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Iron retention in iron-fortified rice and use of iron-fortified rice to treat women with iron deficiency: A pilot study

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ABSTRACT

Objectives: 1. Evaluate the effect of washing and cooking iron-fortified rice on iron retention and bioavailability. 2. Evaluate the effect of iron-fortified rice on women with iron deficiency anemia

Methods: 1. Iron-fortified rice (18 mg/100 g as $FeSO_4$) was cooked in Baton Rouge, Louisiana (C), rinsed and cooked (RC), fried and cooked (FC), cooked with extra water (CW), or soaked and cooked with extra water (SCW), and iron retention was determined. 2. Rice samples were cooked in Kampala, Uganda in a lab (C-Uganda) and households using traditional cooking method (TC-Uganda) and iron retention were determined. 3. Seventeen women with iron deficiency (low iron and/or low ferritin) anemia were randomized to 100 g/d of rice (two cooked 0.75 cup servings) for two weeks containing 18 mg/d iron (supplemented) or 0.5 mg/d iron (un-supplemented). Hemoglobin and hematocrit were evaluated at baseline and 2 weeks with other measures of iron metabolism.

Results: 1. Iron retention, from highest to lowest, was (C), (RC), (FC), (C-Uganda), (CW), (SCW) and (TC-Uganda). 2. Seventeen women were randomized and 15 completed the study (hemoglobin 10.6 ± 1.6 g, hematocrit 33.7 ± 4.1%), 9 in the iron-fortified rice group and 6 in the un-fortified rice group. The iron-fortified group had a greater increase in hemoglobin (0.82 g, p = 0.0035) and Hematocrit (1.83%, p = 0.0248) with directional differences in other measures of iron metabolism favoring the iron-fortified group.

Conclusions: Iron-fortified rice increased hemoglobin and hematocrit in women with iron-deficient anemia. Iron deficiency and anemia are widespread in Southeast Asia and Africa and undermine development in these regions.

1. Introduction

Micronutrient deficiencies in iron, zinc, vitamins A/B, iodine, and folic acid affect > 2 billion people worldwide [1,2]. Micronutrient malnutrition, a common condition in Southeast Asia and Africa, is a risk factor for several diseases and has profound implications for health, cognitive development, education, economic development, and productivity in these regions [3–9]. Food fortification with micronutrients has been recognized as an important health program for the prevention and treatment of micronutrient deficiencies in many countries around the world. Although staple foods such as rice, maize, wheat flour, or cassava fortification can all address the problem of malnutrition, the focus of our study was on iron fortified rice and predicting its efficacy in prevention/treatment of iron deficiency anemia in regions with a rice based diet.

Iron deficiency is a major public health problem particularly across Sub-Saharan Africa where 67.6% of pre-school children and 57.1% of pregnant women suffer from anemia [10]. For example, in Angola, > 56% of children under 5 years of age are anemic [11]. In Cameroon, the prevalence of iron deficiency in women between the ages of 15 and 49 years and children between the ages of 1 and 5 years has been estimated to be 14% to 68% and 12% to 47%, respectively [12]. Iron deficiency is associated with a higher prevalence of pre-term birth and low birth weight babies with lower Appearance, Pulse, Grimace, Activity and Respiration (APGAR) scores [13]. In this region, where rice is a dietary staple, iron fortified rice may provide the solution for

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prevention of iron deficiency.

There are many advantages associated with the use of polished rice for iron fortification. Rice is the staple for an estimated 3 billion people around the world [7], but the iron content in polished rice is very low. The phytate content of polished rice is low compared to wheat or corn flour, the fortification of which is associated with adverse interactions including sensory effects [14]. A significant share of rice coming from rice mills can be fortified before distribution to the market at a cost that is not > 3% of the current rice retail price. Iron treatment has been shown to improve birth weight in a linear fashion [15], and the use of iron supplementation in food has been demonstrated to be better accepted in developing countries than supplements like iron drops or pills. Foods supplemented with iron are cheaper and result in higher compliance to treatment than medication based supplementation [16].

Although fortification of bread through wheat flour has been a successful strategy in Egypt, other African countries like Nigeria have a rice-based economy [17]. Fortifying rice with iron is challenging because iron leaches easily from the surface of rice during washing, and cooking has been reported to decrease the iron concentration of the iron-fortified rice [18]. Rinse resistant fortified rice kernels have been developed using an advanced coating technology that applies concentrated nutrients (iron or multiple nutrients) to rice kernels which can be added to regular rice to produce micronutrient-fortified rice without altering the taste and with high nutrient retention after washing [19].

