

A Randomized Control Trial Using a Fish-Shaped Iron Ingot for the Amelioration of Iron Deficiency Anemia in Rural Cambodian Women

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Received date: August 1, 2015, Accepted date: August 22, 2015, Published date: August 25, 2015

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Abstract

Objective: The objectives were to determine whether cooking food with an iron ingot increases the hemoglobin and serum ferritin of women and whether women would use an iron ingot shaped like a fish considered lucky in Cambodian culture.

Methods: A randomized controlled trial was conducted in three villages in rural Kandal Province, Cambodia. Participants were randomly assigned to the iron ingot, the iron ingot plus nutrition education, or untreated control group. Participants were instructed to use the iron ingot daily by adding it to the cooking pot when preparing soup or boiling drinking water and boiling for at least 10 minutes. Blood samples were taken at baseline and every three months thereafter, over a 12-month trial period.

Results: Overall, a 46% reduction in the prevalence of anemia within the intervention group was noted at the end of the study. Hemoglobin concentrations were greater by 11.8 g/L (95% CI: 9.1, 14.6; P<0.0001) in women in the iron ingot group when compared to controls. Serum ferritin concentrations were 31.0 ng/mL (95% CI: 17.1, 45.0; P<0.0001) greater after 12 months of using the iron ingot when compared to controls. Over 94% of women used the iron ingot at least 3 times per week.

Conclusion: The iron ingot shaped as a lucky fish is a very effective, innovative form of homestead food fortification in a country lacking affordable and accessible means of improving iron intake.

Keywords: Iron deficiency anemia; Cambodia; Iron; Fish; Ingot; Anaemia

Abbreviations:

ANOVA: Analysis of Variance; CRP: C-reactive protein; Fe: Iron; g/L: Grams per liter; Hb: Hemoglobin; LFU: lost to follow up; mg/L: Milligrams per liter; ng/mL: Nanograms per milliliter; Preak Khmeng Village 1 (PK1); Preak Khmeng Village 2 (PK2); SF: Serum ferritin; TT: Tuol Trea Village; µg/g: Micrograms per gram; WHO: World Health Organization

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 public health problem with serious consequences for human health and socio-economic development. The World Health Organization (WHO) estimates that nearly 2 billion people suffer from iron deficiency anemia, with the largest burden of disease in women, children and infants [1].

The long-term consequences of anemia are severe and often irreversible [1]. Mild to moderate anemia leads to weakened immunity, reduced work capacity, reduced cognitive ability and an overall decreased quality of life [2]. 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 [2]. In Cambodia, anemia affects 55% of children, 43% of women of reproductive age, and 50% of pregnant women [3].

Previously, we reported on a pilot study of a uniquely shaped iron ingot that can be used when cooking to provide additional iron to an otherwise deficient diet [4]. The cast iron ingot was designed to resemble a species of fish that is commonly found throughout Cambodia, and is considered lucky in traditional village folklore. The results of the pilot study were promising but not definitive, warranting further investigation [4]. Therefore, the objectives were to determine whether cooking food with an iron ingot increases the hemoglobin and serum ferritin of women. A secondary objective was to determine whether women would use the fish-shaped ingot.

The findings of a randomized controlled trial of an 'iron fish' conducted in rural Cambodia are presented here. The effect of the ingot on hemoglobin and iron status was measured, and data were collected on compliance with the supplementation regime.

Materials and Methods

Study area, population and recruitment

Between June 2010 and June 2011, the parallel longitudinal community, randomised control trial was conducted in Lvea Aem District, Kandal Province, Cambodia. 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 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), intention to migrate before the end of the trial, pregnancy, and use of iron supplements in the previous three months before the trial. Women who started to use iron supplements during the trial were removed from the analysis at the end of the trial period.

Households were selected using systematic random sampling by randomly selecting the first household, and then approaching every fourth household until the pre-determined sample size was met during a two-week period in June 2010. All women in the selected household, who met the inclusion criteria, were eligible to be included in the study. The project was explained and those women who consented to participate were then enrolled and a blood sample was collected for biomarker measurement. A baseline survey that collected data on demographic factors was completed at this time.

