Iron-Biofortified Rice Improves the Iron Stores of Nonanemic Filipino Women\textsuperscript{1,2}

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ABSTRACT Iron deficiency is endemic in much of the world, and food system–based approaches to eradication may be viable with new plant breeding approaches to increase the micronutrient content in staple crops. It is thought that conventional plant breeding approaches provide varieties of rice that have 400–500% higher iron contents than varieties commonly consumed in much of Asia. The efficacy of consuming high-iron rice was tested during a 9-mo feeding trial with a double-blind dietary intervention in 192 religious sisters living in 10 convents around metro Manila, the Philippines. Subjects were randomly assigned to consume either high-iron rice (3.21 mg/kg Fe) or a local variety of control rice (0.57 mg/kg Fe), and daily food consumption was monitored. The high-iron rice contributed 1.79 mg Fe/d to the diet in contrast to 0.37 mg Fe/d from the control rice. The 17% difference in total dietary iron consumption compared with controls (10.16 ± 1.06 vs. 8.44 ± 1.82 mg/d) resulted in a modest increase in serum ferritin (P = 0.10) and total body iron (P = 0.06) and no increase in hemoglobin (P = 0.59). However, the response was greater in nonanemic subjects for ferritin (P = 0.02) and body iron (P = 0.05), representing a 20% increase after controlling for baseline values and daily rice consumption. The greatest improvements in iron status were seen in those nonanemic women who had the lowest baseline iron status and in those who consumed the most iron from rice. Consumption of biofortified rice, without any other changes in diet, is efficacious in improving iron stores of women with iron-poor diets in the developing world.


KEY WORDS: ● biofortification ● iron ● ferritin ● rice ● Philippines

Iron deficiency is the most prevalent nutritional deficiency in the world. The Fourth Report of the World Nutrition Situation issued by the United Nations estimates that 3.5 billion people in developing countries are iron deficient based on levels of blood hemoglobin (Hb)\textsuperscript{7} that reflect anemia (1). Women of reproductive age are among the most vulnerable to iron deficiency, with anemia prevalence estimates of 44% in developing countries. In the Philippines, where this study was conducted, 32% of women between 15 and 49 y of age are anemic and as many as 75% may be iron deficient (2,3). In developing countries, common strategies employed to improve iron nutritional status include public education to improve diets, supplementation with iron pills, capsules, or tonics, and iron fortification of the food supply. However, these all have limitations that influence their effectiveness or long-term sustainability in countries in which resources are scarce (4).

A recent approach that has the potential to overcome many of the shortcomings of the conventional strategies is biofortification of staple foods. This approach involves the development of new varieties of staple food crops that are selectively bred to enhance specific nutritional qualities, such as the levels of biologically available iron. Varieties of such crops as rice, maize, wheat, beans, and cassava were developed with enhanced levels of specific micronutrients such as iron, zinc and \textit{β}-carotene through the HarvestPlus project and its predecessors (5). One of the first crops to be biofortified under this initiative was rice, which is consumed as a staple food by 3 billion people, many of them among the world’s poorest (6). The International Rice Research Institute (IRRI) recently developed a variety of rice for experimental use that has ~400–500% more iron, after processing and cooking, than varieties that are commercially available in the Philippines (7). The Philippine diet is based on rice as a staple, accounting for 41% of daily dietary energy intake and 33% of per capita food consumption (2). This high level of rice consumption

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\textsuperscript{2} Supplemental Table 1 is available as Online Supporting Material with the online posting of this paper at www.nutrition.org.

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\textsuperscript{5} Abbreviations used: AGP, α1 acid glycoprotein; C4, control rice; EAR, estimated average requirement; FA, field assistant; Ft, ferritin; Hb, hemoglobin; Hct, hematocrit; IRRI8144, high-iron rice; IRRI, International Rice Research Institute; pFe, plasma iron; RDA, Recommended Dietary Allowance; TIR, transferrin receptor; TIBC, total iron-binding capacity.
SUBJECTS AND METHODS

Research setting

The study was carried out among young women in training to become religious sisters of the Roman Catholic Church. Subjects were chosen from convents located near Manila, the Philippines. We selected this population because they represent a segment of the population at high risk for iron deficiency, they consume large quantities of rice, and their meals are prepared in a common kitchen and consumed in a common dining area. Their diet is typical of those in the Philippines and their activity pattern similar to that of lay people. Also, long-term observation is possible in this setting and, based on a pilot study, the extensive documentation of dietary intake required for such a study was well tolerated by this population.