The cooking quality of rice is an important attribute that affects consumer acceptance, and cooking methods significantly impact the nutritional values of the cooked rice. There are several ways of cooking rice according to culture and final cooked rice quality preferences. Typical rice cooking methods include (1) rinsing and cooking, (2) frying and cooking without rinsing, (3) cooking with extra water, and (4) soaking and cooking in extra water. The methods commonly used to cook rice include boiling, steaming, and pressure-cooking. Boiling can be performed with a precise volume of water, usually 1.5 to 2 times the weight of rice, and boiling proceeds until all the water is absorbed and some has evaporated. During boiling with extra water, the excess water is discarded when rice has cooked. Each cooking method has a different effect on nutrient retention.

The objective of this study was two-fold, (1) to determine iron retention in iron-fortified rice that was cooked following each one of the cooking methods mentioned above, and (2) to carry out a short-term, double-blind, controlled preliminary evaluation of the Wright Group fortification technology to confirm that it would effectively treat iron deficiency anemia by increasing hemoglobin and hematocrit.

2. Materials and methods

2.1. Rice samples and reagents

Rice samples including control (unfortified rice) and iron-fortified samples were provided by The Wright Group (Crowley, LA). The form of iron used in the fortified rice was food grade ferrous sulfate. Concentrated ferrous sulfate was coated on to the rice kennels to form coated iron fortified rice kernels. Then coated iron fortified rice kernels were added to the regular rice at a 1 to 200 ratio (1 g of coated iron fortified rice kernels was added to 199 g of regular rice). The iron content in the finished iron fortified rice is 18 mg/100 g.

2.2. Rinse test for the analysis of iron retention during rinsing

To assess iron retention during rinsing, a rinse test was performed. Fifteen grams of rice were added to a 250 mL beaker. One hundred mL of deionized water was added. The sample was stirred vigorously for 30 s with a glass rod. The rice was allowed to settle for 30 s. The water was decanted. Two mL of concentrated HCl was added to 98 mL of the rinse water and used for iron analysis by inductively coupled plasma-mass spectrometry (ICP) as described below. All reagents were of analytical grade.

2.3. Rice cooking

Fortified rice cooking was performed at two different locations, in Baton Rouge, LA and in Kampala, Uganda. Rice cooking in Baton Rouge was performed as follows. All cooking methods used 100 g rice samples in triplicates and treated as described below. Sample 1 was used as control uncooked iron-fortified rice. Sample 2 was cooked at boiling temperature (BT) for 20 min in 200 mL water until fully cooked and no water remained (absorption method) without rinsing and referred to as cooked rice (C). Sample 3 was rinsed with 500 mL of water, the rinsing water was saved and the rice cooked at BT for 20 min with 200 mL water with absorption method. This sample was referred to as rinsed and cooked rice (RC). Sample 4 was fried for 1 min in 1 tablespoon of corn oil and cooked at BT for 20 min without rinsing with absorption methods. Sample 4 was referred to as fried and cooked rice (FC). Sample 5 was cooked at BT for 20 min with 500 mL water and referred to as cooked in excess water sample (CW). After 20 min, the remaining water was removed and saved for iron analysis. Sample 6 was soaked for 1 h in 500 mL of water and cooked at BT for 20 min. The excess water was removed and saved for iron analysis. This sample was referred to as soaked and cooked in excess water sample (SWC).

Fortified rice cooking in Kampala, Uganda was also prepared in triplicates and performed in two ways as follows. Approach 1. The rice to water ratio was 1:2. The cooking time of 100 g of rice was about 30 min on low heat after boiling. This was done in the Food Science Department Lab at Makerere University and referred as to rinsed and cooked in Uganda (C-Uganda). Approach 2. Fortified rice samples (500 g triplicates) were given to ten local households. Women in the ten households prepared rice by following their own customs without any control, mainly by boiling in open or covered pans over firewood flame. As the rice boiled, some of the water initially added foamed and poured out of the pan. In many of the cases, more water was added until the rice was fully cooked. The cooking time could be around 30 min or longer. The ratio of rice to water at the beginning of the cooking varied from 1:1 to 1:2.5. The additional amount of water added varied widely and was mainly based on amount required to get the rice well cooked. These various household cooking methods were reported to the researchers of this Uganda study and were referred to as traditional cooking method (TC-Uganda). Cooked rice samples were collected by Makerere University researchers for iron retention analysis.

