Randomized controlled trial assessing the efficacy of a reusable fish-shaped iron ingot to increase hemoglobin concentration in anemic, rural Cambodian women

Aviva I Rappaport,1 Kyly C Whitfield,1,2 Gwen E Chapman,1,3 Rickey Y Yada,1 Khin Meng Kheang,5 Jennie Louise,6 Alastair J Summerlee,7 Gavin R Armstrong,4 and Timothy J Green1,7

1Food, Nutrition and Health, Faculty of Land and Food Systems, University of British Columbia, Vancouver, British Columbia, Canada; 2College of Social and Applied Human Sciences and Biomedical Science, University of Guelph, Guelph, Ontario, Canada; 3Nutrifood Cambodia Phnom Penh, Cambodia; 4University of Adelaide, South Australia, Australia; and 5Healthy Mothers, Babies, and Children Theme, South Australia Health and Medical Research Institute, South Australia, Australia

INTRODUCTION

The prevalence of anemia (hemoglobin concentration <120 g/L) in women of reproductive age (WRA) is ~45% in Cambodia, which is a severe public health problem according to the WHO classification system (1, 2). Globally, iron deficiency is thought to be the most common cause of anemia and accounts for a purported 50% of all cases of anemia (1, 3). Consistent with WHO guidelines, Cambodian health authorities recommend blanket weekly iron (60 mg) and folic acid (2.8 mg) supplementation for WRA (3–5). In Cambodia, as elsewhere, adherence to iron supplements is poor, and there are issues with distribution and procurement (4, 6). As such, alternative approaches are needed to prevent iron-deficiency anemia in Cambodia.

A simple alternative to iron supplements is the use of cast-iron utensils during cooking (7). Iron is leached into the food when prepared in iron pots; transfer is greater in an acidic environment and with a longer cooking duration (8–11). However, the effectiveness of iron pots in treating iron-deficiency anemia in human trials has been inconsistent (12, 13). Moreover, in many low-income countries, aluminum pots are used because they are cheaper, lighter, rust resistant, and more available than are iron pots (14, 15).

Supported by the Danish Red Cross, the University of Guelph, and Grand Challenges Canada (grant CDE-0355-55 LIF). Supplements were provided by Natural Factors. Lucky Iron Fish iron ingots were provided by Lucky Iron Fish Inc.

Supplemental Tables 1–4 are available from the “Online Supporting Material” link in the online posting of the article and from the same link in the online table of contents at http://ajcn.nutrition.org.

Present address for KCW: Department of Applied Human Nutrition, Mount Saint Vincent University, Halifax, Nova Scotia, Canada.

Present address for GEC: College of Social and Applied Human Science, University of Guelph, Guelph, Ontario, Canada.

Address correspondence to TIG (e-mail: tim.green@sahmri.com).

Received January 31, 2017. Accepted for publication May 16, 2017. First published online June 14, 2017; doi: https://doi.org/10.3945/ajcn.117.117.152785.

OBJECTIVE

We sought to determine whether there was a difference in hemoglobin concentrations in rural Cambodian anemic women (aged 18–49 y) who cooked with the iron ingot or consumed a daily iron supplement compared with a control after 1 y.

Design: In Preah Vihear, 340 women with mild or moderate anemia were randomly assigned to 1) an iron-ingot group, 2) an iron-supplement (18 mg/d) group, or 3) a nonplacebo control group. A venous blood sample was taken at baseline and at 6 and 12 mo. Blood was analyzed for hemoglobin, serum ferritin, and serum transferrin receptor. Hemoglobin electrophoresis was used to detect structural hemoglobin variants.

