three studies, one of which was conducted in a narrow socio-economic bracket, providing information on the situation for school-aged children.

In 2004, the Copenhagen Consensus brought together a panel of world renowned development economists to consider and confront the ten most pressing challenges to “global welfare,” including human and environmental health, that we face today.\textsuperscript{40} Micronutrient interventions, including iron fortification, ranked at the top of the list and deemed to be the greatest priority with the highest benefit:cost ratio of any development intervention.\textsuperscript{40} These findings were confirmed in 2008, at the most recent Consensus meeting, where iron and zinc fortification were placed within the top three global challenges.\textsuperscript{41} This prioritization of iron and other micronutrient interventions emphasizes the need for well-designed, sustainable and
effective programming efforts to combat iron deficiency anemia, and related deficiencies in Cambodia and beyond.

In spite of the widely accepted need for universal micronutrient intervention among high-risk population groups, there has been very limited progress to prevent and control anemia in Cambodia. Currently, the only government-sponsored anemia program allows for limited iron supplementation for pregnant women who access local health centres.\textsuperscript{39} Although this approach should theoretically be effective at treating anemia and maintaining hemoglobin levels in the short-term, the impact of this measure has been uneven as both limited access to and insufficient intake of supplements appear to be common problems.

There is no denying that iron supplementation should be an important component of policies aimed at reducing anemia, but there is also a need for more sustainable solutions. Ruel and colleagues suggest that these alternative approaches fall into four broadly defined categories: i) approaches to improve the production of vitamin and nutrient rich foods, such as programs to improve homestead production of produce, aquaculture and livestock; ii) approaches to improve the intake of micronutrient rich foods, including nutrition education to guide consumer food practices; iii) approaches to increase micronutrient bioavailability using homestead processing techniques, food-to-food fortification, and proper food preservation; and finally, iv) through the adoption of selectively-bred plants that are designed to increase iron content and bioavailability of staple grains, including rice.\textsuperscript{42}

Any approach to treatment and prevention of anemia must consider the multi-factorial nature of the condition. Although iron deficiency is one of the most prevalent etiologies, other
contributing factors may require different approaches. Thus a holistic approach to combating anemia should include helminth control, malarial prophylaxis, and provision of safe and affordable reproductive health services to limit blood loss during pregnancy and childbirth. Programming must also target whole families rather than pregnant women, alone. The current nutrition policy that appears to neglect children and adolescents, two important demographic groups that may suffer irreversible functional consequences as a result of anemia must be updated. Additionally, the nutritional status of the elderly in Cambodia is unknown and steps must be taken to evaluate the situation and provide appropriate solutions where necessary.

The adverse effects of anemia on mortality, morbidity and development are abundantly clear. Anemia affects how individuals participate in all areas of life, including work, school and social activities, and this limits the ability to generate income and afford iron-rich sources of food, medical treatment, and school fees. In turn, this leads to inhibited socio-economic development, ultimately contributing to a viscous cycle of poverty that is difficult to overcome. The overall extent of the problem in Cambodia therefore highlights the need for an immediate, sustained and holistic intervention is needed. Long-term measures, including food fortification and production programmes, should be adopted and developed in Cambodia to ensure that all demographic groups can benefit from improved iron intake.

Updating of short-term programmes targeted at high-risk groups, such as pregnant and lactating women, must also be scaled-up to ensure that universal access to the appropriate course of iron supplements is available in both urban and rural areas. Assurance of adequate iron intake and proper nutrition in infants and children, followed by a renewed focus during
adolescence is a pre-requisite for a society’s prosperity; thus, state-funded supplementation should also be considered during these important developmental years. Perhaps most urgently, comprehensive nation-wide surveying on the prevalence and etiology of anemia in Cambodia, including collection of data on iron status, must be conducted in order to direct future programs to prevent and control anemia. A multidisciplinary and synergistic approach is essential to achieve these goals and the continued cooperation of the Cambodian government at the federal-, provincial- and community-level, together with international donor and development agencies will be the key to success.
3.6 References


3.7 Tables

Table 3.7.1: Hemoglobin levels (g/L), below which anemia is present in a person by age, gender and pregnancy status.²

<table>
<thead>
<tr>
<th>Subgroup</th>
<th>Hemoglobin concentration (g/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Children 6 months to 59 months</td>
<td>110</td>
</tr>
<tr>
<td>5 – 11 years</td>
<td>115</td>
</tr>
<tr>
<td>12 – 14 years</td>
<td>120</td>
</tr>
<tr>
<td>Women Non-pregnant women &gt;15 years</td>
<td>120</td>
</tr>
<tr>
<td>Pregnant women</td>
<td>110</td>
</tr>
<tr>
<td>Men &gt; 15 years</td>
<td>130</td>
</tr>
</tbody>
</table>
Table 3.7.2a: Summary of prevalence data on anemia among preschool-aged children in Cambodia.

<table>
<thead>
<tr>
<th>Area</th>
<th>Place</th>
<th>Setting</th>
<th>Study period</th>
<th>Age (yrs)</th>
<th>N</th>
<th>Sampling design</th>
<th>Hb method</th>
<th>Anemia prevalence (severe)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CDHS 2005</td>
<td>U</td>
<td>Nat. Com.</td>
<td>2005</td>
<td>0.50-4.99</td>
<td>400</td>
<td>2-stage cluster sampling within U/R strata</td>
<td>Hemo-Cue</td>
<td>59.7 (0.6)</td>
</tr>
<tr>
<td>CDHS 2005</td>
<td>R</td>
<td>Nat. Com.</td>
<td>2005</td>
<td>0.50-4.99</td>
<td>2758</td>
<td>2-stage cluster sampling within U/R strata</td>
<td>Hemo-Cue</td>
<td>62.2 (0.7)</td>
</tr>
<tr>
<td>CDHS 2000</td>
<td>U</td>
<td>Nat. Com.</td>
<td>2000</td>
<td>0.50-4.99</td>
<td>196</td>
<td>2-stage cluster sampling within U/R strata</td>
<td>Hemo-Cue</td>
<td>57.30 (2.3)</td>
</tr>
<tr>
<td>CDHS 2000</td>
<td>R</td>
<td>Nat. Com.</td>
<td>2000</td>
<td>0.50-4.99</td>
<td>1265</td>
<td>2-stage cluster sampling within U/R strata</td>
<td>Hemo-Cue</td>
<td>64.4 (2.0)</td>
</tr>
<tr>
<td>CDHS 2000</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1461</td>
<td></td>
<td></td>
<td>Total: 63.4 (2.0)</td>
</tr>
</tbody>
</table>
### Table 3.7.2b: Continued.

<table>
<thead>
<tr>
<th>Area</th>
<th>Place</th>
<th>Setting</th>
<th>Study period</th>
<th>Age</th>
<th>N</th>
<th>Sampling design</th>
<th>Hb method</th>
<th>Anemia prevalence (severe)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kenfick</td>
<td>R/U</td>
<td>125 vill.</td>
<td>Com.</td>
<td>1998</td>
<td>0.50-4.99</td>
<td>Multistage random sampling</td>
<td>Hemo-Cue</td>
<td>82.0 (2.8)</td>
</tr>
<tr>
<td>Kenfick</td>
<td>R/U</td>
<td>137 vill.</td>
<td>Com.</td>
<td>2000</td>
<td>0.50-4.99</td>
<td>Multistage random sampling</td>
<td>Hemo-Cue</td>
<td>74.0</td>
</tr>
<tr>
<td>HKI</td>
<td>R</td>
<td>10 pro.</td>
<td>Com.</td>
<td>2000</td>
<td>0.50-4.99</td>
<td>Random multistage cluster sampling</td>
<td>Hemo-Cue</td>
<td>54.0</td>
</tr>
<tr>
<td>Johnston</td>
<td>R/U</td>
<td>124 vill.</td>
<td>Com.</td>
<td>2003</td>
<td>0.50-4.99</td>
<td>Two-stage random sampling</td>
<td>Hemo-Cue</td>
<td>72.15 (1.6)</td>
</tr>
<tr>
<td>Giovannini</td>
<td>R</td>
<td>1 dis., 24 vill.</td>
<td>Com.</td>
<td>2003</td>
<td>0.50 +/- 7 days</td>
<td>Two-stage random sampling CBC (Cell Dyn 3200)</td>
<td>78.8</td>
<td></td>
</tr>
<tr>
<td>Schumann</td>
<td>R</td>
<td>1 pro., 13 vill.</td>
<td>Com.</td>
<td>2003</td>
<td>0.50-2.00</td>
<td>Two-stage random sampling</td>
<td>CBC (Cell Dyn 3200)</td>
<td>74.4</td>
</tr>
<tr>
<td>Helmers</td>
<td>R</td>
<td>40 vill., Com.</td>
<td>1999</td>
<td>0.50-4.99</td>
<td>Two-stage random sampling</td>
<td>Hemo-Cue</td>
<td>67.0 (3.3)</td>
<td></td>
</tr>
<tr>
<td>Talukder</td>
<td>R</td>
<td>N/A</td>
<td>Com.</td>
<td>2005</td>
<td>0.50-4.99</td>
<td>Two-stage random sampling</td>
<td>Hemo-Cue</td>
<td>76.0</td>
</tr>
</tbody>
</table>

**Abbreviations:** U=urban; R=rural; Com.=Community-based; Nat.=National; Vill.=Village; Comm.=Commune; Dis.=District; Pro.=Province; N/A=Not available.
**Table 3.7.3: Summary of prevalence data on anemia among school-aged children in Cambodia.**

<table>
<thead>
<tr>
<th>Area</th>
<th>Place</th>
<th>Setting</th>
<th>Study period</th>
<th>Age</th>
<th>N</th>
<th>Sampling design</th>
<th>Hb method</th>
<th>Anemia prevalence (severe)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Longfils</td>
<td>R</td>
<td>2 vill., 2 dis.</td>
<td>1999</td>
<td>Grade 1 children (6–15 years)</td>
<td>451</td>
<td>All grade 1 children present on sampling day</td>
<td>Lovibond method</td>
<td>64.1</td>
</tr>
<tr>
<td>2008(^{16})</td>
<td></td>
<td>2 primary schools</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>McLean</td>
<td>R</td>
<td>4 pro. 32 secondary schools</td>
<td>2007-2008</td>
<td>9 – 21 years</td>
<td>231</td>
<td>Random multi-stage cluster sampling</td>
<td>CBC (Sysmex KX21)</td>
<td>21.2</td>
</tr>
<tr>
<td>2009(^{17})</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Longfils</td>
<td>R</td>
<td>1 pro. 1 secondary school</td>
<td>2005</td>
<td>6 – 21 years</td>
<td>805</td>
<td>2-stage sampling; purposive sampling to include students with IDA</td>
<td>CBC (CellDyn 3200)</td>
<td>7%*</td>
</tr>
<tr>
<td>2008(^{18})</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Longfils</td>
<td>R</td>
<td>1 pro. 1 primary school</td>
<td>2005</td>
<td>6 – 21 years</td>
<td>699</td>
<td>2-stage sampling; purposive sampling to include students with IDA</td>
<td>CBC (CellDyn 3200)</td>
<td>13%*</td>
</tr>
<tr>
<td>2008(^{18})</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: U=urban; R=rural, IDA= iron-deficiency anemia; Vill.=Village; Comm.=Commune; Dis.=District; Pro.=Province.

*Prevalence of IDA
Table 3.7.4a: Summary of prevalence data on anemia among women in Cambodia.

<table>
<thead>
<tr>
<th>Area</th>
<th>Place</th>
<th>Setting</th>
<th>Study period</th>
<th>Status</th>
<th>Age</th>
<th>N</th>
<th>Sampling design</th>
<th>Hb method</th>
<th>Anemia prevalence (severe)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CDHS 2005</td>
<td>R/U</td>
<td>Nat.</td>
<td>2005</td>
<td>NPNLW</td>
<td>15.00-49.99</td>
<td>6408</td>
<td>2-stage cluster sampling within U/R strata</td>
<td>Hemo-Cue</td>
<td>44.3 (0.9)</td>
</tr>
<tr>
<td>CDHS 2005</td>
<td>R/U</td>
<td>Nat.</td>
<td>2005</td>
<td>PWLW</td>
<td>15.00-49.99</td>
<td>1811</td>
<td>2-stage cluster sampling within U/R strata</td>
<td>Hemo-Cue</td>
<td>55.4 (2.1)</td>
</tr>
<tr>
<td>CDHS 2000</td>
<td>R/U</td>
<td>Nat.</td>
<td>2000</td>
<td>NPNLW</td>
<td>15.00-49.99</td>
<td>2722</td>
<td>2-stage cluster sampling within U/R strata</td>
<td>Hemo-Cue</td>
<td>55.1 (0.7)</td>
</tr>
<tr>
<td>CDHS 2000</td>
<td>R/U</td>
<td>Nat.</td>
<td>2000</td>
<td>PWLW</td>
<td>15.00-49.99</td>
<td>889</td>
<td>2-stage cluster sampling within U/R strata</td>
<td>Hemo-Cue</td>
<td>66.3 (3.5)</td>
</tr>
<tr>
<td>CDHS 2000</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>3634</td>
<td></td>
<td></td>
<td>Total: 57.8 (1.3)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>8219</td>
<td></td>
<td>Total: 46.6 (1.0)</td>
</tr>
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</table>
### Table 3.7.4b: Continued.

<table>
<thead>
<tr>
<th>Area</th>
<th>Place</th>
<th>Setting</th>
<th>Study period</th>
<th>Status</th>
<th>Age</th>
<th>N</th>
<th>Sampling design</th>
<th>Hb method</th>
<th>Anemia prevalence (severe)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Charles 2011&lt;sup&gt;19&lt;/sup&gt;</td>
<td>R</td>
<td>1 dis.</td>
<td>Com.</td>
<td>All women</td>
<td>20.0-74.0</td>
<td>120</td>
<td>2-stage random sampling</td>
<td>CBC (CellDyn 3200)</td>
<td>25.0</td>
</tr>
<tr>
<td>Helmers 1999&lt;sup&gt;14&lt;/sup&gt;</td>
<td>R</td>
<td>40 vill., 20 comm.</td>
<td>Com.</td>
<td>NPW</td>
<td>N/A</td>
<td>924</td>
<td>2-stage random sampling</td>
<td>Hemo-Cue</td>
<td>69.0</td>
</tr>
<tr>
<td>Helmers 1999&lt;sup&gt;14&lt;/sup&gt;</td>
<td>R</td>
<td>40 vill., 20 comm.</td>
<td>Com.</td>
<td>PW</td>
<td>N/A</td>
<td>112</td>
<td>2-stage random sampling</td>
<td>Hemo-Cue</td>
<td>72.0</td>
</tr>
<tr>
<td>Kenfick 1998&lt;sup&gt;9&lt;/sup&gt;</td>
<td>R/U</td>
<td>125 vill.</td>
<td>Com.</td>
<td>NPW</td>
<td>N/A</td>
<td>994</td>
<td>Multistage random sampling</td>
<td>Hemo-Cue</td>
<td>69.0</td>
</tr>
<tr>
<td>Kenfick 1998&lt;sup&gt;9&lt;/sup&gt;</td>
<td>R/U</td>
<td>125 vill.</td>
<td>Com.</td>
<td>PW</td>
<td>N/A</td>
<td>97</td>
<td>Multistage random sampling</td>
<td>Hemo-Cue</td>
<td>74.0</td>
</tr>
<tr>
<td>Kenfick 2000&lt;sup&gt;10&lt;/sup&gt;</td>
<td>R/U</td>
<td>137 vill.</td>
<td>Com.</td>
<td>NPW</td>
<td>N/A</td>
<td>998</td>
<td>Multistage random sampling</td>
<td>Hemo-Cue</td>
<td>61.0</td>
</tr>
<tr>
<td>Kenfick 2000&lt;sup&gt;10&lt;/sup&gt;</td>
<td>R/U</td>
<td>137 vill.</td>
<td>Com.</td>
<td>PW</td>
<td>N/A</td>
<td>116</td>
<td>Multistage random sampling</td>
<td>Hemo-Cue</td>
<td>77.0</td>
</tr>
<tr>
<td>McLean 2009&lt;sup&gt;17&lt;/sup&gt;</td>
<td>R</td>
<td>1 dis.</td>
<td>Com.</td>
<td>WRA</td>
<td>15.0–54.0</td>
<td>231</td>
<td>2-stage random sampling</td>
<td>CBC (Sysmex KX21)</td>
<td>33.3</td>
</tr>
</tbody>
</table>

**Abbreviations:** U=urban; R=rural; Com.=Community-based; Nat.=National; Vill.=Village; Comm.=Commune; Dis.=District; Pro.=Province; NPNLW= non-pregnant, non-lactating women; PWLW=pregnant women or lactating women; NPW=non-pregnant women; PW= pregnant women; WRA=women of reproductive age; N/A=not available
CHAPTER 4: AN INVESTIGATION OF WOMEN’S NUTRITIONAL STATUS AND FOOD-RELATED KNOWLEDGE IN RURAL CAMBODIA

4.1 Introduction

Globally, iron deficiency is the most common micronutrient deficiency and severe iron deficiency is often associated with anemia.¹ Iron deficiency anemia (IDA) predominantly affects reproductive-aged women and children and is responsible for numerous health consequences. These include, but are not limited to: impaired cognitive function, fainting and dizziness, and an increased susceptibility to infection.²³ In particular, pregnant women who are anemic are at an increased risk of adverse pregnancy outcomes, including premature delivery and low birth weight children, which results in increased perinatal morbidity and mortality.⁴

Diets low in iron or bioavailable iron are one of the major causes of IDA.² However, deficiencies in other key micronutrients that influence hemoglobin metabolism can also contribute to anemia.⁵ In particular, vitamin A plays an essential role in hemoglobin synthesis through the mobilization of iron and is required for hematopoiesis.⁵⁶ Beyond its role in maintaining iron status, vitamin A is essential for maintaining the immune system and epithelial tissue. Vitamin A deficiency (VAD) can lead to eye damage and increases the severity of infections.⁷ The primary dietary cause of both iron and vitamin A deficiency is a poor diet that is low in diversity, in particular, an insufficient intake of animal source foods.⁸

Anemia is most prevalent in the developing world, where poverty is widespread and access to healthcare is limited.⁹ The largest population affected by anemia (315 million) resides in South-East Asia.¹⁰ In Cambodia, both anemia and VAD are serious health problems. Estimates
from the most recent Cambodian Demographic and Health Survey (CDHS) show that anemia affects 44% of the total population of Cambodia and approximately 44.9% of women living in Kandal province. The incidence of night blindness, a clinical sign of VAD, is experienced by twice the number of women living in rural areas compared to urban areas.7

Few comprehensive nutritional surveys have been completed in Cambodia. Data on the prevalence of anemia and the types of foods consumed by mothers aged 15-49 were collected through the 2005 CDHS. Among the women surveyed in Kandal province, 98% had consumed iron rich foods, such as meat, fish, shellfish and poultry the day and night prior to the interview, while 88% had consumed vegetables and fruits rich in vitamin A.7 The CDHS did not collect data on the total daily iron and vitamin A intakes, therefore, it is not clear whether or not daily requirements for iron and vitamin A are being met. Moreover, nutrition studies in Cambodia have failed to gain an understanding of both food consumption as well as nutritional knowledge as it relates to anemia and iron- and vitamin A- rich foods. This information is fundamental to producing data that can be used to design dietary modification and education interventions.

The current article reports on research conducted in several villages in rural Kandal Province. Specifically, data were gathered on the mean usual intakes of iron, vitamin A, and energy, as well as the use of iodized salt. In addition, the ways in which food consumption and nutritional knowledge constrain and/or facilitate iron, vitamin A and iodine status were investigated.
4.2 Materials and Methods

4.2.1 Data Collection

4.2.1.1 Study site and population

The study took place in August 2011, during the wet season, in five villages in Kandal Province: Preak Thom and Somrang Thom, Kien Svay District, Preak Khmeng and Veal Thom, Lvea Aem District, and Leuk Dek, located in Leuk Dek District. These villages were chosen because they were involved in programs organized by Resource Development International Cambodia (RDIC), a local, non-governmental organization, and were therefore accessible.

The sample consisted of 67 women aged 25-75; this included 30 women from Preak Thom, Preak Khmeng and Veal Thom who participated in the interactive 24-hour recall and pile sort activities and 37 women from Preak Thom, Veal Thom and Somrang Thom who participated in focus groups (11-14 women per group). In addition, at the outset of the study, three families from Preak Thom were involved in a free-listing activity. Key informants included: farmers; community elders; and women employed as cooks. Women sampled in Preak Thom had received health education. Women sampled in Preak Thom were of the highest socioeconomic status, followed by Somrang Thom, Preak Khmeng, Veal Thom, Leuk Dek.

RDIC identified key informants who were knowledgeable about food for the free-listing activity. Recall and pile sort participants were selected using random walk sampling with the intent to include an equal number of women from three socioeconomic (SES) strata. Potential participants were initially approached based on a preliminary assessment of SES. Women
selected were approached at their homes and through a short back and forth discussion the purpose of the study was explained and the formal SES assessment applied.\textsuperscript{15} It is unlikely that there was any response bias present because all the women approached decided to participate. Typically, rural \textit{Khmer} women are willing to speak about health and nutrition with others (Personal Observation, CVC). Focus group participants consisted of women from already-existing health education groups created by RDIC. For baseline characteristics of the study population, see Table 1.

Data were collected by the primary author and three research assistants employed by RDIC who are native \textit{Khmer} speakers and all study activities were conducted in \textit{Khmer}. In addition, informed verbal consent was obtained from all participants and study procedures were approved by the Research Ethics Board at the University of Guelph, Canada.

\textbf{4.2.1.2 Market surveys}

To determine the prices of iron- and vitamin A-rich foods, two market surveys, spaced two weeks apart were conducted at two markets (\textit{Phsa Moni} and \textit{Kohras}) to identify the average prices for commonly consumed foods rich in vitamin A or iron. Four fish vendors, were selected purposively (one from \textit{Phsa Moni} and three from \textit{Kohras}) and interviewed about the seasonality and price of \textit{chanwa phlieng} (\textit{Esomus longimanus}), a small fish reported to have more than six times the amount of iron compared with most small fish commonly consumed in Kandal Province.\textsuperscript{12}
4.2.1.3 Socioeconomic surveys

A socioeconomic score was calculated for each woman based on the sum of the values of the assets in her household. The asset-based approach as a proxy for household wealth is widely used and is based on the assumption that wealthier households will own a larger number of consumer items.\textsuperscript{15} An index of household socioeconomic status was constructed based on household ownership of 21 possessions such as a working car, bicycle, mobile phone, water tank, livestock (including cows, chickens, goats, adult pigs and growing pigs), and housing building materials (such as concrete, bricks, and iron sheets). For each woman an asset-based socioeconomic survey was completed.

4.2.1.4 Free listing

Prior to beginning the interactive 24-hour recalls and pile sort activity, free listing was completed with key informants in Preak Thom. Free listing assisted in the development of a list of key vitamin A, iron-rich and staple foods that was used to create cards for the pile sort activity. The free listing protocol used was adapted from Blum and colleagues ethnographic protocol for assessing community sources of vitamin A.\textsuperscript{13} The current study was focused on collecting information about key foods rich in vitamin A and/or iron. For this reason, the number of vitamin A-rich foods and staple-foods included on the final list was decreased to allow for the inclusion of iron-rich foods. Informants were asked to list the most common foods consumed in poorer areas in Preak Thom in the wet and dry seasons. A list of the foods listed was kept and 22 key foods were selected according to their iron and vitamin A content, and how commonly they were used.
4.2.1.5 Interactive 24-hour recalls

The interactive 24-hour recall method was used to estimate participants' mean usual intake of iron, energy and vitamin A. Approximately 24 hours before the recall interview, women were asked to observe the types and quantities of food they consumed on the recall day. Each woman was given a pre-calibrated bowl and cup (purchased from Koki Market, Kien Svay District, Kandal Province, Cambodia) to visualize their portion sizes on the recall day. In addition, literate participants were provided with a pencil and paper to mark their food consumption. Participants were reminded to eat normally and avoid eating directly out of communal pots, where possible. On the interview day, participants were asked to recall all foods and drinks they consumed on the previous day, from the morning through the night. Only women who reported feeling “normal” on the recall day were included in the study. The recalls were conducted according to the interactive four-pass method, described in Gibson and Ferguson’s guide. First, the participant was asked to list the foods and drinks consumed, in chronological order. In the second pass, each woman was asked to describe each of the foods and drinks consumed in more detail, including cooking method and brand names where applicable. To record fish consumed, the common name was documented using a previously compiled list of common fish species in the area. In particular, participants were asked whether or not they had consumed chanwa phlieng (Esomus longimanus). Next, the foods and drinks consumed were again reviewed and participants were asked to estimate the portion sizes consumed. In the fourth pass, the foods and drinks consumed were reviewed to probe for forgotten items.
Various methods of portion size estimation were used, including the bowl and cup, measuring spoons (Koki market, Kien Svay District, Cambodia) and measuring cups (Arcoware Limited, London, England), and a tape measure (Jobmate, Maxtech Consumer Products Limited, Waterloo, Canada). All measuring items were laid out and women were asked to indicate which item they felt represented their portion size most accurately. To estimate the individual portion size consumed from recipes made for the entire family, each woman was asked to estimate the amount of each ingredient used and the value estimated was divided by the total number of people who consumed the meal. The quantity of leftover food was estimated and included in the portion size calculation. For foods commonly purchased from vendors such as desserts and bor bor (a rice porridge with meat and vegetables often eaten for breakfast), research assistants estimated the size of each ingredient for a single portion. Where portion size information was not available in grams, the food was purchased at a local market and the amount reported was converted into an average weight using an electronic kitchen scale (Starfrit Ultra Slim Electronic Kitchen Scale, Nashua, USA). In the final discussion with the participant, the interviewer reviewed the recall with each woman to ensure accuracy. On average, each recall lasted 25 minutes. Ten recalls were completed in each of the villages of Preak Thom, Preak Khmeng and Veal Thom.

