

# Iron-deficiency anaemia in rural Cambodia: community trial of a novel iron supplementation technique

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**Background:** More than 3.5 billion people are affected by iron-deficiency anaemia (IDA). Previous studies have shown that the use of iron pots in daily cooking ameliorates IDA. We report a study on the use of a novel treatment to address IDA in rural women in Cambodia, where the use of iron pots is not common. **Methods:** A community-wide randomized controlled trial was conducted in the village of Preak Ruessei, Kandal Province, Cambodia. Rural women ( $n = 189$ ) were enrolled and randomly assigned by household to one of three groups: (i) control, (ii) iron treatment with no follow-up and (iii) iron treatment with follow-up visits to provide IDA education. Haemoglobin, serum iron and C-reactive protein concentrations were measured at baseline, 3 and 6 months. A reusable fish-shaped iron ingot was distributed to the two treatment groups and participants were directed to use them daily for cooking. We hypothesized that iron from the ingot would leach iron into food providing an effective iron source. **Results:** Blood iron levels were higher in women in the iron fish plus follow-up at 3 months compared with controls, but this was not maintained. At 6 months, haemoglobin and serum iron had fallen in all groups and the proportion of anaemic women had increased. **Conclusions:** This study shows that the iron ingot was effective in the short but not longer-term against IDA. Though a novel treatment option, further research is warranted to determine bioavailability of leached iron and whether or not the surface area is large enough for sufficient iron leaching.

**Keywords:** developing country, iron cooking pots, iron-deficiency anaemia, iron ingot, randomized clinical trial

## Introduction

Iron-deficiency anaemia (IDA) is the single most common micronutrient deficiency in the world, affecting >3.5 billion men, women and children.<sup>1</sup> 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.<sup>2</sup> According to the World Health Organization (WHO), anaemia is estimated to affect 66.4 and 57.3% of pregnant and non-pregnant women of reproductive age, respectively.<sup>3</sup> Consequences of IDA include: inhibited growth, impaired cognitive development, poor mental and motor performance, reduced work capacity and an overall decreased quality of life.<sup>4–9</sup>

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.<sup>10–12</sup> Though iron pots do leach iron into food, the acceptability of this supplementation technique appears limited. Prinsen-Geerligs *et al.*<sup>13</sup> 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 follow-up visits affected compliance.

## 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.

### **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 (haematocrit <40%) in a village-wide anaemia screening programme. Haemoglobin (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.

### **Recruitment**

The primary study population consisted of 256 households from Preak Ruessei. 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 (haematocrit <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%.

### **Study completion**

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

### **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.<sup>14,15</sup> 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.

### **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 haematology testing, including Hb and haematocrit; 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 haematocrit <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).

### **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 ( $\geq 120$  g l<sup>-1</sup>), SFe (59–148  $\mu$ g l<sup>-1</sup>) and CRP (<6.0 mg l<sup>-1</sup>) were defined according to Paramed Laboratories (Phnom Penh, Cambodia).

### **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 anaemia). 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  $\geq 130 \mu\text{g l}^{-1}$  or CRP  $\geq 6.0 \text{ mg l}^{-1}$ ; data from women with these values were excluded from the analyses.

## Results

### Recruitment

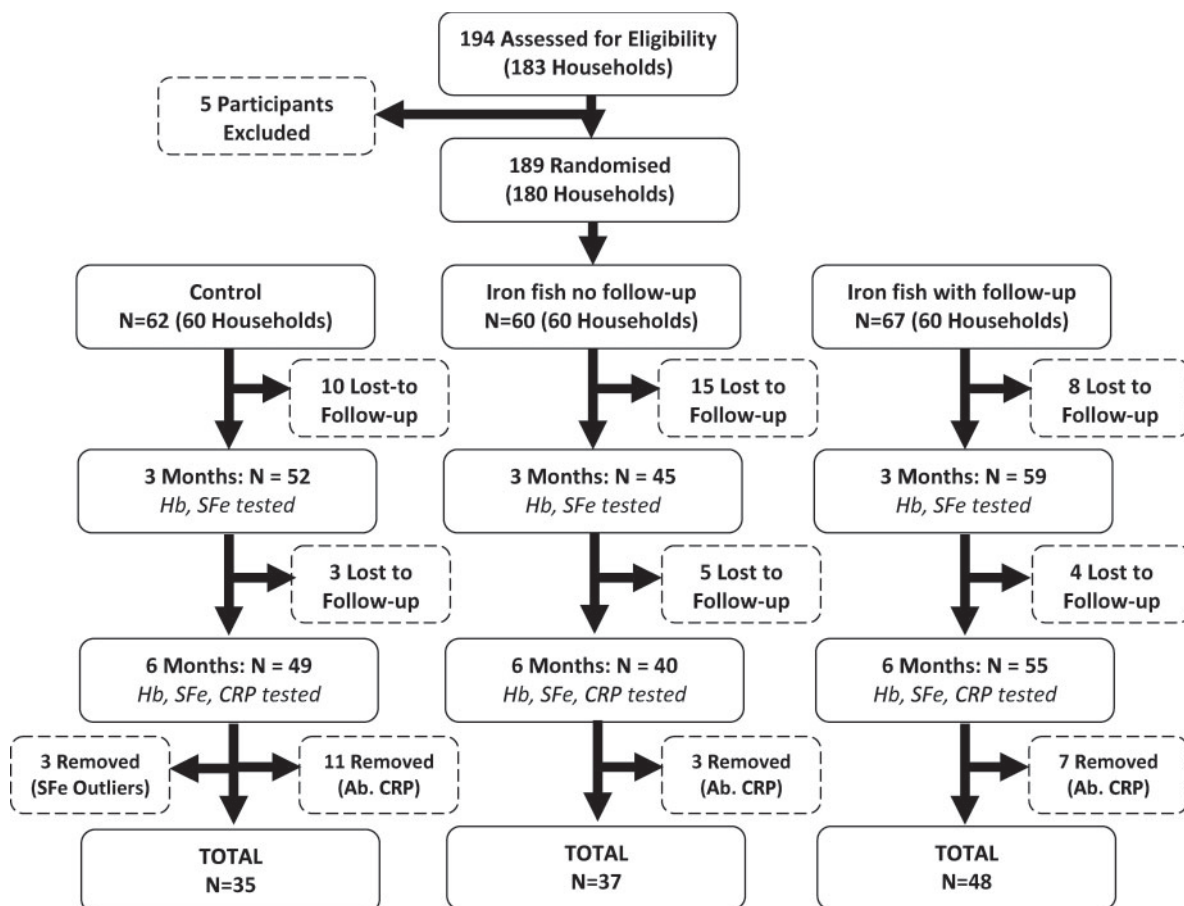
A total of 194 participants from 183 households were assessed for eligibility, from which 189 women from 180 households entered the study (Figure 1). Following baseline blood sampling, five women were identified as severely anaemic and were excluded. Of the 180 households recruited into the study, 177 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, haematological 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).