RESULTS: Anemia prevalence was 44% with the use of a portable hemoglobinometer during screening. At baseline, prevalence of iron deficiency was 9% on the basis of a low serum ferritin concentration. There was no significant difference in mean hemoglobin concentrations between the iron-ingot group (115 g/L; 95% CI: 113, 118 g/L; P = 0.850) or iron-supplement group (115 g/L; 95% CI: 113, 117 g/L; P = 0.998) compared with the control group (115 g/L; 95% CI: 113, 117 g/L) at 12 mo. Serum ferritin was significantly higher in the iron-supplement group (73 µg/L; 95% CI: 64, 82 µg/L; P < 0.002) than in the control group at 6 mo; however, this significance was not maintained at 12 mo (73 µg/L; 95% CI: 58, 91 µg/L; P = 0.176).

Conclusions: Neither the iron ingot nor iron supplements increased hemoglobin concentrations in this population at 6 or 12 mo. We do not recommend the use of the fish-shaped iron ingot in Cambodia or in countries where the prevalence of iron deficiency is low and genetic hemoglobin disorders are high. This trial was registered at clinicaltrials.gov as NCT02341586.


Keywords: anemia, Cambodia, hemoglobin, inflammation, iron deficiency, iron ingot, Lucky Iron Fish, randomized controlled trial, serum ferritin, women of reproductive age
The reusable fish-shaped iron ingot was designed for use during cooking in the home (14) and is shaped like a fish, which is believed to be lucky by Cambodians (14). The iron-ingot stems from the same principle as cast-iron cookware (16). Two randomized controlled trials of the iron ingot were carried out in Kandal province, Cambodia (14, 17), but had inconsistent findings. The first trial had a duration of 6 mo (n = 189 WRA) and measured serum iron as the biomarker of iron status. However, serum iron is a poor biomarker of iron because it changes diurnally and is affected by inflammation (14, 18). In addition, the study had a high attrition rate of ~40% over 6 mo (14). The second trial had a duration of 12 mo (n = 310 WRA) and reported a large reduction in anemia of ~46% in the intervention group who cooked with the iron ingot despite a low prevalence of iron-deficiency anemia at baseline, which was 13% on the basis of a ferritin concentration <15 μg/L (17). Therefore, a larger trial that compares the efficacy of this iron ingot with iron supplementation, which is the current method to address anemia, was warranted. In addition, it is important to address the known high prevalence of genetic hemoglobin disorders and inflammation in an efficacy study of the iron ingot (19–21). Genetic hemoglobin disorders result in a decreased or defective production of hemoglobin regardless of iron status (22).

The primary aim of the current study was to determine whether hemoglobin concentrations differed in anemic Cambodian women (18–49 y old) who were randomly assigned to a group who cooked with the iron ingot, a group who took a daily iron supplement, or a control group after 1 y. Secondary objectives were to assess changes in iron status with the use of more robust biomarkers [serum ferritin and serum transferrin receptor (sTfR)] with appropriate corrections for inflammation and to assess the prevalence of genetic hemoglobin disorders in these 3 groups of WRA in Preah Vihear.

METHODS

This was a parallel, 3-arm, randomized controlled trial that was conducted between April 2015 and May 2016 in the Rovieng district, Preah Vihear province, Cambodia. The Cambodian National Ethics Committee for Health Research [(NECHR) 0319NECHR], the University of British Columbia’s Clinical Research Ethics Board (H14-02551), and the University of Guelph’s Research Ethics Board (13NV022) approved the study. Informed written consent was obtained from all women. The trial was registered at clinicaltrials.gov as NCT02341586.

Participants

A 2-stage recruitment process was used to assess eligibility. A convenience sample of nonpregnant WRA was recruited for screening in consultation with the village health center chief and through village-level sensitization by community health workers. Women were eligible for screening with a portable hemoglobinometer (HemoCue Hb 301) if they were between 18 and 49 y of age, were the female head of their household, and self-reported as being healthy, not pregnant, not currently taking medications or iron supplements, not currently participating in any other active nongovernmental nutrition interventions or programs, and not planning to relocate. Women who met these eligibility requirements were screened for anemia with the use the portable hemoglobinometer; (HemoCue Hb 301); women with mild-to-moderate anemia (hemoglobin concentration 80–119 g/L) were eligible to participate. Women with severe anemia (hemoglobin concentration <80 g/L) were excluded and referred to the health center for treatment.