4.2.1.6 Pile sort activity

Pile sorts were conducted to understand local concepts of food combinations and attributes associated with iron-rich, vitamin A-rich and staple foods. Pictures of each food and its Khmer word were placed on laminated numbered cards. Following each interactive 24-hour
recall, the cards were laid out for participants. Participants were prompted to sort according to food expense, commonality of use, recipes, likes and dislikes, and general similarities and differences among the foods. However, women were not restricted to sorting into these categories, rather these category headings were suggestions that could be used to initiate the activity. Participants were allowed to sort the cards into as many piles as desired in one or more categories. Once each woman finished sorting, the primary author asked her to discuss the meaning behind each pile. Each pile sort activity lasted approximately 15 minutes.

4.2.1.7 Focus groups

Focus groups were carried out in Preak Thom, Leuk Dek and Samrong Thom with participants recruited through previously-formed health education groups. In Preak Thom, women had been exposed to health education sessions conducted by RDIC. In Leuk Dek and Samrong Thom, health education groups had recently formed through RDIC but education sessions had not yet begun. Between 11 and 14 women participated in each focus group. The objective for each focus group was to understand how health education groups impact women’s nutritional knowledge. The focus group guide presented women with two hypothetical scenarios about community members with symptoms of iron deficiency anemia and *kwak moin* (night blindness), and asked them to identify possible causes and treatment options. The scenario on severe vitamin A deficiency resulting in night blindness was adapted from Blum and colleagues’ ethnographic protocol. Questions were posed to explore the relationship between health and nutrition, and community-recommended diets for pregnant women, adolescent girls, breastfeeding women, and growing children. In addition, to
investigate food security, women were asked what worried them in everyday life. They were probed about whether or not they worried about having enough food and asked to explain the reasons for a lack of food.

4.2.2 Data Analysis

A socioeconomic score was calculated for each woman based on the sum of the values of the assets in her household based on a previously validated test. A value for each asset was created by calculating the proportion of the study households who owned one or more of that asset and then taking the reciprocal of the proportion to create a weighted-score. Each weighted-score was multiplied by the number of units of the item owned by that particular household, and all of the products were summed to produce a final socioeconomic score for each home. Housing materials were assigned a binary score (0 = not used, 1 = used). The distribution of the asset scores (11-101) was divided into three parts representing low (11-41), medium (41-71) and high scores (71-101) and recalls and pile sorts were assigned to the appropriate category. Averages of household asset scores were calculated for participants in each focus group. Prices obtained from the market surveys were averaged and the prices of foods were compared to determine which foods were both inexpensive and rich in either vitamin A or iron.

Intakes of energy, iron and vitamin A were calculated using NutriSurvey (Nutrisurvey for Windows, Dr. Juergen Erhardt, University of Indonesia, Jakarta, Indonesia). The standard NutriSurvey food database was complemented using USDA and Thai food databases available on the NutriSurvey website (www.nutrisurvey.de). For the iron content of local fish, the
common name of the fish consumed was documented during the recall and compared with Roos and colleagues’ set of values for the iron content of common Cambodian fish species. When the iron content of the fish species consumed had been documented, this value was entered along with the Nutrisurvey values for the vitamin A and energy content of cooked fish. In instances where the iron content of the fish species had not been documented, Nutrisurvey values for cooked fish was used. Nutrient values for ginger leaf (consumed by three participants), lime leaf, rice paddy herb, Java feroniella, Roselle and Ricefield rat (consumed by one participant each) were not found in the existing databases or the literature and were omitted from the nutrient analysis. Nutrient values for moringa were added to the database (see Table 4.7.5 for a list of the common and scientific names of foods referred to in this article). The iron intake generated by NutriSurvey was converted into bioavailable iron by taking into account the iron content of foods, as well as the presence of iron enhancers (heme iron and ascorbic acid) and inhibitors (phytate and tea) using Murphy’s algorithm. Total iron consumption for each meal was separated into heme and non-heme sources for each meal and summed. The total heme iron per meal was multiplied by an availability factor of 0.25. To determine the non-heme availability factor, intakes of ascorbic acid and meat, fish and poultry protein for each meal were converted into nutrients per 1000kcal and Murphy’s algorithm was applied to determine the percent availability. The resulting non-heme iron value was multiplied by a tea factor determined from a tea scale. The tea factors in this study ranged from 1 (no tea) to 0 (greater than 1000ml of tea). Vitamin A and iron intakes for each woman were compared to USDA dietary reference intakes (DRIs) and energy intakes were compared to Health Canada’s estimated energy requirements for active individuals. In addition, nutrient
intakes among women of low, medium and high socioeconomic status were averaged and compared.

Pile sort data was coded thematically under the following categories: recipes, deserts and snacks, likes and dislikes, expensive, cheap, eaten often, easy to make, easy to find and healthy. Frequencies for each food and theme were recorded and data were entered into SPSS Statistical Package for the Social Sciences (SPSS v.19.0, IBM Corporation, Armonk, USA). Multidimensional proximity scaling (MDS) and hierarchical clustering analysis was used to determine patterns in food consumption and practices. Specifically, dendrograms and MDS output for each SES group and the total population were examined. Pairs of related foods were reviewed to determine which characteristics were used to group them. Characteristics listed by five or more women in the pile sorts for both foods were recorded. A conceptual diagram (Figure 4.8.1) was then constructed to explain the relationship within and between different theme categories. Focus group data was compiled for each question and the answers given by each focus group were compared and contrasted. Emerging themes for each question were recorded and described.

4.3 Results

4.3.1 Market survey

The results of the market survey are shown in Table 4.7.2. Chicken and fish (particularly small fish) are inexpensive, rich sources of iron. Morning glory and papaya are inexpensive, vitamin A-rich food sources. One kilogram of *chanwa phlieng*, the iron-rich fish, is cheaper than
the price per kilogram of other small fish. Most vendors reported that the availability of *chanwa phlieng* peaks in November and December.

### 4.3.2 Energy and nutrient intakes

In general, women did not meet daily-recommended nutrient intakes. The average consumption of iron and vitamin A was well below the USDA DRI. The majority of women (97%) did not meet their daily-recommended intake of iron; seventy-seven percent consumed less than one half of the iron they required. Most women (70%) did not meet their DRI of vitamin A. In addition, 83% of women did not meet their daily energy requirement. Only seven women (23%) reported using iodized salt. No participants reported consuming *chanwa plieng* (*Esomus longimanus*). Intakes of calories, vitamin A and iron were not associated with socioeconomic status. See Table 3 for a summary of intakes of energy and micronutrients by age. No women were pregnant or nursing.

### 4.3.3 Pile sort activity

A conceptual diagram using information from the MDS and cluster analyses is displayed in Figure 4.8.1; the descriptors that link foods together are shown in Table 4.7.4. For the total population (including women from all SES groups), foods were primarily classified using the descriptors: eaten often, cheap, easy to find, healthy, expensive, dessert, or recipe. “Eaten often” is used to describe foods that are eaten at least a number of times a week, for example, rice is eaten with every meal and it is common for small fish to be eaten daily. Cheap foods are often linked with the phrase “easy to find”; foods that are “easy to find” are often grown around the house and are available at no cost. Foods such as mango, papaya, morning glory, ivy gourd.
and ginger leaf are labeled as both cheap and easy to find. Foods associated with health are
sweet potato, soy nut, mango and papaya. In particular, sweet potato, mango and papaya are
associated with energy and papaya was associated with reducing constipation. A number of
iron-rich foods, including pork intestine, beef, prawns, chicken and soybeans are labeled
expensive, whereas pork meat was considered to be inexpensive. Tea is also frequently
considered to be expensive. In addition, two vitamin A-rich foods, carrots and sweet potato, are
also included in the “expensive category”. The category “dessert” describes foods that are eaten
after or in between meals and included sweet potato, mango and papaya. Foods included in the
recipe category, such as morning glory and moringa are described as cooked often with other
foods in a stir-fry or soup. For participants in low, medium and high socioeconomic groups, the
descriptions associated with each food did not change appreciably.

4.3.4 Focus groups

4.3.4.1 Nutrition and health

Overall, women in the focus groups made a strong link between food and health.
Women stated that people get sick from not having enough “good food”, in particular, green
vegetables and meat. Consequences of poor nutrition discussed by women were sickness,
weakness and failure to grow. For children, women stated that sickness, feeling tired, diarrhea,
failure to grow and be smart, resulted from poor nutrition. Furthermore, women strongly felt
that poor nutrition and a lack of food were caused by a lack of money. Food insecurity was a
daily concern for many women. As participants stated, it is “all about the money”, and “if we
have money, we can buy good food”. A lack of food was also linked to flooding; women
discussed how vegetables could not be grown in these conditions. In addition, some women
Preak Thom discussed the inaccessibility of the market. Some women in Somrang Thom linked
being unhealthy to fate; here, fate likely refers to the Buddhist notion of karma.

4.3.4.2 Responses to iron and vitamin A deficiency scenarios

In response to Scenario 1, which described a woman with the symptoms of iron
deficiency anemia and asked participants to identify possible causes and treatment options,
women from each group linked the symptoms of iron deficiency anemia to a problem with
nutrition, in particular, a deficiency in vitamins. However, a specific deficiency in iron was not
mentioned. Participants in Preak Thom and Somrang Thom both suggested that a woman with
these symptoms should eat green vegetables. In Somrang Thom, women also suggested beef,
fish and meat. In Leuk Dek, participants stated that the individual should just eat healthy food
and/or use traditional medicine.

When presented with the vitamin A deficiency scenario, participants from each group
conveyed that the problem was vitamin A deficiency and linked the symptoms presented
(diarrhoea, frequent infections and kwak moin) with a lack of vegetables. Participants in Preak
Thom suggested the woman eat more foods rich in vitamin A, for example, pumpkin, sweet
potato, carrots, and green vegetables. Women in Somrang Thom stated that the woman should
eat more green vegetables and yellow fruits, and participants in Leuk Dek discussed traditional
medicine.
4.3.4.3 Iodized salt

Women in every group discussed how iodized salt was important to prevent goiters and to ensure the development of proper mental capacity in children. Some women stated that they had heard about the importance of iodized salt on television. However, participants expressed concerns that they had no money to buy the salt, or that it was not available in their area.

4.3.4.4 Breastfeeding and complementary foods

In all groups, women stated that in the past, some may have discarded breastmilk and fed infants other liquids, such as sugar water. However, in general, women stated that now they know that the first milk is healthy, and that one should breastfeed as soon as they are able. They attributed this knowledge to the recent broadcast of public service announcements on television. When asked how long one should breastfeed for, participants had a variety of answers, ranging from three months to five years, although the median response was 18 months. Women in all focus groups agreed that complementary foods should be introduced after six months. In general, participants stated these foods, such as healthy vegetables, fruits and meat, should be added to the infant’s bor bor (rice porridge, a common homemade complementary food). Participants agreed that women should eat differently when breastfeeding. Several foods were considered to be harmful to pregnant women. These included certain types of fish such as Kry fish, Kahel fish, Broma fish and Khgoeng fish. In addition, chek nuon (a type of banana) and salty foods such as Prahok (fermented fish paste)
were described to be harmful women’s health. Spicy hot foods like ginger and “peppered foods” were listed as good foods to eat.

**4.3.4.5 Pregnant women and adolescent girls**

All groups stated that adolescent girls should eat beans, peanuts and sesame seeds. In particular, women recommended that girls follow a vegetarian diet for three to six months after beginning menstruation for the first time. One group also discussed how women also may not be allowed to leave the house while menstruating. However, it was stated by one group that these practices around menstruation are diminishing. In general, participants stated that pregnant women should avoid drinking alcohol, and smoking and should eat normally. However women stated that spicy foods like chili should be avoided “because it will make your child hot inside you”. In addition, women discussed how salty foods like *prahok* should be avoided during pregnancy.

**4.4 Discussion**

The current study is the first in Cambodia to assess the status of food security together with nutrition practices of rural women. By confronting the research question using a mixed methods approach, we revealed that the vast majority of women do not meet daily nutrient intakes. Nutritious food was widely available in the marketplace, but women either chose not to, or could not afford to purchase and consume these foods.
4.4.1 Market surveys

The results of the market survey confirm Roos and colleagues’ finding that *chanwa phlieng* is an inexpensive, rich source of iron. Roos and colleagues report that consumption of the fish peaks in July-November.\(^1\) However, in this study, vendors reported that the availability of the fish peaks closer to the end of the year, in November and December. Additionally, although the study took place in August, none of the recall participants reported consuming the fish. Since villagers often raise small fish, further studies on *chanwa phlieng* should be completed to confirm its seasonality and explore the potential to raise the fish in communities year round. Preservation of the fish through salting or fermentation might also be an option, and this should investigated in due course. If *chanwa phlieng* could be made available in communities year round, it would provide a significant source of iron for individuals.

4.4.2 Energy and nutrient intakes

There are severe health consequences for women with deficiencies in vitamin A, iron, energy and iodine. Iron deficiency can lead to iron deficiency anemia, which can result in increased susceptibility to infection, decreased cognitive function and adverse pregnancy outcomes.\(^2\)-\(^4\) A diet low in vitamin A can increase the severity of infections, lead to eye damage and exacerbate iron-deficiency anemia.\(^5\)-\(^7\) Furthermore, insufficient intakes of energy in adults can lead to marasmus, which causes weight loss, and decreases immune function and cognitive ability.\(^2\) Finally, using non-iodized salt can lead to iodine deficiency disorders (IDD) for women in the study region; IDD cause high rates of impaired mental capacity and goiter as well as low
birth weight, stillbirth cretinism, increased risk of perinatal and infant mortality in pregnant women.\textsuperscript{12,20}

Similar to the findings of the CDHS conducted in 2005,\textsuperscript{7} our study reveals that women frequently consume iron rich foods, especially fish and pork, at each meal. However, the results of the current study demonstrate that although iron-rich foods are consumed frequently, these foods are not eaten in sufficient quantities for women reach their DRI of iron. Only 13% of participants consumed tea with meals, and typically these women were of a higher socioeconomic status. Nevertheless, any tea consumption significantly reduces the availability of non-heme iron.\textsuperscript{17}

In contrast to the finding of our study, where less than one half of the women consumed vitamin A-rich foods, the 2005 CDHS observed that 88% of women consumed vegetables and fruits rich in vitamin A.\textsuperscript{7} However, it is notable that only 30% of participants met their vitamin A requirement in the 24 hour period prior to the interview. This suggests that foods rich in vitamin A (for example, morning glory, ivy gourd, pumpkin, and sweet potato) can be accessed by women, but are not eaten in sufficient quantities.

While the most recent Cambodia Demographic and Health Survey (2010) reports that 86.3% of households in Kandal Province had some iodine in their salt,\textsuperscript{11} only 23% of women in the current 24-hour recalls reported using iodized salt. This discrepancy may be accounted for because women were unknowingly using iodized salt, though we did not perform chemical analysis of salt samples so we cannot be certain. It has been suggested that fish sauce may be made with iodized salt since the introduction of the iodized salt law in 2003, however this has
not been confirmed. Nonetheless, work in Thailand has shown that fish sauce is used widely and can be easily and cost-effectively fortified with iodine, and thus fortified products have the potential to be included in national programs for iodine deficiency in South-East Asia. Ninety-three percent of women reported to cook with fish sauce at least once during the recall period. Thus, the current study reveals that fish sauce is widely used in Cambodian households and could therefore make a good vehicle for iodine fortification.

One limitation of the recall method used is that the relative validity of recall data was not assessed because a reference data set is not available. This clearly highlights the need for food consumption studies in the region. Recall data will have reduced accuracy if participants eat atypically during the recall period, despite instructions to the contrary. However, it is unlikely that atypical eating habits were a large source of bias in the recall data as these women are comfortable with speaking openly about food and health practices (personal observation, CVC). Nevertheless, this limitation cannot be avoided entirely. Finally, due to time and funding constraints it was not possible to obtain a sample representative of the population of Kandal province. A representative food consumption study should be completed to build upon this study and validate the findings.

The quality of the 24-hour recall data depends on the quality of the measurements taken. Compared to non-interactive methods, the interactive 24-hour recall is more reliable. This is because the 24-hour interactive recall includes several procedures that minimize the major sources of error that typically occur in 24-hour recalls, increasing the quality of the data received. For example, through this protocol, women were provided with pre-calibrated cups
and bowls for the recall days, discouraging them from eating from a common pot and allowing for a more accurate visualization of the amount of food consumed. In addition, the four-phase technique of the interactive 24-hour recall provided participants with many opportunities to remember foods that may have been forgotten in the initial phase.

4.4.3 Pile sort activity

The results of the pile sorts corroborate findings from the market surveys and 24-hour recalls. For example: tea was placed in the “expensive category” in the pile sort activity and only women in the 24-hour recall reported drinking tea which indicates that for the majority of women, tea would probably not affect iron intake negatively; vitamin A-rich foods were placed in the “cheap category”, were present in the 24-hour recall of the women in the lower socioeconomic groups, were grown in home gardens and were readily available in the markets; finally, both the pile sort and market survey results confirm that small fish are a cheap and accessible source of iron, whereas beef is considered expensive. Although in the pile sort activity chicken meat was associated with being expensive and pork meat was associated with being cheap, the market surveys show that the price of chicken is less than that of pork. In addition, although pork intestine was considered more expensive than pork meat in the pile sorts, we observed that pork intestine was generally cheaper than meat at the time of the survey. The reason for this observation is not clear, though it may be that participants might have been confused about the meaning behind the pile sort card for pork because the card presented a graphic of a whole pig, rather than pork meat specifically.
4.4.4 Focus groups

Overall, groups recognized the importance of iodized salt, breastfeeding and the symptoms of vitamin A deficiency. This may be due to televised health education campaigns, in particular, on iodized salt and breastfeeding, and the existence of *kwak moin*, a Khmer term for night blindness. Although women realize the importance of iodized salt, focus group data suggests that households may not have access to iodized salt or that they are unknowingly consuming a product fortified with iodized salt. This may be due to the high cost of iodized salt (up to five times greater than that of non-iodized salt) and low level of iodized salt available in Cambodia\textsuperscript{20} however there are reports that iodized salt is used in fish sauce and women may be unaware that they are using a fortified product. Initiatives that lower the cost of iodized salt for households and increase the coverage of iodized salt, specifically, those that encourage the compliance of salt producers and distributors to the universal salt iodization law of 2003, should be explored.\textsuperscript{20}

Despite the similarities in knowledge about breastfeeding and iodized salt among all three groups of women, according to level of health education and socioeconomic status, there are some apparent differences in nutritional knowledge. Focus group data showed that women in Preak Thom, who had previously received health education and whose village had the highest mean socioeconomic status, are more nutritionally knowledgeable than those in Somrang Thom, where women received no education and had a moderate socioeconomic status. In turn, women in Somrang Thom were more knowledgeable than participants in Leuk Dek, who received no health education and were of lower socioeconomic status. For example, in response to the vitamin A deficiency scenario, participants in Preak Thom stated that the
woman should consume specific vegetables high in vitamin A, while the other groups provided a more general response or recommended traditional medicine.

Focus group data on the time of initiation of breastfeeding is consistent with results from the 2010 CDHS. In general, participants stated that women should initiate breastfeeding as soon as possible because the first milk is healthy. Similarly, the 2010 CDHS report states that among last-born children born in the 2 years prior to the survey, 66.5% of women reported initiating breastfeeding within an hour of birth and 83.8% started to breastfeed within a day. These results suggest that the majority of Cambodian women and infants are receiving the benefits of early initiation of breastfeeding, including a high source of nutrition and antibodies for infants and decreased post partum blood loss for women.

Moreover, women’s knowledge about the duration of breastfeeding also concurred with the 2010 CDHS results. The median duration of breastfeeding reported in this study in Kandal Province was 18 months and in the CDHS, it was 20 months. Finally, most participants reported adding complementary foods after six months; this is consistent with the 2010 CDHS finding that 75% of children in Cambodia are exclusively breastfed under 6 months and with UNICEF and WHO guidelines for infant and young child feeding practices. Since some women in the current study sourced their knowledge about breastfeeding to televised health campaigns, this suggests that these types of health campaigns are successful in this context.

In many parts of South East Asia, including Cambodia, humoral theory guides women’s perspectives of the causes of health and illness and practices for upholding health. In humoral theory, health is defined by a state of balance and pregnancy is described using a hot-cold
distinction. During pregnancy, a woman is in a hot state, therefore, consuming hot foods can make her child too hot. The loss of hot blood during delivery leads to a cool postpartum state. Due to this cold, vulnerable state following delivery, Cambodian women practice roasting (known as Ang Pleung) in which they are physically heated by lying on a bed above a stove or charcoal fire following birth. During this period, women also consume hot food to restore heat to their bodies.\textsuperscript{23} Therefore, adherence to the avoidance of hot, spicy foods during pregnancy and consumption of these foods during the postpartum state can be explained by the incorporation of humoral theory into the Khmer medical worldview. In addition, Khmer women experience culture-bound postpartum complications, which include priey krawlah pleung and toas. Priey krawlah pleung causes symptoms such as loss of consciousness, abnormal behaviour, seizures and fainting. Cambodian women often describe the condition to be a result of a spirit that attacks women while roasting or a result of severe swelling during pregnancy. Trained midwives report that the condition is a hypertensive disorder exacerbated by eating salty foods. Khmer women often attribute postpartum relapses in health to toas, a disorder that commonly occurs from eating the “wrong food”. Specific foods considered dangerous include different varieties of fish, often poisonous and certain species of banana.\textsuperscript{22} Thus, the avoidance of salty foods like prahok during both pregnancy and in the postpartum state and certain types of fish and banana are likely explained by priey krawlah pleung and toas respectively. If Cambodia is to take action to preserve the health of reproductive-aged women, further studies should be completed to investigate Khmer food classifications and the impact of accepted and restricted foods on nutritional status during adolescence, pregnancy, and the postpartum state.
Food restrictions during pregnancy and after delivery are common cross-culturally and have been shown to affect daily nutrient intakes.25-27

4.5 Conclusions

Findings indicate that women in rural Kandal province, Cambodia are likely deficient in Vitamin A, iron, energy and iodine. Deficiencies in energy and micronutrients have severe health consequences and may decrease a woman’s earning potential, exacerbating household poverty. Although women who have previously received health education are likely more nutritionally knowledgeable, the mere provision of health education does not appear to be sufficient to change practice. Most women stressed that they did not have enough money to purchase healthy food. Interventions to combat nutrition must be comprehensive. Programs should combine health education with other initiatives that address detrimental social and economic power relations that prevent women from putting nutritional knowledge into action. In addition to education, initiatives to assist women in increasing household income should be implemented. For example, community food production programs that work with women to organize food production at the household and village level could create small farms for poultry, livestock and fish and year-round gardens with micronutrient-dense foods. This type of community program would allow women to gain nutritional knowledge and allow for the improved nutritional status of both them and their families.
4.6 References

4.7 Tables

Table 4.7.1: Average age, number of pregnant women, and socioeconomic asset score* (per village) of 67 women who participated in an investigation of nutritional status and knowledge in Kandal Province, Cambodia.

<table>
<thead>
<tr>
<th>Village</th>
<th>Age^</th>
<th>Asset score^</th>
<th>Recall</th>
<th>Focus Group</th>
<th>Pile Sort</th>
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</thead>
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<td>52(11)</td>
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<td>✓</td>
<td>✓</td>
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<tr>
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<td>34(12)</td>
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<tr>
<td>Leuk Dek</td>
<td>54(15)</td>
<td>29(23)</td>
<td></td>
<td>✓</td>
<td></td>
</tr>
<tr>
<td>Somrang Thom</td>
<td>46(14)</td>
<td>39(28)</td>
<td>✓</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

✓ Surveys women participated in, by village
^ Reported as: Mean (Standard Deviation)

* Asset scores for focus group participants in Preak Thom were determined using scores for women who participated in recalls and pile sorts in Preak Thom
Table 4.7.2: Average year-round cost of vitamin A- and iron-rich foods in Kandal Province, Cambodia compiled through two market surveys in August 2011.