### Outcomes

Baseline blood parameters and demographics did not differ significantly between groups ( $P < 0.05$ ) (Table 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



**Figure 1** Trial profile for a randomized clinical trial of an iron ingot as a means of iron supplementation in women in rural Cambodia. Hb, haemoglobin; SFe, serum iron; CRP, C-reactive protein; Ab. CRP, abnormal C-reactive protein ( $\geq 6.0 \text{ mg l}^{-1}$ ); SFe outliers, serum iron outliers ( $\geq 130 \mu\text{g l}^{-1}$ )

**Table 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 ( $n = 120$ )

Characteristic	Treatment group			P-value
	Control (N = 35)	NFU (N = 37)	FU (N = 48)	
Age (years)	42.1 ± 13.2	45.9 ± 13.5	47.4 ± 12.7	0.18
Post-menopausal (%)	12 (34)	14 (38)	19 (40)	0.62
Number of pregnancies	3.7 ± 2.3	4.1 ± 2.8	4.1 ± 2.9	0.76
Baseline Hb (g l <sup>-1</sup> )	12.4 ± 1.3	12.6 ± 1.2	12.8 ± 0.9	0.33
Baseline SFe (µg l <sup>-1</sup> )	107.8 ± 24.2	117.7 ± 25.5	109.1 ± 26.4	0.20

Data are mean ± standard deviation or count (and column percentage)

**Table 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

Treatment group	Blood parameter	Baseline (B) evaluation	Evaluations during and at end of intervention		Significance of difference versus Baseline	
			3 months (3M)	6 months (6M)	P-value B versus 3M	P-value B versus 6M
Control	Hb	12.41 ± 1.32	12.05 ± 1.17	12.87 ± 1.32	0.20	0.14
	SFe	107.80 ± 24.17	73.71 ± 18.53	84.43 ± 17.40	<0.0001	<0.0001
NFU	Hb	12.57 ± 1.23	12.51 ± 1.06	12.59 ± 1.30	0.74	0.93
	SFe	117.68 ± 25.53	77.89 ± 14.70	76.11 ± 17.67	<0.0001	<0.0001
FU	Hb	12.79 ± 0.94	13.02 ± 0.95	12.87 ± 1.09	0.23	0.62
	SFe	109.13 ± 26.44	86.17 ± 16.20	78.15 ± 14.81	<0.0001	<0.0001

Data are mean ± standard deviation; significance determined by paired *t*-test comparing baseline with follow-up at 3 and 6 months.

**Table 3** Differences in blood parameters between three different treatment groups 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

	Time of evaluation	FU versus Control		NFU versus Control		FU versus NFU	
		β	P	β	P	β	P
Hb	3 months	0.96	0.0001	0.46	0.07	0.50	0.03
	6 months	0.18	0.51	-0.12	0.67	0.30	0.25
SFe	3 months	12.45	0.0009	-4.18	0.28	8.27	0.02
	6 months	-4.47	0.23	6.68	0.09	2.21	0.53

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.

6-month evaluation (Table 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 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 (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 (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.

## Discussion

We studied the effect of a supplemental iron technique as a practical, household intervention on haematological 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.<sup>13,16</sup> 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 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.<sup>10–12,17</sup> 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.<sup>13</sup> Secondly, the iron pots rust quickly and this reduces acceptability.<sup>13</sup> 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.<sup>18</sup> 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.<sup>18</sup> Unfortunately, SFe is also affected by infection and inflammation<sup>19</sup> 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.<sup>18</sup>

Despite the promising data in the first half of the trial for the group using the fish with follow-up visits, the elevated haematological 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.<sup>20</sup> 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.

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*Conflicts of interest:* None declared.

## Key points

- In contrast to the generally low acceptability of iron cooking pots for the amelioration of IDA, the small, lightweight, reusable and cost-effective iron ingot developed for this study was widely accepted.
- The sequestering of iron as a result of ground water contamination should be considered in the development of adventitious iron sources, like the iron ingot.
- Due to the limited access to conventional treatments throughout much of rural Cambodia, IDA remains a significant public health problem that requires immediate attention and the development of novel iron supplementation techniques.

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