Study design

This study was a prospective, randomized, controlled, double-blind, longitudinal (9 mo), intervention trial involving 317 women. The study had 2 arms: low-iron rice and high-iron rice, which were the exclusive sources of rice consumed for 9 mo. Randomization was done according to 2 strata based on ferritin (Fr) and Hb concentrations. At each of 10 research sites women, who had a Hb concentration < 120 g/L or a Fr concentration < 20 mg/L at baseline, were randomly allocated to 1 of 4 rice groups, followed by randomization of all remaining women at the site. For the duration of the study, all of the participants, as well as the field workers, were unaware of the subjects’ rice group assignments. Each rice type was randomly assigned 2 different colors in each convent. Colors used were green, blue, gray, and cream. Rice was delivered in bins of these colors, cooked in containers with these matching colors, and served in bowls of the corresponding color.

We assumed that in a high percentage of subjects, anemia was caused by factors other than iron deficiency; thus, their Hb would not be expected to increase in response to increases in iron in the diet. However, increases in serum Ft could be expected in response to increases in dietary iron in subjects whose Hb synthesis was adequate (i.e., not anemic) but whose body iron stores were depleted. Therefore, at the outset, we proposed to study serum Ft, and not Hb, as the major response indicator of iron status in nonanemic women. For the study, all of the participants, as well as the field workers, were unaware of the subjects’ rice group assignments. Each rice type was randomly assigned 2 different colors in each convent. Colors used were green, blue, gray, and cream. Rice was delivered in bins of these colors, cooked in containers with these matching colors, and served in bowls of the corresponding color.

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Recruitment and screening

Convents were identified through the Philippine Roman Catholic Directory and contacted about participation. There are 286 convents registered with this directory, with 56 convents in the greater Manila area (Fig. 1); 25 of these convents, identified as potential research sites based on size and accessibility, were sent a formal letter explaining the study and requesting participation. A total of 15 convents agreed to participate. Interested religious sisters from within the convents were given a full explanation of the study and scheduled for screening. Written informed consent was obtained from each subject. Screening involved the assessment of Hb by HemoCue with blood obtained from a finger puncture, administration of a health history questionnaire, and a brief physical exam. Subjects were excluded from analysis if they had a history of gastrointestinal or hematological disorders or if they were taking medications that could interfere with hematopoiesis or dietary iron absorption. Subjects also had to be between 18 and 45 y old. After screening, 10 convents were chosen to participate in the 9-mo feeding trial. The other 5 convents were excluded because they did not have enough eligible women to justify the expense of feeding and monitoring for 9 mo.

Even though 288 subjects were known at baseline to be qualified for the study, we elected to randomize treatment to all 317 sisters in the 10 eligible convents. Individual subjects were randomly assigned to consume either the high-iron rice (IR68144) or the control rice (C4). The 29 ineligible subjects were excluded because of severe anemia (Hb < 105 g/L), current or previous health history, or stated uncertainty about their ability to participate for the full 9-mo trial. Of the 288 remaining subjects, 96 had insufficient data to permit statistical analysis, including 58 subjects who dropped out either because they transferred to a nonparticipating convent or discontinued religious training, 11 who consumed >1000 mg supplemental iron during the study, 16 with α-1 acid glycoprotein (AGP) values > 30 g/L indicating acute infection, and 11 who had missing or compromised blood data. The final sample for statistical analysis included 192 subjects, 92 in the high-iron group and 100 in the control group.

The procedures were reviewed and approved by the Institutional Review Boards for use of human subjects in research at The Pennsylvania State University, Cornell University, and the University of the Philippines, and were in accordance with the Helsinki Declaration of 1975 as revised in 1983.

Deworming

Before screening and again after 4–5 mo, all women in each of the participating convents were treated for intestinal parasites. Even though the prevalence of intestinal parasites was suspected to be very low, deworming ensured that no subject would have reduced ability to respond to additional dietary iron due to parasitic infection. Albenda-
zole (Smith Kline Beecham) was given to each subject daily (200 mg) for 3 d, after which a fecal sample was collected from each woman. Fecal samples were tested for roundworm (Ascaris lumbricoides), hookworm (Necator americanus, Ancylostoma duodenale), whipworm (Trichuris trichiura), and tapeworm (Diphyllobothrium latum) using the Kato thick smear technique (9). After the initial treatment, 9 subjects tested positive for intestinal parasites and were given a second course of Albendazole, after which no subjects remained infected.

