Micronized ferric pyrophosphate supplied through extruded rice kernels improves body iron stores in children: a double-blind, randomized, placebo-controlled midday meal feeding trial in Indian schoolchildren1–3

Madhari S Radhika, Krishnapillai M Nair, Rachakulla Hari Kumar, Mendu Vishnuvardhana Rao, Punjal Ravinder, Chitty Gal Reddy, and Ginnela N V Brahmam

ABSTRACT

Background: Micronized ferric pyrophosphate (MFPP) in extruded rice kernels mixed in a rice-based meal could be an effective strategy for improving iron status of children in India.

Objective: The objective was to determine the impact of MFPP supplied through extruded rice kernels in a rice-based meal on iron status of children participating in the midday meal (MDM) scheme in India.

Design: The sensory characteristics of cooked rice containing MFPP in extruded rice kernels, in vitro availability, and loss of iron during cooking from a typical MDM consisting of 125 g rice (dry weight) containing 19 mg iron [fortified rice (FR); normal rice mixed with Ultra Rice (extruded kernels containing MFPP of \( \sim 3.14-\mu m \) mean particle size)] in comparison with unfortified rice (UFR) were tested. A double-blind, 8-mo, placebo-controlled trial was conducted in 5–11-y-old schoolchildren (n = 140) who were randomly assigned to receive either an FR-MDM or an UFR-MDM. Average consumption amounts of the MDM, height, weight, hemoglobin, ferritin, and C-reactive protein were measured at baseline and at 8 mo.

Results: The sensory qualities of cooked FR and UFR were similar. The in vitro iron availability from FR-MDM (1.3%) was significantly \((P < 0.05)\) lower than that from UFR-MDM (3.3%). Providing FR-MDM to the schoolchildren for 8 mo improved ferritin significantly \((P < 0.001)\), by 8.2 \(\mu g/L\). However, the increase in ferritin was similar between groups (FR: 0.99 \(\pm\) 0.10 g/dL; UFR: 1.15 \(\pm\) 0.10 g/dL), suggesting that other factors beyond additional iron intake had a large influence on ferritin concentration. The prevalence of iron deficiency decreased significantly \((P < 0.05)\) in the FR group (33–14%) and increased marginally in the UFR group (31–37%). The prevalence of anemia and iron deficiency anemia was similar between groups at baseline and at 8 mo.

Conclusion: Regular intake of 19 mg iron/d in MFPP supplied through extruded rice kernels improves iron stores and reduces iron deficiency among schoolchildren in India. Am J Clin Nutr doi: 10.3945/ajcn.110.007179.

INTRODUCTION

Anemia is a major micronutrient deficiency disorder of public health concern in developing countries worldwide, including India, that affects mostly women of reproductive age and young children (1). Large-scale population-based nutrition and health surveys in India have shown that the prevalence of anemia is \(\sim 70–80\%\) among different age, sex, and physiologic groups (2, 3). In young children and adolescents, ID4 even without anemia impairs cognition, immune status, growth, and development (4, 5). IDA is also high among these vulnerable groups in Asia (6). The major etiologic factor of anemia is dietary inadequacy of iron (7). Other important contributory factors are the presence of high amounts of inhibitors such as phytates and tannins (8) that limit iron absorption and inadequate intake of dietary promoters of iron absorption or use such as heme-iron, vitamin C, vitamin A, riboflavin, folic acid, and vitamin B-12 (9–12).

Fortification of commonly consumed staples with iron is an effective strategy for reducing ID and IDA, because the requisite iron can be provided through the diet without altering the prevailing dietary preferences of the population. Technology for fortification of wheat flour with iron has been developed and adopted by several flour millers, which benefits the wheat-eating populations in India (13). However, because rice forms the major staple in the majority of the population in the southern and eastern parts of peninsular India, similar technology for fortifying rice is warranted.