In both approaches used in Uganda, cooking was mainly done on charcoal stoves, and it was difficult to estimate the cooking temperature. The cooking time varied according to the temperature of the stove in the Approach 2 (see Table 1).

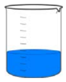
2.4. Iron analysis in cooked rice samples

To assess iron retention, uncooked control and cooked rice samples were lyophilized, ground into powder, passed through a 20 mesh US Standard screen (0.841 mm), mixed well, and 2 g samples were added to 5 mL of 5% nitric acid. The samples were mixed well, allowed to settle and the supernatant was filtered through 0.22 μm filter. The mineral analysis was performed using inductively coupled plasma-optic emission spectroscopy (ARCOS, Spectro, Germany for LSU, Baton Rouge, LA, USA and Perkin Elmer 2380 at Makerere University, Kampala, Uganda). All reagents were of analytical grade.

2.5. Clinical study

Originally 20 women with iron deficiency determined by a serum ferritin (Siemens Immulite 2000) and/or a serum iron value (Beckman Coulter DXC600) below the lower limits of normal for the local laboratory (5 ng/mL and 40 μg/mL, respectively) but otherwise healthy

Table 1
Cooking methods used in this study: 1. unfortified uncooked control (UUC); 2. cooked (C); 3. rinsed and cooked (RC); 4. fried and cooked (FC); 5. cooked with extra water (CW); 6. soaked and cooked with excess water (SCW). Two Uganda cooking methods: 7. and 8; two traditional cooking methods (TC).

 Rice	 Water	 Cook	 Cook	 Rinse	 Fry	 Soak	Description
1. 100 g							UUC
2. 100 g	200 mL	20 min					C
3. 100 g	200 mL	20 min		500 mL			RC
4. 100 g		20 min			1 min		FC
5. 100 g	500 mL	20 min					CW
6. 100 g	200 mL	20 min				500 mL	SWC
7.100 g	118–296 mL		30 min				TC-Uganda
8. 500 g	1183 mL or more	> 30 min					TC-Uganda

between the ages of 18 and 50 years of age were to be recruited from Baton Rouge, LA area and randomized to receive 100 g of rice (0.75 cups of cooked rice in two servings) per day in a 1:1 ratio of fortified rice (18 mg iron/100 g rice) or un-fortified rice (0.5 mg iron/100 g rice) for 2 weeks. The rice dishes were prepared in the Pennington metabolic kitchen and dispensed frozen in an insulated chest to the subjects weekly in lots of 14 with instructions to heat and consume one rice dish twice a day. The subjects were asked to return the dishes with any remaining food with the insulated chest each week. Compliance was measured by counting the empty containers. Whether the rice dishes were made with fortified or unfortified rice was blinded to the investigators and the study participants, since the rinse did not alter the appearance or taste of the rice. Women who were pregnant, nursing, taking an iron supplement or taking a chronic medication that had not been stable for 1 month or longer were excluded. Although subjects were initially recruited from a study of women with irregular menses, recruitment eventually slowed. The study was halted due to slow recruitment, the blind was broken and analysis performed after randomization of 17 subjects and completion of 15. In addition to the measuring serum iron and ferritin at baseline and 2 weeks, hemoglobin, hematocrit, mean corpuscular volume, red blood cell number, reticulocytes (Beckman Coulter Unicel DxH 800) and transferrin (Beckman Coulter Immage 800) were also measured.

2.6. Statistical analysis

Differences between the iron content in control rice and rice cooked using different cooking methods were calculated using ANOVA with a p-value < 0.05 being considered statistically significant. Categorical data like race was analyzed by the chi squared test. The normally distributed data like the iron-associated laboratory values were analyzed by *t*-test and an alpha of 0.05 was considered to be statistically significant. This was a pilot study to power a definitive study and a power analysis was not performed.

3. Results

3.1. Iron retention

Rice is typically not a good source of iron. Fortification enhanced the iron content of rice without changing the color or taste. The results of iron retention in iron-fortified rice samples after cooking using the different cooking methods mentioned above compared with uncooked iron-fortified rice sample are presented in Fig. 1.