Iron sources

Two types of rice were used in this study. One type was a commercially available, low-iron rice (C4) used as the control rice. The other type, IR86144–2B–2–3–3, was developed at the IRRI for experimental use, and is a high-yielding, high-iron rice (7). Analysis of milled samples immediately after harvest indicated an ∼500% difference in iron content between the 2 varieties (1.8 vs. 9.8 mg/kg, dry weight). In 3 different locations throughout the Philippines (2 sites in Mindanao and 1 close to Los Baños), >40 tons of this rice was grown specifically for this study. Although loss of iron occurs during milling, IR86144 was shown to retain more of its iron content after milling than many other rice varieties (7), and careful control of the milling process ensured less iron loss than might be expected from commercial mills. Before using the IR86144 variety in the feeding trial, sensory evaluation was undertaken in 2 nonintervention convents to ensure acceptability following the protocol employed by the Philippine Rice Varietal Improvement Program (10). A consumer panel of 60 women aged 18–50 y assessed the qualities of rice samples prepared following standardized procedures. Cooked rice samples were presented to each panelist in coded containers according to a randomized design. The panelists evaluated the cooked rice samples in terms of aroma, color, taste, tenderness and texture, and gloss. A standardized scorecard was used and overall consumer acceptability and preference for each of the samples were computed. The acceptability level of the high-iron rice was 90% compared with 80% for a commercially available rice variety similar to the one chosen as the control rice. The 2 types of rice were preferred equally by this consumer panel.

Study protocol

The following procedures were followed to implement the research protocol and ensure quality of data collection.

Field assistants. Each convent housed a female member of the research team [Field Assistant (FA)] for the duration of the study. The FAs were all recent college graduates from the Institute of Human Nutrition and Food at the University of the Philippines, Los Baños. These FAs underwent 1 wk of training in rice preparation and cooking, weighed dietary intakes, administration of questionnaires, administration of deworming medication, collection of the fecal samples, and how to adapt to life in the convents. The FAs were in the convent at all times and collected all of the daily data.

Delivery of rice. Rice was stored at IRRI and delivered to the convents once every 2 wk. For each convent, the 2 rice varieties were randomly allocated into plastic bins of 4 different colors before delivery to the convents; 2 bins contained the control rice and 2 bins contained the high-iron rice. The color coding was retained for the duration of the study for each convent, and the resident FA did not know the color code assignments for her convent.

Rice bowl assignment. Each sister within the convent was assigned a bowl color (corresponding to the randomization scheme) and instructed to eat rice from that bowl color exclusively for the duration of the study. All women living in the convents and consuming meals in the communal dining room were assigned a specific bowl color, even if not a qualified study participant.

Cooking/sampling of rice. Rice cooks (one for each assigned color of rice container) were provided to each of the convents for a total of 4 rice cookers per convent. The FA in each convent was responsible for cooking the rice for every meal. To monitor the iron content of the rice, every other week, the FA collected 3 representative samples of cooked rice from each of the rice cooks. These samples (188 samples/convent) were placed into acid-washed Petri dishes and kept in the refrigerator until they were transported to IRRI when the next batch of rice was delivered.

Weighed food intake. Meals in the convent were served family style with all of the women eating their meals at the same time. A common kitchen prepared all of the food and the women were allowed to eat as much as they desired. The only component of the meal that was controlled was the type of rice consumed by each woman. Every portion of rice consumed at all meals was weighed. In addition, weighed intakes of the entire diet were collected from each of the study participants on 3 random days (including 1 weekend day) every 2 wk for a total of 56 daily food intake measurements from each woman. Conversion of weighed food items to nutrients was made using Philippine food composition tables (11).

Collection of weekly forms. A self-administered weekly activities form was collected from each woman to determine general level of physical activity and to record menstrual cycle data, incidence of illnesses, and medication/supplement use.

Blood samples

Blood samples were collected at baseline, midpoint (4.5 mo), and endpoint (9 mo) using blood from an antecubital vein. This blood was analyzed for the following characteristics: complete blood counts including Hb and hematocrit (Hct) (Coulter Model-S), plasma iron (pFe) and total iron-binding capacity (TIBC), serum Fe (Diagnostic Products), soluble transferrin receptor (TfR) (12), AGP (Kent Laboratories), serum folate, and vitamin B-12 (ICN Pharmaceuticals), serum zinc by atomic absorption spectrophotometry (13), and serum retinol by HPLC with a modification described by Gieng et al. (14). Plasma transferrin saturation was calculated as the ratio of pFe/TIBC × 100 and body iron was calculated using the method of Cook et al. (15). Additionally, Hb was assessed at 2.5 mo and 7.5 mo using a HemoCue to ensure that the Hb concentrations of the women had not dropped to a level <105 g/L. The 17 women diagnosed as iron deficient were given supplemental iron (30 mg elemental iron/d for 4 wk), continued with their assigned rice, but were excluded from the data analyses.