<table>
<thead>
<tr>
<th>Name of Food</th>
<th>Unit</th>
<th>Price (KHR)*</th>
<th>Price (USD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Beef</td>
<td>1kg</td>
<td>26000</td>
<td>0.65</td>
</tr>
<tr>
<td>Carrot</td>
<td>1kg</td>
<td>5000</td>
<td>1.25</td>
</tr>
<tr>
<td><em>Esomus longimanus</em></td>
<td>1kg</td>
<td>5000</td>
<td>1.25</td>
</tr>
<tr>
<td>Chicken</td>
<td>1kg</td>
<td>15000-16000</td>
<td>3.75-4.00</td>
</tr>
<tr>
<td>Chicken egg</td>
<td>10 eggs</td>
<td>5000-6000</td>
<td>1.25-1.50</td>
</tr>
<tr>
<td>Fish (big)</td>
<td>1kg</td>
<td>13000-14000</td>
<td>3.25-3.50</td>
</tr>
<tr>
<td>Fish (medium-sized)</td>
<td>1kg</td>
<td>8000-14000</td>
<td>2.00-3.50</td>
</tr>
<tr>
<td>Fish (small)</td>
<td>1kg</td>
<td>8000-10000</td>
<td>2.00-2.50</td>
</tr>
<tr>
<td>Mango</td>
<td>1kg</td>
<td>300</td>
<td>0.750</td>
</tr>
<tr>
<td>Moringa</td>
<td>1 bunch</td>
<td>300</td>
<td>0.080</td>
</tr>
<tr>
<td>Morning glory</td>
<td>1 bunch</td>
<td>200-500</td>
<td>0.050-0.125</td>
</tr>
<tr>
<td>Mung bean</td>
<td>100g</td>
<td>1000</td>
<td>0.250</td>
</tr>
<tr>
<td>Papaya</td>
<td>1kg</td>
<td>300-600</td>
<td>0.075-0.150</td>
</tr>
<tr>
<td>Pork</td>
<td>1kg</td>
<td>20000-22000</td>
<td>5.00-5.50</td>
</tr>
<tr>
<td>Pumpkin</td>
<td>1kg</td>
<td>2000-2500</td>
<td>0.50-0.625</td>
</tr>
<tr>
<td>Soybean</td>
<td>100g</td>
<td>100000</td>
<td>2.50</td>
</tr>
<tr>
<td>Sweet Potato</td>
<td>1kg</td>
<td>1500-2000</td>
<td>0.375-0.500</td>
</tr>
</tbody>
</table>

*KHR = Khmer Riel; 4000 KHR = $1 USD
Table 4.7.3: Percent of women who met the daily recommended intakes (DRI) for iron and vitamin A, estimated average requirement of energy and used iodized salt in 30 interactive 24-hour recalls in Kandal Province, Cambodia.

<table>
<thead>
<tr>
<th>Age</th>
<th>Total # Women</th>
<th>Iron DRI (mg/d)</th>
<th>% met iron needs</th>
<th>Vitamin A DRI (ug/d)</th>
<th>% met Vit A needs</th>
<th>Energy EAR* (kcal/d)</th>
<th>% met energy needs</th>
<th>Iodized salt</th>
</tr>
</thead>
<tbody>
<tr>
<td>19-30</td>
<td>7</td>
<td>8.1</td>
<td>0</td>
<td>500</td>
<td>1</td>
<td>2350</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>31-50</td>
<td>10</td>
<td>8.1</td>
<td>0</td>
<td>500</td>
<td>5</td>
<td>2250</td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td>51-70</td>
<td>12</td>
<td>5</td>
<td>0</td>
<td>500</td>
<td>2</td>
<td>2100</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>&gt;70</td>
<td>1</td>
<td>5</td>
<td>1</td>
<td>500</td>
<td>1</td>
<td>2000</td>
<td>1</td>
<td>0</td>
</tr>
</tbody>
</table>

* EAR = estimated average requirement
Table 4.7.4: Characteristics associated with groupings of vitamin A-rich, iron-rich and staple foods gathered from multidimensional scaling and hierarchical clustering analysis of a pile sort activity by 30 Cambodian women in Kandal Province.

<table>
<thead>
<tr>
<th>Foods</th>
<th>Recipe</th>
<th>Dessert</th>
<th>Expensive</th>
<th>Healthy</th>
<th>Easy to find</th>
<th>Cheap</th>
<th>Eaten often</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sweet potato, soy nut</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mango, papaya</td>
<td></td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pork intestine, beef</td>
<td></td>
<td></td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kale, pumpkin, pork</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>X</td>
</tr>
<tr>
<td>Morning glory, ivy gourd, ginger leaf</td>
<td>X</td>
<td></td>
<td></td>
<td>X</td>
<td>X</td>
<td></td>
<td>X</td>
</tr>
<tr>
<td>Large fish, moringa</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td>X</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Shrimp, peanuts, chicken, carrot</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td>X</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Small fish, noodles</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>X</td>
</tr>
<tr>
<td>Tea</td>
<td></td>
<td></td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rice</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>X</td>
</tr>
</tbody>
</table>
Table 4.7.5: Common and scientific names of foods from the pile sort investigation, market survey and interactive 24-hour recalls referenced in this article.

<table>
<thead>
<tr>
<th>Common Name</th>
<th>Scientific Name</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carrot</td>
<td><em>Daucus carota</em></td>
</tr>
<tr>
<td>Mango</td>
<td><em>Mangifera indica</em></td>
</tr>
<tr>
<td>Moringa</td>
<td><em>Moringa oleifera</em></td>
</tr>
<tr>
<td>Morning glory</td>
<td><em>Ipomoea aquatica</em></td>
</tr>
<tr>
<td>Mung bean</td>
<td><em>Vigna radiata</em></td>
</tr>
<tr>
<td>Papaya</td>
<td><em>Carica papaya</em></td>
</tr>
<tr>
<td>Pumpkin</td>
<td><em>Curcubita sp.</em></td>
</tr>
<tr>
<td>Soybean</td>
<td><em>Glycine max</em></td>
</tr>
<tr>
<td>Sweet potato</td>
<td><em>Ipomoea batatas</em></td>
</tr>
<tr>
<td>Malabar Spinach</td>
<td><em>Basella alba</em></td>
</tr>
<tr>
<td>Ginger leaf</td>
<td><em>Zingiber zerumbet</em></td>
</tr>
<tr>
<td>Lime leaf</td>
<td><em>Citrus hystrix</em></td>
</tr>
<tr>
<td>Rice paddy herb</td>
<td><em>Limnophila chinesis</em></td>
</tr>
<tr>
<td>Java feroniella</td>
<td><em>Feroniella lucida</em></td>
</tr>
<tr>
<td>Roselle</td>
<td><em>Hibiscus sabdariffa</em></td>
</tr>
<tr>
<td>Ricefield rat</td>
<td><em>Rattus argentiventer</em></td>
</tr>
<tr>
<td><em>Chanwa phlieng</em></td>
<td><em>Esomus longimanus</em></td>
</tr>
<tr>
<td>Ivy gourd</td>
<td><em>Coccinia indica</em></td>
</tr>
<tr>
<td>Chinese kale</td>
<td><em>Brassica alboglabra</em></td>
</tr>
</tbody>
</table>
4.8 Figures

Figure 4.8.1: Aggregated multidimensional scale diagram of a pile sort activity completed by 30 Cambodian women, including 22 key iron-rich, vitamin A-rich and staple foods.

CHAPTER 5: IRON DEFICIENCY ANEMIA IN RURAL CAMBODIA: COMMUNITY TRIAL OF A NOVEL SUPPLEMENTATION TECHNIQUE

5.1 Introduction

Iron-deficiency anemia (IDA) is the single most common micronutrient deficiency in the world, affecting >3.5 billion men, women and children. Iron deficiency (ID) is inherently associated with poverty, so it is particularly prevalent in the developing world where the problem is often exacerbated by limited access to appropriate healthcare and treatment. According to the World Health Organization (WHO), anemia is estimated to affect 66.4 and 57.3% of pregnant and non-pregnant women of reproductive age, respectively. Consequences
of IDA include: inhibited growth, impaired cognitive development, poor mental and motor performance, reduced work capacity and an overall decreased quality of life.\textsuperscript{4–9}

Randomized controlled trials in Ethiopia, Brazil and Malawi demonstrate that the use of a cast iron pot for cooking leads to improved blood parameters and decreased levels of IDA in women and children.\textsuperscript{10–12} Though iron pots do leach iron into food, the acceptability of this supplementation technique appears limited. Prinsen-Geerligs et al.\textsuperscript{13} reported that a number of problems exist with iron cooking pots, including: aesthetic and health concerns regarding rusting; increased weight and decreased manufacturing quality when compared with aluminum pots; ease with which the pot can be cleaned, with aluminum pots perceived as being easier to clean; and a perceived change in taste and appearance of food when left in the pot overnight.

Currently a vast majority of Cambodians use aluminum instead of cast iron pots (Pers. Obs.) so it would not be possible to explore this option to ameliorate IDA. Therefore, the objective of the current study was to evaluate whether or not the use of a small, lightweight iron ingot placed in aluminum cooking pots in Cambodia could serve to ameliorate IDA. One of the critical challenges with various options for iron replacement is compliance so the current study was designed to investigate whether or not a series of follow-up visits affected compliance.

5.2 Materials and methods

This longitudinal community-wide trial was conducted in the village of Preak Ruessei, Kampong Kong Commune, Kaoh Thum District, Kandal Province, Cambodia, between September 2008 and February 2009. A control group and two treatment groups included: (i) an
intervention group that received an iron fish with an introductory session and no follow-up (NFU), and (ii) a second intervention group that received an iron fish, an introductory session and three weekly follow-up (FU) meetings immediately after distribution. Follow-up visits attempted to enhance compliance by addressing concerns with the treatment and providing suggestions on how to incorporate the iron fish into daily cooking routines. Study procedures were approved by the Research Ethics Board at the University of Guelph, Canada.

5.2.1 Study site and population

Participants in the village of Preak Russei had been previously surveyed by a local non-governmental agency, Resource Development International Cambodia (RDIC), during June 2008, as part of ongoing interventions to improve health status. At this time, IDA was treated as needed with 30 days of oral iron treatment (Ferrovit Softgel Capsules, Mega Lifesciences Ltd, Samutprakarn, Thailand) for all women aged >16 years who were found to be anaemic (hematocrit < 40%) in a village-wide anemia screening programme. Hemoglobin (Hb) was not measured. In total, 88.4% of the women were classified as anaemic and were given a one-month course of iron pills. Villagers were approached 2 months after the distribution of iron pills to determine willingness to participate in the current study and were enrolled if they agreed to the conditions described in the written informed-consent process.

5.2.2 Recruitment

The primary study population consisted of 256 households from Preak Russei. The village was divided into four socio-economic strata based on crude observation of housing condition, belongings and clothing. Within each stratum, three clusters of approximately 15
homes were identified and women were invited to participate in the study. All eligible women who wished to participate within the selected households were enrolled in the trial, for a total of 183 households. Four households, whose owners did not wish to participate, were replaced by others in geographic proximity. Baseline blood samples were collected to eliminate severely anaemic women (hematocrit < 30%, n = 5). After a baseline data collection period of 3 weeks (one sampling round per household), 60 households each were randomly assigned to one of the three treatment groups using a random digit generator (Microsoft Excel 2007, Redmond, Washington, USA).

Group sizes of 60 women allowed a power of 99% (alpha 0.05), however we expected some dropouts. The final sample size of approximately 35 women per group provided a power of 90%.

5.2.3 Study completion

Baseline blood parameters and demographics were compared among those participants who completed the study and those who dropped out prior to completion.

5.2.4 The intervention – iron fish

Each household in the NFU and FU groups received a cast iron fish to place in the daily cooking pot. The iron fish were designed and produced locally, using readily available scrap iron at a small factory in Kandal Province. The iron fish was designed to resemble a local fish species that is considered lucky in village folklore. Previous research shows that iron uptake is greatly enhanced by the concurrent consumption of ascorbic acid and the avoidance of concurrent
consumption of polyphenols and phytates. Most soups in the Cambodian diet are soured with ascorbic acid and soup represents a significant component of the diet, therefore soup was chosen as the primary vehicle for iron supplementation in this study. Some participants preferred to use the fish while boiling their drinking water, and were therefore encouraged to add a small amount of citrus fruit juice to the boiled drinking water prior to consumption. Participants were instructed to cook soup or boil water with the fish for at least 10 minutes and daily use was encouraged.

5.2.5 Data collection

Baseline demographic data were collected at the start of the study through face-to-face interviews. Blood samples were collected at baseline (September 2008) and at 3 (December 2008) and 6 (February 2009) months during the study period. Blood samples (2 ml in EDTA-coated tubes and 1 ml for serum collection; Source: Greiner Bio-One, Shanghai, China) were collected for routine hematology testing, including Hb and hematocrit; serum iron (SFe) levels were also measured (Paramed Laboratoire D’Analyses Medicales, Phnom Penh, Cambodia). For the first 3 weeks of the study, participants in the FU group were visited once per week by the primary author and a translator to encourage the use of the iron fish and to respond to any questions. No participants identified any major issues during these visits. Participants were visited at 3 months and a second blood sample was collected. Results of this blood test were used to assess continued eligibility of the participant in the study; if the participant was found to have a hematocrit <30% he/she would be removed from the study and treated with
conventional oral iron supplements. The final blood sample was taken at the end of the study period when samples were also tested for C-reactive protein (CRP).

5.2.6 Outcomes

The Hb and SFe levels were compared at baseline, 3 and 6 months after the intervention. Data collected on CRP levels were used to exclude participants assumed to have an inflammatory condition (n = 20). Normal levels for hb (≥120 g l−1), SFe (59–148 µg l−1) and CRP (<6.0 mg l−1) were defined according to Paramed Laboratories (Phnom Penh, Cambodia).

5.2.7 Statistical analysis

The study used households as the unit for randomization and intervention, and individual women were the unit of data collection. Because nearly all households (98.3%) included only one subject, it was assumed that the potential within-household influence was minimal and that all participating women could effectively be treated as independent. Therefore, individual women were used as the primary unit of data analysis.

Data were entered into Statistix Version 7 (Analytical Software, Tallahassee, FL, USA) for statistical analysis. To ensure effective randomization, univariate analyses were used to examine outcome variables and covariates among treatment groups at the start of the study. One-way analysis of variance followed by a least significant difference test were used to compare Hb, SFe, age of participants and number of pregnancies per participant in each group; the proportion of post-menopausal women was tested with a chi-square test. Because loss-to-follow-up occurred over the 6-month study, a Student’s t-test was used to compare baseline Hb
and SFe of women who dropped out prior to completion compared with those that completed the study.

Preliminary analyses involved examining data using descriptive statistics, frequency distribution tables and histograms. To compare outcomes (3 and 6 month values of Hb and SFe) within women and among groups the following statistical tests were used: paired t-tests were used to examine within woman changes from baseline to 3 months and from 3 to 6 months for Hb and SFe; two-sample t-tests were used to compare changes in Hb and SFe among groups and also to compare values among groups at a given time in the study; McNemar chi-square tests were used to compare the proportion of women with normal values of Hb and SFe within woman over time; and chi-square tests were conducted to compare the proportion of women with normal values of Hb and SFe among groups at a given time in the study. The level of significance for all statistical analysis was set at P < 0.05.

Multi-variable analyses were conducted by regressing the outcome using multiple linear regressions: either Hb or SFe on treatment while controlling for the covariates age, post-menopausal status and number of pregnancies. These analyses used multiple linear regression for numeric dependent variables (e.g. Hb and SFe levels) or logistic regression for dichotomous variables (e.g. normal Hb versus anemia). Models were built using a backward elimination selection process whereby covariates were dropped one at a time based on the highest P-value and retained only if P < 0.05. Confounding was determined by identifying variables whose coefficients changed by 20% and/or became either significant or insignificant as another
covariate left the model. Outliers were identified if SFe was $\geq 130$ µg l$^{-1}$ or CRP $\geq 6.0$ mg l$^{-1}$; data from women with these values were excluded from the analyses.

5.3 Results

5.3.1 Recruitment

A total of 194 participants from 183 households were assessed for eligibility, from which 189 women from 180 households entered the study (Figure 5.8.1). Following baseline blood sampling, five women were identified as severely anaemic and were excluded. Of the 180 households recruited into the study, 177 households had only one participant, three households had two participants and two households had three participants. Because loss-to-follow-up was observed in each group, hematological data were collected for only 120 participants; 23 participants were removed from analysis because of abnormal CRP or outlier SFe.

There was no significant difference in Hb and SFe concentrations between those participants who dropped out of the study prior to completion compared with those who did complete the study (data not shown).

5.3.2 Outcomes

Baseline blood parameters and demographics did not differ significantly between groups ($P < 0.05$) (Table 5.7.1). On average, in each of the three treatment groups, there was no significant change in Hb levels either from baseline to the 3-month evaluation or from baseline to the 6-month evaluation (Table 5.7.2). In contrast, average SFe values decreased significantly
from baseline to the 3-month evaluation, in all three treatment groups. The decreases in SFe were still significant at 6 months, in all three groups.

After adjusting for covariates (CRP at 6 months) and baseline values, the mean Hb and mean SFe were significantly higher in the FU group at the 3-month evaluation, compared with the control and NFU groups (Table 5.7.3). There were, however, no such differences at the 6-month evaluation. The NFU group showed no such differences compared with the control group. There were no significant changes in Hb or SFe in any group, between the 3- and 6-month evaluations (data not shown).

Women in the FU group were 10.4 times [95% confidence interval (CI): 3.1–35.2, \( P < 0.0001 \)] more likely to have normal Hb levels and 8.0 times (95% CI: 1.6–39.7, \( P = 0.004 \)) more likely to have normal SFe levels than the control group at 3 months. Similarly, women in the FU group at 3 months were 4.1 times (95% CI: 1.2–14.3, \( P = 0.02 \)) more likely to have normal Hb levels than the NFU group. These same relationships were not significant at 6 months.

5.4 Discussion

We studied the effect of a supplemental iron technique as a practical, household intervention on hematological indices in women of rural Cambodia. The results were promising at 3 months of the study: regular use of an iron ingot placed in the cooking pot was effective at elevating Hb and SFe levels, relative to a control group, and the proportion of anaemic women was lower in the study group using the iron supplement technique with follow-up visits compared with controls and no follow-up. However, the beneficial effects of the iron ingot did not persist: by 6 months, the levels of Hb and SFe had fallen in all groups and the proportion of
anaemic women had increased. These findings suggest that the iron ingot was effective in the first 3 months of the trial but that either the efficacy of the intervention waned or there was change in environmental parameters in the second half of the study.

Appropriate design of the iron ingot was a major consideration during the preparatory stage of the trial. Acceptability of the treatment was vital for compliance during the trial.\textsuperscript{13,16} It was important to design an ingot that was relatively lightweight, easy to clean and attractive. In addition, there was an interest in designing an ingot that would be intriguing to the women. The final design was an ingot cast in the shape of a fish that is considered lucky in Cambodian culture. The fish was engineered to a relatively flat design to maximize the exposed surface areas in the cooking pot to allow for a greater degree of iron leaching. The fish-shaped ingot was produced by a local metal worker (Kean Svay Commune, Kandal Province, Cambodia) from scrap metal and preliminary tests were completed to ensure that the fish were contaminant free and safe to use. The acceptability of the fish in the community was pre-tested through discussion with village elders and community members.

The efficacy of iron supplementation using iron cooking pots in the prevention and/or treatment of IDA has been reported in a number of different developing country communities.\textsuperscript{10-12,17} A number of these studies indicated positive or a trend towards positive results but two significant challenges were noted. First, the acceptability of using an iron cooking pot is limited: the weight of the pot and in some cases the cultural preference to use aluminum or other pots made compliance difficult.\textsuperscript{13} Secondly, the iron pots rust quickly and this reduces acceptability.\textsuperscript{13} In Cambodia, the cost of the iron pots is prohibitive in most rural
communities. The fish ingot overcame these problems. Each fish cost \$1.50 (USD) to produce, which is considerably cheaper than supplying each household with an iron pot. To set the economic impact of this technique in context, conventional oral supplements in Cambodia cost \$2.50 per person per month. Studies have yet to be conducted on the effective life usage of the fish ingots but it is likely to be at least several years, making the ingots an effective and economically viable approach. To date, no data have been gathered on the bioavailability of iron from the ingots but experiments are currently underway.

Blood parameters used in the current study to determine iron status were a compromise based on the practical settings. The WHO suggests that Hb concentration should be measured to determine ID and IDA. Tests for serum ferritin or transferrin receptors were not available in the field and would have been too costly. The current study, in addition to reporting on Hb levels, also tested for SFe levels. SFe represents a fraction of the total iron in the body that is circulating and bound primarily to transferrin. Unfortunately, SFe is also affected by infection and inflammation so CRP was measured in the participants at the end of the trial to exclude individuals that might have elevated SFe levels in conjunction with concurrent inflammation. This approach has been recommended by the WHO as an effective means of eliminating potentially erroneous and confounding data.

Despite the promising data in the first half of the trial for the group using the fish with follow-up visits, the elevated hematological parameters and improvement in the proportion of anaemic women were not maintained. It is possible that the quality of the water used in the cooking changed over the period of the trial. The study was conducted from September 2008 to
February 2009. The monsoon season is from May to October each year with the heaviest rain occurring between September and October. The monsoon rain is stored during this period of the heavy rainfall and for approximately one month following the monsoon, rural villages effectively use monsoon water for drinking and daily cooking. However, beginning in December as the dry season commences, it is common to switch from harvested rain water to water from a variety of other sources including tube wells. Although villagers are discouraged from using the tube well water, they use it as a necessity. The tube well water in many parts of Cambodia contains high levels of arsenic and manganese and the levels of the anions are particularly high in the study site. Both elements are known to complex iron (B. Parker, 19 July 2009, personal communication), which may have effectively sequestered the iron available not only from the diet but also from the fish ingot. Hb levels remained relatively unchanged throughout the study in all groups but SFe levels fell. This could suggest that total body stores of iron were being depleted to maintain Hb levels and that iron was simply not available from the diet despite the use of the fish ingot because the iron was being sequestered. This intriguing possibility deserves further investigation because it could certainly confound the results of the study and explain the deterioration of the effect reported in the first half of the study.

The initial promising results from the trial suggest that the fish ingot may be a useful iron supplement technique to pursue further. Moreover, the participants reported beneficial symptoms of using the fish on exit interview and the acceptability of the fish was clearly high. Coupled with relatively cheap cost of this intervention, it would be important to follow up this trial with a more intensive study with more data on bioavailability of iron and in a location
where the possible problems from arsenic and manganese contamination of water could be avoided.
5.5 References


5.7 Tables

Table 5.7.1: Baseline characteristics of women in three treatment groups in a community trial of a novel iron supplementation technique using an iron ingot in cooking pots (n=120).

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Control (N=35)</th>
<th>Treatment Group</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>NFU (N=37)</td>
<td>FU (N=48)</td>
<td>P-value</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>42.1 ± 13.2</td>
<td>45.9 ± 13.5</td>
<td>47.4 ± 12.7</td>
<td>0.18</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Post-menopausal</td>
<td></td>
<td>14 (38%)</td>
<td>19 (40%)</td>
<td>0.62</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. of pregnancies</td>
<td>3.7 ± 2.3</td>
<td>4.1 ± 2.8</td>
<td>4.1 ± 2.9</td>
<td>0.76</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline hemoglobin (g/L)</td>
<td>12.4 ± 1.3</td>
<td>12.6 ± 1.2</td>
<td>12.8 ± 0.9</td>
<td>0.33</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline serum iron (µg/L)</td>
<td>107.8 ± 24.2</td>
<td>117.7 ± 25.5</td>
<td>109.1 ± 26.4</td>
<td>0.20</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

NFU – iron ingot with no follow-up; FU – iron ingot with follow-up

* Data are mean ± standard deviation or count (and column percentage)
Table 5.7.2: Change in blood parameters in treatment groups compared at three points in time in 120 women from Preak Ruessei, Cambodia who participated in a community trial using an iron ingot in the cooking pot or an iron ingot in the cooking pot plus follow-up visits.

<table>
<thead>
<tr>
<th>Treatment Group</th>
<th>Blood Parameter</th>
<th>Baseline (B) Evaluation</th>
<th>Evaluations during and at end of intervention</th>
<th>Significance of difference vs. baseline</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>3 Months (3M)</td>
<td>6 Months (6M)</td>
</tr>
<tr>
<td>Control</td>
<td>Hb</td>
<td>12.41 ± 1.32</td>
<td>12.05 ± 1.17</td>
<td>12.87 ± 1.32</td>
</tr>
<tr>
<td></td>
<td>SFe</td>
<td>107.80 ± 24.17</td>
<td>73.71 ± 18.53</td>
<td>84.43 ± 17.40</td>
</tr>
<tr>
<td>NFU</td>
<td>Hb</td>
<td>12.57 ± 1.23</td>
<td>12.51 ± 1.06</td>
<td>12.59 ± 1.30</td>
</tr>
<tr>
<td></td>
<td>SFe</td>
<td>117.68 ± 25.53</td>
<td>77.89 ± 14.70</td>
<td>76.11 ± 17.67</td>
</tr>
<tr>
<td>FU</td>
<td>Hb</td>
<td>12.79 ± 0.94</td>
<td>13.02 ± 0.95</td>
<td>12.87 ± 1.09</td>
</tr>
<tr>
<td></td>
<td>SFe</td>
<td>109.13 ± 26.44</td>
<td>86.17 ± 16.20</td>
<td>78.15 ± 14.81</td>
</tr>
</tbody>
</table>

Abbreviations: NFU – iron ingot with no follow-up; FU – iron ingot with follow-up; Hb – hemoglobin; SFe – serum iron

* Data are mean ± standard deviation; significance determined by paired t-test comparing baseline with follow-up at 3 and 6 months.
Table 5.7.3: Differences in blood parameters between three different treatment groups in 120 women from Preak Ruessai, Cambodia who participated in a community trial using an iron ingot in the cooking pot or an iron ingot in the cooking pot plus follow-up visits.