PATH (Seattle, WA) owns the technology (14) for fortification of rice using Ultra Rice (Bon Dente International), which consists of extruded rice kernels made from rice flour fortified with MFPP

1 From the Division of Community Studies (MSR, RHK, CGR, and GNVB), the Division of Micronutrient Research (KMN and PR), and the Department of Biostatistics (MVVR), National Institute of Nutrition, Indian Council of Medical Research, Hyderabad, India.

2 Supported by the Department of Biotechnology, New Delhi, India. The Program for Appropriate Technology in Health (PATH), Seattle, WA, provided the Ultra Rice premix.

3 Address correspondence to GNV Brahman, Division of Community Studies, Jamai Osmania, Hyderabad – 500 007, Andhra Pradesh, India. E-mail: gnvbrahmam@yahoo.com.

4 Abbreviations used: CRP, C-reactive protein; FR, fortified rice (normal rice mixed with Ultra Rice); ID, iron deficiency; IDA, iron deficiency anemia; MDM, midday meal; MFPP, micronized ferric pyrophosphate; NCHS, National Center for Health Statistics; PATH, Program for Appropriate Technology in Health; UFR, unfortified rice.

Received October 28, 2010. Accepted for publication August 3, 2011. doi: 10.3945/ajcn.110.007179.

Copyright (C) 2011 by the American Society for Nutrition
The MDM consisted of cooked rice equivalent to \( \sim 125 \text{ g rice} \) (dry weight) per child per meal along with regular side dishes provided under the government-implemented MDM program on all school working days, excluding Sundays and school holidays. Rice served in combination with a typical liquid preparation of pulse, locally called \textit{sambar} (prepared by combining dehusked split pigeon peas, tamarind pulp, and vegetables, eg, bottle gourd, cucumber, brinjal), or a semiliquid preparation of the same pulse with green leafy vegetables (eg, spinach, amaranth, sorrel), locally called \textit{aakukoora pappu}. Another variation in the menu included rice seasoned with tamarind pulp, locally called \textit{pulihora}. The main ingredients used on a daily basis were tomatoes, onions, peanuts, red chilies, salt, and peanut oil. The rice and side dishes were cooked in wide-mouthed aluminum vessels covered with a lid, and firewood was used for fuel. A boiled egg was provided once per week. To avoid monotony and maintain interest, the 3 meals were prepared and served to the children in repeated sequence in a typical week during the academic year.

Fortification of rice

Rationale for setting the amount of fortification

The Recommended Dietary Allowance of iron for Indian children in the age group 5–11 y is 28 mg/d (17); their mean dietary intakes were estimated to be about 10 mg/d (18). Therefore, for the present study, the deficit of \( \sim 18 \text{ mg iron} \) was provided through a single MDM containing 125 g FR, on a dry-weight basis. The FR was prepared by mixing MFPP-fortified extruded rice kernels into normal rice just before use in the kitchen.

Production of MFPP-fortified extruded rice kernels

The extruded kernels were produced by a cold-extrusion process. The MFPP and other stabilizing ingredients were added to the rice flour and then a dough was prepared and extruded in a pasta-manufacturing machine by using die with rice-shaped inserts by a contract manufacturer under an agreement with Camil Alimentos SA (Sao Paulo, Brazil). The extruded rice premix contained 9.6 mg Fe/g as MFPP with a mean particle size of \( \sim 3.14 \mu m \) (AKSELL Única). The other constituents in the premix were soybean oil, sodium alginate citric acid, sodium tetra polyphosphate, butylated hydroxyl anisole, butylated hydroxyl toluene, and calcium chloride. All constituents used were in conformity with Codex Alimentarius standards. The formulation, production, and supply of the extruded rice kernels were coordinated by PATH and transported to the study headquarters as a single consignment of 60 kg, sufficient for the entire study duration.