Iron supplements and the iron ingot

The Lucky Iron Fish iron ingot (Bowmanville Foundry) was designed by the University of Guelph, weighs 178 g, and has a surface area of 143 cm² (16). These reusable fish-shaped ingots were tested for metal contaminants and, when boiled in water, have been reported to meet WHO drinking water quality standards (16). Depending on the acidity, ~30–80 mg Fe (as ferrous and ferric iron)/L of water is released during the cooking process (16).

A supplement dose of 18 mg elemental Fe/d day as ferrous sulfate (57 mg; Natural Factors) was formulated to correspond to that which might be provided by the iron ingot during normal use (16). This dose also corresponds to the Recommended Dietary Allowance for iron for WRA in the United States and Canada (23).

Study procedures

After enrollment, women were randomly assigned to 1 of 3 treatment arms with the use a random-digit generator (Excel 2010; Microsoft Corp.). Women who were randomly assigned to the iron-ingot group received training on the use of the ingot including best practices for the cooking time, cooking environment (e.g., adding lemon juice), and hygiene and care of the ingot. Lemon juice is known to enhance the absorption of nonferrous iron, and therefore, its addition during cooking improves the bioavailability of iron that already exists in the diet (24).

Women who were randomly assigned to the iron-supplement group were instructed to take 1 tablet/d and were given training on how to open the bottles and about the common side effects of iron supplements. The control group did not receive a placebo. Within 1 mo of baseline, standard nutrition education on the basis of principles outlined by the Cambodian National Nutrition Program (including dietary sources of iron and causes and signs of iron deficiency and anemia) was provided to all study participants, including the control group.

Data and blood collection took place at local health centers at baseline, 6, and 12 mo. At baseline, height and weight were measured with the use standardized techniques (25), and a questionnaire was administered to attain sociodemographic information. At screening and 6 and 12 mo, the hemoglobin concentration of capillary blood was assessed with the use of a portable hemoglobinometer (HemoCue Hb 301) according to standardized procedures and with appropriate quality-control solutions (Hemotrol Level 1, 2, and 3 solutions; Eurotrol E.V.) (26). At baseline, 6, and 12 mo, nonfasting venous blood samples were collected into evacuated tubes containing EDTA and trace-element–free tubes containing no anticoagulant (Vacutainer; Becton Dickinson). Samples were transported daily on ice to the Siem Reap Provincial Hospital for processing. The blood in the
tube containing EDTA was used for a complete blood count to determine the hemoglobin concentration and other red blood cell variables. The trace element free tubes were centrifuged (3000 × g for 10 min at 4°C), and serum was separated, aliquoted, and stored at −80°C until analyzed. At 6 mo, an additional blood sample was collected into a tube containing EDTA and centrifuged (3000 × g for 15 min), and the erythrocytes and buffy coat were transported at 4°C to the National Pediatric Hospital in Phnom Penh for hemoglobin electrophoresis.

Laboratory analysis

A complete blood count was performed with the use an automated hematology analyzer (Sysmex XP-100; Sysmex Corp.) at Siem Reap Provincial Hospital. Hemoglobin electrophoresis was conducted on a SEBIA MINICAP analyzer (Sebia) with the use the Hemoglobin (E) program to detect hemoglobin E, CS, H, Bart, or F variants. Serum ferritin, serum transferrin (sTfR), C-reactive protein, and α-1 acid glycoprotein were assessed with the use of a sandwich ELISA at the Erhardt Laboratory (VitMin Laboratory) (27).

Data analysis

The primary outcome was the hemoglobin concentration. As such, the sample size was determined on the basis of having 90% power to detect a difference at a significance level of a 2-sided α = 0.05 if the true difference in hemoglobin concentrations between groups was 5 g/L. This determination was based on the assumption that the SD of the response variable (hemoglobin) was 11 g/L (28). As such, a sample size of n = 90 women in each arm was required. To account for dropouts, loss to follow-up, and missing data, we increased the number of women in each arm by 20%. Therefore, the final sample size that was required was n = 110 in each trial arm for a total of 330 women.