<table>
<thead>
<tr>
<th>Time of Evaluation</th>
<th>Hb</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>FU vs. Control</td>
<td>NFU vs. Control</td>
<td>FU vs. NFU</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>β</td>
<td>P</td>
<td>β</td>
<td>P</td>
<td>β</td>
<td>P</td>
</tr>
<tr>
<td>3 Months</td>
<td>0.96</td>
<td>0.001</td>
<td>0.46</td>
<td>0.07</td>
<td>0.50</td>
<td>0.03</td>
</tr>
<tr>
<td>6 Months</td>
<td>0.18</td>
<td>0.51</td>
<td>-0.12</td>
<td>0.67</td>
<td>0.30</td>
<td>0.25</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Time of Evaluation</th>
<th>SFe</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>FU vs. Control</td>
<td>NFU vs. Control</td>
<td>FU vs. NFU</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>β</td>
<td>P</td>
<td>β</td>
<td>P</td>
<td>β</td>
<td>P</td>
</tr>
<tr>
<td>3 Months</td>
<td>12.45</td>
<td>0.0009</td>
<td>-4.18</td>
<td>0.28</td>
<td>8.27</td>
<td>0.02</td>
</tr>
<tr>
<td>6 Months</td>
<td>-4.47</td>
<td>0.23</td>
<td>6.68</td>
<td>0.09</td>
<td>2.21</td>
<td>0.53</td>
</tr>
</tbody>
</table>

Abbreviations: NFU – iron ingot with no follow-up; FU – iron ingot with follow-up; Hb – hemoglobin; SFe – serum iron

*β coefficient and P-value for between group differences determined by multiple linear regression model including a covariate, CRP at 6 months, and baseline values.
5.8 Figures

Figure 5.8.1: Trial profile for a randomised clinical trial of an iron ingot as a means of iron supplementation in women in rural Cambodia.

Abbreviations: Hb – hemoglobin; SFe – serum iron; CRP – C-reactive protein; Ab. CRP – abnormal C-reactive protein (>6 mg/L); SFe Outliers – serum iron outliers (>129 μg/L)
CHAPTER 6: IRON CONTENT OF CAMBODIAN FOODS WHEN PREPARED IN COOKING POTS CONTAINING AN IRON INGOT

6.1 Introduction

Iron deficiency (ID) that causes iron deficiency anemia (IDA) is the most common micronutrient deficiency. Globally, anemia affects more than 2 billion people,\(^1\) the vast majority of whom live in the developing world where access to conventional medicine is limited and costly and plant food is a large portion of the diet.\(^1,2\) Women and children are particularly burdened by the disorder: According to the WHO, anemia affects 66.4% of pregnant and 57.3% of non-pregnant women of reproductive age.\(^1\) Because the health and socioeconomic effects of anemia can be severe and often irreversible, there is a great need for a cost-effective prevention and control strategy.\(^1\)

In the use of adventitious iron sources, such as cooking with iron pots and iron utensils, iron is leached from iron cooking equipment, often in great quantities.\(^3-11\) Iron leached from iron cooking equipment is bioavailable to humans and rodents.\(^12-14\)

Despite this advantage, iron cooking equipment is generally not well accepted because it is prone to rusting, heavier, more difficult to clean and of poorer manufacturing quality than other pots and food left in the pot overnight is perceived to change in taste and appearance.\(^7\) Aluminium pots are perceived as easier to clean and less expensive than iron pots; thus, they are more common in many countries, including Cambodia.
Previously, we conducted a community trial to determine the long-term effectiveness of cooking with an iron ingot. Study participants were instructed to place an ingot in the daily cooking pot when preparing soup and/or when boiling drinking water to which citrus juice was added. Participants who cooked with an iron ingot had a lower prevalence of anemia three months after the start of the trial than women who cooked without the ingot.

Although field research is needed to assess the value of an intervention under different settings, laboratory studies are also required to see whether, and to what extent, iron is leached from the ingot. The aim of the current study was to determine the iron content of several water/food preparations, including two commonly consumed Cambodian soups, when prepared in glass and aluminium pots that contained an iron ingot during the cooking process. The meals were selected after qualitative fieldwork that identified commonly used recipes.

### 6.2 Materials and methods

The iron content of five water or food preparations, cooked in two types of pots either with or without an iron ingot, was determined. Three batches of water or food were prepared for each pot type and ingot combination, from which three samples from each batch were taken for iron content analysis. All samples were prepared on the same day, and the same ingot was used for each replicate batch. The pH of each sample was recorded prior to iron content analysis (Accumet Basic AB15, Fisher Scientific Intl., Hampton, NH, USA).

The iron ingots were designed and produced using readily available scrap iron at a factory in Kandal Province, Cambodia. To enhance compliance to the treatment regime at the household level, the ingots were designed to resemble a local fish species that is considered
lucky in village folklore (Figure 6.7.1). This design with its relatively flat profile also combined maximal surface area (142.5 cm$^2$) with light weight (177.5 g). To ensure safety of use, quality assurance tests using acidified water (HCl to pH=2) were conducted to determine the presence of metal contaminants. A random sub-sample of the ingot batch was boiled in varying aliquots of distilled water (from 0.5l to 3l) over varying time periods (from 10 min to 2 h). These samples were then analysed appropriately for manganese, arsenic, nickel, mercury, copper, zinc, lead, iron and magnesium. All samples met WHO drinking water quality standards.$^{16}$

Aluminium pots are most commonly used throughout Cambodia and were therefore selected for this study (Thermalloy Aluminium®, Browne Halco Inc., Union, NJ, USA). However, metal contaminants may be leached from the metal pot; therefore, we also used a glass pot for comparison (CorningWare®, World Kitchens Inc., Reston, VA, USA). Both pot types had 1.5l capacity, were un-insulated, flat-bottomed and had one side handle and a lid. Two identical, unused glass pots and two identical, unused aluminium pots were used. One glass pot and one aluminium pot were chosen for use with the iron ingot, resulting in four experimental set-ups: (i) glass pot (control), (ii) glass pot with iron fish, (iii) aluminium pot (control) and (iv) aluminium pot with iron ingot.

Five food preparations were used in the experiment: distilled water, weakly acidic water (lemon water), saline water and two commonly consumed Khmer soups (Table 6.6.1) prepared according to recipes from a rural Cambodian village (personal observation, CVC). The first soup was lemon fish soup ($sngor chouk trey$), and the second was sweet and sour pineapple and pork soup ($salor machu manor nung s’ung chrouk$). All foods were prepared using non-metal
utensils. Where possible, fresh, authentic ingredients from a large Asian market in Toronto were used. After weighing out the correct portions of each ingredient, a 1000-ml sample was prepared in each pot. The ingredients for the meals were purchased at the same time, and all experiments were performed within 48 h of purchasing the ingredients.

After preparing each sample batch, the cooking pots were cleaned with water and a plastic scouring pad and dried with a paper towel. The pots were then rinsed with distilled water and left to air-dry. All samples were cooked on identical, previously unused electric burners (Better Chef Better Results®, Vernon, CA, USA). The water samples and soups were each cooked on the maximum setting of the burner (120 °C) for 20 min. The cooking time mimicked conditions in the community as determined in consultation with residents of a rural Cambodian village. In the pilot study, we had instructed participants to use the iron ingot when cooking for at least 10 min;¹⁵ but we later observed that participants typically cooked fish for 20 min or longer (personal observation, CVC).

For analysis, 15 ml samples of the boiled waters and the liquid portion of each soup were extracted from the pot using a glass eye dropper fitted with a paper filter to prevent sampling of food particles. To account for potential bias from extraction of only the liquid portion of the soup, three replicates from each batch were collected, weighed and analysed; there were no significant differences in the weight of the samples, suggesting that they were comparable.

Inductively coupled plasma optical emission spectroscopy (ICP–OES) was used for analysis of total iron content of each prepared sample. All samples first underwent microwave-
assisted digestion. Next, they were brought to volume using Nanopure water, and then, the clear extract supernatant was removed and analysed in duplicate without further processing. Deionized water (used as blanks), reagents and SRM 8435, were prepared similarly, and subjected to the same analytical procedure for quality control. The Division of Laboratory Services at the University of Guelph performed all elemental analyses.

The iron content of the water and food preparations was averaged over the replicate samples to determine the mean and standard deviation of the iron content for each. The lower detection limit for the method of analysis was <0.40 μg iron/g. Observations with a result of <0.40 μg iron/g were replaced by 0.20 μg iron/g for statistical analysis. Total iron content in each sample was regressed on the putative factors using linear regression. The factors examined comprised of the use of the iron ingot; pot type; inclusion of an acidifying agent (for the water samples); fish soup vs. pork soup. For the purposes of this experiment, control samples are defined as those that were prepared without an iron ingot present during the cooking process.

6.3 Results

Table 6.6.2 shows that water with lemon, prepared with an iron ingot, had 76.3 μg iron/g more iron (P < 0.001) control samples prepared without an ingot. Similarly, Khmer fish soup contained 3.3 μg more iron/g (P = 0.006) and Khmer pork soup 32.6 μg more iron/g (P < 0.001). The iron content of the distilled water and saline samples prepared with an iron ingot did not differ from controls. Neither did the use of glass and aluminium pots have an effect on the iron content of any of the preparations and thus did not has an effect on the amount of iron
leaching from the ingot (P = 0.67). The coefficient of variation in the weakly acidic preparation was higher than 20%, indicating that the laboratory test had some variability. Lemon water prepared with an iron ingot had the highest iron content of any preparation; 43.7 μg more iron/g than pork soup (P < 0.001) and 73.0 μg more than fish soup (P < 0.001).

6.4 Discussion

Iron deficiency and IDA pose a severe public health problem in Cambodia. The most recent estimates suggest that 61.9% of pre-school-aged children, 44.3% of non-pregnant women and non-lactating women, 53.6% of lactating women and 57.1% of pregnant and lactating women are anaemic. This study demonstrates that cooking with an iron ingot, regardless of cooking vessel, increases the iron content of water and food preparations. Lemon water and two varieties of soup cooked with an iron ingot contained more iron than ingot-free preparations (P-values < 0.001). In distilled water and saline, the iron ingot made no difference, highlighting the importance of acidity in iron leaching. Our previous research showed that the iron ingot is an acceptable and at least partially effective treatment. The study confirms the potential impact of iron ingots as an adventitious iron source.

Studies on the influence of iron pots and utensils on iron content in a variety of foods have consistently shown that foods cooked in iron pots and/or with iron utensils have significantly higher iron content than foods cooked with non-iron equipment. Both crude and available iron content appear to be positively influenced by iron cooking equipment: twice as much crude iron and five times more available iron in meat and vegetable preparations was observed when preparing foods in iron pots when compared to controls. Liu et al. reported an
estimated increase in iron intake of about 14.5 mg for adults and 7.4 mg for children when iron pots were used. Continuous use of adventitious iron utensils has negligible effect (P < 0.05). The effect of life usage of the ingot has not been determined but should be investigated in a field trial over several years.

Iron requirements vary according to age, gender, menstrual status and pregnancy status (Table 6.6.3). Because iron bioavailability is dependent on a number of factors, most dietary iron guidelines are based on an estimate of 10% absorption from total dietary iron. Based on the estimated bioavailability, greater percentages of daily iron requirements are met by drinking water and soup samples prepared with the iron ingot. Importantly, based on a daily water intake of 1.0 l, approximately 76.5% of the iron requirements are met in children, adult males and post-menopausal women. Although the approximate iron contribution of the Khmer soup appears low (Table 6.6.3), the soup is only one meal per day, and together with the iron contribution from lemon water, daily iron requirements could easily be met. Water and soup intake may vary among individuals, and in particular, children may consume less than adults; thus, the overall benefit may vary accordingly.

Iron overload owing to increased dietary iron has only been recognized in some areas of sub-Saharan Africa; therefore, the risk of overload resulting from the use of the iron ingot is limited. However, the long-term effect of the use of the iron ingot or other iron utensils should be assessed.

The effectiveness of this supplementation technique depends on two factors: the amount of iron that is leached during cooking and the amount of leached iron that is absorbed.
Both factors are, in turn, influenced by a number sub-factors (Figure 6.7.2). Iron that is leached from both the iron ingot and iron cooking equipment is referred to as contaminant iron.\textsuperscript{19} Contaminant iron sources are non-heme in nature and are typically in either the ferrous (Fe$^{2+}$) or ferric (Fe$^{3+}$) form as these oxidation states are stable in an aqueous medium.\textsuperscript{20} Ferrous iron is rapidly oxidized to the ferric form when exposed to oxygen.\textsuperscript{20} Thus, the form of iron presented to the body depends on both the iron source(s) and the degree of oxidation during cooking and/or storing the food. We do not know what type of iron the ingot adds, which makes it difficult to determine how the leached iron varies in solubility and affinity for reaction with other compounds, specifically the enhancers and inhibitors of iron absorption.

Iron leaching is dependent on the acidity, moisture content and cooking time of the food preparation. More acidic, moister foods and foods cooked longer have higher iron content than controls when cooked with an iron ingot or pot.\textsuperscript{5,8} Incorporation of iron into food also depends on the regularity of use of an iron utensil or ingot.\textsuperscript{5} During the first use, more iron leaches than at later uses, so the iron ingot would be most effective during the first few uses. The effective lifespan of the ingot remains to be determined.

After consumption of dietary iron, an unknown percentage of the mineral enters a common pool of non-heme iron while the rest is not readily absorbed. In the end, only 1–25% of the iron from the non-heme iron pool is absorbed.\textsuperscript{19} This percentage depends, among other factors, on meal constituents (presence/absence of iron absorption inhibitors and enhancers) and the size of the iron stores.
Our findings correlate well with the previous research on the iron content of foods prepared with iron pots: More acidic, moister foods cooked in iron vessels have a higher iron content than controls. Lower pH correlates well with higher iron content. Distilled water with a small amount of lemon juice had a pH of 3.20 and leached the most iron from the ingot. The two soup preparations were also acidic: the fish soup had a pH of 4.45 and the pork soup had a pH of 4.58. Both had significantly higher iron concentrations than controls. Pork soup contained significantly more iron than fish soup (Table 6.6.4), which may be accounted for by the higher iron content of pork meat.\textsuperscript{21} Conversely, the least acidic preparations had the lowest iron concentrations, even when cooked with an iron ingot: Distilled water had a pH of 7.06, while saline had a pH of 5.80.

In the preliminary community trial, participants were encouraged to cook with the ingot for a minimum of 10 min per day, preferably when preparing soup,\textsuperscript{15} the use of the ingot when boiling water was given as an alternative only. In the current experiment, water with lemon juice was the most acidic preparation (pH = 3.20) and accordingly had the highest iron content when prepared with an ingot. Thus, instructions for use should be modified, as it is now clear that benefit is maximized when using the iron ingot to prepare boiled drinking water with lemon, followed by preparation of acidified soups. Boiled drinking water would be consumed in greater quantities than soup; thus, an individual would benefit more using the ingot to prepare drinking water. Both boiled drinking water with citrus juice and soup are particularly advantageous for two reasons: the acidity of these preparations is important for maximizing iron leaching, and both are acidified with ascorbic acid, an enhancer of iron absorption.\textsuperscript{22}
Iron deficiency and IDA in Cambodia constitute a severe public health problem requiring immediate attention from the government and organizations responsible for community health. The iron ingot and other interventions that utilize adventitious iron sources to improve iron intake may provide an alternative to the relatively costly and unsustainable supplementation programs currently in place. Importantly, the ingot was widely accepted by household members; it is cheap, lightweight and easy to use and therefore a good alternative to iron cooking pots. The ingot must undergo further testing in representative households and communities to determine both the efficacy and effectiveness of this possible preventive option.
6.5 References


6.6 Tables

Table 6.6.1: Required ingredients and sources for 3 different water preparations and 2 different Khmer soups prepared for a laboratory study of iron content when using an iron ingot during cooking.

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Fish Soup</th>
<th>Pork Soup</th>
<th>Distilled Water</th>
<th>Lemon Water</th>
<th>Saline Solution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Distilled water(^a)</td>
<td>1000 ml</td>
<td>1000 ml</td>
<td>1000 ml</td>
<td>1000 ml</td>
<td>1000 ml</td>
</tr>
<tr>
<td>Lemon juice concentrate(^b)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>5 ml</td>
<td>-</td>
</tr>
<tr>
<td>Non-iodized, coarse salt(^c)</td>
<td>-</td>
<td>2.5 ml</td>
<td>-</td>
<td>-</td>
<td>5 mg</td>
</tr>
<tr>
<td>Catfish</td>
<td>680 g</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Pork riblets</td>
<td>-</td>
<td>455 g</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Jasmine Rice</td>
<td>30 ml</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Fresh pineapple</td>
<td>-</td>
<td>355 ml</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Green Onion</td>
<td>2 stalks</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Bean sprouts</td>
<td>-</td>
<td>475 ml</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Tomato</td>
<td>-</td>
<td>240 ml</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Sweet basil</td>
<td>120 ml</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Asian coriander</td>
<td>120 ml</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Fish sauce(^d)</td>
<td>15 ml</td>
<td>30 ml</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Fresh tamarind paste</td>
<td>-</td>
<td>15 ml</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Lemon grass</td>
<td>1 stalk</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Garlic</td>
<td>-</td>
<td>2 cloves</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Fresh lemon</td>
<td>Juice of 1</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Black pepper(^e)</td>
<td>1.25 ml</td>
<td>1.25 ml</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Sugar(^f)</td>
<td>7.5 ml</td>
<td>30 ml</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Fresh chili pepper</td>
<td>1 small</td>
<td>2 small</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Sources: \(^a\): Canadian Springs, Toronto, Canada; \(^b\): No Name Brand\(^\textregistered\), Loblaws Inc., Toronto, Canada; \(^c\): Sifto Canada Corporation, Mississauga, Canada; \(^d\): Squid Brand, Thai Fishsauce Factory Co. Ltd., Bangkok, Thailand; \(^e\): Kirkland Signature, Costco Wholesale Canada Ltd., Ottawa, Canada; \(^f\): Red Path Sugar Ltd., Toronto, Canada
Table 6.6.2: Iron content [mean (standard deviation)] and pH of water and food samples when prepared both with and without an iron ingot, in both glass and aluminium cooking vessels (μg/g).

<table>
<thead>
<tr>
<th>Sample Type</th>
<th>Glass Pot</th>
<th>Glass Pot with Iron Ingot</th>
<th>Aluminium Pot</th>
<th>Aluminium Pot with Iron Ingot</th>
</tr>
</thead>
<tbody>
<tr>
<td>Distilled water (pH = 7.06)</td>
<td>&lt;0.40 (0)</td>
<td>&lt;0.40 (0)</td>
<td>&lt;0.40 (0)</td>
<td>&lt;0.40 (0)</td>
</tr>
<tr>
<td>Saline water (pH = 5.80)</td>
<td>&lt;0.40 (0)</td>
<td>&lt;0.40 (0)</td>
<td>&lt;0.40 (0)</td>
<td>&lt;0.40 (0)</td>
</tr>
<tr>
<td>Weakly acidic distilled water (pH = 3.20)</td>
<td>&lt;0.40 (0) Y</td>
<td>84.7 (15.04) a,x</td>
<td>&lt;0.40 (0) Y</td>
<td>68.3 (18.82) a,x</td>
</tr>
<tr>
<td>Khmer fish soup (pH = 4.45)</td>
<td>&lt;0.40 (0) Y</td>
<td>4.1 (3.01) b,x</td>
<td>&lt;0.40 (0) Y</td>
<td>2.8 (1.70) b,x</td>
</tr>
<tr>
<td>Khmer pork soup (pH = 4.58)</td>
<td>&lt;0.40 (0) Y</td>
<td>41.3 (7.50) c,x</td>
<td>&lt;0.40 (0) Y</td>
<td>27.7 (11.72) c,x</td>
</tr>
</tbody>
</table>

a,b,c: within column superscripts differ at P<0.001; x,y: within row superscripts differ at P<0.001.
Table 6.6.3: Iron requirements (by demographic group) and theoretical iron intake when preparing water and Cambodian food samples with an iron ingot in an aluminum pot.

<table>
<thead>
<tr>
<th>Group</th>
<th>Iron Requirements (mg per day)</th>
<th>Lemon Water (1.0 L per day)</th>
<th>Khmer Fish Soup (0.5 L per day)</th>
<th>Khmer Pork Soup (0.5 L per day)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Fe</td>
<td>Daily %*</td>
<td>Fe</td>
<td>Daily %*</td>
</tr>
<tr>
<td>Children (1.0-9.9 years)</td>
<td>10</td>
<td>76.5</td>
<td>1.7</td>
<td>17.3</td>
</tr>
<tr>
<td>Males (10.0-17.9 years)</td>
<td>12</td>
<td>76.5</td>
<td>63.8</td>
<td>1.7</td>
</tr>
<tr>
<td>Males (18.0+ years)</td>
<td>10</td>
<td>76.5</td>
<td>76.5</td>
<td>1.7</td>
</tr>
<tr>
<td>Females (10.0-49.9 years)</td>
<td>15</td>
<td>76.5</td>
<td>51.0</td>
<td>1.7</td>
</tr>
<tr>
<td>Females (50.0+ years)</td>
<td>10</td>
<td>76.5</td>
<td>76.5</td>
<td>1.7</td>
</tr>
</tbody>
</table>

* Assuming 10% iron bioavailability (Herbert 1987; Hurrell and Egli 2010).
Table 4: Predicted increase of iron content after cooking soup, water or acidified water with an iron ingot during preparation.

<table>
<thead>
<tr>
<th>Factor</th>
<th>Coefficient (β)</th>
<th>Standard Error of β</th>
<th>P-value</th>
</tr>
</thead>
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<tr>
<td>Glass pot vs. aluminum pot</td>
<td>2.8</td>
<td>6.43</td>
<td>0.67</td>
</tr>
<tr>
<td>Lemon water with ingot vs. lemon water</td>
<td>76.3</td>
<td>7.21</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Fish soup with ingot vs. fish soup</td>
<td>3.3</td>
<td>0.94</td>
<td>0.006</td>
</tr>
<tr>
<td>Pork soup with ingot vs. pork soup</td>
<td>32.6</td>
<td>4.83</td>
<td>0.0001</td>
</tr>
<tr>
<td>Pork soup with ingot vs. fish soup with ingot</td>
<td>29.4</td>
<td>4.92</td>
<td>0.0001</td>
</tr>
<tr>
<td>Lemon water with ingot vs. distilled water with ingot</td>
<td>76.3</td>
<td>7.21</td>
<td>&lt;0.0001</td>
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<tr>
<td>Lemon water with ingot vs. fish soup with ingot</td>
<td>73.0</td>
<td>7.27</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Lemon water with ingot vs. pork soup with ingot</td>
<td>43.7</td>
<td>8.68</td>
<td>0.0005</td>
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</tbody>
</table>
6.7 Figures

Figure 6.7.1: Iron ingot designed to resemble a species of Cambodian fish for use during preparation of water and food samples (surface area, 142.5 cm$^2$; mass, 177.5 grams).

Figure 6.7.2: Factors influencing the leaching and absorption of iron from dietary and contaminant iron sources.
CHAPTER 7: ANEMIA IN CAMBODIA: A CROSS-SECTIONAL STUDY OF ANEMIA, SOCIOECONOMIC STATUS AND OTHER ASSOCIATED RISK FACTORS IN RURAL WOMEN

7.1 Introduction

Despite the efforts of governments, multilateral agencies and nongovernmental organizations worldwide, anemia remains a major public health problem. An estimated 3.6 billion people are iron deficient and of these more than two billion are anemic, especially women of reproductive age, infants and young children.¹

Iron is an essential mineral for human development and function and is vital for the production of oxygen-carrying hemoglobin (Hb). Inadequate iron stores result in underproduction of red blood cells, ultimately leading to anemia.² Iron status flows from iron deficiency anemia (IDA), to iron deficiency (ID) in the absence of anemia, to normal iron status, and finally to iron toxicity.³ Globally, the most significant contributor to the onset of anemia is iron deficiency, which is estimated to be responsible for approximately 50% of all cases.³

The functional consequences of iron deficiency and anemia have been well documented, including delayed motor and cognitive development in childhood, and increased risk of maternal mortality due to hemorrhage.³ In economic terms, the elimination of anemia in adults can result in productivity increases up to 17%, equivalent to 2% of gross domestic product in the worst effected countries.⁴
Iron deficiency (ID) is inherently associated with poverty, and is therefore particularly prevalent in the developing world where the problem is often exacerbated by limited access to appropriate healthcare and treatment, poor sanitation, widespread disease, and inadequate education. Women in particular have a high burden of anemia due to increased iron requirements during pregnancy, hemorrhage during childbirth, and regular blood loss associated with menstruation. The most recent Cambodian Demographic and Health Survey (CDHS) reports a country-wide prevalence of anemia of 44.4% in women of reproductive age and 55.1% in children aged 6-59 months. Consequently, anemia is considered to be a severe public health problem in Cambodia and requires immediate attention. To date, the prevalence of iron deficiency is unknown and there is a dearth of information describing the nutrition situation and associated factors in Cambodia. Baseline data of this sort is needed in order to determine the extent of the public health problem and is essential for the development of effective and targeted treatment and prevention programmes.