Randomization

Participants within each village were randomized to one of three treatment groups: (i) an intervention group receiving an iron ingot (A), (ii) a second intervention group receiving an iron ingot plus six follow-up sessions where nutritional education was provided (B) and (iii) a control group (C) which did not receive any intervention but was followed throughout the study. Random allocation to the intervention involved the use of a random digit generator (Microsoft Excel 2007, Redmond, USA) that assigned a unique ID to each participant and then assigned each participant into one of three groups, stratified by village. The randomization was done by the primary author and given the nature of the intervention; treatment allocation was not blinded.

Intervention

The iron ingots were designed and produced in Kandal Province, Cambodia. Methods and process were published previously [5]. Briefly, readily available scrap iron was sourced from a small metalworking factory, processed and subjected to quality control procedures to ensure the absence of heavy metals (lead, arsenic, cadmium, mercury, manganese, magnesium and aluminum) and other contaminants.

The ability of the ingot to leach iron into food and water samples has been reported elsewhere [5]. Briefly, under realistic cooking conditions approximately 76 µg/g more iron was released in slightly acidified water when compared to a control, correlating to nearly 75% of iron requirements for women 10-49 years of age with daily requirements of 15 mg iron and with an estimated 10% bioavailability [5-7]. When the iron ingot was used in non-acidified water, the iron content was negligible (<0.40 µg/g), highlighting the need for an

acidifier like citrus juice in order to enhance both iron leaching and absorption.

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 daily, and is also often soured with fruits containing ascorbic acid. For those women who did not consume soup on a daily basis we encouraged use of the ingot when boiling drinking water, to which the addition of a small amount (approximately 1 teaspoon) of citrus juice (high concentration of ascorbic acid) was encouraged, both prior to boiling and again just before consumption. Participants in the control group were also asked to boil their water with 1mL of citrus juice to ensure that the only difference between the groups was the presence of the fish. After use, participants were instructed to remove the ingot from the pot, rinse with clean water, towel dry and store in open air until the next use.

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 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, 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.

Following enrolment and baseline sampling, participants were visited at their homes by trained fieldworkers every three months for one year. At each visit, a blood sample was taken as above and pre-coded structured questionnaires regarding usage, compliance, and exclusion criteria were completed. Participants in the iron ingot groups were asked when they had last used the ingot for cooking and the typical frequency of use. Field enumerators were not blinded to the intervention allocation.

At each sampling round, all participants were asked to complete a short questionnaire about any changes in their diet or eating habits and, for the women in the iron ingot groups, to ask questions about use of the fish. In addition, participants in the group B met for approximately five to ten minutes with a member of the research team and received a short 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.

Data were initially recorded by hand onto coded spreadsheets and later transcribed into Microsoft Excel 2007 (Redmond, USA). Double-data entry was used to ensure accuracy.

Outcomes

The primary outcomes included iron deficiency and anemia. 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 [2]. Iron deficiency anemia was indicated when both anemia and iron deficiency were present. These outcomes were assessed at baseline, every three months thereafter, and at the end of the study (12 months post-intervention).

Serum ferritin was the biomarker chosen to assess iron stores because it is the most specific biochemical test that correlates with relative total body iron stores [8]. Serum ferritin is an acute-phase reactant, which is elevated in response to any infectious or inflammatory process. Following WHO recommendations, a

measurement of CRP concentration was included, and a CRP concentration >10 mg/L was selected to represent a state of inflammation. Participants who had both abnormal hemoglobin levels (Hb<120 g/L) and abnormal CRP concentration (CRP>10 mg/L) were excluded from analysis [8,9].

Sample size

Sample size was calculated in order 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% [10]. Thus, at least 76 participants per group were needed. Approximately 25% loss-to-follow-up was anticipated over the 12-month study and sufficient participants were included to account for this anticipated loss. For details on precise numbers in each group (Figure 1).