Fortification

Every 2 weeks, 100 kg rice supplied for the MDM to the school through the public distribution system of the government of Andhra Pradesh, India, was obtained and divided into 2 equal portions. One portion was blended with the extruded rice premix and the other was used as UFR. The process of blending the rice with the premix was done through manual mixing in 50-kg batches outside rice mills or normal packaging facilities, for the sole purpose of use in the school study. The premix (0.8 kg) was mixed with normal rice (49.2 kg), ie, a dilution of 1:61.5, so as to provide 15 mg iron per 100 g FR (ie, 18.8 mg iron per 125 g FR), on a dry-weight basis. Briefly, the rice was formed into a cone or pile. The cone was flattened and divided into 4 equal segments and formed into 4 separate piles. The premix was similarly divided into 4 equal parts. Each part of the premix was added to each pile of the rice and mixed thoroughly. The diagonally opposite piles were combined and mixed again. This process was repeated 3 times to ensure uniform mixing. The FR and UFR were packed in color-coded, 10-kg, high-density polyethylene bags and supplied to the school for MDM preparation.

Sensory evaluation

The sensory evaluation in terms of color, appearance, texture, odor, taste, and overall acceptability was determined in cooked rice by using a 5-point (5, very good; 4, good; 3, fair; 2, bad; 1, very bad) hedonic scale (19) on 2 consecutive days. The panel consisted of 132 children (56 boys, 76 girls) aged 8–11 y from one of the government primary schools, situated in Keesara, a rural village of Keesara Mandal, Ranga Reddy District, Andhra Pradesh, India, which is located 30 km from our study headquarters, the National Institute of Nutrition. The children were randomly allocated to 2 groups (\( n = 66, 28 \text{ boys and 38 girls in each group} \)) and blinded to the study procedures. The study was conducted at 1200 h under uniform lighting conditions and each child was seated separately while participating in the study. The investigators explained to the children how to evaluate the food samples and record results on a pretested sensory evaluation.
sheet prepared in the local language. Both types of coded rice were washed in potable drinking water and cooked separately in the school kitchen. Only plain, cooked rice was served without any side dishes for testing the sensory qualities. On the first day, one group of children received 1 of the 2 types of rice and the second group received the other type of rice to test the sensory qualities. On the second day, the study was repeated on the same children by interchanging the type of rice given to each group. The children were allowed to consume the side dishes only after they had completed the sensory evaluation of the cooked rice.

Study site for the intervention study

The intervention study was carried out in an adjacent government primary school in the same village of Keesara, where the MDM program is in operation for children in the age group 5–11 y.

Study design

The study was a double-blind, randomized, placebo-controlled feeding trial conducted in a primary school, in children in the age group 5–11 y.

Sample size

Assuming an overall mean increment in hemoglobin of 0.56 g/dL with an SD of 1.04 g/dL (based on a pilot study), 95% CI, significance of 0.05 (2-tailed), 80% power of estimate, and 20% attrition, the sample size calculated was 70 children per group.

Screening

Informed written assent from children and consent from their parents were obtained for screening. The school had 230 children (85 boys and 145 girls) aged 5–14 y, who were screened for inclusion in the study. The criteria for inclusion were age 5–11 y; hemoglobin \( \geq 7 \) g/dL; weight for age, height for age, and/or weight for height of at least the median minus 3 SD of NCHS reference values, and regular participation in MDM. Height, weight, and hemoglobin were measured in 228 children. A total of 146 children (56 boys and 90 girls) were eligible for inclusion in the intervention study. Eighty-four children (29 boys and 55 girls) were excluded from the study: 2 children with age/sex strata, the children (n = 140, 54 boys and 86 girls) were randomly allocated to 2 groups (n = 70, 27 boys and 43 girls in each group) to receive the rice containing MFPP-fortified extruded kernels (FR group) or the unfortified rice (UFR group) in the MDM each day. In this process a total of 6 unmatched children were excluded. Individual color-coded photo identity cards were prepared and distributed to the children. All of the participating children were dewormed with 100 mg mebendazole (Mebex; Cipla Limited) twice daily for 3 d, 1 wk before initiating the feeding trial.