The current paper describes a baseline observational study that was conducted to investigate the prevalence of iron deficiency and associated anemia in rural Cambodian women aged 16 and older. Particular emphasis has been placed on understanding the role that socioeconomic status has on the prevalence of anemia and the degree of meat consumption in the study population. This study represents the baseline assessment prior to execution of a novel intervention to address iron deficiency and anemia in rural populations in one region of rural Cambodia.
7.2 Materials and methods

7.2.1 Study site and subjects

The study was conducted in Tuol Trea and Preak Khmeng villages in Preak Khmeng Commune, Lvea Aem District, Kandal Province, Cambodia. The study sites are located along tributaries of the Mekong River and experience severe flooding during the monsoon season. In turn, the land is not considered highly valuable, typically only one rice crop per year is cultivated, and thus the residents of the district are of low socioeconomic status when compared to neighbouring areas. Approximately 2,313 people live within the study area of Preak Khmeng Commune, with 1,563 in Preak Khmeng Village and 750 in Tuol Trea Village. For the purposes of the current study, Preak Khmeng village was considered as two distinct study sites, Preak Khmeng 1 (PK1) and Preak Khmeng 2 (PK2). These two areas within Preak Khmeng are separated geographically by a river system, have distinct village councils, experience different degrees of flooding and drought, and for all intents and purposes can be examined as two distinct locales.

Households were selected using a systematic random sample by approaching every fourth household until the pre-determined sample size was met during a 2-week period in June 2010, the beginning of the monsoon season. The project was explained briefly and those women who consented to participate were then surveyed and a blood sample was collected for biomarker measurement. Women were included if they lived in the study area and did not intend to move in the following 12-months and were 16 years of age and older; exclusion criteria included unwillingness to give a blood sample. Sample size was calculated to produce a
power of 95% (α=0.05) with an allowable error of 5%; the calculation was performed to detect a difference in hemoglobin concentration following a dietary intervention of 10 g/L (not described herein). A total of 310 women first completed a structured, coded survey, after which a blood sample was taken to establish anemia status and the size of iron stores.

7.2.2 Survey procedures

Trained village health personnel from a local non-governmental organization, Resource Development International Cambodia (RDI) were employed to conduct the survey and to collect the biomarker data. All staff were Cambodian nationals and conducted the survey in the Khmer language. At each household, after explaining the study procedures and obtaining consent, a questionnaire was administered. Data were collected on the size and demographics of the household, as well as the participant’s pregnancy status and history, history of anemia and infectious disease, education level attained, literacy, common water, and household possessions. Frequency of the consumption of meat and meat products (including poultry, pork, beef, goat, buffalo, and rodent) was assessed through a series of open-ended questions. The frequency of common health ailments was investigated through a series of survey questions that the women responded to by describing the frequency of their occurrence. A detailed socioeconomic analysis was also performed and was based on information collected on household possessions, housing quality and livestock possession. The socioeconomic asset score was derived using Morris and colleagues approach. First, a weight equal to the reciprocal of the proportion of the study households who owned one or more of that item was assigned.
Next, this weight value was multiplied by the number of that asset. Finally, the product values were summed over all possible assets. 7

7.2.3 Laboratory methods

A 3 mL sample of blood was collected following venipuncture (Greiner Bio-One, Shanghai, China) for each participant. A portable hemoglobin analyzer (HemoCue AB, Angelholm, Sweden) was used on-site to assess hemoglobin level; a single drop of blood from the syringe was placed on a piece of acetate and the HemoCue microcuvette was then used for collection. The remaining blood sample was placed into a tube containing a clot-activator (microscopic silica particulate) and then immediately placed into a cooler with a freezer pack to maintain sample integrity. The first author or his Cambodian research partner to a laboratory in Phnom Penh transported the samples within a four hour time period. Analysis of the collected blood sample was conducted daily at Paramed Laboratory (Paramed Laboratoire D’Analyses Medicales, Phnom Penh, Cambodia). Serum ferritin (SF) was measured using an Abbott AxSYM system (Abbott Laboratories, North Chicago, IL, USA) and serum C-reactive protein (CRP) was assessed by an ELISA (AssyMax Human CRP ELISA Kit, St. Charles, USA); appropriate controls and reference standards were used to ensure accuracy of the laboratory analysis.

Serum ferritin was the biomarker chosen to assess iron stores because it most closely correlates with relative total body iron stores. 3 Serum ferritin is an acute-phase reactant protein, which is raised in response to infection or inflammation. A CRP concentration > 10 mg/L indicated infection/inflammation. 8 Anemia was indicated with hemoglobin concentration < 120 g/L, and iron deficiency with a SF level < 15 ng/mL, according to World Health
Organization (WHO) recommendations for non-pregnant women greater than 15 years of age. Anemia of chronic disease was identified in women who had abnormal hemoglobin levels (Hb < 120 g/L) together with abnormal CRP concentration (CRP > 10 mg/L), and these women were excluded from detailed analysis. The overall socioeconomic status scores were also broken down into quartiles for detailed analysis.

7.2.4 Statistical analysis

Data were initially recorded by hand onto coded spreadsheets and later transcribed into PAWS Statistics statistical software package version 18.0 (SPPS Inc., Chicago, IL, USA). Hemoglobin, SF and socioeconomic score were evaluated to determine whether they were normally distributed using normality plots and the Shapiro-Wilk test for normality of data. Hemoglobin values were normally distributed, facilitating use of the arithmetic mean. Serum ferritin and asset scores values were right-skewed, and were therefore log-transformed.

Descriptive statistics were used to examine baseline demographic information. Baseline demographics, Hb and log-transformed serum ferritin were compared among villages using linear regression for continuous variables (age, height, weight, total number of pregnancies, education level, and literacy) and chi-square tests for binary variables (pregnancy status, menopausal status, prior use of iron pills, and previous diagnosis of anemia). Additional descriptive analysis was used to examine biomarker data and meat consumption. The geometric mean was calculated for SF and asset score data, and the median meat consumption was determined. The baseline demographic data was compared among the three study sites to determine if village should be included as a fixed effect.
Serum ferritin values were further classified into borderline (15-30 ng/mL), low (<30 ng/mL), and very low (<15 ng/mL), while meat consumption was ranked according to high (>9 servings per month), medium (2-9 servings per month), and low (<2 servings per month) intake. These values, together with socioeconomic status and a self-reported history of infectious disease (malaria and hookworm) were compared between women found to be anemic versus those who were non-anemic at baseline using univariate logistic regression incorporating the clustering effect of village.

Multiple regression analysis was used to evaluate the associations between either hemoglobin or log-transformed SF and meat consumption, the demographic variables of age, education level attained, number of pregnancies, socioeconomic status and menopausal status after controlling for village as a fixed effect. Linear regression was also used to determine the association between socioeconomic status and meat consumption. The assumptions of the final models were tested by examining the standardized residuals and ensuring homogeneity of variances.

7.2.5 Ethical considerations

After introducing the research project and explaining the time commitment involved, oral informed-consent was obtained. All study procedures and materials received ethics approval from the Research Ethics Board at the University of Guelph, Canada prior to implementation. In addition, study procedures were reviewed by the International Development Research Centre, Canada. Consent was also obtained from the district and
commune health offices, as well as village leaders, and all parties were updated of the progress of the project as required.

7.3 Results

The demographic data for the 310 women included in the study are presented in Table 7.7.1. Preliminary analysis confirmed that PK1 and PK2 should be considered as distinct study sites, as baseline hemoglobin levels and CRP levels differed significantly. Of the 310 women entered into the study, 13 were omitted from detailed analysis: six were pregnant, while seven had both abnormal CRP (>10mg/L) and abnormal Hb concentrations, signalling anemia of chronic disease.9

The mean age of the study participants was 42.0 (95% CI: 40.29-43.61) and women had a mean 4.8 (95% CI: 4.39-5.18) total number of pregnancies over their lifetime. The majority of participants (47.7%) had some education, although more than one-third of all those surveyed had none at all. Many women (24% - 47%) experienced clinical symptoms that were suggestive of anemia such as headache, dizziness, insomnia and fatigue regularly (4-6 times per month). Approximately 17 - 36% of those women surveyed reported experiencing these symptoms more frequently (>2-3 times per week).

The baseline survey was used to collect information on food, water and hygiene practices (Table 7.7.2). While Preak Khmeng village (PK1 and PK2) is located along a main river tributary, Tuol Trea is located further from this river system. There was a high degree of variation in the water source used during the wet and dry seasons, and also among the three study sites. During the dry season (December to June), the river system was the primary source
of water for residents in PK1 (95.6%) and PK2 (87.4%), while ground water collected from a tube well was most often used in Tuol Trea (81.0%). During the monsoon season (July to November), however, the majority of villagers in all three study sites relied heavily upon stored rain water (72.9%), which is typically collected in large, concrete tanks. Water treatment was typically reported to be achieved through boiling drinking water (85.8%), with a small subset reliant upon chemical treatment or the use of water filters. Few women regularly used latrines 0.97% (3/310). Approximately one-third of the participants consumed meat at least once a week, while almost half (45.8%) reported to consume meat only on special occasions (i.e., festivals, weddings, funerals). Soup was most commonly eaten four to five times per week, and was considered a staple food preparation in all three study sites.

Detailed analysis was performed on a truncated data set (N=297), not including pregnant women or women with anemia of chronic disease (Table 7.7.3). The prevalence of anemia, as defined by hemoglobin concentration < 120 g/L, was 51.2% (152/297); of those affected, 44.2%, 6.7% and 0.3% had mild (119-100 g/L), moderate (70 - 99 g/L) and severe (<70 g/L) anemia, respectively. The prevalence of iron deficiency, defined by SF concentration <15 ng/mL was 12.1% (36/297); an additional 16.8% (50/297) were found to be borderline iron deficient (15-30 ng/mL). The prevalence of iron deficiency anemia, that is both abnormal hemoglobin and SF levels, was observed in 9.8% (29/297) of the participants. Borderline iron deficiency anemia, taking into account those individuals with both abnormal hemoglobin and decreased iron stores (<30 ng/mL) included an additional 18.5% (55/297) of the women surveyed.
A comparison of iron status, animal-source protein consumption, socioeconomic status and infectious disease between anemic and non-anemic populations is shown in Table 7.7.4. The mean indices of iron status in the anemic group were different from those in the non-anemic group. Of the 152 anemic participants analyzed, 29 (19.1%) had very low SF levels (<15 ng/mL), compared with 7/145 (4.8%) who were not anemic (OR=0.22, 95% CI: 0.09-0.53, P=0.001). Women with low serum ferritin levels (<30 ng/mL) may be at a higher risk of developing iron deficiency and/or IDA than the general study population given that iron status can change rapidly, therefore this group was analyzed separately. Approximately 36% of anemic participants, compared to 21.4% of non-anemic participants were found to have low SF levels (<30 ng/mL) (OR=0.51, 95% CI: 0.30-0.86, P=0.01). No difference in borderline iron deficiency (15-30 ng/mL) between the non-anemic and anemic women was observed. Women in the non-anemic group were two times more likely (OR=1.97, 95% CI: 1.17-3.32, P=0.01) to have normal SF values (> 30 ng/mL) than women in the anemic group.

The proportion of participants consuming meat between two and nine times per month was greater among the non-anemic population (OR=1.80, 95% CI: 1.11-2.92, P= 0.02) than the anemic population. Low meat consumption (less than the median of two times per month) and high meat consumption (more than nine servings per month) did not differ by anemia status (P>0.05). A greater number of participants declaring a prior history of hookworm was found in the non-anemic group (OR=1.91, 95% CI: 1.11-3.28, P=0.02) than the anemic group. The proportion of women in the various socioeconomic status groups did not differ between the anemic and non-anemic populations (P>0.05).
The results of multiple regression analyses of various risk factors on hemoglobin and SF concentrations are given in Table 7.7.5. There was a negative association between the demographic variables age and menstrual status (pre-menopausal) with hemoglobin and iron stores (P-values<0.05). As women aged by five years, their Hb level was predicted to decrease by 2.1 g/L, while serum ferritin increased by 4.9 ng/mL. Post-menopausal women had an Hb level that was predicted to be 6.1 g/l lower, and serum ferritin level that was 0.48 ng/mL higher than pre-menopausal women. Additionally, increasing socioeconomic status (P=0.03) and total number of pregnancies (P=0.03) were associated with higher SF concentrations. Although low meat consumption was not associated with decreased hemoglobin or iron indices compared to high meat consumption (>9 servings per month), women consuming meat 2 – 9 times per month had higher Hb than those consuming meat >9 times per month (P=0.016).

Socioeconomic status was associated with water source and treatment practices. Increasing socioeconomic status was associated with treatment of water (P=0.04). The use of river water, rather than rain water, in the wet (P=0.02) and dry (P=0.04) season was associated with lower socioeconomic status. Participants in the medium and high meat consumption groups were in higher socioeconomic status groups (Table 7.7.6).

7.4 Discussion

To our knowledge, the current study is the first to describe the prevalence of anemia, iron deficiency and associated risk factors in Preak Khmeng Commune, Lvea Aem District in Kandal Province, Cambodia. We found a prevalence of anemia and iron deficiency of 51.2% and 12.1%, respectively, in the 297 women who were included in the analysis. Anemia in this study
site, in keeping with the overall national prevalence, is therefore considered to be a severe public health problem according to WHO criteria.\textsuperscript{10} The prevalence of iron deficiency anemia, that is anemia with low iron stores, was found in 9.7% of those women surveyed, while borderline iron deficiency (abnormal hemoglobin with iron stores <30 ng/mL) was observed in a further 18.5% of the study participants. Because nutritional anemia is the result of severe iron deficiency, these findings highlight the multi-faceted nature of anemia and suggest that some other etiology(s) may be responsible for the high prevalence of anemia without iron deficiency in the study area. Helminths, including hookworm infection, inherited hemoglobin disorders, malaria, and other micronutrient deficiencies are well-known to exist in rural Cambodia and may be responsible for some of the anemia observed in the study area.\textsuperscript{11}

Iron deficiency was found in 12.1% of those surveyed, though nearly one-third of the women had low iron stores (<30 ng/mL). These individuals, although not all meeting the WHO-defined serum ferritin cut-point for iron deficiency at the time of survey, can be considered to be in a high-risk group for developing clinical iron deficiency. Iron deficiency was observed to be more prevalent among the anemic study population, with approximately 20% of anemic patients found to have iron stores less than 15 ng/mL. Abnormally low SF concentrations were associated with women of low socioeconomic status and those who had a low number of pregnancies. It is not clear why increasing number of pregnancies is associated with increasing SF concentration, but it may be that pregnant women had access to iron supplements and were therefore able to improve overall iron status. Menopausal women are at risk of anemia especially if they are consuming a diet low in iron, which may account for the decreased Hb concentrations observed in this group.
Lower socioeconomic status was associated with lower SF concentration, possibly implicating that poorer women were unable to afford a diet rich in iron. Our data would appear to substantiate these findings, and this may account for low SF concentrations observed in those women belonging to the lower socioeconomic group. In addition, a poor understanding of nutrition has been shown to result in the failure to consume nutritious, iron-rich foods.\textsuperscript{12, 13} In fact, we found that women belonging to the higher socioeconomic group were just as likely to be anemic as those in lower status groups. This finding would suggest that women who have more money do not choose to spend it on improving the household diet by purchasing iron-rich foods, again implicating poor nutritional knowledge.

The main limitation of the current study was the choice of biomarker for iron stores. The WHO recommends the measurement of SF for assessment of iron status in populations, though cautions that SF is an acute-phase protein and may be increased in situations where infection is present. Therefore we also measured CRP, a marker of inflammation, and removed those women with abnormally high CRP values from detailed analysis in an attempt to ameliorate this concern. Unfortunately, there is no absolute cut-off point for CRP concentration at which infection is deemed to be present, and we therefore adopted a cut-off value that has been used in previous studies.\textsuperscript{7, 8} Detailed statistical analysis was performed on a truncated data set, after removing those that were found to have irregular CRP levels (N=7). For logistical reasons, we were unable to measure additional biomarkers of iron status, though this may have revealed different findings. This limitation highlights the need in the short-term to investigate a standard CRP cut-off value that is used in similar population-based studies, and in the longer term, to
develop an easily measurable and cost-effective means of assessing iron status in field situations where infection may be present.

In this study, we found a higher proportion of individuals with a self-reported history of hookworm infection in the non-anemic group. Hookworm is a well-established cause of anemia, resulting in intestinal bleeding and the subsequent loss of red blood cells. The reason for this finding is unclear, however it may be that women suffering from infection sought medical treatment at a local health facility and were tested and/or treated for hookworm and/or anemia. Similarly, it may be that participants who were able to afford medical treatment were either from a higher socioeconomic group and/or had more health knowledge, and were subsequently more likely to seek diagnosis and treat anemia when appropriate, though this was not investigated directly in the current study.

Many participants reported regularly experiencing various adverse health events, and most reported suffering from one or multiple symptoms approximately four to six times per month. These events are known symptoms of iron deficiency and IDA and taken together with the high prevalence of anemia in the study population these self-reported symptoms highlight the need for better prevention and control of anemia in the area. The regular occurrence of these adverse health events may contribute to an overall poor quality of life, and may also affect work capacity and earning potential.

Consumption of iron-rich foods was also investigated. Meat, including chicken, pork, beef, water buffalo, goat, several rodent species, and associated organ meat was not found to be a main constituent of the diet. Nearly one half of the participants reported meat
consumption on special occasions only, and the median meat consumption was two servings per month. We found that those participants who consumed meat between two and nine times per month were less likely to be anemic than those who consumed meat less frequently. It is unclear why those women consuming meat >9 times per month were not protected against anemia and/or iron deficiency but this finding in an observational study may indicate a cause of anemia in these women other than iron deficiency. In contrast, the 2005 CDHS revealed that 98.4% of women aged 15-49 consumed meat, fish, and/or eggs on the day before the survey.\textsuperscript{15} These findings would suggest that fish and eggs, which have lower iron content,\textsuperscript{16} are much more commonly consumed than meat and poultry, though we did not investigate this directly. Previous research in the area has shown that per capita consumption of fish, including both fresh and processed, is 71.1 kg, placing Cambodia among the top fish-consumption countries in the world.\textsuperscript{17}

In addition to describing the prevalence of clinical anemia and iron deficiency in the study population, information on the use of latrines, treatment of water, and primary water source during the monsoon and dry season was collected (Table 7.7.6). The presence of many intestinal parasites can be attributed to improper water and sanitation conditions, and infection may lead to interruption of iron absorption and ultimately anemia. Latrine use in the villages was extremely low with less than 1% of the women surveyed indicating that they owned or used a latrine regularly. Typically, villagers in the area use either the river or surrounding rice fields for defecation.\textsuperscript{18} Given the incredibly low latrine use, it is surprising that the overall reported history of hookworm infection was not higher, though many women may have been
unknowingly infected. Future research in the area should investigate hookworm infection as an important cause of anemia through laboratory assessment of stool hookworm egg count.

The current study also found that increasing socioeconomic status was associated with increased use of water treatment techniques, including boiling of drinking water (Table 7.7.6). Further, low socioeconomic status was associated with regular use of heavily polluted river water during the wet (P=0.015) and dry seasons. Preak Khmeng village is situated along a river that is used for urination, defecation, clothes cleaning, washing of cattle and other livestock, along with fish-rearing, thus the use of this untreated water for drinking and cooking highlights the possibility for infection with various bacteria and parasites, including those that are known to cause anemia. These findings underscore the need for an integrated approach to anemia control in the area, with targeted interventions to improve water and sanitation practices.

The current study confirmed the severe public health problem of anemia in women in Cambodia. Poor diet, including limited meat consumption, together with infection are contributing factors to anemia and must be considered in programming efforts to ameliorate iron deficiency and IDA in this region. Additional research on the etiology of anemia in the area would benefit the development of health intervention strategies.
7.6 References


7.7 Tables

Table 7.7.1: Demographic data for women in two villages of Lvea Aem District, Kandal Province, Cambodia

<table>
<thead>
<tr>
<th>Category</th>
<th>Variable</th>
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<th>PK2</th>
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<tbody>
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<td>119</td>
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<td>310</td>
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<td>(38.18-43.93)</td>
<td>(42.08-47.54)</td>
<td>(40.29-43.61)</td>
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<td>151</td>
<td>153</td>
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<tr>
<td></td>
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<td>(152.1-154.7)</td>
<td>(149.8-152.9)</td>
<td>(152.1-153.6)</td>
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<tr>
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<td>49</td>
<td>49</td>
<td>51</td>
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<tr>
<td></td>
<td>(45.7-67.1)</td>
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<td>(47.3-50.9)</td>
<td>(48.0-54.4)</td>
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</tr>
<tr>
<td>Anemia risk factors</td>
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<td>4 (4.4%)</td>
<td>2 (1.7%)</td>
<td>0</td>
<td>6 (1.9%)</td>
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<tr>
<td></td>
<td>Total number</td>
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<td>4.4</td>
<td>4.8</td>
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<tr>
<td></td>
<td>(4.41-6.05)</td>
<td>(3.82-5.01)</td>
<td>(4.12-5.52)</td>
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<tr>
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<td>Pre-Menopausal</td>
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<td>80 (67.2%)</td>
<td>52 (52.0%)</td>
<td>201 (64.8%)</td>
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<td></td>
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<td>7 (7.7%)</td>
<td>3 (2.5%)</td>
<td>2 (2%)</td>
<td>12 (3.9%)</td>
</tr>
<tr>
<td></td>
<td>Taken iron pills</td>
<td>41 (45.1%)</td>
<td>62 (52.1%)</td>
<td>36 (36%)</td>
<td>139 (44.8%)</td>
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<tr>
<td></td>
<td>Diagnosed hookworm</td>
<td>13 (14.3%)</td>
<td>38 (31.9%)</td>
<td>31 (31%)</td>
<td>82 (26.5%)</td>
</tr>
<tr>
<td></td>
<td>Latrine use</td>
<td>0 (0.0%)</td>
<td>1 (0.0032%)</td>
<td>2 (0.0065%)</td>
<td>3 (0.97%)</td>
</tr>
<tr>
<td></td>
<td>Diagnosed malaria</td>
<td>2 (2.2%)</td>
<td>7 (6.3%)</td>
<td>7 (7%)</td>
<td>16 (5.2%)</td>
</tr>
<tr>
<td>Education</td>
<td>None</td>
<td>38 (41.8%)</td>
<td>28 (23.5%)</td>
<td>37 (37%)</td>
<td>103 (33.2%)</td>
</tr>
<tr>
<td></td>
<td>Grade 1-5</td>
<td>39 (42.9%)</td>
<td>70 (58.9%)</td>
<td>39 (39%)</td>
<td>148 (47.7%)</td>
</tr>
<tr>
<td></td>
<td>Grade 6-9</td>
<td>9 (9.9%)</td>
<td>14 (11.8%)</td>
<td>21 (21%)</td>
<td>44 (14.2%)</td>
</tr>
<tr>
<td></td>
<td>&gt; 9th grade</td>
<td>5 (5.5%)</td>
<td>7 (5.9%)</td>
<td>3 (3%)</td>
<td>15 (4.8%)</td>
</tr>
<tr>
<td>Literacy</td>
<td>None</td>
<td>44 (45.0%)</td>
<td>39 (32.8%)</td>
<td>42 (42.0%)</td>
<td>125 (40.3%)</td>
</tr>
<tr>
<td></td>
<td>Low</td>
<td>25 (27.5%)</td>
<td>58 (48.7%)</td>
<td>34 (34.0%)</td>
<td>117 (37.7%)</td>
</tr>
<tr>
<td></td>
<td>High</td>
<td>22 (24.2%)</td>
<td>22 (18.5%)</td>
<td>24 (24.0%)</td>
<td>68 (21.9%)</td>
</tr>
</tbody>
</table>

*Mean (95% CI).
Table 7.7.2: Water practices in Preak Khmeng Commune, Kandal Province, Cambodia collected from a cross-sectional survey in June 2010.