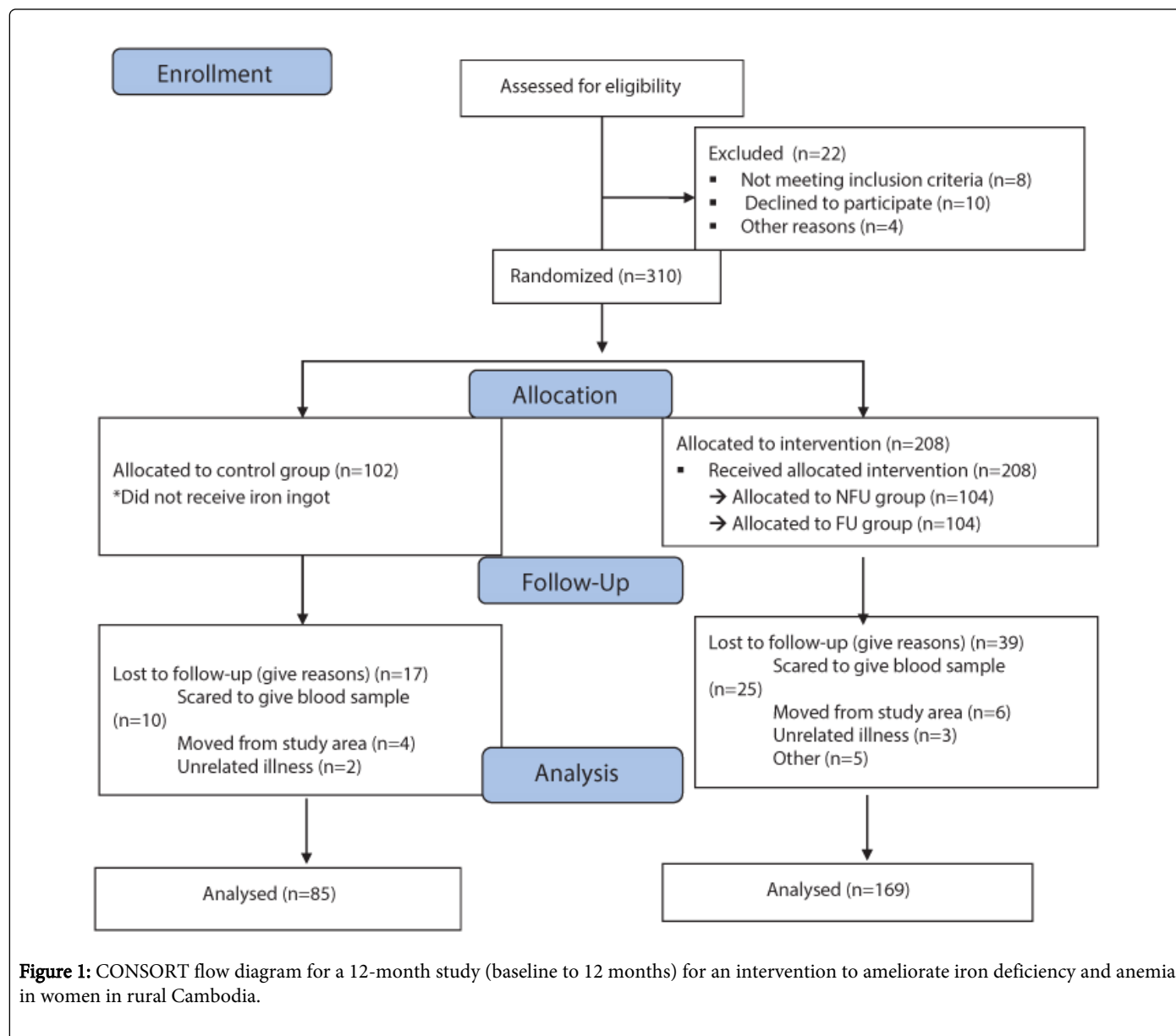


Figure 1: CONSORT flow diagram for a 12-month study (baseline to 12 months) for an intervention to ameliorate iron deficiency and anemia in women in rural Cambodia.