Statistics

All data were analyzed using the SAS system for Windows version 8e (SAS Institute). Iron status measures were evaluated for normality against a standard normal distribution by using the Shapiro-Wilk test. Natural log transformation of Ft, TfR, folate, and vitamin B-12 values was required to normalize these measures for statistical testing. Standard descriptive statistics were determined by type of rice consumed (control or high-iron). A t test was performed to test for treatment group differences at baseline, and for differences between participants and nonparticipants. Analysis of covariance (ANCOVA) with mixed models was used to test for differences in nutritional status indicators between rice groups (fixed effect) at both baseline and endpoint. At baseline, convent identifier codes were included as a random effect variable in the model. When testing for rice group differences in Hb, Ft, and body iron at endpoint, convent was a random effect, whereas baseline nutritional status, daily rice intake, and BMI were included as covariates. Differences were considered significant at P < 0.05. Mixed models were also run to test for statistical interactions of rice group by convent and rice group by baseline values.

RESULTS

Baseline and descriptive statistics. Descriptive statistics for the 192 sisters with complete data and those with incomplete data (Table 1) indicate that age, body size, time in the convent, and baseline iron status did not differ between those who had complete data and those who did not. Of the subjects with incomplete data, 55 were originally assigned to the high-iron rice group and 41 were assigned to the control group. Background measures did not differ by rice treatment group (data not shown). The subjects in this study were 2.5 cm taller
and 2.5 kg lighter than the general Philippine population of women of similar age (2).

The nutritional profile for the 192 eligible subjects (Table 2) analyzed by the type of rice consumed during the trial indicated that the rice groups did not differ for any of the anthropometry. The dietary data are based on 56 d of weighed food intakes over 9 mo and daily weighings of all rice consumed. On average, 53% of daily food energy was consumed from rice. Among the macronutrients, the groups differed only in carbohydrate intake, which is explained by the additional 79 g of rice consumed daily by the control group ($P < 0.001$). The 2 rice varieties differed significantly in the concentration of iron ($P < 0.001$). The high-iron rice contained 3.21 mg/kg iron, whereas the control rice contained 0.57 mg/kg, measured from multiple samples collected after cooking from each treatment group throughout the study period. The 1.42 mg/d additional iron from biofortified rice accounted for 83% of the difference in daily iron intake between the high-iron and control groups ($P < 0.001$). Although meat consumption was low in this population, the high-iron group did consume 0.11 mg/d more iron from meat ($P = 0.015$) than the controls.

The unadjusted mean values for various blood indicators of micronutrient status (zinc, folate, vitamin B-12, and vitamin A) for these 192 subjects did not differ between groups at baseline, nor were there significant changes in these indicators within treatment groups over 9 mo (data not shown; see Supplemental Table 1). At baseline, 31% of the control group (n = 31) and 25% of the high-iron group (n = 23) were anemic (Hb < 120 g/L). Iron deficiency based on Ft values < 12 µg/L was present in 32% of the control and 25% of the high-iron subjects at baseline, whereas 40 subjects in each group (40% and 43%, respectively) had Ft values < 20 µg/L. Concentrations of other micronutrients were moderate to low for the 192 subjects: folate (20% < 2 µg/d), vitamin B-12 (6% < 250 ng/L), and zinc (23% < 500 g/L). No woman had a serum retinol concentration < 200 g/L. Means for these nutritional status indicators did not differ between treatment groups at the endpoint as tested by ANCOVA after controlling for covariates. The interaction between treatment group and Hb status (anemic vs. nonanemic) was also tested to determine whether there was a differential response as a function of baseline anemia status. There was no rice treatment effect on Hb values, either for the whole sample ($P = 0.59$) or when baseline anemia status was tested in an interaction with treatment group ($P = 0.85$). For the analysis of final Ft values, the natural logarithm (ln) of Ft was used to normalize the distribution. Ft tended to differ between groups ($P = 0.10$).

Further analysis considered anemic and nonanemic subjects separately and tested the statistical interaction between baseline anemia status and rice treatment group for a differential effect of the high-iron rice on final Ft. The interaction tended to be significant ($P = 0.07$). The least-square means generated from this analysis indicate a significantly greater final Ft in the high-iron compared with the control group for the nonanemic women ($t = 2.41, P = 0.02$) and no treatment group difference for the anemic women ($t = 0.59, P = 0.55$). To assess whether the effect on Ft could be extended to a more global measure of iron status, the same analysis was performed on the calculated body iron values. The high-iron group tended to have more body iron at the end of the feeding trial than the control group (0.73 mg/kg difference, $t = 1.92, P = 0.06$) after controlling for baseline values and rice intake. The test for a modifying effect of baseline anemia status indicated that nearly all of the effect in the total sample was due to the effect in nonanemic women, with the high-iron rice group having 0.89 mg/kg more body iron than the control group ($t = 1.99, P = 0.05$). There was no rice treatment effect on body iron in the anemic subjects ($t = 0.14, P = 0.89$).