Analyses were conducted both on complete cases and on imputed data. For imputed analyses, multiple imputation with the use of the fully conditional specification ( chained equations) method was used to create 100 complete data sets including all women with a measure at any time point (29). Imputation was carried out separately by treatment group.

Linear regression with generalized estimating equations to account for correlation that were due to repeated measures over time were performed. A time-by-treatment interaction term was used in these models to test for differences in the change over time between treatment groups. Analyses were adjusted for the presence or absence of a hemoglobin variant. For participant characteristics at baseline, means ± SDs are reported for continuous data. For categorical data, n (percentages) are reported. For the primary outcome variable of the hemoglobin concentration, means (95% CIs) were compared between intervention and control groups at 6 and 12 mo. For the secondary objective, serum ferritin and sTfR, data were corrected for inflammation on the basis of established cutoffs with the use the biomarkers C-reactive protein and α-1-acid-glycoprotein (30). Serum ferritin and sTfR were not normally distributed and, thus, were transformed with the use a natural logarithmic scale. Estimates were back transformed and are presented as geometric means (95% CIs) at each time point.

Tests were 2 tailed, and a significance level of P < 0.05 was set. Data were analyzed with the use Stata version 14 software (StataCorp LP).

RESULTS

Of 1049 women who were recruited and underwent screening, 281 women were ineligible. Of the remaining 768 women who were screened for anemia with the use a portable hemoglobinometer (HemoCue Hb 301), 428 women were excluded because they had normal hemoglobin (>120 g/L). The remaining 327 women were randomly assigned to treatments. At 6 mo, 281 women remained; attrition (n = 46; 14%) was similar across the groups and mostly occurred because women migrated or were pregnant or planning on becoming pregnant. At 12 mo, 240 women remained, which gave an overall attrition rate of 27% (Figure 1).

Participant characteristics are given in (Table 1). The mean age was 32 y, and almost all women had completed some schooling. Most women had a normal BMI (in kg/m2; 18.5–24.9); however, the control group had a higher proportion of overweight women (19%), and the iron-ingot group had the greatest number of underweight women (16%). The mean household size was similar across the 3 groups with 5–6 people living in each home. Most households (n = 253; 78%) earned <$1800 in the previous 12 mo. At baseline, the prevalence of iron deficiency differed by biomarker (9% with serum ferritin, and 30% with sTfR). The majority of women (69%) had a structural hemoglobin variant. However, of the n = 87 women (31%) without a structural hemoglobin variant, 52 of the 87 women may have had the α- or β-thalassemia trait on the basis of a low mean corpuscular volume and adequate iron stores, but these instances could not be ruled out or assessed with the use of hemoglobin electrophoresis. There were no significant differences in the mean corpuscular volume between groups at any time point (Supplemental Table 1).

In the imputed analyses, there was no significant difference (P = 0.934) in the mean hemoglobin concentration between groups at any time point (Table 2). The estimated mean hemoglobin concentration at 12 mo was 115 g/L (95% CI: 113, 117 g/L) in the control group (n = 109), 115 g/L (95% CI: 113, 117 g/L) in the iron-supplement group (n = 109), and 115 g/L (95% CI: 113, 118 g/L) in the iron-ingot group (n = 109). A complete case analysis of hemoglobin concentrations is shown in Supplemental Table 2. The analysis of complete cases did not differ markedly from the imputed analysis and did not affect their interpretations.

For hemoglobin concentrations that were assessed with a portable hemoglobinometer (HemoCue Hb 301), there was a temporal change in hemoglobin concentrations across all groups such that hemoglobin concentrations were ~10 and ~6 g/L higher than at screening at 6 and 12 mo, respectively, regardless of treatment (Supplemental Table 3).