For rice samples cooked without rinsing and at two different locations (Baton Rouge and Kampala), there was no significant difference in iron retention. These rice samples retained > 80% of iron after cooking. There was no significant difference in iron retention between cooked and rinsed and cooked rice. Similarly, the difference between

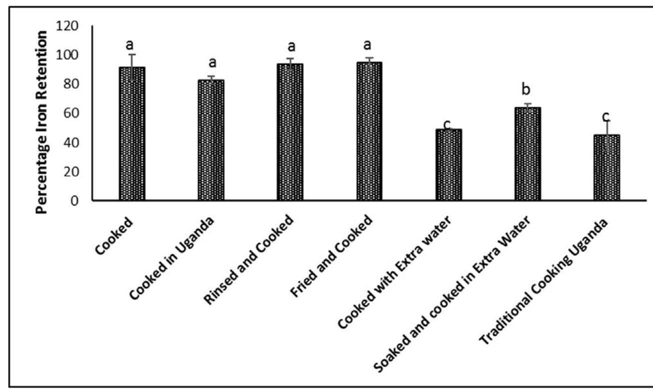


Fig. 1. Iron retention in iron-fortified rice cooked using different cooking techniques. Different letters indicate statistical significance ($p < 0.05$).

fried and cooked and cooked rice was not significant. However, there was significant difference in iron retention between cooked and cooked in extra water. There was also significant difference between cooked in extra water and soaked and cooked in extra water. The iron retention of cooked in extra water was about 50% while that of soaked and cooked with extra water was about 67%. When rice was cooked using the Ugandan traditional way where excessive water is added to rice and the rice is cooked while water is spilling over the pot, iron retention was < 45%. These results suggest that significant amount of iron was lost with discarded or spilled cooking water. For better iron retention, cooking rice by absorbing cooking water is the best cooking approach.

3.2. Clinical study

This trial was approved by the Pennington Biomedical Research Center IRB, all participants signed an informed consent and the trial was registered on www.clinicaltrials.gov (NCT01658488). Seventeen subjects were randomized to the study and 15 completed. One subject changed her mind about participating in the trial. Another subject did not return and could not be contacted. Of the 15 subjects that completed the study, 9 were in the iron-supplemented group and 6 were in the un-supplemented group (Fig. 2). All subjects in the trial had at least one of the following tests at or below the normal limits at screening: ferritin (6 of 15), iron (7 of 15), hemoglobin (12 of 15) or hematocrit (10 of 15).

All subjects were menstruating women and the two groups were well-matched. The racial distribution, age, weight, body mass index (BMI), systolic blood pressure, diastolic blood pressure and resting heart rate can be seen in Table 2.

Adverse events included intermittent mild palpitations, mild fatigue and mild insomnia that were ongoing at the end of the study and all of which were in the un-supplemented group. An episode of severe