Age, measures of body size, and time in the convey for the nonanemic subjects did not differ from the values for the total sample of eligible subjects reported in Table 1. The treatment groups did not differ in any of these measures. Dietary intakes of energy and iron for nonanemic subjects were essentially the same as those reported in Table 2 for the entire sample of eligible women. For the nonanemic subjects, the daily consumption of control rice was 70 g greater than that of high-iron rice, and consumption of both types of rice over the 9-mo.
TABLE 3

<table>
<thead>
<tr>
<th>Iron status indicator</th>
<th>Control rice (n = 100)</th>
<th>High-iron rice (n = 92)</th>
<th>Covariates and interactions (P-value)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ferritin, In-μg/L</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total sample</td>
<td>3.12 ± 0.63</td>
<td>3.26 ± 0.65</td>
<td></td>
</tr>
<tr>
<td>Anemic</td>
<td>3.27 ± 0.10</td>
<td>3.18 ± 0.12</td>
<td></td>
</tr>
<tr>
<td>Nonanemic</td>
<td>3.06 ± 0.07</td>
<td>3.28 ± 0.07*</td>
<td></td>
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</table>

Body iron, μmol/kg

| Total sample          | 78.7 ± 6.6             | 91.7 ± 6.8              |                                     |
| Anemic                | 83.2 ± 9.6             | 85.0 ± 11.1             |                                     |
| Nonanemic             | 77.6 ± 7.1             | 93.5 ± 7.1*             |                                     |

Hemoglobin, g/L

| Total sample          | 126 ± 1.8              | 127 ± 1.9               |                                     |
| Anemic                | 115 ± 2.6              | 118 ± 2.8               |                                     |
| Nonanemic             | 130 ± 1.7              | 131 ± 1.7               |                                     |

1 Values are least square means ± SE after adjusting for covariates. * Different from the control group, P < 0.05 (t test).
2 Sample size for the control group: anemic subjects = 31, nonanemic = 69; Sample size for high-iron group: anemic subjects = 23, nonanemic = 69.

feeding trial remained reasonably constant. Relative to the control group, the high-iron group consumed 1.41 mg/d more iron from rice and 20% more iron from the total diet (both P < 0.001). Dietary iron intake of this population is low, with ~8.0 mg/d obtained from sources other than rice (Fig. 2). This represents ~44% of the recommended dietary allowance (RDA) of 18 mg/d set by the U.S. Food and Nutrition Board for nonpregnant women of reproductive age (16). These levels of dietary iron intake suggest that ~50% of the women in the control group reached their estimated average requirement (EAR) of 8.1 mg/d set by the Food and Nutrition Board. The control rice accounts for 0.36 mg/d additional iron, or 2% of the RDA. In contrast, the 1.77 mg/d from the biofortified rice accounts for 10% of the RDA, but total iron intake was still well below the RDA.

Effects of high-iron rice on the nutritional status of nonanemic women. In an analysis of group differences in iron status indicators for the 138 nonanemic subjects, none of the indicators of nutritional status differed between the groups at baseline (Table 4). After 9 mo, the only measures that differed between groups were Ft (t = 2.58, P = 0.011) after controlling for nonenrolled (random effect), baseline Ft status (P < 0.001), and rice intake (P = 0.099). The antilogs of mean log Ft values after 9 mo were 26.0 μg/L for the control group and 31.5 μg/L for the high-iron group. The computed body iron after 9 mo was 20% greater in the high-iron group compared with the control group (4.99 vs. 6.03 mg/kg, respectively, t = 2.27, P = 0.025; after controlling for baseline body iron (Ft < 0.001, and rice intake, P = 0.47).

To test the plausibility of these results, we performed several analyses of internal consistency. Recognizing that individual response to dietary iron depends on the amount of biofortified rice consumed, we tested the relation between iron intake from rice and change in serum Ft (Fig. 3). Overall, Ft values changed over 9 mo from a decline of 61 μg/L to a gain of 55 μg/L. The control group had a very narrow range of iron ingested from rice (0.22–0.35 mg/d) and a wide range of change in Ft, with 33% experiencing a decline in Ft > 10 μg/L. The high-iron group had a wider range of iron ingested from rice (0.56–2.7 mg/d) with 13% experiencing a decline in Ft > 10 μg/L. In the high-iron group, there was a significant positive correlation between the iron intake from rice and change in serum Ft (r = 0.31, P < 0.01).