At baseline, mean serum ferritin concentrations were normal at 59, 53, and 51 μg/L for the control, iron-ingot, and iron-supplement groups, respectively. The iron-supplement group had a significantly higher mean serum ferritin concentration of 73 μg/L (95% CI: 64, 82 μg/L) at 6 mo (P = 0.002) compared with that of the control group 52 μg/L (95% CI: 44, 62 μg/L). However, this difference was not maintained at 12 mo (73 μg/L.
(95% CI: 58, 91 μg/L) in the iron-supplement group and 59 μg/L (95% CI: 49, 72 μg/L) in the control group (P = 0.176) (Table 3). A complete case analysis of serum ferritin concentrations did not markedly alter these findings and is shown in Supplemental Table 4.

There were no significant differences in the mean sTfR concentration at 6 or 12 mo by treatment group. At 12 mo, mean sTfR concentrations were 6.6 mg/L (95% CI: 6.0, 7.3 mg/L), 6.6 mg/L (95% CI: 5.9, 7.4 mg/L), and 6.1 mg/L (95% CI: 5.6, 6.6 mg/L) in the control, iron-ingot, and iron-supplement groups, respectively. A complete case analysis of sTfR is shown in Supplemental Table 4.

Monitoring was conducted monthly to measure adherence. In the iron-supplement group, women were considered adherent if they took >80% of pills in their supplement bottle for the month (assessed via a pill count). For the iron-ingot group, adherence was calculated on the basis of whether the iron ingot was used in the previous day and if this pattern was a typical routine for the woman. Adherence was high in both treatment groups at >86% and 83% in the iron-supplement group and 83% and 90% in the iron-ingot group in months 1–6 and months 7–12, respectively.

**DISCUSSION**

The reusable fish-shaped iron ingot was developed to address anemia in Cambodia because it was expected to have better adherence and longer-term sustainability than iron supplements. Results from this randomized controlled trial do not support the use of this iron ingot in Cambodia. There were no significant differences in hemoglobin concentrations in the iron-supplement or iron-ingot groups than in the control group after 6 mo or 1 y as measured with either the portable hemoglobinometer or an automated hematology analyzer. The lack of an effect of either the iron ingot or the iron supplement on anemia may have been due to the low prevalence of iron deficiency at baseline on the basis of serum ferritin concentrations in this population. This low prevalence of iron deficiency, despite high anemia, is consistent with the findings of other recent research that has been conducted in Cambodia (19, 33).

The finding that the iron ingot did not reduce anemia is consistent with earlier studies that showed that iron pots were not effective in anemia management (12, 13). However, our findings are not consistent with previous research that was conducted on the iron ingot in Cambodia. In the first study (n = 189 WRA), the mean ± SD hemoglobin concentration in the ingot group who...
received follow-up was 130 ± 10 g/L compared with 125 ± 11 g/L in the ingot group without follow-up and 121 ± 12 g/L in the control group (P = 0.001) after 3 mo. These observed differences in hemoglobin concentrations were not maintained at 6 mo (14). The use of the iron ingot resulted in higher hemoglobin at some points but not at others. The authors suggested that this variance was due to the different availabilities of iron-rich foods in the wet and dry seasons. The authors suggested that the iron ingot works only when iron in the food supply is low. We did not see any difference between treatment groups at either 6 or 12 mo that corresponded to the dry and wet seasons, which suggested that this postulation was not the case.