Preparation and serving of the MDM

During the intervention period, the 2 types of coded rice were weighed separately (at 125 g per child) depending on the day’s attendance of children and cooked in 2 similar-looking coded vessels in the school kitchen by the MDM cooks, using the recipes mentioned above. The side dishes were cooked commonly for both groups. The meal was served ad libitum to the children with local serving spatulas in stainless steel serving plates along with potable water. The group assignment of the children was identified by using the color-coded photo identity cards, and the children consumed their respective meals in 2 different large halls. Care was taken to ensure that the children did not share their meal or exchange their plates with other participants. The entire process of cooking, serving, and consumption was directly monitored by the project’s field staff each day. Unannounced study site visits by the research investigators were also conducted to monitor compliance.

Estimation of meal intakes

Institutional diet survey by weighing method (20) was performed at baseline and at 8 mo, on 2 consecutive days with different menus (pulihora on day 1 and rice with sambar and boiled egg on day 2). Individual intake of MDM was measured on the same days among a subsample of 24 randomly selected children belonging to different age and sex strata (12 from each of the study group). The edible portions of all foods were weighed before and after preparation by using a mechanical column weighing scale with an accuracy of 50 g (Seca 710) and at the time of consumption by using an electronic kitchen scale with an accuracy of 1 g (Soehnle Vera). Visible leftovers in the serving dishes and on individual plates were weighed and recorded. The data on nutrient intake from MDM were derived from Indian food composition tables (21) with the use of an in-house computer program.

Data collection

The study period was from 1 July 2007 to 15 April 2008. The baseline and final measurements (at 8 mo) were done for height and weight and for biochemical estimation of hemoglobin, ferritin, and CRP. The FR and UFR samples (uncooked and cooked) were collected in duplicates from the school kitchen, twice monthly on random days, for estimation of the iron content.

Anthropometric measurements

The height and weight of the children were measured by using an anthropometer rod with an accuracy of 1 mm (AP-BGC) and a compact, digital, personal weighing scale of 100 g accuracy (Seca 813), respectively (22). The nutritional status of each child
was determined according to SD classification (23) using NCHS reference values (24).

Biochemical analysis

Two milliliters of blood from the antecubital vein was collected in heparinized evacuated tubes. Hemoglobin was estimated on the same day of blood collection by the cyanmethemoglobin method (25) and the plasma was separated and divided into aliquots and stored at −70°C until analysis for ferritin and CRP. An indigenous sandwich ELISA developed against human liver ferritin and previously validated against recombinant human ferritin (WHO International Reference Material 94/572; National Institute for Biological Standards and Control, United Kingdom) with an assay sensitivity of 2 ng/mL (26) was used to measure ferritin concentrations. CRP was estimated by a commercial ELISA kit (Abazyme, LLC) according to the manufacturer’s instructions (27), and a value ≥0.8 mg/L was considered to be an elevated CRP value.

In children aged 5–11 y, anemia (mild to moderate) was defined as a hemoglobin concentration between 7 and 11.5 g/dL, ID was defined as ferritin <15 μg/L, and IDA was defined as hemoglobin between 7 and 11.5 g/dL and ferritin <15 μg/L (6).

Iron content and iron retention

The iron content in the extruded rice premix, FR, and UFR was estimated. The iron content was analyzed in triplicate by the bathophenanthroline method in mineral solution after microwave digestion (28).

In vitro availability of iron

In vitro iron availability from cooked FR and UFR rice along with the side dish sambar was estimated. For this estimation, the food samples were lyophilized and subjected to gastric digestion with pepsin at pH 2 followed by intestinal phases of digestion with pancreatin and bile salts at pH 6.8 (29, 30), and the amount of iron dialyzed was estimated by the bathophenanthroline method (28).