Additional analysis examined the effect of baseline total body iron content on response to biofortified rice (Fig. 4). Although the interaction between baseline body iron content and rice group was not significant (P = 0.21) in a model that included nonenrolled and the amount of rice consumed, the least-square mean values calculated at selected percentiles of baseline values indicated that the most significant effect of iron treatment was in the subjects whose initial body iron was low (at the 25th percentile); for these women, the final values were 43% greater in the high-iron group compared with the controls (P = 0.036) at the same value of baseline body iron. There was also a significant group difference at the 50th percentile (P = 0.032), but not at the 75th percentile of baseline body iron.

DISCUSSION

This study was designed to test the biological effects of consuming additional dietary iron from biofortified rice. It
employed a design that randomly assigned one of 2 rice varieties to individual women living in 10 different religious convents. The randomization was successful to the degree that all measures of nutritional status, body size, and other background characteristics did not differ between the 2 rice groups for eligible women at the start of the feeding trial. Although the study began with 288 women, the 96 subjects excluded for eligible women at the start of the feeding trial. Although ground characteristics did not differ between the 2 rice groups all measures of nutritional status, body size, and other back-

covariates. The randomization was successful to the degree that
distributions did not differ between the 2 rice groups.

The efficacy trial was conducted among religious sisters who consumed diets typical of the Philippines. These diets include relatively large amounts of rice, which account for >50% of daily dietary energy intake, and low amounts of iron due to heavy reliance on plant foods and limited meat in the diet. The subjects were chosen because they represented a segment of the population, women of reproductive age, that is at high risk of iron deficiency and therefore most likely to respond biologically to increased iron in their diets. The research was conducted in convents where the research team could exercise considerable control over the research environment, including continuous supervision and measurement of dietary intake, and assurance of compliance with the rice group allocations.

![FIGURE 3](image)

**FIGURE 3** Changes in serum Ft according to daily intake of iron from rice over 9 mo for nonanemic women who consumed control or high-iron rice. Regression line generated from analysis that controls for convent (random effect), baseline Ft (P < 0.001), total energy intake (P < 0.01), and BMI (P < 0.05). Slope for “iron from rice” is significant (t = 2.89, P = 0.005, SE = 0.05), after adjusting for covariates. The slopes do not differ for the 2 rice groups.

![TABLE 4](image)

**TABLE 4** Rice group differences for baseline and final blood values of nonanemic women who consumed control or high-iron rice

<table>
<thead>
<tr>
<th>Variable</th>
<th>Baseline</th>
<th>Final</th>
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<tbody>
<tr>
<td></td>
<td>Control</td>
<td>High-iron</td>
</tr>
<tr>
<td>Serum Ft, μg/L</td>
<td>30.6 ± 2.0</td>
<td>24.8 ± 1.1</td>
</tr>
<tr>
<td>Log Ft, ln μg/L</td>
<td>3.4 ± 0.1</td>
<td>3.2 ± 0.1</td>
</tr>
<tr>
<td>Serum TIR, mg/L</td>
<td>4.3 ± 0.2</td>
<td>4.2 ± 0.2</td>
</tr>
<tr>
<td>Body iron, μmol/kg</td>
<td>100.0 ± 8.9</td>
<td>91.1 ± 8.9</td>
</tr>
<tr>
<td>Transferrin saturation, %</td>
<td>30.2 ± 1.4</td>
<td>32.1 ± 1.1</td>
</tr>
<tr>
<td>Hb, g/L</td>
<td>128 ± 1</td>
<td>130 ± 1</td>
</tr>
<tr>
<td>Hct, vol fraction</td>
<td>0.385 ± 0.02</td>
<td>0.390 ± 0.02</td>
</tr>
<tr>
<td>Plasma</td>
<td></td>
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</tr>
<tr>
<td>Folate, nmol/L</td>
<td>6.6 ± 1.8</td>
<td>7.0 ± 2.0</td>
</tr>
<tr>
<td>Vitamin B-12, pmol/L</td>
<td>0.45 ± 0.05</td>
<td>0.46 ± 0.05</td>
</tr>
<tr>
<td>Retinol, μmol/L</td>
<td>1771 ± 47.5</td>
<td>1779 ± 49.9</td>
</tr>
<tr>
<td>Zinc, μmol/L</td>
<td>8.7 ± 0.15</td>
<td>8.6 ± 0.15</td>
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<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>High-iron</th>
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</table>

1 Values are least-square means ± SE, n = 69 for each group, from mixed model ANCOVA; the random effect is convent, the fixed effect is Control vs. High-iron group, with baseline blood value (P < 0.001) and rice consumed throughout the study (P = 0.047–0.099) as covariates. * Different from the control group, P < 0.05.