LFU = Lost to Follow-up

Statistical analysis

The trial used households as the unit of randomization and intervention. The individual women were the unit of data collection and analysis. Data were transcribed from the spreadsheet into IBM SPSS V19.0 (SPSS Inc., Chicago, USA) for analysis. Statistical significance was set at $P < 0.05$.

One-way analysis of variance (ANOVA) followed by a least significant difference analysis was used to compare Hb, SF, participant age 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 (LFU) occurred over the 12-month study, a Student's t-test was used to determine whether LFU was associated with baseline iron status or not; no significant difference was detected (data not shown).

Statistical analyses involved examining descriptive statistics, frequency tables and histograms. Analysis of the data was blinded and repeated by two independent assessors. To compare outcomes, chi-square tests were conducted to compare the proportion of women by anemia, iron deficiency, and iron deficiency anemia status among the treatment groups. One-way ANOVA was also used to compare changes in Hb and SF over time between the control and intervention groups.

Multivariable analyses were conducted by regressing the outcomes on the treatment and covariates using multiple linear regressions. The outcomes Hb and SF at 12 months, and the difference from baseline 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 $P < 0.20$. Confounding was determined by identifying variables whose coefficients changed by 20% and/or changed in significance as another covariate left the model.

Ethics approval

Ethics approval was obtained from the Research Ethics Board at the University of Guelph, Canada (REB #10FE022), and the Ministry of Rural Development, Kingdom of Cambodia. The International Research Development Centre, Canada also reviewed study procedures. Prior to the intervention, informed consent was obtained from all participants. Obtaining oral rather than written consent is common practice in Cambodia and was approved by the Research Ethics Board due to the limited literacy in some rural areas. Consent was obtained from each participant and recorded in the trial database.

Results

The initial analyses revealed that the levels of Hb and SF by sampling round did not differ between the two groups receiving the ingot (A and B); therefore, the data from the two groups receiving the iron ingot were combined into a single intervention group for some components of the statistical analysis.

The detailed statistical analysis provides a comparison of women in the control group with those receiving an iron ingot (including A and B). The preliminary analysis also showed that the three study sites had different baseline Hb and CRP values (data not shown); therefore 'Village' (i.e. study site) was included as a fixed effect dummy variable in the regression analysis. Serum ferritin was right-skewed and was therefore log-transformed for analysis.

Recruitment and baseline characteristics

The trial profile is shown in Figure 1. A total of 304 women from 299 households were recruited into the study at baseline. The unit of analysis was participating women despite the unit of randomization being household. Loss-to-follow-up was observed in all trial groups, however overall loss was less than the 25% accounted for in the sample size calculation.

At baseline six participants were identified as pregnant. Given their unique iron requirements these women were not included in the study and were referred to their local Health Centre for antenatal care. Thus, 104 participated in the study as the control group, and 200 participated in the two intervention groups (100 participants for each A and B). No new pregnancies were reported following commencement of the trial.

The baseline demographic characteristics of the participants are shown in Table 1. These data did not differ significantly between control and intervention groups ($P > 0.05$). The mean household size was 5.8 (range: 1-12), and did not differ by treatment group.

	Treatment Group	
	Control (n=84)	Intervention (n=164)
Age	43.5 (40.2, 46.9)	41.2 (39.0, 43.4)
Post-menopausal	31 (36.9%)	58 (35.4%)
Total number of pregnancies	5.5 (4.70, 6.38)	4.7 (4.18, 5.26)
Height, cm	153.3 (152.0, 154.5)	153.0 (152.05, 153.9)
Weight, kg	51.0 (49.0, 53.0)	52.8 (46.8, 58.7)
Baseline Hemoglobin, g/L	118.9 (116.3, 121.6)	116.5 (114.3, 118.6)
Baseline Serum Ferritin, ng/mL	53.8 (46.4, 61.2)	59.3 (51.2, 67.5)
Baseline C-Reactive Protein, mg/L	4.0 (2.82, 5.1)	3.9 (3.25, 4.65)

Table 1: Baseline demographic characteristics of women in a randomized controlled trial of a novel iron supplementation technique for one year (n=248).