<table>
<thead>
<tr>
<th>Water source</th>
<th>PK1</th>
<th>PK2</th>
<th>Tuol Trea</th>
<th>Combined</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N 91</td>
<td>119</td>
<td>100</td>
<td>310</td>
</tr>
<tr>
<td>River</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wet: 35 (38.5%)</td>
<td>Dry: 87 (95.6%)</td>
<td>Wet: 11 (9.2%)</td>
<td>Dry: 104 (87.4%)</td>
<td>Wet: 4 (4.0%)</td>
</tr>
<tr>
<td>Tube well</td>
<td>Wet: 0 (0%)</td>
<td>Dry: 1 (1.1%)</td>
<td>Wet: 4 (3.4%)</td>
<td>Dry: 5 (4.2%)</td>
</tr>
<tr>
<td>Stored rain water</td>
<td>Wet: 56 (61.5%)</td>
<td>Dry: 2 (2.2%)</td>
<td>Wet: 104 (87.4%)</td>
<td>Dry: 8 (6.7%)</td>
</tr>
<tr>
<td>Bottled</td>
<td>Wet: 0 (0%)</td>
<td>Dry: 1 (1.1%)</td>
<td>Wet: 0 (0%)</td>
<td>Dry: 2 (1.7%)</td>
</tr>
<tr>
<td>Water treatment</td>
<td>None 25 (27.5%)</td>
<td>7 (5.9%)</td>
<td>9 (9.0%)</td>
<td>41 (13.2%)</td>
</tr>
<tr>
<td></td>
<td>Boil 65 (71.4%)</td>
<td>111 (93.3%)</td>
<td>90 (90.0%)</td>
<td>266 (85.8%)</td>
</tr>
<tr>
<td></td>
<td>Chemical/ filter 1 (1.1%)</td>
<td>1 (0.8%)</td>
<td>1 (1.0%)</td>
<td>3 (1.0%)</td>
</tr>
</tbody>
</table>
Table 7.7.3: Hemoglobin, iron indices, and meat consumption for 297 women surveyed in Lvea Aem District, Kandal Province, Cambodia.

<table>
<thead>
<tr>
<th></th>
<th>PK1</th>
<th>PK2</th>
<th>Tuol Trea</th>
<th>Combined</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>82</td>
<td>115</td>
<td>100</td>
<td>297</td>
</tr>
<tr>
<td>Hemoglobin (g/L)</td>
<td>Mean (95% CI)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>123.5 (120.42-126.56)</td>
<td>115.2 (112.46-117.94)</td>
<td>117.9 (115.20-120.62)</td>
<td>118.4 (116.74-120.06)</td>
</tr>
<tr>
<td>Proportion anemic (%)</td>
<td>&lt;12 g/L (anemic)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>39.0 (28.2, 49.8)</td>
<td>59.1 (50.0, 68.3)</td>
<td>52.0 (42.0, 62.0)</td>
<td>51.2 (45.5, 56.9)</td>
</tr>
<tr>
<td></td>
<td>&lt;10 g/L (moderate)</td>
<td>1.2 (-1.2, 3.6)</td>
<td>12.2 (6.1, 18.2)</td>
<td>5.0 (0.70, 9.3)</td>
</tr>
<tr>
<td></td>
<td>&lt;7 g/L (severe)</td>
<td>0 (-0.9, 2.6)</td>
<td>0.9 (0.70, 9.3)</td>
<td>0.0 (-0.3, 1.0)</td>
</tr>
<tr>
<td>Ferritin (ng/mL)</td>
<td>Mean of raw data (95% CI)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>76.7 (53.07-100.34)</td>
<td>57.0 (49.76-64.20)</td>
<td>62.07 (51.31-71.82)</td>
<td>64.2 (55.23-72.05)</td>
</tr>
<tr>
<td>Geometric mean for log data (95% CI)</td>
<td>51.3 (42.66-61.66)</td>
<td>41.7 (35.48-50.12)</td>
<td>44.7 (37.15-52.48)</td>
<td>45.7 (40.74-50.12)</td>
</tr>
<tr>
<td>Proportion &lt; 15 ng/mL</td>
<td>9.7% (3.20, 16.32)</td>
<td>14.8% (8.20, 21.37)</td>
<td>11.0% (4.76, 17.24)</td>
<td>12.1% (8.39, 15.85)</td>
</tr>
<tr>
<td>Proportion &lt; 30 ng/mL</td>
<td>20.7% (11.77, 29.69)</td>
<td>33.0% (24.32, 41.77)</td>
<td>31.0% (21.78, 40.22)</td>
<td>29.0% (23.77, 34.14)</td>
</tr>
<tr>
<td>Proportion 15 – 30 ng/mL</td>
<td>11.0 (0.41, 0.18)</td>
<td>18.3% (11.1, 25.4)</td>
<td>20.0% (12.0, 28.0)</td>
<td>16.8% (12.6, 21.2)</td>
</tr>
<tr>
<td>Iron deficiency anemia</td>
<td>Anemic women with low ferritin (&lt; 15 ng/mL)</td>
<td>6.1% (0.81, 11.39)</td>
<td>12.2% (6.11, 18.24)</td>
<td>10.0% (4.02, 15.98)</td>
</tr>
<tr>
<td></td>
<td>Anemic with borderline-low ferritin (&lt; 30 ng/mL)</td>
<td>12.2% (4.96, 19.43)</td>
<td>22.6 (14.85, 30.37)</td>
<td>19.0% (11.18, 26.82)</td>
</tr>
<tr>
<td>Infection</td>
<td>Mean CRP concentration (95% CI)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>5.4 (4.14, 6.58)</td>
<td>2.8 (2.23, 3.33)</td>
<td>2.7 (2.41, 3.01)</td>
<td>3.5 (3.04, 3.90)</td>
</tr>
<tr>
<td>Meat consumption</td>
<td>Mean meat serves per month (95% CI)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>4.88 (3.95, 5.81)</td>
<td>5.15 (4.25, 6.05)</td>
<td>5.30 (4.12, 6.48)</td>
<td>5.12 (4.54, 5.71)</td>
</tr>
<tr>
<td></td>
<td>Median (range)</td>
<td>2 (0-20)</td>
<td>2 (0-20)</td>
<td>2 (0-20)</td>
</tr>
</tbody>
</table>
Table 7.7.4: Proportion of iron deficiency, socioeconomic status, low meat consumption, prior history of malaria and hookworm by anemia classification (Hb < 120 g/L) in women from 3 villages in rural Cambodia.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Proportion Non-Anemic</th>
<th>Proportion Anemic</th>
<th>P-value</th>
<th>Odds Ratio</th>
<th>95% CI of Odds Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>145</td>
<td>152</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ferritin Percent</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ferritin very low (&lt;15 ng/mL)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Proportion</td>
<td>4.8% (7)</td>
<td>19.1% (29)</td>
<td>0.001</td>
<td>0.22</td>
<td>0.09-0.53</td>
</tr>
<tr>
<td>Percent ferritin low</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(&lt;30 ng/mL)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Proportion</td>
<td>21.4% (31)</td>
<td>36.2% (55)</td>
<td>0.011</td>
<td>0.51</td>
<td>0.30-0.86</td>
</tr>
<tr>
<td>Percent ferritin borderline</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(15 to &lt;30 ng/mL)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Proportion</td>
<td>16.6% (24)</td>
<td>17.1% (26)</td>
<td>0.92</td>
<td>1.03</td>
<td>0.56-1.92</td>
</tr>
<tr>
<td>Percent normal ferritin</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>78.6% (114)</td>
<td>63.8% (97)</td>
<td>0.011</td>
<td>1.97</td>
<td>1.17-3.32</td>
</tr>
<tr>
<td>Socioeconomic status*</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Very low (Q1)</td>
<td>30.3% (44)</td>
<td>19.7% (30)</td>
<td>0.30</td>
<td>1.37</td>
<td>0.76-2.47</td>
</tr>
<tr>
<td>Low (Q2)</td>
<td>24.1% (35)</td>
<td>25.7% (39)</td>
<td>0.86</td>
<td>0.95</td>
<td>0.56-1.62</td>
</tr>
<tr>
<td>Medium (Q3)</td>
<td>22.8% (33)</td>
<td>27.6% (42)</td>
<td>0.68</td>
<td>0.89</td>
<td>0.52-1.53</td>
</tr>
<tr>
<td>High (Q4)</td>
<td>22.8% (33)</td>
<td>27.0% (41)</td>
<td>0.71</td>
<td>0.90</td>
<td>0.52-1.56</td>
</tr>
<tr>
<td>Meat consumption</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;2 servings per month</td>
<td>5.5% (8)</td>
<td>19.5% (16)</td>
<td>0.17</td>
<td>0.54</td>
<td>0.22-1.31</td>
</tr>
<tr>
<td>2 to 9 servings per month</td>
<td>69.7% (101)</td>
<td>55.3% (84)</td>
<td>0.02</td>
<td>1.80</td>
<td>1.11-2.92</td>
</tr>
<tr>
<td>&gt;9 servings per month</td>
<td>24.8% (36)</td>
<td>34.2% (52)</td>
<td>0.09</td>
<td>0.64</td>
<td>0.38-1.07</td>
</tr>
<tr>
<td>Infectious disease</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diagnosed with malaria</td>
<td>5.5% (8)</td>
<td>5.3% (8)</td>
<td>0.77</td>
<td>1.16</td>
<td>0.42-3.23</td>
</tr>
<tr>
<td>Diagnosed with hookworm</td>
<td>31.0% (45)</td>
<td>21.7% (33)</td>
<td>0.02</td>
<td>1.91</td>
<td>1.11-3.28</td>
</tr>
</tbody>
</table>

*For this analysis, socioeconomic status was treated as categorical, determined by the quartiles.
Table 7.7.5: Multiple regression analysis of risk factors (demographic, socioeconomic status, and meat consumption) associated with hemoglobin and iron stores in women from 3 villages residing in rural Cambodia.

<table>
<thead>
<tr>
<th></th>
<th>Hemoglobin</th>
<th></th>
<th>Serum Ferritin*</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Coefficient (SE)</td>
<td>P</td>
<td>Coefficient (SE)</td>
<td>P</td>
</tr>
<tr>
<td>Age (years)</td>
<td>-0.42 (0.095)</td>
<td>&lt;0.001</td>
<td>0.98 (1.0)</td>
<td>0.004</td>
</tr>
<tr>
<td>Education (years)</td>
<td>1.38 (1.03)</td>
<td>0.18</td>
<td>1.0 (1.07)</td>
<td>0.92</td>
</tr>
<tr>
<td>Number of pregnancies (N)</td>
<td>0.34 (0.28)</td>
<td>0.22</td>
<td>1.04 (1.02)</td>
<td>0.033</td>
</tr>
<tr>
<td>Socioeconomic status</td>
<td>-0.003 (0.016)</td>
<td>0.874</td>
<td>1.0 (1.0)</td>
<td>0.025</td>
</tr>
<tr>
<td>Pre-menopausal</td>
<td>-6.09 (2.49)</td>
<td>0.015</td>
<td>0.49 (1.17)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Less than 2 meat servings per month**</td>
<td>1.95 (3.17)</td>
<td>0.54</td>
<td>1.15 (1.22)</td>
<td>0.49</td>
</tr>
<tr>
<td>2 – 9 meat servings per month**</td>
<td>4.33 (1.78)</td>
<td>0.016</td>
<td>1.09 (1.12)</td>
<td>0.45</td>
</tr>
</tbody>
</table>

* Log-transformed serum ferritin used for analysis (back-transformed values shown)

**Referent group: high meat consumption (9+ servings per month).
CHAPTER 8: “TRY SA’BAY” – IRON FISH FOR THE AMELIORATION OF IRON DEFICIENCY ANEMIA IN RURAL CAMBODIA, A RANDOMIZED COMMUNITY TRIAL

8.1 Introduction

Despite a concerted effort by government and civil society in recent years, malnutrition, hunger and food insecurity continue to plague the developing world. Anemia, largely resulting from iron deficiency, is a serious public health problem with major consequences for human health and socio-economic development. The World Health Organization (WHO) estimates that nearly 2 billion people are anemic, with the largest burden of disease in women, children and infants.¹

Anemia is a condition with a multi-factorial etiology, which may account for the relatively low impact of interventions to date.² Severe blood loss, acute and chronic infections, the presence of other micronutrients deficiencies, and inherited defects resulting in abnormal hemoglobin production are known causes. That said, nutritional anemia resulting from inadequate iron intake and/or poor absorption of iron is a leading cause.¹ Throughout the world, iron deficiency and anemia are serious problems, and strategies to improve iron intake are needed.

The long-term consequences of anemia are severe and often irreversible.¹³ Mild to moderate anemia leads to weakened immunity, reduced work capacity, reduced cognitive ability and an overall decreased quality of life. Severe anemia (hemoglobin < 70 g/L) reduces a woman’s ability to survive bleeding during and after childbirth and is considered a major cause
of maternal morbidity and mortality. Anemia during pregnancy is also associated with increased risk of low birth-weight, premature delivery, and perinatal mortality. Delayed cognitive development and limitation in intellectual development is also a major concern in children and adolescents.

In Cambodia, anemia is a critical public health problem. Recent estimates suggest that 55% of children, 43% of women of reproductive age, and 50% of pregnant women are affected. Although Cambodia is rice sufficient, a lack of crop diversification and production of nutrient-rich food results in inadequate dietary iron intake leading to iron deficiency and eventually anemia. Further, the majority of women lack access to processed iron-fortified food products and other sources of high bioavailable iron. Oral iron supplements are supplied to pregnant women, but not to people in other high-risk groups. Therefore, Cambodia must adopt widely accessible and sustainable approaches to prevent and control iron deficiency and anemia in order to reduce this high burden of condition.

In this paper, we describe the development and evaluation of a novel, cost-effective iron supplementation technique that has the potential to provide a long-term solution to iron deficiency in the developing world. Previously, we reported on a pilot study of a uniquely shaped iron ingot that can be used during cooking to provide additional iron to an otherwise deficient diet. The results of the pilot study were promising but not definitive, as a change in the season mid-way through the study resulted in a shift in use of rainwater to well water, which is contaminated with heavy metals that inhibit iron absorption. As such, we reported amelioration of anemia in the first half of the study, followed by a plateau coinciding with the
change in drinking water. Despite this, we found the iron ingot to be acceptable at the household-level, warranting further research on the effectiveness at improving iron status.

The current paper presents the findings of a randomized controlled community trial that was conducted in rural Cambodia from June 2010 to June 2011. Investigation of the acceptability and effect of continued use of the iron ingot in three communities in rural Kandal Province was performed. The effect of the ingot on hemoglobin and iron status was measured and data was collected on compliance with the supplementation regime. In consideration of the challenges experienced in the pilot study, this community trial was conducted in several small villages which do not use tube well water, and included a 12-month follow-up to encompass both seasons in their entirety, thereby accounting for any seasonal variation.

8.2 Materials and methods

8.2.1 Study area, population and recruitment

Between June 2010 and June 2011, the longitudinal community trial was conducted in Lvea Aem District, Kandal Province, Cambodia. Kandal province is estimated to have a population of 1.25 million living in approximately 260,000 households, with the vast majority situated in rural areas of the province. Anemia is estimated to affect 56.4% of children 6-59 months of age and 44.9% of women of reproductive age (15-40 years) throughout the province, approximately the same as the national prevalence estimates.

Three geographically distinct study sites, Tuol Trea (TT), Preak Khmeng 1 (PK1), and Preak Khmeng 2 (PK2) were selected for the trial. These three sites comprise Preak Khmeng
Commune, Lvea Aem District and are separated by a complex of roadways and rivers. All women living within the study area and who met the criteria listed below formed the sampling frame. Inclusion criteria included informed consent, residence in the study area, and age greater than 16 years at the time of enrolment; exclusion criteria included severe anemia (hemoglobin < 70 g/L) and intention to migrate before the end of the trial. Households were selected using systematic random sampling by approaching every fourth household until the pre-determined sample size was met during a 2-week period in June 2010, the beginning of the monsoon season. The project was explained and those women who consented to participate were then enrolled and a blood sample was collected for biomarker measurement. These data represented baseline values for the remainder of the study.

The study was a randomized field trial. Participants within each village were randomized to one of three trial arms: (i) an intervention group receiving an iron ingot (non-follow-up, NFU), (ii) a second intervention group receiving an iron ingot plus six follow-up sessions where nutritional education was provided (follow-up, FU), and (iii) a control group which did not receive any intervention but was followed throughout the study. Randomization was performed by the primary author using a random digit generator (Microsoft Excel 2007, Redmond, WA, USA). The cast iron ingot was designed to resemble a species of fish that is common throughout Cambodia, and was called ‘try sa’bay’, roughly translated as ‘happy fish’. Further details of the design and production of the iron ingot have been previously reported.5

Participants in the iron ingot groups were instructed to use the ingot every day. Soup was chosen as one vehicle for supplementation because it is typically eaten every day, and is
also often soured with fruits containing ascorbic acid, a well-known enhancer of iron absorption.\textsuperscript{7} For those women that did not consume soup on a daily basis we encouraged use of the ingot when boiling drinking water, to which addition of a small amount of citrus juice was encouraged, both prior to boiling and again just before consumption.

8.2.2 Data collection and processing

A 3 mL venous blood sample was collected from each participant, one drop of which was used to measure hemoglobin (Hb) concentration using a HemoCue portable hemoglobin analyzer (HemoCue AB, Angelholm, Sweden). The remaining blood sample was placed into a tube (Greiner Bio-One, Shanghai, China) containing a clot-activator (microscopic silica particulate) and then immediately placed into a cooler with a freezer pack to maintain sample integrity. Samples were transported within four hours to Paramed Laboratories (Paramed Laboratoire D’Analyses Medicales, Phnom Penh, Cambodia) for serum ferritin (SF) and C-reactive protein (CRP) analysis. Serum ferritin (SF) was measured using an Abbott AxSYM system (Abbott Laboratories, North Chicago, IL, USA) and serum C-reactive protein (CRP) was assessed by an ELISA (AssyMax Human CRP ELISA Kit, St. Charles, USA). Appropriate controls and reference standards were used on each day of testing to ensure accuracy of the laboratory analysis.

Following enrolment and baseline sampling, participants were visited at their homes by trained fieldworkers every three months for one year, for a total of five sampling visits. At each visit, a blood sample was taken (as above) and pre-coded structured questionnaires regarding
usage and compliance were completed. Participants in the iron ingot groups were asked when they had last used it for cooking and the typical frequency of use.

At each sampling round, participants in the iron ingot plus follow-up group met for approximately 5-10 minutes with a member of the research team and received a lesson about nutrition. The lessons were given in order to enhance compliance to the treatment regime by repeatedly encouraging use of the ingot with suggestions for incorporation into the daily cooking routines. In addition, basic nutrition education was provided, including a discussion of food groups, the availability of iron-rich foods, and the negative health outcomes associated with inadequate nutrition. Initially, we hypothesized that those women who received the follow-up visits would have better compliance (as their questions and concerns about the ingot could be voiced more regularly) and that their iron status would increase above that of the non-follow-up group, both because of improved compliance, but also because of the increase in nutritional knowledge. Simple handouts using photographs and diagrams to explain the concepts were left with the women for future reference.

Data were initially recorded by hand onto coded spreadsheets and later transcribed into Microsoft Excel 2007 (Redmond, WA, USA); after entry, data were checked for accuracy.

Anemia was defined as hemoglobin concentration < 120 g/L and iron deficiency as a serum ferritin level < 15 ng/mL, according to WHO recommendations for non-pregnant women > 15 years of age.8

Serum ferritin was chosen to assess iron stores because it most closely correlates with relative total body iron stores. Serum ferritin is an acute-phase reactant protein, which is raised
in response to infection or inflammation. Following WHO recommendations, we therefore included measurement of C-reactive protein. A CRP concentration > 10 mg/L was selected to represent a state of infection/inflammation. Anemia of chronic disease was identified in women who had abnormal hemoglobin levels (Hb < 120 g/L) together with abnormal CRP concentration (CRP > 10 mg/L), and these women were excluded from analysis.\textsuperscript{9,10}

\subsection*{8.2.4 Sample size}
Sample size was calculated to be able to detect a difference in hemoglobin of 5 g/L at the end of the study with a 95% confidence level (\(\alpha=0.05\)) and a power of 80\%.\textsuperscript{11} Thus, a minimum of 76 participants per group was needed. Approximately 25\% loss-to-follow-up was anticipated over the 12-month study duration and we therefore included sufficient samples to account for this loss. A total of 310 women completed the baseline survey and laboratory evaluation.

\subsection*{8.2.5 Statistical analysis}
The trial used households as the unit of randomization and intervention. The individual women were the unit of data collection and analysis because only two homes included more than one participant. Data were transcribed from the spreadsheet into IBM SPSS Statistics statistical software package version 19.0 (SPSS Inc., Chicago, IL, USA) for analysis. The level of significance for all statistical analysis was set at \(P < 0.05\).

One-way analysis of variance followed by a least significant difference analysis was used to compare Hb, SF, age of participants and number of pregnancies per participant in each
group; the proportion of post-menopausal women was tested with a chi-square test. Because loss-to-follow-up occurred over the 12-month study, a Student’s t-test was used to compare baseline Hb and SF of women who withdrew prior to completion compared with those who completed the study to ensure that loss-to-follow-up was not associated with baseline iron status. Hemoglobin and SF were assessed for normality.

Preliminary analyses involved examining descriptive statistics, frequency tables and histograms. To compare outcomes, the following tests were used: i) paired t-tests to examine within woman changes over time (baseline, 3 months, 6 months, 9 months, 12 months) for Hb and SF; ii) one-way ANOVA was used to compare changes in Hb and SF among groups and to compare values among groups at a given time in the study; iii) and chi-square tests were conducted to compare the proportion of women with normal values of Hb and SF among groups at a given time in the study and the use of the iron ingot between the NF and NFU groups.

Multivariable analyses were conducted by regressing the outcome on the treatment and covariates using multiple linear regressions. Hb and SF at 12 months (endline), and the difference from baseline to endline for both biomarkers were included in separate analyses. Models were built using a manual backward elimination selection process whereby covariates were dropped one at a time based on the highest value and retained only if $P < 0.20$. The covariates pregnancy and village were included for both Hb and SF models, and CRP was also included for the SF models. Confounding was determined by identifying variables whose coefficients changed by 20% and/or changed in significance as another covariate left the model.
8.2.6 Ethics approval

Ethics approval was obtained from the research ethics board at the University of Guelph, Canada, and the International Research Development Centre, Canada reviewed study procedures. The district health officials and the local commune office in Cambodia also provided study approval. Prior to the intervention, oral informed consent was obtained from all participants.

8.3 Results

Hemoglobin values were normally distributed, facilitating use of the arithmetic mean. Serum ferritin concentrations were right-skewed and were log-transformed for successive analyses. There were only 2 homes with multiple participants (5/254 participants) and within-household variance was minimal and was therefore not included in the analyses.

A preliminary analysis revealed that the levels of Hb and SF by month did not differ between the two groups receiving the ingot; therefore, the data from the two groups receiving the iron ingot were combined for further analysis. The detailed statistical analysis provides a comparison of women in the control group with those receiving an iron ingot (including both NFU and FU) groups. Preliminary analysis confirmed that the three study sites had different baseline Hb and CRP values; therefore ‘village’ was included as a dummy variable in the analyses.
8.3.1 Recruitment

The trial profile is shown in Figure 8.7.1. A total of 310 women from 305 households were recruited into the study at baseline. Of these, 102 were allocated to the control group, and 104 were allocated to each the NFU and FU groups. Loss-to-follow-up was observed in all trial groups (Figure 8.7.1), however overall loss was less than the 25% that was accounted for in the sample size calculation thereby preserving the power of this study.

8.3.2 Baseline characteristics

The baseline demographic characteristics of the participants are shown in Table 8.6.1. The data did not differ significantly between control and ingot group (P values > 0.05). Pregnancy at baseline was the only characteristic that tended to be higher in the iron ingot group than the control group (P = 0.05) but there were only 6 pregnant women in total at the start of the study (baseline).

8.3.3 Outcomes

Hemoglobin concentrations differed significantly between the iron ingot group and the control group. A two-fold reduction in the prevalence of anemia was observed at endline in the iron ingot group when compared to controls (P<0.0001). There was a gradual increase in Hb concentration from baseline to endline in the group using the iron ingot (P<0.0001). The difference in Hb concentration from baseline to endline was 11.6 g/L higher in the iron ingot group compared to control group (Table 8.6.2). Similarly, SF concentrations differed from baseline to endline, with the iron ingot groups displaying an improvement of 30.8 ng/mL over
the controls. An increase in SF concentration was also noted from 9 months to endline in the iron ingot group, but not at the previous sampling rounds.

The change in Hb and SF values over time is shown in Table 8.6.3. The Hb and back-transformed values of SF display a trend of gradually increasing concentration over time. Significant differences in Hb concentration within group began at 9 months and were still present at endline, while the treatment group SF values differed significantly at endline only.

The findings of the multivariable regression analysis are displayed in Table 4. After adjusting for the covariates pregnancy (at baseline) and village, the mean Hb concentrations differed significantly between control and treatment group at endline ($P<0.0001$). Similarly, the difference in Hb from baseline to endline was also significantly different between groups ($P<0.0001$). After controlling for the additional covariate CRP concentration, significant differences were observed for SF values at endline, and for the difference between the baseline and endline.

Compliance to the supplementation regime throughout the study was high in both ingot groups (Table 8.6.5). Upwards of 80% of participants used the ingot daily and few participants used the ingot every 3 to 4 days. Participants who reported never using the ingot were lost-to-follow-up in both groups, so that by endline only participants who used the ingot regularly were remaining. There was no difference in iron ingot usage between the NFU and FU groups.