In another study (n = 310), in which nearly one-third of the participants were postmenopausal, the same authors reported a significantly higher (P < 0.05) hemoglobin concentration in the groups who received the iron ingot than in the control group at 9 and 12 mo (17). Similar to our study, the authors reported a low prevalence (11%) of iron deficiency in this population (17). Anemia was reduced by 34% and 46% at 9 and 12 mo, respectively, despite the low prevalence of iron deficiency at baseline. The authors showed a significant difference in the mean serum ferritin concentration in the iron-ingot group (102 μg/L; 95% CI: 89, 116 μg/L) after 1 y than in the control group (66 μg/L; 95% CI: 57, 76 μg/L) in contrast with the results that are presented in this study in which no significant differences in serum ferritin for the iron ingot was shown as 6 mo (P = 0.639) and 12 mo (P = 0.781) (17). Also, we showed that serum ferritin was significantly different (P = 0.002) at 6 mo and approached significance at 12 mo (P = 0.176) with ferrous sulfate supplementation, whereas the iron ingot had no effect on ferritin.
TABLE 2
Imputed analysis for estimated differences in hemoglobin concentrations that were assessed via venous blood on an automated hematology analyzer and tests for treatment- and control-group differences1

<table>
<thead>
<tr>
<th>Outcome for hemoglobin</th>
<th>n</th>
<th>g/L</th>
<th>Difference2 g/L</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control, mo</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>109</td>
<td>117.4 (115.2, 119.5)</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>6</td>
<td>109</td>
<td>115.3 (113.3, 117.3)</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>12</td>
<td>109</td>
<td>115.0 (112.8, 117.1)</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Iron ingot, mo</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>109</td>
<td>118.3 (116.0, 120.5)</td>
<td>0.91 (−2.19, 4.01)</td>
<td>0.564</td>
</tr>
<tr>
<td>6</td>
<td>109</td>
<td>117.8 (115.6, 120.1)</td>
<td>2.53 (−0.51, 5.58)</td>
<td>0.102</td>
</tr>
<tr>
<td>12</td>
<td>109</td>
<td>115.3 (112.7, 117.9)</td>
<td>0.32 (−3.01, 3.74)</td>
<td>0.850</td>
</tr>
<tr>
<td>Iron supplement, mo</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>109</td>
<td>117.2 (115.6, 119.1)</td>
<td>−0.11 (−2.89, 2.68)</td>
<td>0.941</td>
</tr>
<tr>
<td>6</td>
<td>109</td>
<td>116.4 (114.0, 118.8)</td>
<td>1.10 (−1.98, 4.18)</td>
<td>0.483</td>
</tr>
<tr>
<td>12</td>
<td>109</td>
<td>115.0 (112.8, 117.1)</td>
<td>0.003 (−3.12, 3.12)</td>
<td>0.998</td>
</tr>
</tbody>
</table>

1Generalized linear model was used to assess differences between groups. Adjustments were made for the baseline hemoglobin concentration and the presence of a hemoglobin variant with a significance level of 0.05 for all analyses.
2 Compared with control.
3 Estimated mean; 95% CI in parentheses (all such values).

The findings in this trial are in line with those reported for cast-iron cookware. Sharief et al. (13) conducted a randomized controlled trial in Benin that compared groups of women and children who consumed food from 1 of 2 different types of iron cookware with a control group who received iron supplements or micronutrient powders in a population with 50% anemia. As in our study, after 1 year, there was no significant difference in hemoglobin concentrations in the 3 groups (13). Cast-iron cookware has a larger surface area than that of the iron ingot. Therefore, if cast-iron cookware cannot affect biomarkers of iron status, it was not surprising that the iron ingot did not have an impact on these biomarkers either, thereby questioning the biological plausibility of this mechanism of iron transfer.

The aim of this clinical trial was to address anemia caused by iron deficiency. A finding of this study was that, although iron deficiency was low, it varied considerably by biomarker. Approximately 9% of WRA were iron deficient when the ferritin deficiency was low, it varied considerably by biomarker. Ap- proximately 9% of WRA were iron deficient when the ferritin deficiency was low, it varied considerably by biomarker. Therefore, if cast-iron cookware cannot affect biomarkers of iron status, it was not surprising that the iron ingot did not have an impact on these biomarkers either, thereby questioning the biological plausibility of this mechanism of iron transfer.