Values are Mean (95% Confidence Interval) or n (% of total)

Outcomes

At the end of the trial, participants in the control group were 4.1 times more likely to be anemic than those in the intervention groups (Table 2). Similarly, participants in the control group were more than 2.8 times more likely to be iron deficient and more than 4.6 times more likely to have iron deficiency anemia, when compared to the treatment group (Table 2).

The change in Hb and SF values over time is shown in Table 3. Hemoglobin concentrations differed significantly between the intervention and control groups at 9 ($P = 0.002$) and 12 months ($P < 0.0001$), while SF concentrations differed at 12 months only ($P = 0.001$).

	Anemia		Iron Deficiency		Iron Deficiency Anemia	
	Control (n=84)	Intervention (n=164)	Control (n=84)	Intervention (n=164)	Control (n=84)	Intervention (n=164)
Baseline	38 (45.2%)	93 (56.7%)	7 (8.3%)	26 (15.9%)	6 (7.1%)	22 (13.4%)
3 months	45 (53.6%)	64 (39.0%)	6 (7.1%)	21 (12.8%)	4 (4.8%)	14 (8.5%)
6 months	41 (48.8%)	70 (42.7%)	6 (7.1%)	18 (11.0%)	5 (6.0%)	13 (7.9%)
9 months	40 (47.6%)	38 (23.2%)*	11 (13.1%)	12 (7.3%)	9 (10.7%)	7 (4.3%)*
12 months	38 (45.2%)	18 (11.0%)*	7 (8.3%)**	5 (3.0%)	7 (8.3%)	3 (1.8%)*

Table 2: Anemia, iron deficiency and iron deficiency anemia in women participating in a randomized controlled trial of an iron ingot intervention over a 12-month period.

Values are number of women (Percent)

*Different from Control, P<0.05

**Tend to differ from Control, P=0.06

	Hemoglobin, g/L		Serum Ferritin, ng/mL	
	Control (n =84)	Intervention (n = 164)	Control (n =84)	Intervention (n = 164)
Baseline	119 (116, 122)	117 (114, 119)	54 (46, 61)	59 (51, 68)
3 Months	118 (116, 121)	120 (118, 122)	59 (50, 61)	67 (58, 76)
6 Months	119 (116, 122)	121(119, 123)	62 (54, 71)	72 (60, 83)
9 Months	118 (116, 121)	123* (122, 125)	61 (51, 71)	75 (63, 87)
12 Months	120 (117, 123)	130* (128, 132)	66 (57, 76)	102* (89, 116)

Table 3: Association between iron ingot use and hemoglobin and serum ferritin values over one year for 248 women from Lvea Aem District, Kandal Province, Cambodia who participated in a community trial using an iron ingot in the cooking pot.

Values are Mean (Confidence Interval) using the control group as the referent

*Different from Control, P<0.05.

The results of the multivariable regression analysis are displayed in Table 4. After adjusting for the covariate 'village', the mean Hb concentrations differed by 11.8 g/L between control and treatment group at 12 months (P<0.0001). Differences of 31.0 ng/mL were also observed for SF values at 12 months (P <0.0001).

Variable		Coefficient (β)	Standard Error of β
12 months Hemoglobin, g/L	Intervention	9.5* (95% CI: 6.2, 12.8)	1.8
	PK1 ¹	0.5 (95% CI: -0.3, 4.3)	1.9
	PK2 ²	3.3 (95% CI: -0.6, 7.2)	2.0
12 months Hemoglobin Difference, g/L	Intervention	11.8* (95% CI: 9.1, 14.6)	1.4
	PK1 ¹	-1.4 (95% CI: -4.5, 1.8)	1.6
	PK2 ²	3.7* (95% CI: 0.5, 7.0)	1.6
12 months Serum Ferritin, ng/mL	Intervention	1.5* (95% CI: 1.2, 1.9)	0.05
	PK1 ¹	-0.03 (95% CI: -0.1, 0.07)	0.05
	PK2 ²	-0.088 (95% CI: -0.2, 0.02)	0.05
12 months Serum Ferritin Difference, ng/mL	Intervention	31.0* (95% CI: 17.1, 45.0)	7.1
	PK1 ¹	-13.0 (95% CI: -28.9, 2.9)	8.1
	PK2 ²	-22.6* (95% CI: -39.0, -6.2)	8.4

Table 4: The association between the use of an iron ingot and hemoglobin (Hb) and serum ferritin (SF) values at 12 months, after controlling for village, in a community trial of an iron ingot, where women randomized to the control group were the referent category.