8.4 Discussion

The current paper presents the findings of a novel and effective supplementation technique used to enhance dietary iron content in poor, rural areas of Cambodia. Women who
were assigned an iron fish to use while cooking were found to have significantly improved hemoglobin and serum ferritin values over a 12-month longitudinal trial. The amelioration of serum ferritin concentrations would suggest that more iron was absorbed than was needed for immediate erythropoiesis. These data would imply that regular use of a simple, fish-shaped ingot not only supplies iron, but also that this iron is bioavailable and effective in amelioration iron deficiency. Given that it is very difficult to improve iron status after entering pregnancy, the ability to improve and maintain normal levels of both circulating and stored iron would be significant for women of reproductive age.12

Anemia is a condition that is not quickly ameliorated. The absorption of iron intensifies as iron deficiency and anemia becomes more severe.13 Oral iron supplements can take several weeks and up to three months before an effect on biomarkers is observed, and repletion of iron stores may take six months or more.2 Nutritional interventions to improve iron status require behavioural change and therefore require time to see an effect and eventual repletion. Thus, it was not surprising that an effect on Hb and SF was not detectable until nine months.

The iron fish was developed in an effort to supply iron at a slow but steady rate to a deficient diet. Oral iron supplements remain the standard of care in acute cases of IDA, but prevention and control of IDA is an important public health consideration. In effect, the iron ingot may prove to be an innovative form of homestead food fortification in a country that is otherwise lacking affordable and accessible enriched food products.

Previously, we showed that iron readily leached from the ingot into different types of soured soup and acidified boiling drinking water.14 The acidity of the preparation was an
important factor influencing the amount of iron released from the ingot therefore, if the ingot was recommended for widespread use, it would be important to include advice to use the ingot in preparations that are slightly acidic. In the current study, we encouraged women to use the ingot every day when preparing consumables which are slightly acidic. Women were urged to use the ingot when preparing soup that is soured with fruit juice containing ascorbic acid: a staple component of Khmer cuisine. We also recommended that the ingot should be used when boiling drinking water, and that a small amount of citrus juice should be added both prior to boiling and just before consumption. Participants and the research team commented that this slightly acidic drinking water was similar to consuming a glass of water with a slice of lemon.

One of the principal challenges with adventitious iron intervention studies is compliance,\textsuperscript{15} therefore several design considerations were included at the outset. The ingot was designed to resemble a species of fish, which is both widely consumed and considered to be lucky in Khmer folklore. Residents in the study area commented on the attractive design of the smiling fish, and highlighted this appeal as a reason for continued use over the study duration. In addition, the ingots were designed to be light-weight, with maximal surface area to enhance iron leaching, and were easy-to-clean-- an important consideration to avoid the build-up of rust which could potentially discolour food/water and possibly deter women from using the ingot over time. All iron ingots were produced locally by a metalworker with a small metal shop in Kean Svay Commune, Kandal Province, Cambodia. The ingots were produced from scrap metal and were subjected to quality control procedures to ensure that heavy metals and other contaminants were not present in concentrations exceeding WHO standards.\textsuperscript{16} The
acceptability of the design was pre-tested in a pilot study, and was subsequently assessed with key informants and village officials in the current study site just prior to manufacturing.

Compliance to the supplementation regime was high in both the NFU and FU groups. In studies that have examined the use of iron pots as a method of improving nutritional status, compliance ranged from approximately 30 – 70% over time.\(^{17,18}\) In contrast, over 90% of women who were allocated an iron ingot used the ingot regularly (i.e., more than 5 times per week) throughout the study. In discussion with the women over the 12-month trial period, the research team observed that there was some reluctance to use the ingot, but eventually the fish was incorporated into the daily cooking routine. Initially a number of women kept the iron fish in a plastic bag in the sleeping area of their homes, which is the customary place to keep various medicines. This practice resulted in lower use in the first few weeks of the study. After suggesting that the women should think of the iron fish as a cooking implement and keep the ingot in the cooking area of the home, many participants volunteered that they remembered to use the iron fish every time they boiled drinking water and/or prepared soup.

By endline only a few women were reluctant to use the iron ingot and participants in the ingot groups who did not use the ingot throughout the study were eventually lost-to-follow-up for various reasons. Loss-to-follow-up was typically associated with fear of giving a blood sample. No participant cited reluctance to use the ingot as a reason to withdraw from the study. Taken together, these findings would suggest that after habituating to use, women were comfortable with using the ingot regularly.
Initially, we expected that women in the iron ingot group plus follow-up nutritional education group (i.e., FU) would have a greater improvement in iron status and compliance compared with those in the ingot without education group (i.e., NFU), but this was not observed. There are a number of possible explanations for this lack of difference: it is possible that women in the FU groups were either sharing the nutritional education with other residents in the study area (including women assigned to control and NFU groups), or that the women in the FU group were not able to follow the discussion as well as we had planned; it is also possible that women were already aware of the suggestions made on nutrition but that they did not have the financial means to purchase nutrient-dense foods such as meat, or enhancers of iron absorption such as citrus fruits. We did not collect data to confirm or refute these ideas and this should be the subject of future research.

There is a history of using iron from extraneous sources as a dietary supplement. Beginning in the 1980s, formative research was conducted which determined that iron cookware not only increased the iron content of food, but that this iron was bioavailable.\textsuperscript{19-21} Since then, several randomized community trials have been conducted in Brazil and Sub-Saharan Africa, with varying results.\textsuperscript{11,17,18,22} Although the use of iron cooking vessels should theoretically be effective, cost, weight, lack of familiarity, alteration of food colour and taste, and/or some other culturally-specific concerns limit the value of this supplementation technique in practice.\textsuperscript{15} The iron ingot appears to overcome such concerns: it is inexpensive, light-weight, can be used in previously purchased cooking pots regardless of construction material; the ingot does not alter the colour and taste of food as the ingot can be removed once the food is cooked; the ingot is relatively cheap (approximately $1.50 USD and probably
has a life-span of more than 10 years compared to monthly supplements that cost $2-4 USD per person); and the appearance of the fish appeared to encourage women to use the ingot. The iron ingot therefore represents a much more sustainable option.

Families in the study area live in simple wooden and bamboo homes with immediate and extended family members who typically share food, water and other aspects of daily living (Personal Observation, C. Charles). As is common throughout much of the country, residents in this village depend on farming, with the main crop and complementary food being rice, which is low in bioavailable iron. One of the benefits of use of the iron ingot, when compared to alternative nutritional supplements, is that the whole family benefits from its use. Women are typically responsible for cooking in the Khmer household. When the ingot is used to prepare family meals, men, children and the elderly, although not included in the current trial, may have benefited from ingot’s ability to enhance dietary iron content.

This study highlights both the acceptability and effectiveness of iron fish as a means of improving dietary iron content, and in the amelioration of iron deficiency and anemia. Scaling-up of the iron fish project, including widespread distribution of the ingot would likely have significant, beneficial impacts on the lives of women, and by proxy, their families, in rural Cambodia. Although not investigated directly in the current study, there is no reason to believe that the iron fish would not be acceptable to communities in neighbouring countries throughout the region. Fundamentally, this research provides evidence that cooking with a specially designed iron ingot is useful in the prevention and control of iron deficiency and anemia, and the design of the ingot itself – whether it be a fish, a lotus flower, or some other
meaningful shape – is important for the acceptability at the household level. Future research should investigate social marketing techniques and various ingot designs to assess cultural relevance in new settings.
8.5 References


### 8.6 Tables

Table 8.6.1: Baseline demographic characteristics of women in a randomized controlled trial of a novel iron supplementation technique using an iron ingot in cooking pots (N=254).

<table>
<thead>
<tr>
<th></th>
<th>Control (N=85)</th>
<th>Iron Ingot (N=169)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>43.4 (15.25)</td>
<td>40.8 (13.97)</td>
<td>0.16</td>
</tr>
<tr>
<td>Post-menopausal</td>
<td>54 (63.5%)</td>
<td>111 (65.7%)</td>
<td>0.74</td>
</tr>
<tr>
<td>Pregnant at baseline</td>
<td>1 (1.2%)</td>
<td>5 (3.0%)</td>
<td>0.05</td>
</tr>
<tr>
<td>Baseline Hb (g/L)</td>
<td>118.6 (12.48)</td>
<td>116.3 (13.77)</td>
<td>0.21</td>
</tr>
<tr>
<td>Baseline SF (ng/mL)</td>
<td>53.3 (34.17)</td>
<td>58.5 (52.58)</td>
<td>0.41</td>
</tr>
<tr>
<td>Baseline CRP (mg/L)</td>
<td>4.0 (5.22)</td>
<td>3.9 (4.48)</td>
<td>0.90</td>
</tr>
</tbody>
</table>

*Abbreviations: Hb = Hemoglobin, SF = Serum ferritin, CRP = C-reactive protein*
Table 8.6.2: Differential increase in hemoglobin and serum ferritin for women who used an iron ingot compared to women in a control group for 254 women from Lvea Aem District, Kandal Province, Cambodia who completed a community trial of an iron ingot for use in the cooking pot.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Coefficient (β)</th>
<th>Standard Error of β</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hb 3 Months – Hb Baseline</td>
<td>3.6</td>
<td>.89</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Hb 6 Months – Hb Baseline</td>
<td>4.0</td>
<td>.97</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Hb 9 Months – Hb Baseline</td>
<td>7.0</td>
<td>1.13</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Hb Endline – Hb Baseline</td>
<td>11.6</td>
<td>1.39</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>SF 3 Months – SF Baseline</td>
<td>2.4</td>
<td>5.35</td>
<td>0.65</td>
</tr>
<tr>
<td>SF 6 Months – SF Baseline</td>
<td>5.0</td>
<td>6.01</td>
<td>0.41</td>
</tr>
<tr>
<td>SF 9 Months – SF Baseline</td>
<td>6.9</td>
<td>1.13</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>SF Endline – SF Baseline</td>
<td>30.8</td>
<td>7.07</td>
<td>&lt; 0.0001</td>
</tr>
</tbody>
</table>

Abbreviations: Hb = Hemoglobin, SF = Serum ferritin

*Referent is the control group
Table 8.6.3: Association between iron ingot use and hemoglobin and serum ferritin values over one year for 254 women from Lvea Aem District, Kandal Province, Cambodia who participated in a community trial using an iron ingot in the cooking pot.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Iron Fish</th>
<th>Control</th>
<th>β (SE)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hb Baseline</td>
<td>116.3 (13.77)</td>
<td>118.6 (12.48)</td>
<td>-1.9 (1.78)</td>
<td>0.28</td>
</tr>
<tr>
<td>Hb 3 Months</td>
<td>119.4 (12.62)</td>
<td>118.0 (12.89)</td>
<td>1.7 (1.70)</td>
<td>0.31</td>
</tr>
<tr>
<td>Hb 6 Months</td>
<td>120.9 (12.73)</td>
<td>119.0 (13.14)</td>
<td>2.1 (1.73)</td>
<td>0.23</td>
</tr>
<tr>
<td>Hb 9 Months</td>
<td>123.2 (11.05)</td>
<td>118.2 (12.62)</td>
<td>5.0 (1.56)</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Hb 12 Months</td>
<td>130.0 (12.01)</td>
<td>120.1 (13.04)</td>
<td>9.7 (1.66)</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>SF Baseline</td>
<td>58.5 (52.58)</td>
<td>53.3 (34.17)</td>
<td>1.0 (0.051) §</td>
<td>0.95</td>
</tr>
<tr>
<td>SF 3 Months</td>
<td>66.1 (56.93)</td>
<td>58.6 (44.19)</td>
<td>1.0 (0.053) §</td>
<td>0.79</td>
</tr>
<tr>
<td>SF 6 Months</td>
<td>70.5 (71.66)</td>
<td>61.6 (40.00)</td>
<td>1.0 (0.051) §</td>
<td>0.68</td>
</tr>
<tr>
<td>SF 9 Months</td>
<td>74.0 (74.78)</td>
<td>60.4 (44.47)</td>
<td>1.2 (0.049) §</td>
<td>0.06</td>
</tr>
<tr>
<td>SF 12 Months</td>
<td>101.3 (87.86)</td>
<td>66.3 (44.35)</td>
<td>1.5 (0.045) §</td>
<td>&lt; 0.0001</td>
</tr>
</tbody>
</table>

Abbreviations: Hb = Hemoglobin, SF = Serum ferritin

Mean (SD); *Referent is control group; §Exponentiated (backtransformed) values of SF
Table 8.6.4: The association between the use of an iron ingot and endline hemoglobin (Hb) and serum ferritin (SF), after controlling for pregnancy and village, in a community trial of an iron ingot, where women randomized to the control group were the referent category.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Coefficient (β)</th>
<th>Standard Error of β</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Endline Hb</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Treatment</td>
<td>9.7</td>
<td>1.66</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Pregnancy</td>
<td>-0.6</td>
<td>5.16</td>
<td>0.92</td>
</tr>
<tr>
<td>PK1</td>
<td>0.7</td>
<td>1.90</td>
<td>0.71</td>
</tr>
<tr>
<td>PK2</td>
<td>3.2</td>
<td>1.95</td>
<td>0.10</td>
</tr>
<tr>
<td>Endline Hb Difference</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Treatment</td>
<td>11.6</td>
<td>1.39</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Pregnancy</td>
<td>8.8</td>
<td>4.34</td>
<td>0.044</td>
</tr>
<tr>
<td>PK1</td>
<td>-1.01</td>
<td>1.60</td>
<td>0.53</td>
</tr>
<tr>
<td>PK2</td>
<td>3.4</td>
<td>1.64</td>
<td>0.041</td>
</tr>
<tr>
<td>Endline SF</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Treatment</td>
<td>1.5</td>
<td>.045</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>CRP Endline</td>
<td>1.02</td>
<td>.005</td>
<td>0.059</td>
</tr>
<tr>
<td>Pregnancy</td>
<td>0.9</td>
<td>.14</td>
<td>0.67</td>
</tr>
<tr>
<td>PK1</td>
<td>0.9</td>
<td>.052</td>
<td>0.57</td>
</tr>
<tr>
<td>PK2</td>
<td>0.8</td>
<td>.053</td>
<td>0.13</td>
</tr>
<tr>
<td>Endline SF Difference</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Treatment</td>
<td>30.8</td>
<td>7.07</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>CRP Endline</td>
<td>1.5</td>
<td>.82</td>
<td>0.069</td>
</tr>
<tr>
<td>Pregnancy</td>
<td>4.9</td>
<td>22.07</td>
<td>0.82</td>
</tr>
<tr>
<td>PK1</td>
<td>-13.0</td>
<td>8.11</td>
<td>0.11</td>
</tr>
<tr>
<td>PK2</td>
<td>-21.8</td>
<td>8.33</td>
<td>0.009</td>
</tr>
</tbody>
</table>

*Referent group is control group using a linear regression model; Village 3 (TT) is referent village*
Table 8.6.5: Reported usage of an iron ingot over the previous 3 months as assessed at each sampling round in a randomized controlled trial of a novel iron supplement conducted in Kandal Province, Cambodia.

<table>
<thead>
<tr>
<th></th>
<th>NFU Group</th>
<th></th>
<th>FU Group</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Daily</td>
<td>3-4 days</td>
<td>Rarely</td>
<td>Never</td>
</tr>
<tr>
<td>3 months (N=185)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>80.0% (75/94)</td>
<td>10.6% (10/94)</td>
<td>4.3% (4/94)</td>
<td>5.3% (5/94)</td>
</tr>
<tr>
<td>6 months (N=177)</td>
<td>90.1% (82/91)</td>
<td>5.5% (5/91)</td>
<td>1.1% (1/91)</td>
<td>3.3% (3/91)</td>
</tr>
<tr>
<td>9 months (N=172)</td>
<td>90.0% (80/89)</td>
<td>5.6% (5/89)</td>
<td>2.2% (2/89)</td>
<td>2.2% (2/89)</td>
</tr>
<tr>
<td>Endline (N=169)</td>
<td>92.0% (80/87)</td>
<td>5.7% (5/87)</td>
<td>0.0% (0/87)</td>
<td>2.3% (2/87)</td>
</tr>
</tbody>
</table>
8.7 Figures

Figure 8.7.1: CONSORT flow diagram for a 12-month study (baseline to endline) for an intervention to ameliorate iron deficiency and anemia in rural Cambodia.
CHAPTER 9: CONCLUSIONS AND RECOMMENDATIONS

9.1 Summary

In the developing world, current strategies to prevent and correct iron deficiency and anemia have met with little success. Food-based strategies, including general or targeted food fortification, have been difficult to implement in many regions where centralized food processing is not typical and where cost is prohibitive. It is imperative to develop simple, cost-effective solutions that are accessible to rural populations and are widely accepted.

Currently, pregnant women (deemed to be in the highest risk category) are the prime targets for iron supplementation, but this narrow focus is inadequate. More than half of rural women of reproductive age are iron deficient and pregnancy simply makes their condition worse. In fact, once pregnant it is unlikely that it will be possible to restore their body iron reserves. It is vital to make a radical shift in thinking. Women of reproductive age – before they become pregnant – should be targeted and public health efforts should be switched to prevention long before pregnancy intervenes.

Infants, children and adolescents are also largely neglected by supplementation and fortification programs, despite the devastating consequences that iron deficiency can have in these important stages of life. If food and nutrition security is to be achieved, policies and programmes must target whole families, rather than focusing on specific groups. Fieldwork conducted for this thesis revealed that many rural women were not ingesting sufficient quantities of vitamin A nor iron in order to meet daily-recommended intakes. A lack of nutritional knowledge was also identified, and women typically did not have a clear
understanding of which foods they should be consuming in order to provide a well-balanced diet and promote good health. Therefore, education and social marketing are crucial components of national, municipal and community efforts for sustained improvements in food and nutrition security and evidence-based practices must be adopted.

The current thesis examined the use of a cost-effective and long-lasting means of improving the dietary iron content at the household level in rural Cambodia. It has been shown in controlled laboratory research that iron is readily leached from the ingot in substantial quantities.\(^4\) Even after taking into account the limited bioavailability of consumed iron, nearly three-quarters of daily iron requirements should theoretically be met by consuming acidified boiled drinking water which was prepared with the ingot. Various soups, a staple of the Khmer diet, are also effective food vehicles. In randomized controlled field conditions, the iron ingot proved to be effective at not only increasing hemoglobin levels, a measure of circulating iron, but also contributing to iron storage. The ability of the iron ingot to alter stored iron concentrations suggests that more iron can be absorbed than needed for red blood cell production, and that the additional iron can be stored for use in times when dietary iron is insufficient, or when iron deficiency occurs for some other reason.

Perhaps the most interesting aspect of the present research is the design of the iron ingot as a species of fish that is considered to be lucky in Khmer culture. In the early stages of the project, a number of prototypes were produced – including a simple disc, a lotus flower, and several fish designs. Ultimately, the ‘happy fish’ design proved to be the most readily accepted by rural Cambodians and this was important for the uptake of the iron ingot at the
household-level. The biggest challenge of any intervention, and nutritional interventions in particular, is to create lasting behaviour change. Although a number of different ways to introduce iron to the diet have been tried, the vast majority of the techniques failed because people were not willing to change their behaviour needed for success. In contrast to other interventions, users report that the iron fish is simple in concept, easy-to-use, and does not require significant behaviour change in order for it to be effective, and this is the principal reason why it was so readily accepted. The concept of a lucky iron fish design does not pander to superstition, but instead provides a culturally relevant solution to a common public health problem.

The findings of the current thesis show that the iron fish is an innovate form of homestead food fortification in a country that is otherwise lacking affordable and accessible means of improving iron intake. Unlike traditional oral iron supplements, the iron fish can be used in Khmer homes where families eat and drink from common pots, and would therefore benefit not just women, but all members of the family. Scaling-up of the iron fish project, including widespread distribution of the ingot would likely have significant, beneficial impacts on the lives of women and their families. Moreover, there is no reason to believe that the iron fish would not be acceptable to communities in neighbouring countries throughout the region.
9.2 Recommendations

Based on the work of this research, the following recommendations are made:

- Research must be conducted to collect information on the prevalence and etiology of iron deficiency and anemia in various age groups and by gender in different socioeconomic groups in order to better inform nutritional intervention programs;

- Cultural and behavioural practices that may predispose individuals to iron loss and/or iron deficiency should be investigated and this information should be used for the development of public health policy;

- The possibility of environmental conditions, specifically heavy metal contamination of drinking water, should be investigated as a possible reason for the high prevalence of anemia in the Kingdom of Cambodia;

- The government and civil society organizations should explore ways in which to educate families, particularly in rural areas, about the importance of good nutrition – including nutritional advice on food selection, food combinations, and food preparations to enhance nutrient absorption – and the ways in which iron can be easily and effectively incorporated into the diet;

- Given that logistical challenges with blood sampling were the most problematic experienced by the research team, the scientific community should develop methods of assessing iron status that are both inexpensive and easy to use in field conditions, so that better estimates of the prevalence of iron deficiency and the effectiveness of interventions can be widely assessed;
The effectiveness of the iron fish for the amelioration of this widely prevalent nutritional condition should be shared with the United Nations Standing Committee on Nutrition and other civil society organizations as the supplement should prove to be appropriate worldwide, though perhaps with an alternate design and social marketing strategy;

Finally, local, provincial and federal public health decision-makers should use the results published from the present thesis to consider use of adventitious iron as an effective preventive for iron deficiency and anemia.

9.3 Suggestions for further research

Targeted interventions to prevent and control iron deficiency and anemia have been well researched in the past, but often research findings do not translate well in practice. Thus, it is important to have a comprehensive understanding of the problem in the particular context, and how an intervention can be tailored to meet the needs of the particular community.

Aspects related to sustainability, continued use of, and successful delivery of the iron fish need to be explored. As a result, the following is a list of recommendations for future areas of research related to the iron fish.

- Long-term (5+ years) studies evaluating long term use, sustainability and effectiveness of the iron fish at ameliorating iron deficiency and anemia;
- Conduct a cost:benefit analysis to quantify the value of use of the iron fish when compared to alternative prevention and control measures;
- The effect of consumption of ingot-fortified food on the health and nutrition status of children, including anthropometric effects, should be explored;
- Examine methods to enhance the social marketability of the iron fish at community-, household-, and individual-levels;
- Investigate quality assurance methodology which can be used to ensure safe production of the iron fish using readily available scrap metals;
- Explore the potential for the iron fish as a means of providing multiple micronutrients, including vitamin A, zinc and/or copper;
- Investigate the potential use of the iron fish outside of the Cambodian context, including need, acceptability, and alternative design and social marketing considerations.

### 9.4 Final remarks

The final conclusions of this can research can best be summarized in the words of Maggie Black:

“There can be no recipe for development, only many potential recipes for different contexts... But true development is about people, and social beings do not function mechanistically. There is no common prescription. To be of genuine use to people, development has to grow organically, building on existing knowledge and systems, and engaging emphatically with modern ideas. Is this really so impossible?”

---

5
9.5 References


APPENDICES

Appendix I. Permission to include published journal articles in a thesis

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<td>TITLE OF PROJECT:</td>
<td>Investigation of a Novel Supplementation Technique for Iron-Deficiency Anemia in Rural Cambodia</td>
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### CHANGES:

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SPONSOR: CIHR
IDRC
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CHANGES:
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SPONSOR: INTERNAL DEPARTMENTAL FUNDS
TITLE OF PROJECT: Investigation of Nutritional Status and Food-Related Knowledge, Beliefs and Practices in Rural Cambodia

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Appendix III. Household survey tools

Figure 10.3.1a: Baseline survey used in a community trial for a novel iron supplementation technique conducted in June 2010.

<table>
<thead>
<tr>
<th>Household Characteristics:</th>
<th>Total # Within Household</th>
</tr>
</thead>
<tbody>
<tr>
<td># of people in household</td>
<td></td>
</tr>
<tr>
<td>Total # of males</td>
<td></td>
</tr>
<tr>
<td>Total # of females</td>
<td></td>
</tr>
<tr>
<td>Total # in school</td>
<td></td>
</tr>
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</table>

<table>
<thead>
<tr>
<th>Age</th>
<th>Total # Within Household</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;15</td>
<td></td>
</tr>
<tr>
<td>15-49</td>
<td></td>
</tr>
<tr>
<td>50-64</td>
<td></td>
</tr>
<tr>
<td>65+</td>
<td></td>
</tr>
</tbody>
</table>

Health:

1. Are you currently pregnant? Y N
   □ If yes, how many months?
2. Total number of pregnancies in lifetime:________
3. Are you currently menstruating (pre-menopausal)? Y N
   □ If no, at what age did you stop?
4. Have you ever been diagnosed with anemia? Y N
   □ If yes, when?
5. Have you ever taken iron pills? Y N
   □ If so, when?________ for how long?
6. Have you ever had a blood transfusion? Y N
7. Have you ever suffered from hookworm? Y N
8. Have you ever suffered from malaria? Y N
9. Do you suffer from any of the following?
   a) Headache
   b) Dizziness
   c) Nausea
   d) Constipation
   e) Black stool
   f) Stomach pain
   g) Insomnia
   h) Heavy bleeding
   i) Fatigue

<table>
<thead>
<tr>
<th>Never</th>
<th>Sometimes 1-3 times per month</th>
<th>Often 4-6 times per month</th>
<th>All of the time</th>
</tr>
</thead>
<tbody>
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</tbody>
</table>

Education:

10. What is your highest level of education?
   □ a) No education
   □ b) Grade 1-5
   □ c) Grade 6-9
   □ d) >9th Grade
Figure 10.3.1b: Baseline survey continued.