2 Value is the antilog of mean for log transformed values; SE determined as $e^{se(logX_i)}$, where $se$ = estimate standard error and $Xi$ = least square mean.

![FIGURE 4](image)

**FIGURE 4** Body iron levels in nonanemic women after 9 mo of consuming high-iron or control rice at 3 levels of baseline body iron status. Baseline values represent the 25th, 50th and 75th percentiles of the distribution for the total sample of subjects. Bars are least square means ± SE, estimated from a mixed effects regression model that tested for the fixed effect of rice type after controlling for the random effect of convent, and significant covariates of mean daily rice intake (P < 0.05) and baseline body iron (P < 0.001). * Different from the control group, P < 0.05, n = 69 for each group; NS, P = 0.05) at each level of baseline status after considering the interaction between rice group and baseline body iron status (P = 0.21). The effect of rice group was significant (P = 0.025) in a mixed model that did not include the interaction. The numbers within the bars represent the change in body iron from baseline values in μmol/kg.
These research conditions were essential because the additional iron found in the biofortified rice was a small 2.80 mg/kg improvement over commercially available rice, based on wet weight of processed rice. This additional iron intake occurred despite an 11% lower rate of rice consumption by the high-iron group. Although the preference and acceptability testing gave us some assurance that the biofortified rice would be consumed, the slightly gray color may have influenced the consumption patterns. This would bias the results away from seeing a significant treatment effect, a situation that was partially resolved by using rice intake as a covariate in the analysis reported in Table 3. The resulting iron intake of an additional 1.41 mg/d for the high-iron group represents ~8% of the 18 mg/d RDA for these subjects. The additional iron from the high-iron rice raised the mean iron intake up to 10 mg, which is ~56% of the RDA. Both the control and high-iron groups had iron-deficient diets, which are within the range of the intakes found throughout the Philippines. Overall mean iron intake in the Philippine diet, as reported from the 4th National Nutrition Survey in 1993, is only 10.1 mg Fe/d with a low of 8.5 mg/d in the Bicol region and a high of 12.1 mg/d in southern Mindanao (2). Given these research conditions, the study had to be conducted over 9 mo to allow for measurable improvements in iron status to be observed.

The choice of serum Ft as the primary indicator of iron status may seem unusual given that most iron intervention trials use Hb concentration for this purpose. Hb reflects the most abundant pool of iron in the body, and its synthesis is highly conserved under conditions of iron depletion. Hb concentration falls to body levels diagnosed as anemia only after body iron stores are nearly depleted. Therefore, low Hb reflects the most severe stage of iron deficiency and is responsive to iron treatment when other causes of anemia are not present. We anticipated multiple causes of anemia in this population. There were 54 subjects diagnosed as marginally anemic at baseline (Hb 100–119 g/L). Closer inspection revealed that 31 of these subjects had low Ft levels (~<20 μg/L), and 20 had indications that nutrients other than iron could be potential sources of the low Hb (low folate, vitamin B-12, and/or zinc). Given the possibility of multiple causes of anemia in addition to iron deficiency, analysis was performed for rice treatment effects on serum Ft and body iron as well as Hb. We chose serum Ft because it is a well-established proxy for storage iron and is not influenced by the many factors that affect Hb in this population. Because Ft levels generally increase with increased iron ingestion after anemia is corrected, we chose to measure the Ft response in nonanemic women. This decision was supported by the analysis of Hb and Ft response in the entire sample of 192 women, in which Ft levels improved marginally, whereas no improvement in Hb occurred. Further analysis showed that the Ft response was significant only in the nonanemic subjects and that Hb did not improve, even in the subjects who were initially anemic. The analysis for a Hb response in the 11 anemic subjects whose Ft was low, but whose folate, vitamin B-12, and vitamin A concentrations were normal, was not practical because only 3 of these subjects consumed the high-iron rice.