In our study at 6 mo, mean serum ferritin was higher in the group who received the iron-supplement (73 μg/L; 95% CI: 64, 82 μg/L) than in the iron-ingot group (49 μg/L; 95% CI: 41, 59 μg/L) and control group (52 μg/L; 95% CI: 44, 62 μg/L). At 12 mo, although ferritin was 14 μg/L higher in the iron-supplement group than in the control group, it was no longer significant (P = 0.176). Our study was not powered to detect differences in serum ferritin concentrations, which may explain the nonsignificant findings at 12 mo.

Our study has some limitations. First, anemia in Cambodia and elsewhere is generally treated with an elemental iron supplement containing 30–60 mg Fe (3, 5). However, the dose of 18 mg Fe/d was chosen in this study to parallel the dose of iron that was received by the women who cooked with the iron ingot. Although this dose is lower than a 60-mg/d iron supplement, it is greater than the suggested weekly dose of 60 mg. Second, we did not account for other hemoglobin variants (i.e., α^3.7 thalassemia) or combinations of variants that are known to exist in this population. These variants are also known to effect iron indexes. However, because this was a randomized control trial, we expected that these variants would be distributed evenly across the treatment groups. Also, after screening for anemic women, the prevalence of anemia as assessed via capillary blood on the portable hemoglobinometer was 100%. At baseline, the prevalence of anemia that was assessed via venous blood on the automated hematology analyzer was only 50%. Regression to the mean and differences in variability between capillary and venous blood likely account for this difference (35). Finally, we did not correct for multiple comparisons for our secondary outcomes.

TABLE 3
Imputed analysis for estimated means and estimated differences in biomarkers of iron status (serum ferritin and sTIR) and tests for treatment- and control-group differences1

<table>
<thead>
<tr>
<th>Outcome</th>
<th>n</th>
<th>Value</th>
<th>Difference2</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum ferritin,3 μg/L</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control, mo</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>109</td>
<td>59.57 (50.24, 68.29)</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>6</td>
<td>109</td>
<td>52.12 (44.07, 61.64)</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>12</td>
<td>109</td>
<td>59.17 (48.75, 71.83)</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Iron ingot, mo</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>109</td>
<td>52.78 (44.76, 62.24)</td>
<td>0.90 (0.72, 1.13)</td>
<td>0.365</td>
</tr>
<tr>
<td>6</td>
<td>109</td>
<td>49.03 (40.52, 59.34)</td>
<td>0.94 (0.73, 1.21)</td>
<td>0.639</td>
</tr>
<tr>
<td>12</td>
<td>109</td>
<td>56.73 (45.25, 71.12)</td>
<td>0.96 (0.71, 1.29)</td>
<td>0.781</td>
</tr>
<tr>
<td>Iron supplement, mo</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>109</td>
<td>50.82 (43.73, 59.05)</td>
<td>0.87 (0.70, 1.08)</td>
<td>0.195</td>
</tr>
<tr>
<td>6</td>
<td>109</td>
<td>72.54 (64.06, 82.14)</td>
<td>1.39 (1.13, 1.71)</td>
<td>0.002</td>
</tr>
<tr>
<td>12</td>
<td>109</td>
<td>72.66 (58.08, 90.90)</td>
<td>1.23 (0.91, 1.65)</td>
<td>0.176</td>
</tr>
<tr>
<td>sTIR, mg/L</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control, mo</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>109</td>
<td>6.66 (6.89, 8.52)</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>6</td>
<td>109</td>
<td>6.89 (5.92, 7.34)</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>12</td>
<td>109</td>
<td>6.60 (5.97, 7.31)</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Iron ingot, mo</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline, mo</td>
<td>109</td>
<td>6.74 (6.93, 8.42)</td>
<td>1.00 (0.86, 1.15)</td>
<td>0.971</td>
</tr>
<tr>
<td>6</td>
<td>109</td>
<td>6.70 (5.97, 7.51)</td>
<td>1.02 (0.87, 1.19)</td>
<td>0.846</td>
</tr>
<tr>
<td>12</td>
<td>109</td>
<td>6.61 (5.94, 7.35)</td>
<td>1.00 (0.86, 1.16)</td>
<td>0.997</td>
</tr>
<tr>
<td>Iron supplement, mo</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline, mo</td>
<td>109</td>
<td>7.73 (7.10, 8.41)</td>
<td>1.01 (0.88, 1.16)</td>
<td>0.898</td>
</tr>
<tr>
<td>6</td>
<td>109</td>
<td>6.14 (5.65, 6.66)</td>
<td>0.93 (0.81, 1.07)</td>
<td>0.304</td>
</tr>
<tr>
<td>12</td>
<td>109</td>
<td>6.06 (5.58, 6.59)</td>
<td>0.92 (0.81, 1.05)</td>
<td>0.203</td>
</tr>
</tbody>
</table>