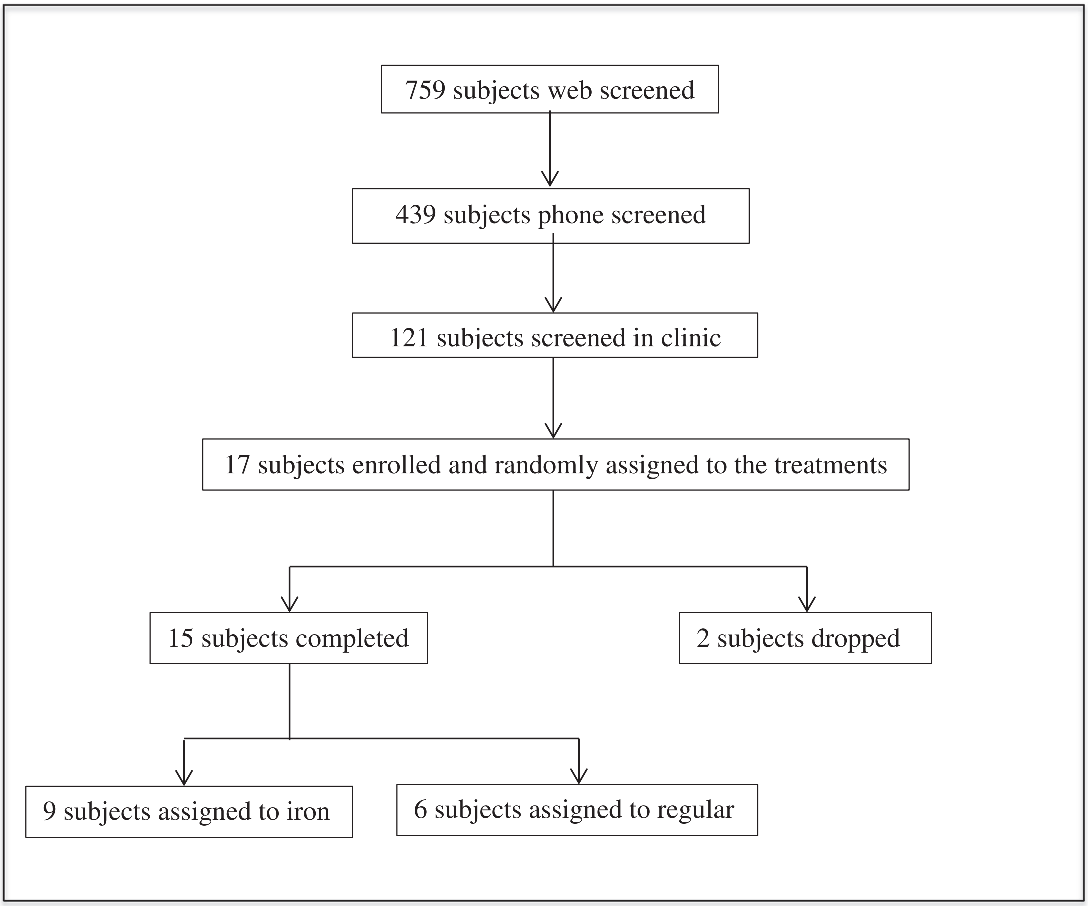


Fig. 2. Consort diagram showing the disposition of the subjects that screened and participated in the study.

Table 2
Demographics of the participants in the trial.

Variable	Iron rice	Regular rice	p-Value
Number completed	9	6	
Gender			N/A
Female	9	6	
Male	0	0	
Race			0.8557
White	4	3	
Black or African American	3	2	
Asian	1	1	
Other	1	0	
Age (years)	28.3	30.20	0.6676
SD	6.52	9.74	
Weight (kg)	69.72	62.72	0.4322
SD	18.86	11.39	
Body Mass Index (kg/m^2)	25.61	23.63	0.5291
SD	6.90	3.36	
SBP (mm Hg)	112.67	115.67	0.6697
SD	13.92	11.27	
DBP (mm Hg)	74.22	76.00	0.6730
SD	7.96	7.56	
Resting HR (bpm)	69.1	65.3	0.2837
SD	6.17	6.77	

migraine headache and an episode of mild sinus congestion resolved during the study, both of which were in the un-supplemented group. Three study participants in the iron supplemented group were on stable doses of medication. One was on Amlodipine 5 mg/d and Hydrochlorothiazide 25 mg/d, another took a birth control pill and eye drops containing ciprofloxacin and prednisolone acetate one drop four times a day and the third used an albuterol inhaler and an antihistamine-decongestant twice a day. One subject in the non-supplemented group took amlodipine 5 mg/d and metoprolol 25 mg/d. The baseline hemoglobin, hematocrit, ferritin, iron and their standard deviations were 10.6 ± 1.6 g, 33.7 ± 4.1%, 7.36 ± 7.87 ng/mL and 33.5 ± 18.5 μg/dL, respectively. Subjects in the iron fortified group had a statistically significant increase compared to placebo in hemoglobin (0.82 g, $p = 0.0035$), hematocrit (1.83%, $p = 0.0248$) and a statistical trend toward an increase in ferritin (2.52 ng/mL, $p = 0.0804$). There were directional differences between the iron-supplemented and non-supplemented groups that favored the supplemented group in other aspects of iron metabolism (Table 3).

Calculating from the height and weight of the women in the trial and using the data from Brown et al., the red blood cell volume was approximately 1.5 L [20]. Since the amount of iron is 3.47 mg/g of hemoglobin and the increase in hemoglobin above control was 0.82 g, the approximate amount of iron absorbed from the 14 days of 18 mg/d of iron fortification of the rice was 2.845 mg. This means that approximately 1.13% of the iron in the rice was absorbed and converted to hemoglobin.

4. Discussion

The cooking method did not affect iron retention when no extra water was involved, but extra water decreased the retained iron. This suggests that iron-fortified rice prepared by the coating technology can be used by different cultures that use the cooking method mentioned above even with washing, but that extra water should be minimized. Since these methods represent the majority of the world cultures, iron-fortified rice with rinse resistance can be offered to populations in Sub-Saharan Africa and Southeast Asia where iron deficiency is pronounced

Table 3
Change in iron metabolism.