11. Are you able to...?
   | Yes | Just a bit | No |
--- | --- | --- | ---
...read Khmer  |     |     |     |
...write Khmer |     |     |     |

Socioeconomic Status:

12. Do any of the males work outside the household? Y N Jobs: ______________________
13. Do any of the females work outside the household? Y N Jobs: ______________________

14. Possessions:

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<thead>
<tr>
<th>Item</th>
<th>How many</th>
<th>Item</th>
<th>How many</th>
</tr>
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<tbody>
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<td>Working clock</td>
<td>Electricity</td>
<td></td>
</tr>
<tr>
<td>Motorcycle</td>
<td>Working radio</td>
<td>Piped water</td>
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</tr>
<tr>
<td>Bicycle</td>
<td>Working TV</td>
<td>Watertanks</td>
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</tr>
<tr>
<td>Tractor</td>
<td>Mobile phone</td>
<td>Working latrine</td>
<td></td>
</tr>
<tr>
<td>Boat</td>
<td>Karaoke machine</td>
<td>Glass windows</td>
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<tr>
<td>Motor boat</td>
<td>Sewing machine</td>
<td>Generator</td>
<td></td>
</tr>
<tr>
<td>Animal cart</td>
<td>Hand cart</td>
<td>Sleeping rooms</td>
<td></td>
</tr>
</tbody>
</table>

15. Housing material (check all that apply):
- ☐ Wood
- ☐ Ceramic tile
- ☐ Mud
- ☐ Bamboo
- ☐ Iron sheets
- ☐ Grass
- ☐ Concrete
- ☐ Bricks

16. Livestock:

<table>
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<th>Animal</th>
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<th>How many</th>
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<td>Cows</td>
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<td>Growing pigs (&gt;8 weeks)</td>
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<tr>
<td>Chickens</td>
<td></td>
<td>Goats</td>
<td>Other (specify)</td>
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Food and Water:

17. Where do you usually get your drinking/cooking water in the WET season?
- ☐ River
- ☐ Stored rain water
- ☐ Tube well
- ☐ Other: ______________________

18. Where do you usually get your drinking/cooking water in the DRY season?
- ☐ River
- ☐ Stored rain water
- ☐ Tube well
- ☐ Other: ______________________

19. How do you typically treat your water before using it?
- ☐ No treatment
- ☐ Strained through cloth
- ☐ Boil
- ☐ Ceramic or sand filter
- ☐ Bleach/chlorine/alum
- ☐ Other: ______________________

20. How many times per week do you usually eat soup?
- ☐ Every day
- ☐ 2-3 times per week
- ☐ 4-5 times per week
- ☐ 1 or fewer times per week

21. How often do you eat meat (beef, pork, chicken)?
- ☐ Daily
- ☐ Few times per week
- ☐ Rarely
- ☐ Few times per month
- ☐ Never

22. Fuel source:

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<td>Gas</td>
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Figure 10.3.2: Follow-up survey used in a community trial for a novel iron supplementation technique used to assess compliance to the treatment regime. The survey was used at 3, 6, and 9 months of the study at the relevant sampling round.

**Question for Iron Fish Follow-up:**

1. When was the last time that you used the iron fish?
   - A. Today
   - B. This week
   - C. 2-3 weeks ago
   - D. 1-2 months ago
   - E. >3 months ago
2. Typically, how often do you use the iron fish?
   - A. Daily
   - B. >1 time per day
   - C. Every 2-3 days
   - D. Every 4-5 days
   - E. Every week
3. How do you use the iron fish?
   - A. Boil water
   - B. Prepare soup
   - C. Both
   - D. Other: ________
Figure 10.3.3a: Endline survey used in a community trial for a novel iron supplementation technique, used in June 2011 to assess effectiveness and compliance to the treatment regime.

<table>
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<th>Sample #</th>
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<th>Survey #</th>
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1. When was the last time that you used the iron fish?
   - (1) Today
   - (2) This week
   - (3) 2-3 weeks ago
   - (4) 1-2 months ago → go to question 5
   - (5) > 3 months ago → go to question 5
   - (6) Never used → go to question 5

2. Typically, how often do you use the iron fish?
   - (1) Daily
   - (2) > 1 time per day
   - (3) Every 2-3 days
   - (4) Every 4-5 days
   - (5) Every week

3. Do you feel better when you use the iron fish regularly?
   - (1) Yes
   - (2) Sometimes
   - (3) No
   - (4) Don't know

4. How do you typically use the iron fish?

<table>
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<td></td>
</tr>
<tr>
<td>Boiling water</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Other (specify)</td>
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</tr>
</tbody>
</table>

5. What is the primary reason that you didn’t use the iron fish?
   - (1) Forgot to use
   - (2) Don’t know how to use
   - (3) Don’t know how often to use
   - (4) Health concern with iron fish
   - (5) Iron fish is too rusty to use
   - (6) Lost the iron fish
   - (7) Iron fish is broken
   - (8) Did not find the iron fish helpful
   - (9) Too busy to use, explain: ______________
   - (10) Other: ______________

6. Are there additional reasons that you didn’t use the iron fish?
   - (1) Forgot to use
   - (2) Don’t know how to use
   - (3) Don’t know how often to use
   - (4) Health concern with iron fish
   - (5) Iron fish is too rusty to use
   - (6) Lost the iron fish
   - (7) Iron fish is broken
   - (8) Did not find the iron fish helpful
   - (9) Too busy to use, explain: ______________
   - (10) Other: ______________

7. Are you currently pregnant?
   - (0) No
   - (1) Yes
   - (2) Don’t know

8. Have you been pregnant in the last year (June 2010)?
   - (0) No
   - (1) Yes, when did you give birth? ______________
   - (2) Yes, when did you abort? ______________

9. Have you taken iron pills in the last year (June 2010)?
   - (0) No
   - (1) Yes, when did you start? ______________
   - (2) When did you stop? ______________
Figure 10.3.3b: Endline survey continued.

<table>
<thead>
<tr>
<th>Question</th>
<th>Options</th>
</tr>
</thead>
<tbody>
<tr>
<td>10. In the last month, did you suffer from the following?</td>
<td>e) Headache</td>
</tr>
<tr>
<td></td>
<td>b) Dizziness</td>
</tr>
<tr>
<td></td>
<td>c) Nausea</td>
</tr>
<tr>
<td></td>
<td>d) Constipation</td>
</tr>
<tr>
<td></td>
<td>e) Black stool</td>
</tr>
<tr>
<td></td>
<td>f) Stomach pain</td>
</tr>
<tr>
<td></td>
<td>g) Insomnia</td>
</tr>
<tr>
<td></td>
<td>h) Heavy bleeding</td>
</tr>
<tr>
<td></td>
<td>i) Fatigue</td>
</tr>
<tr>
<td>11. Since the first time that we began working with you (June 2010 survey), are you doing anything differently?</td>
<td>Are you eating differently?</td>
</tr>
<tr>
<td></td>
<td>Do you prepare food differently?</td>
</tr>
<tr>
<td></td>
<td>Did you learn anything?</td>
</tr>
<tr>
<td>12. Do you have any concerns with the iron fish?</td>
<td>0) No</td>
</tr>
<tr>
<td></td>
<td>1) Rusting</td>
</tr>
<tr>
<td></td>
<td>2) Too heavy</td>
</tr>
<tr>
<td></td>
<td>3) Changes the taste of food</td>
</tr>
<tr>
<td></td>
<td>4) Changes the colour of food</td>
</tr>
<tr>
<td></td>
<td>5) Other: __________________________________________________________________</td>
</tr>
<tr>
<td>13. How could we make the fish better?</td>
<td></td>
</tr>
<tr>
<td>14. Height</td>
<td></td>
</tr>
<tr>
<td>15. Weight</td>
<td></td>
</tr>
</tbody>
</table>
Figure 10.3.4: Focus group discussion guide used in a study to determine food consumption practices and beliefs in rural Cambodia.

Focus Group Guide

A. Scenarios

Scenario 1: Nary lives in a village like this one. She is 41 and is married with 4 children. For the past year, Nary has not been feeling well. She cannot work for a long time because she gets tired very easily. She is often dizzy and has a headache. People say she looks very pale. When she had her last child three months ago, it was a difficult pregnancy. The baby was smaller than normal. She knows that she is not well but she does not know what to do. What is wrong with Nary? What should she do? What are the causes of this problem?

Scenario 2: Sopheap is 31 years old. She does not eat a lot of green or orange vegetables. Recently she has experienced a number of health problems. She has had constant diarrhea and a bad respiratory infection. In addition, she noticed that she cannot see at night. As the sun sets she was unable to do anything. Last week she also noticed a white spot on her eye. The spot has become bigger and very dry. What is wrong with her? What is the cause of her health problems? What should she do?

B. Relationship between health and nutrition

1. Is what a person eats related to their health?
   a. If so how? What should women eat to be healthy?

2. What causes a person to have poor nutrition?

3. What are the consequences of poor nutrition for women?
   a. For children?

4. Is it important to consume iodized salt? Why?

C. Patterns of food use – how do diets change according to important life stages?

Pregnant women

1. What should adolescent girls eat?
2. Should a woman eat differently when she is pregnant?

3. Can what a woman eat while she is pregnant affect her pregnancy?
   a. Why or why not?

4. Is there anything she should not eat while pregnant?

**Lactating women/infants**

1. When should a woman start breastfeeding?
   a. For how long should she breastfeed?

2. Should a woman who is breastfeeding eat differently?
   a. What should a woman who is breastfeeding eat?
   b. Is there anything she should not eat?

3. When should the child be given other foods besides breastmilk?

4. What kind of food should this be in the first year?

**Children (6 mos-6 yrs)**

1. What types of foods are good for children to grow well? Why?

2. What snacks do children eat between meals?

3. How often do children eat meat?

4. How often do children eat fish?

**D. Food Security**

1. What are the things that worry you in everyday life?
   a. Are there times when you worry about not having enough food?

2. What are common causes of a lack of food?

3. What are your main income sources?
   a. Which ones are most important?

4. Who in the household decides how money for food is spent?
Figure 10.3.5: Recording sheet for collection of pile sort activity data used to assess the food consumption practices and beliefs of Khmer women.

<table>
<thead>
<tr>
<th>Card Number</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1</td>
<td></td>
</tr>
<tr>
<td>Group 2</td>
<td></td>
</tr>
<tr>
<td>Group 3</td>
<td></td>
</tr>
<tr>
<td>Group 4</td>
<td></td>
</tr>
<tr>
<td>Group 5</td>
<td></td>
</tr>
<tr>
<td>Group 6</td>
<td></td>
</tr>
<tr>
<td>Group 7</td>
<td></td>
</tr>
<tr>
<td>Group 8</td>
<td></td>
</tr>
</tbody>
</table>

Date __________  Name ______________________

ID#___________  Location___________________
Appendix IV. Supplemental statistical analysis and study findings

Table 10.4.1: Demographic characteristics of households in Lvea Aem District, Kandal Province, Cambodia

<table>
<thead>
<tr>
<th>Variable</th>
<th>PK1</th>
<th>PK2</th>
<th>Tuol Tre</th>
<th>Combined</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total household size*</td>
<td>6.04 (1-12)</td>
<td>6.23 (1-12)</td>
<td>5.09 (1-11)</td>
<td>5.81 (1-12)</td>
</tr>
<tr>
<td>Males*</td>
<td>2.96 (0-8)</td>
<td>2.91 (0-7)</td>
<td>2.44 (0-8)</td>
<td>2.77 (0-8)</td>
</tr>
<tr>
<td>Females*</td>
<td>3.09 (1-7)</td>
<td>3.32 (0-8)</td>
<td>2.65 (1-6)</td>
<td>3.04 (0-8)</td>
</tr>
<tr>
<td>Mean female : male ratio</td>
<td>1.04</td>
<td>1.14</td>
<td>1.09</td>
<td>1.10</td>
</tr>
<tr>
<td>Total in school*</td>
<td>1.3 (0-5)</td>
<td>1.2 (0-4)</td>
<td>1.1 (0-5)</td>
<td>1.2 (0-5)</td>
</tr>
<tr>
<td>Age &lt; 15*</td>
<td>2.3 (0-6)</td>
<td>2.2 (0-6)</td>
<td>1.6 (0-5)</td>
<td>2.0 (0-6)</td>
</tr>
<tr>
<td>Age 15 – 49*</td>
<td>3.1 (0-8)</td>
<td>3.4 (0-8)</td>
<td>2.6 (0-7)</td>
<td>3.0 (0-8)</td>
</tr>
<tr>
<td>Age 50 – 64*</td>
<td>0.4 (0-2)</td>
<td>0.5 (0-3)</td>
<td>0.6 (0-3)</td>
<td>0.51 (0-3)</td>
</tr>
<tr>
<td>Age 65 +*</td>
<td>0.2 (0-2)</td>
<td>0.2 (0-2)</td>
<td>0.3 (0-2)</td>
<td>0.3 (0-2)</td>
</tr>
<tr>
<td>Households with formal employment</td>
<td>9 (9.9%)</td>
<td>12 (10.1%)</td>
<td>6 (6%)</td>
<td>27 (8.7%)</td>
</tr>
<tr>
<td>Possession of livestock</td>
<td>8 (8.8%)</td>
<td>9 (7.6%)</td>
<td>21 (21%)</td>
<td>38 (12.3%)</td>
</tr>
</tbody>
</table>

*Mean (Range)
Table 10.4.2: Food and water practices in Preak Khmeng Commune, Kandal Province, Cambodia.

<table>
<thead>
<tr>
<th></th>
<th>PK1</th>
<th>PK2</th>
<th>Tuol Tre</th>
<th>Combined</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>N</strong></td>
<td>91</td>
<td>119</td>
<td>100</td>
<td>310</td>
</tr>
<tr>
<td>Proportion of women who consume meat</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Weekly</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PK1</td>
<td>28 (30.8%)</td>
<td>40 (33.6%)</td>
<td>26 (26%)</td>
<td>94 (30.3%)</td>
</tr>
<tr>
<td>Monthly</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PK1</td>
<td>16 (17.6%)</td>
<td>16 (13.4%)</td>
<td>14 (14%)</td>
<td>46 (14.8%)</td>
</tr>
<tr>
<td>Special occasions only</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PK1</td>
<td>41 (45.1%)</td>
<td>49 (41.2%)</td>
<td>52 (52%)</td>
<td>142 (45.8%)</td>
</tr>
<tr>
<td>Never</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PK1</td>
<td>6 (6.6%)</td>
<td>14 (11.8%)</td>
<td>8 (8%)</td>
<td>28 (9.0%)</td>
</tr>
<tr>
<td>Soup consumption</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number of serves per week</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PK1</td>
<td>5.3 (4.89-5.64)</td>
<td>4.8 (4.45-5.08)</td>
<td>4.7 (4.35-5.09)</td>
<td>4.9 (4.69-5.10)</td>
</tr>
</tbody>
</table>
Table 10.4.3: Frequency of common health ailments in women participating in a nutritional intervention reported at baseline and endline in three villages in rural Cambodia.

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Iron fish</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Never</td>
<td>1-3 times / month</td>
</tr>
<tr>
<td><strong>Baseline</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Headache</td>
<td>1 (1.2%)</td>
<td>24 (28.2%)</td>
</tr>
<tr>
<td>Dizziness</td>
<td>6 (7.1%)</td>
<td>20 (23.5%)</td>
</tr>
<tr>
<td>Nausea</td>
<td>34 (40.0%)</td>
<td>30 (35.3%)</td>
</tr>
<tr>
<td>Constipation</td>
<td>56 (65.9%)</td>
<td>18 (21.2%)</td>
</tr>
<tr>
<td>Black stool</td>
<td>55 (64.7%)</td>
<td>20 (23.5%)</td>
</tr>
<tr>
<td>Stomach pain</td>
<td>23 (27.1%)</td>
<td>32 (37.6%)</td>
</tr>
<tr>
<td>Insomnia</td>
<td>22 (25.9%)</td>
<td>22 (25.9%)</td>
</tr>
<tr>
<td>Heavy bleeding</td>
<td>72 (84.7%)</td>
<td>11 (12.9%)</td>
</tr>
<tr>
<td>Fatigue</td>
<td>5 (5.9%)</td>
<td>19 (22.4%)</td>
</tr>
</tbody>
</table>

| **Endline**    |           |             |                 |             |       |             |                 |             |
| Headache       | 17 (20.0%)| 24 (28.2%)  | 39 (45.9%)      | 5 (5.9%)    | 35    | 65 (26.6%)  | 66 (39.1%)      | 2 (1.2%)    |
| Dizziness      | 24 (28.2%)| 20 (23.5%)  | 37 (43.5%)      | 4 (4.7%)    | 58    | 63 (37.3%)  | 43 (25.4%)      | 4 (2.4%)    |
| Nausea         | 55 (64.7%)| 25 (29.4%)  | 5 (5.9%)        | 0 (0%)      | 104   | 52 (30.8%)  | 12 (7.1%)       | 0 (0%)      |
| Constipation   | 66 (77.6%)| 12 (14.1%)  | 6 (7.1%)        | 1 (1.2%)    | 137   | 28 (16.6%)  | 4 (2.4%)        | 0 (0%)      |
| Black stool    | 69 (81.2%)| 14 (16.5%)  | 1 (1.2%)        | 0 (0%)      | 126   | 41 (24.3%)  | 1 (0.6%)        | 1 (0.6%)    |
| Stomach pain   | 24 (28.2%)| 44 (51.8%)  | 15 (17.6%)      | 2 (2.4%)    | 57    | 82 (48.5%)  | 26 (15.4%)      | 4 (2.4%)    |
| Insomnia       | 44 (51.8%)| 23 (27.1%)  | 9 (10.6%)       | 9 (10.6%)   | 64    | 53 (31.4%)  | 49 (29.0%)      | 3 (1.8%)    |
| Heavy bleeding | 75 (88.2%)| 9 (10.6%)   | 1 (1.2%)        | 0 (0%)      | 156   | 11 (6.5%)   | 2 (1.2%)        | 0 (0%)      |
| Fatigue        | 21 (24.7%)| 26 (30.6%)  | 35 (41.2%)      | 3 (3.5%)    | 32    | 76 (50.0%)  | 55 (32.5%)      | 6 (1.6%)    |
Appendix V. Nutrition education materials

The following resources were developed in order to increase nutritional knowledge throughout rural Cambodia. The resources were constructed first in English and then translated into the Khmer language. By maximizing the use of photos and pictures, villagers with limited literacy were better able to understand the message. The resources were not used independently, but were paired with 5 to 10 minute long consultations in order to enhance comprehension. The focus of these lessons was to describe the importance of overall meal composition and dietary diversity, in addition to consumption of nutrient-rich foods.
Figure 10.5.1: Anemia and iron fish informational handout that was distributed to women participating in the pilot study (2008-2009). The handout was translated into Khmer and copies were left with participants.

Iron Fish

Energy like you have never known!

What is anemia?
- Anemia is an illness from low stores of iron in the body
- Anemia is usually caused by a diet that is low in iron

Why is anemia bad?
- Anemia can make us dizzy and tired, and can give us headaches and insomnia
- If children are anemic, they will be small and weak
- Children with anemia have weak immune systems and are not as smart as other children

What is the iron fish?
- The iron fish is a supplement that can potentially treat anemia
- The iron fish can be used for many years by the whole family
- The iron fish does not dissolve and should not rust if you clean it properly

How do I use the iron fish?
- Use the iron fish every day
- Put the iron fish in the pot when making soup and cook for at least 10 minutes
- Put the iron fish in the pot when boiling drinking water and cook for at least 10 minutes

Is it bad to have blood taken by a doctor?
- It is not bad to have blood taken by a doctor
- Taking small amounts of blood cannot make you feel sick
- In Europe and America, it is common to have blood taken by the doctor for testing
Figure 10.5.2a: Informational handout describing anemia and the correct usage of the iron fish. The handout was translated into Khmer for distribution to study participants in June 2010.

Lucky Iron Fish
Make your family HEALTHY!

**WHY USE?:**
- Treat anemia (low iron in your body)
- Make your family smart, strong, energetic and healthy
- Is good for a long time and will not disappear when you use it

**HOW TO USE:**
- Use every day
- Put in pot for 10 minutes when cooking
- Use when you make soup and when you boil water (add citrus juice)

**RUST:**
- Will not hurt you
- Clean rust off and keep iron fish dry

**KEEP CLEAN:**
- Rinse iron fish with water
- Dry the fish with a cloth

---

**What is anemia?**
- Low iron in your body
- This makes you sick:
  - Small, weak muscles
  - Tired, headache, dizzy, insomnia
  - Make your children’s brain small

**Why need iron?**
- Help you breathe
- Make you strong, smart
- Give you energy
- Reduce sickness
- Iron comes from meat, green vegetables and the Lucky Fish
Figure 10.5.2b: Khmer version of informational handout.

មន្ត្រីសម្រួសសារ

ឈូរចំណាត់ថ្នាក់សម្រែលសម្រែល

ដំណើរការសម្រែលសម្រែល

- ស្វែងរកមេដឹកនិច ប្រការចិនតាមក្រុមអ្នកប្រការចិនតាមក្រុម
- ស្វែងរកប្រេតុងសម្រែលដែលមានយុត្តិប្រយោជន៍ និងសម្រែលដែលមានយុត្តិប្រយោជន៍
- ស្វែងរកប្រេតុងសម្រែលដែលមានយុត្តិប្រយោជន៍នៅពេលត្រឹមត្រូវ

រូប៖
- រូប៖ រឿងទូរស័ព្ទប្រការចិនតាមក្រុម
- ផលិតផលសម្រែលដែលមានយុត្តិប្រយោជន៍

សម្រែលសម្រែល
- អ្នកដ្ឋាននៅក្នុងរឿងទូរស័ព្ទប្រការចិនតាមក្រុម
- អ្នកដ្ឋាននៅក្នុងរឿងទូរស័ព្ទប្រការចិនតាមក្រុម

មន្ត្រីសម្រួសសារ

ការរស់នៅក្នុងមន្ត្រីសម្រួសសារ

- ការរស់នៅក្នុងមន្ត្រីសម្រួសសារ
- ការរស់នៅក្នុងមន្ត្រីសម្រួសសារ
- ការរស់នៅក្នុងមន្ត្រីសម្រួសសារ
- ការរស់នៅក្នុងមន្ត្រីសម្រួសសារ

ហើយមានការរស់នៅក្នុងរឿងទូរស័ព្ទប្រការចិនតាមក្រុម
- រឿងទូរស័ព្ទប្រការចិនតាមក្រុម
- ផលិតផលសម្រែលដែលមានយុត្តិប្រយោជន៍
- សម្រែលសម្រែលដែលមានយុត្តិប្រយោជន៍

អាហារនៅក្នុងមន្ត្រីសម្រួសសារ
- អាហារនៅក្នុងមន្ត្រីសម្រួសសារ
- អាហារនៅក្នុងមន្ត្រីសម្រួសសារ
- អាហារនៅក្នុងមន្ត្រីសម្រួសសារ
- អាហារនៅក្នុងមន្ត្រីសម្រួសសារ

水资源管理

维护和使用水资源

- 维护和使用水资源
- 维护和使用水资源
- 维护和使用水资源
- 维护和使用水资源
Figure 10.5.3: Nutritional education material used in June 2010 for the iron fish plus follow-up group to inform participants about foods which should be consumed in order to prevent anemia, and on the reverse, those foods which should be avoided. The handout was also translated into Khmer for distribution.
Figure 10.5.4: Nutritional education material used in September 2010 for the iron fish plus follow-up group to inform participants about the signs and symptoms of iron deficiency and anemia. The Khmer version is also displayed.

Iron Fish Keeps You Healthy!

Eat food with high iron and use iron fish everyday! Without anemia, your family will be smart, strong, athletic, tall, energetic and healthy!

Healthy

Not Healthy

Khmer:

铷ེ་ཝན་བཟོ་ེ་བཤོས་པའི་ི་ང་གོང་།

འོ་དོན་ཐལ་བཤོད་ེ་ཝན་འི་ཤོད་ང་། གནས་ཐལ་དོན་་ཤོད་མ་ རིན་ཤོད་མ་ ཅེས་ཐལ་ཅིང་།

Khmer Healthy

Khmer Not Healthy
Figure 10.5.5: Nutritional education material used in December 2010 for the iron fish plus follow-up group to inform participants about i) iron-rich foods and ii) the importance of eating a well-balanced diet including meat, starch and vegetables. The handout was also translated into Khmer for distribution.
Figure 10.5.6: Nutritional education material used in March 2011 for the iron fish plus follow-up group to inform participants about the importance of eating a well-balanced meal including food from the various food groups.