Values are Coefficient (Confidence Interval) using the control group as reference and Village 3 (TT) as reference village.

1PK¹ = Preak Khmeng Village 1

2PK² = Preak Khmeng Village 2

*Different from Control, P < 0.05

Compliance to the treatment regimen

Women reported that they had not used iron supplements at any time in the trial. Further, other nutritional interventions implemented by Non-governmental Organizations, the Ministry of Health or the Ministry of Rural Development were not reported in the study area during this time. No changes in the overall diet of the women during the trial period were noted.

Compliance to the iron ingot throughout the study was high in the intervention group (Table 5). Approximately 94% of participants used the ingot daily by the end of the study. Participants who reported never using the ingot were lost-to-follow-up, so that by the end of the

trial only participants who used the ingot regularly were remaining. There was no difference in iron ingot usage between groups A and B.

	Regularity of Use			
	Daily	3-4 days	Rarely	Never
3 months (n=182)	146 (80.2%)	23 (12.6%)	6 (3.3%)	7 (3.8)
6 months (n=164)	145 (88.4%)	10 (6.1%)	4 (2.4%)	5 (3.0%)
9 months (n=164)	150 (91.5%)	10 (6.1%)	4 (2.4%)	0
12 months (n=164)	154 (93.9%)	7 (4.3%)	3 (1.8%)	0

Table 5: Reported usage of an iron ingot over the previous three months as assessed at each sampling round in a randomized controlled trial of a novel iron supplement conducted in Kandal Province, Cambodia.

Values are Number (Percent)

Discussion

Currently, we present 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 ingot to use while cooking had higher hemoglobin and serum ferritin values over a 12-month longitudinal trial than the women in the control group. The amelioration of serum ferritin concentrations would suggest that more iron was absorbed than was needed for immediate erythropoiesis. These data imply that regular use of a simple, fish-shaped ingot not only supplies iron, but also that this iron is bioavailable and effective in reducing the prevalence of iron deficiency. Given that it is very difficult to improve iron status during pregnancy, the ability to improve and maintain both circulating and stored iron within normal levels is critical for women of reproductive age.

Women using the fish showed improved iron status with or without follow-up education sessions. We had expected that education would make a difference to the impact of using the intervention however, recent evidence from a six-country study suggests that education does not necessarily provide benefits beyond providing users with an intervention that they find useful [11].

One of the limitations of the current study is that it did not exclude post-menopausal women (approximately 1/3 of all participants). In many rural areas of Cambodia, mass migration of women of reproductive age to pursue employment opportunities in peri-urban and urban centers is common, and this may have impacted our sampling frame. Approximately 2/3 of the participants in the current study were pre-menopausal and the findings would suggest that using the iron fish is advantageous for women in this group as well. In fact, no association between outcomes and menstrual status was found (data not shown).

One of the principal challenges with dietary intervention studies is compliance; therefore several design considerations were included at the outset: i) the ingot was designed to resemble a species of fish, which is both widely consumed and considered to be lucky in Khmer folklore; ii) a cartoon-like smile was displayed by the fish of the iron ingot [12], which proved to be attractive to the study participants; iii) the ingot was designed to be lightweight, with maximal surface area to

enhance iron leaching; iv) the ingot was designed to be easy-to-clean, an important consideration to avoid the build-up of rust which could discolor or alter the taste of food/water and possibly deter women from using the ingot; and finally, v) the ingot was designed to release iron at a slow but consistent rate, fortifying the diet gradually, and therefore not causing many of the side effects that commonly deter people from taking oral iron tablets. Women were provided with advice during recruitment on how to dry and clean the fish carefully after use in order to prevent rust formation.