Although the increase in daily dietary iron was small, there is evidence that the observed improvement in iron status can be attributed to the iron in biofortified rice. First, we observed in the high-iron group a significant linear relationship between the amount of iron consumed from rice and the change in serum Ft over 9 mo (Fig. 3). This positive dose response suggests that for every mg of iron consumed daily from biofortified rice there would be a 13.8 μg/L increase in serum Ft after 9 mo. Second, an analysis of the concordance of absorbed iron from rice and the change in body iron content was determined, given assumptions about the percentage of absorption of iron and the calculation of the percentage of absorption times total iron consumed from rice over 270 d of the feeding trial. Assuming conservatively 10% absorption (17,18) and adjusting for body weight, the control group would have absorbed (mean ± SD) 0.20 ± 0.06 mg/kg iron over 270 d, whereas the high-iron group would have absorbed 0.99 ± 0.26 mg/kg over the same period, for a net gain of 0.79 mg/kg (14.1 μmol/kg). This compares favorably with the net increase in body iron between the 2 rice groups of 0.75 mg/kg (13.3 μmol/kg). Although these theoretical calculations support an estimated 10% efficiency in the transfer of iron from rice to body iron stores, the actual bioavailability of the IR68144 rice variety after processing and in the context of a typical Philippine diet has not been determined. Hallberg et al. (18) reported bioavailability as high as 20% for iron from rice consumed in Asian diets. However, this study did not consider the specific amounts and combinations of enhancers and inhibitors to iron absorption found in the typical Philippine diet. In vitro analysis of IR68144 suggests equivalent absorption relative to other varieties of rice and much greater relative absorption than some high-iron Vietnamese varieties (19). Although the results of the current research are encouraging, more research is warranted to determine the exact chemical form and bioavailability of biofortified rice in the typical diets consumed by various subgroups of the Philippine population.

A greater consumption of iron from meat was observed in the high-iron rice group, which could have contributed to the improvement in iron status in this group through a combination of the higher bioavailability of heme iron and the effect of enhanced iron absorption from a “meat factor” (20). However, when iron intake from meat was used as a covariate in the regression models, it was not a significant predictor of change in iron status. Also, it did not change the significance of coefficients for treatment group effects nor the group differences in least-square means reported in Tables 3 and 4, and the adjusted values shown in Figures 2 and 3.

Additional plausibility of the positive results of consuming biofortified rice is indicated by the greater response in body iron content for the women with the lower baseline body iron (Fig. 4). Women at the 25th percentile of baseline body iron would have experienced a 22 μmol/kg increase in body iron, whereas women with higher baseline body iron maintained their baseline values through 9 mo of consuming the high-iron rice. In contrast, the women consuming the control rice maintained their baseline level if it was initially low, but showed increasing loss of body iron as their baseline values increased, to the point that control women at the 75th percentile of baseline body iron actually lost 15 μmol/kg of body iron. Thus, the high-iron rice seems to have benefited those who were originally iron deficient by adding to their iron stores while preventing the loss of body iron in those who had normal or high iron stores.

The implications of this research for the general population must be considered in the context of how the current study subjects reflect the dietary and health conditions of women in developing countries. The study subjects were nominally healthy, free of intestinal parasites, consumed a generally balanced diet without experiencing periods of food insecurity, had relatively low levels of physical activity, and were not anemic. Under these conditions, consumption of the high-iron rice was able to increase iron status significantly, with the greatest effect among those who had initially poor iron status, similar to what would be seen in less privileged women in developing countries. Although this study tested the benefits
of additional iron in replenishing the iron stores of nonanemic women only, it is reasonable to assume that significant benefits would accrue to anemic women as well. We were not able to test the effects of high-iron rice consumption on Hb concentration or anemia prevalence in this sample, because very few anemic subjects could be identified as having iron deficiency as the sole cause of their anemia. However, for any individual with poor iron status and a diet that is deficient in iron, the additional iron from biofortified rice, even if a small amount, would be beneficial.

Although consumption of the high-iron rice did reduce the gap between typical iron intake and iron sufficiency based on the RDA for the United States, it did not eliminate that gap. This may be an unrealistic expectation given the limited potential for increasing iron content of rice through conventional selective breeding. However, by closing the gap between normal iron intake and sufficiency, it is likely that some women who had marginally insufficient iron intakes would have crossed the threshold to sufficiency. Based on data from the Dietary Reference Intakes for iron (16), an increase in mean iron intake of 1.41 mg/d from the 8.3 mg/d seen in the control group, would have shifted the percentage meeting their EAR from 53 to 71%. The upward shift in the distribution of iron intake also improves the possibility that other interventions will close the dietary deficiency gap further.

This feeding trial of Filipino religious sisters, to our knowledge, is the first human study to demonstrate that improvements in the iron content of a staple food through plant breeding result in a measurable improvement in nutritional status. It provides encouragement to plant breeders around the world that the breeding strategy promoted by HarvestPlus has the potential to improve the diets of the poor in developing countries. Nutritional interventions like the one in this study are important to the development of practical and sustainable strategies to reduce the levels of micronutrient malnutrition in the developing world. The rice breeding approach employed by IRRI in the Philippines resulted in increasing dietary nutrient intakes through biofortification, while maintaining acceptable crop yields and cost to the consumer, adequate nutrient bioavailability, and favorable organoleptic properties of the rice as consumed. These properties require further study to evaluate and improve the effectiveness of biofortified rice as it is incorporated into the food system of the general population.

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