1Generalized linear model was used to assess differences between groups. Adjustments were made for baseline serum ferritin and sTIR and the presence of a hemoglobin variant with a significance level of 0.05 for all analyses. Data were log transformed before the analysis. Estimates were back transformed to the original scales. sTIR, serum transferrin receptor.
2 Compared with control.
3 Inflammation biomarkers (C-reactive protein and α-1-acid-glycoprotein) were used to correct serum ferritin for inflammation stage, incubation, and early or late convalescence (30).
4 Geometric estimated mean; 95% CI in parentheses (all such values).
which is consistent with recommendations (36–38). Adjustment for multiple comparisons is done to reduce the probability of making a type I error. The adjustment affects the P value by increasing it but has no effect on treatment means or their variance.

In our study, only one secondary outcome was significant, and thus, adjustment for multiple comparisons would not have influenced the findings or their interpretation. Serum ferritin at 6 mo was significantly higher in the iron-supplement group than in the control group (P = 0.002); however, even if we had used the most conservative approach to adjust for multiple comparisons, the P value would still have been significant.

In conclusion, the primary objective of this study was to determine whether the iron ingot and iron supplements could increase the hemoglobin concentration in nonpregnant WRA in rural Cambodia. After 1 y, the hemoglobin concentration did not significantly differ between the control group and either the iron-ingot or iron-supplement group. An additional objective of this research was to determine the impact of the iron ingot and iron supplements on biomarkers of iron status. Despite the low prevalence of iron deficiency, the results show a significant difference in serum ferritin concentrations in women consuming iron supplements after 6 mo. This result suggests that, in WRA in Cambodia, biomarkers of iron status can be affected by a low dose (18 mg/d) of ferrous sulfate with monthly visits to monitor adherence. The same trend was not seen in the iron-ingot group, suggesting that the ingot does not have the same impact on WRA as iron supplements do. We do not recommend the use of the fish-shaped iron ingot in Cambodia or in other countries where the prevalence of iron deficiency in nonpregnant WRA is low, and the prevalence of genetic hemoglobin disorders is high.

The authors’ responsibilities were as follows—AIR, KCW, and TJG designed the study; AIR, GEC, RYY, AJS, GRA, and TJG: further refined and finalized the research protocol; AIR and KMK: coordinated and led the data collection; JL, AIR, and TJG: conducted the data analysis; AIR:drafted the research manuscript; TJG: had primary responsibility for the final content of the manuscript; and all authors: read and approved the final manuscript.

Natural Factors (supplier of vitamin supplements) and Lucky Iron Fish Inc. (supplier of the iron ingots) had no role in the study design, implementation, or interpretation of the study findings. AJS (board member of Lucky Iron Fish plier of the iron ingots) had no role in the study design, implementation, or collection; JL, AJS, and KMK: coordinated and led the data collection; AIR: conducted the data analysis; AJS: finalized the research protocol; AIR and KMK: coordinated and led the data collection; AFL, PCH, KMK: conducted the data analysis; AIR: drafted the research manuscript; TJG: had primary responsibility for the final content of the manuscript; and all authors: read and approved the final manuscript.

REFERENCES