Compliance was found to be high in both groups A and B. Over 90% of women allocated an iron ingot used the ingot at least 3 times per week throughout the study. It was used daily by 94% of the 164 women in the study at the end of the trial. In discussion with these women over the 12-month trial period, the research team observed that initially there was some reluctance to use the ingot, but that 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 medicine and valuable objects. This practice resulted in decreased 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. Taken together, these findings would suggest that after habituating to use, women were comfortable with using the ingot regularly.

The possibility of sharing of the iron fish among households, resulting in contamination of control participants, is unlikely and was investigated in discussions with intervention participants. Typically, it is not the custom of Cambodian people to share medicine, meals or other similar items with members outside of their direct family (defined as those eating from the same cooking pot). No evidence was recorded on changes in diet or eating habits among the participants throughout the study.

Initially, we expected that women in group B would have a greater improvement in iron status and compliance compared with those in the ingot without nutrition counseling group (group A), but this was not observed. Most likely, women understood the nutrition lessons, but did not have the financial means to purchase nutrient-dense foods such as animal-source foods, although this was not specifically investigated.

There is a history of using iron from extraneous sources as a dietary supplement. Beginning in the 1980s, formative research was conducted suggesting that iron cookware not only increased the iron content of food, but that this iron was bioavailable [13-15]. Since then, several randomized community trials have been conducted in Brazil and Africa, with varying results [10,16-18]. The results of a systematic review of these early studies reveal that 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 [18]. The iron ingot appears to overcome such concerns: it is inexpensive, (approximately \$1.50 USD and probably has a life-span of more than five years, compared to monthly iron supplements that cost \$2-4 USD per person); light-weight; can be used in any 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; and the appearance of the fish appeared to encourage women to use it regularly. The development of the ingot

followed careful field-testing of various prototype designs, and overwhelmingly focus groups preferred the current design (data not shown). This iron ingot therefore represents a more sustainable and feasible option for iron supplementation compared with conventional approaches.

Safety concerns related to the iron ingot are negligible when compared to oral iron supplements. If a high quality source of iron can be established and appropriate quality control procedures developed, the iron ingot provides a safe, sustainable means of supplying iron. Importantly, children are not able to ingest the ingot itself because of its size relative to the mouth of a child, therefore concerns with iron toxicity seen in children who may accidentally consume large quantities of conventional oral iron supplements are not present. While it is possible that some children may suck on the iron ingot, it is unlikely that this may result in accidental overexposure, as previous research has shown that conditions such as an acidic state, prolonged cooking time (10 minutes), high temperature and appropriate medium (water or soup) is essential for the release of meaningful quantities of iron [5]. Adverse effects of using the ingot were neither reported by the participants, nor observed by the research team. The current study highlights both the acceptability and effectiveness of iron fish for the amelioration of iron deficiency and anemia. Expanding the iron fish project, including widespread distribution of the ingot would likely have, beneficial impacts on the lives of women, and by proxy, their families, in rural Cambodia and perhaps elsewhere in South-East Asia. 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 is important for the acceptability at the household level [19].

Conclusion

Regular use of a small iron ingot shaped like a fish during cooking resulted in a 46% reduction in the prevalence of anemia in women in rural Cambodia. By the end of the 12-month trial, hemoglobin levels (a measure of circulating iron) and serum ferritin levels (a measure of stored iron) were significantly ($P < 0.0001$) elevated compared with controls. The compliance rate was high: by the end of trial 94% of the women reported using the fish at least three times a week. Taken together and contrasted with the effectiveness and compliance of taking iron supplements, these data suggest that the iron fish may provide an alternative and effective preventive for iron deficiency anemia.

Statement of author's contributions:

CVC, CED, AH, and AJS designed the research project. CVC, CH and SC provided study oversight and conducted the research. CVC and CED analyzed the data. CVC wrote the paper with input from CED and AJS. CVC had primary responsibility for the final content. All authors have read and approved the final manuscript.

Acknowledgements:

The authors wish to thank the participants of the trial.

Funding:

Provided by the University of Guelph, Canadian Institutes of Health Research and the International Development Research Centre.