Variable	Baseline value/normal	Iron rice	Regular rice	p-Value
Number completed		9	6	
Hemoglobin (HGB) g/dL				
W2-W0	10.7 ± 1.6	0.52	−0.30	0.0035***
SD	nl. 12–16 g/dL	0.51	0.27	
Hematocrit (HCT) %				
W2-W0	33.3 ± 4/1	1.13	−0.72	0.0248*
SD	nl. 37–47%	1.41	1.33	
Red blood cell # (RBC) 10ˆ6				
W2-W0	4.3 ± 0.4	0.09	−0.09	0.1340
SD	nl. 3.8–5.4 × 10^6 cells/μL	0.25	0.11	
Reticulocytes (RETIC) %				
W2-W0	1.2 ± 0.6	0.25	−0.02	0.2681
SD	nl. 0.9–2.3%	0.53	0.26	
Iron (FE) μg/dL				
W2-W0	33.5 ± 18.5	8.44	3.17	0.6574
SD	nl. 40–150 μg/dL	25.20	15.79	
Transferin (TRF) mg/dL				
W2-W0	340.9 ± 47.8	−6.11	−5.83	0.9773
SD	nl. 202–336 mg/dL	22.14	8.77	
FE/TRF μg/dL/ mg/dL				
W2-W0	0.098	−0.35	−0.26	0.9527
SD	nl. 0.25–0.35 (25–35%)	1.97	3.70	
Ferritin (FERR) ng/mL				
W2-W0	7.4 ± 7.9	2.50	−0.02	0.0804
SD	nl. 5–148 ng/mL	3.11	1.01	
MCV fL				
W2-W0	78.2 ± 10.1	0.91	−0.27	0.1090
SD	nl. 81–99 fL	1.20	1.44	

Values are difference of means and standard deviation (SD).
* $p < 0.05$.
*** $p < 0.005$.

with the limitation that iron retention will be less in places where rice is cooked with extra water. Fortunately, the concentration of the rinse can be adjusted to the cooking method of a region without altering taste or appearance to compensate for a reduction in the iron fortification.

Retention of five micronutrients including vitamin A, iron, zinc, folic acid, and vitamin B12 in fortified rice was investigated using different cooking methods and three different methods of fortification, hot extrusion, cold extrusion and coating [21]. Micronutrient retention was between 75% and 100% regardless of fortification method or the cooking method which included cooking with or without washing, washing before cooking, cooking in excess water, and frying. This study also concluded that rice fortification by coating technology was not inferior to other fortification methods such as extrusion.

Although this study was terminated early due to recruiting problems, it demonstrated with statistical significance that an iron-fortified rice delivering 18 mg of iron per day increased hemoglobin and hematocrit compared to the un-fortified rice containing 0.5 mg of iron per day. There were no adverse events in the supplemented group during the trial, demonstrating that the iron-fortified rice was well tolerated. Thus, this study demonstrates that an iron supplement applied in a fortified rice format which withstands cooking is capable of treating iron deficiency anemia in women.

Wheat flour has been the typical vehicle for iron supplementation, since iron can be added without having impact on the food's color or taste. Rice, however, is the staple food for nearly half of the world's population. In fact, rice accounts for at least one-third of the daily caloric intake (200 g or more per day) in 17 countries in Asia and the Pacific, 6 countries in sub-Saharan Africa, a country in Latin America and one in the Caribbean [22]. Although iron supplementation has been attempted using fish sauce in Vietnam, rice appears to be an underutilized vehicle to deliver iron in countries that have a rice-based diet [22]. The method of fortifying rice with iron used in this study applies a concentrated iron compound, ferrous sulfate, to the rice through an advanced coating technology. The resulting iron fortified kernels were then added to the regular rice at a 1 to 200 ration to form the finished iron fortified rice that does not change the color or taste of the fortified rice and can be washed before cooking. Iron fortified rice using this coating technology has been tested in a pilot scale commercialization effort in the Philippines, and anemia was decreased from 17.5% to 12.8% in children [23]. Our present study, however, is the first double-blinded, controlled clinical trial testing the effect of this iron supplementation method to treat iron-deficiency anemia. Iron supplementation using this method adds 2% to 5% to the cost of the rice [22].

This demonstration of safety and efficacy of iron-fortified rice to treat iron deficiency anemia in our trial justifies the introduction of this type of iron supplementation into countries with a high prevalence of iron deficiency and a rice-based diet. A safe and inexpensive method of introducing iron supplementation into food in these developing countries should help to improve the quality of life of the women with iron deficiency by reversing the fatigue and apathy that often accompanies anemia. This rice fortification with iron should also reduce the incidence of pre-term, low birth weight babies with a low APGAR scores and may also improve the brain development of these babies, something that could improve their quality of life over their entire lifetime.

Transparency document

The http://dx.doi.org/10.1016/j.bbacli.2017.09.001 associated with this article can be found, in online version.

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