Conflict of interest:

Authors declared no conflict of interest.

References

1. World Health Organization (2008). Worldwide prevalence of anemia 1993-2005. Available from: <http://www.apps.who.int/iris/handle/10665/43894> [cited June 2, 2010].
2. World Health Organization (2001) Iron deficiency anemia: assessment, prevention and control. Available from: http://www.who.int/nutrition/publications/micronutrients/anemia_iron_deficiency/WHO_NHD_01.3/en/ [cited May 1, 2011].
3. National Institute of Statistics, Directorate General for Health, and ICF Macro, 2011. Cambodian Demographic and Health Survey. Phnom Penh, Cambodia and Calverton, Maryland, USA: National Institute of Statistics Directorate General for Health and ICF Macro. Available at: <http://dhsprogram.com/pubs/pdf/FR249/FR249.pdf> [cited December 22, 2014].
4. Charles CV, Dewey CE, Daniell WE, Summerlee AJ (2011) Iron-deficiency anaemia in rural Cambodia: community trial of a novel iron supplementation technique. *Eur J Public Health* 21: 43-48.
5. Charles CV, Summerlee AJ, Dewey CE (2011) Iron content of Cambodian foods when prepared in cooking pots containing an iron ingot. *Trop Med Int Health* 16: 1518-1524.
6. Herbert V (1987) Recommended dietary intakes (RDI) of iron in humans. *Am J Clin Nutr* 45: 679-686.
7. Hurrell R, Egli I (2010) Iron bioavailability and dietary reference values. *Am J Clin Nutr* 91: 1461S-1467S.
8. Joint World Health Organization/Centers for Disease Control and Prevention Technical Consultation on the Assessment of Iron Status at the Population Level (2007). Assessing the iron status of populations: including literature reviews. Geneva, Switzerland: World Health Organization; Report No.: 9789241596107.
9. Weiss G, Goodnough LT (2005) Anemia of chronic disease. *N Engl J Med* 352: 1011-1023.
10. Sharieff W, Dofonsou J, Zlotkin S (2008) Is cooking food in iron pots an appropriate solution for the control of anaemia in developing countries? A randomised clinical trial in Benin. *Public Health Nutr* 11: 971-977.
11. Banerjee A, Duflo E, Goldberg N, Karlan D, Osei R, et al. (2015) Development economics. A multifaceted program causes lasting progress for the very poor: evidence from six countries. *Science* 348: 1260799.
12. Charles CV (2012) Happy fish: a novel supplementation technique to prevent iron deficiency anemia in women in rural Cambodia. PhD. Thesis, University of Guelph, Guelph, ON, Canada.
13. Brittin HC, Nossaman CE (1986) Iron content of food cooked in iron utensils. *J Am Diet Assoc* 86: 897-901.
14. Liu DY, Chen ZG, Lei HQ, Lu MQ, Li R, et al. (1990) Investigation of the amount of dissolved iron in food cooked in Chinese iron pots and estimation of daily iron intake. *Biomed Environ Sci* 3: 276-280.
15. Martinez FE, Vannucchi H (1986) Bioavailability of iron added to the diet by cooking food in an iron pot. *Nutr Res* 6:421-428.
16. Adish AA, Esrey SA, Gyorkos TW, Jean-Baptiste J, Rohhani A (1999) Effect of consumption of food cooked in iron pots on iron status and growth of young children: a randomised trial. *Lancet* 353: 712-716.
17. Borigato EV, Martinez FE (1998) Iron nutritional status is improved in Brazilian preterm infants fed food cooked in iron pots. *J Nutr* 128: 855-859.
18. Geerligs PD, Brabin BJ, Omari AA (2003) Food prepared in iron cooking pots as an intervention for reducing iron deficiency anaemia in developing countries: a systematic review. *J Hum Nutr Diet* 16: 275-281.
19. Geerligs PP, Brabin B, Mkumbwa A, Broadhead R, Cuevas LE (2003) The effect on haemoglobin of the use of iron cooking pots in rural Malawian households in an area with high malaria prevalence: a randomized trial. *Trop Med Int Health* 8: 310-315.