Statistical analysis

Data were entered into Microsoft Excel 2003 (Microsoft Corporation) and analyzed with SPSS for Windows (version 15.0, 2006; SPSS Inc) to assess the impact of intervention on iron status of children. Descriptive statistics were computed to analyze the central tendencies and dispersions. The geometric mean and CI for ferritin were also computed, as the values were not normally distributed. The effect of time and treatment was assessed for all outcome variables. The difference in the mean values for antheropometric measurements, hemoglobin, and CRP within a study group was tested by means of the paired t test and between study groups with the independent Student’s t test. Because the ferritin values were not normally distributed, the nonparametric test (Wilcoxon signed rank test) was used to test the differences between baseline and postintervention (at 8 mo) values within study groups. The difference in mean ferritin between groups was tested with the nonparametric Wilcoxon Mann-Whitney U test. Efficacy analysis for hemoglobin and ferritin was also performed after exclusion of the data on children with elevated CRP values at baseline, at 8 mo, or at both time points. Proportions were tested with the proportion t test.

RESULTS

Characteristics of rice containing MFPP extruded rice kernels

The mean scores for the sensory attributes tested for both the cooked FR and the cooked UFR were >4.0 on a 5-point scale and were similar (Table 1). The overall acceptability of FR and UFR was 86% and 97%, respectively.

In vitro iron availability from the FR-MDM was 1.3% and that from the UFR-MDM was 3.3%.

The mean (±SD) iron content (on a dry-weight basis) of the uncooked and cooked FR was 19.2 ± 0.44 mg/100 g and 19.0 ± 0.39 mg/100 g, respectively, and that of the UFR samples was 2.0 ± 0.25 mg/100 g and 1.8 ± 0.16 mg/100 g, respectively. Minimal variation in iron concentrations in both uncooked and cooked FR and UFR samples was observed over the duration of the study.

Compliance

Of the 140 children recruited, 128 completed the study (Figure 1). Seven children from the FR group and 5 from the UFR group left the school during the study period because of migration of their families. During the intervention period of 8 mo (244 d),
there were a total of 166 effective feeding days after excluding
Sundays (35 d), school holidays (35 d), and days when MDM
was not prepared because of meetings of the teaching staff (8 d).
The mean (±SD) number of feeding days was 138 ± 23.78 in
the FR group and 133 ± 29.33 in the UFR group.

Meal intakes

The intake based on the institutional diet survey and individual
intake from MDM were similar; the intake of rice was ~110 g
and 111 g per child per meal (on a dry-weight basis) and that of
iron was ~2 mg and 21 mg (of which 19 mg were from MFPP)
per child per meal in the UFR and FR groups, respectively. The
meal intakes were comparable at baseline and at 8 mo, within
and between the groups.

Characteristics of the study group

The mean (±SD) age (7.7 ± 1.50 y) and the sex-wide dis-
tribution of children were similar in both the groups. The mean
heights and weights were comparable during the study period.
The mean CRP values and the proportion of children with ele-
vated CRP values (CRP ≥0.8 mg/L) remained similar between
and within groups both at baseline and at 8 mo (Table 2).

Impact on hemoglobin and ferritin

The mean hemoglobin and ferritin concentrations among chil-
dren were comparable between the 2 study groups at baseline. After
8 mo, the mean increase in hemoglobin values in the FR and the
UFR groups was ~1 g/dL. There was a significant (P < 0.001)
difference in the change in mean ferritin concentrations between
groups, with an increase of 8.2 ± 2.10 μg/L in the FR group and
a decrease of 3.0 ± 1.70 μg/L in the UFR group (Table 2). Similar
changes in hemoglobin and ferritin concentrations were also ob-
erved when the data on children with elevated CRP values were
excluded from each group (Figure 2).

To assess the impact of intervention, the change in hemoglobin
and ferritin values were analyzed by the 3 different methods of
regression (Table 3). In the FR group, the increase in ferritin

FIGURE 1. Flowchart showing details at each stage of the study. FR, fortified rice (normal rice mixed with Ultra Rice); MDM, midday meal; NCHS, National Center for Health Statistics; UFR, unfortified rice.
concentrations was significant \((P < 0.001)\), whereas the change in hemoglobin in both the groups was not different by any of the 3 regression methods.

**DISCUSSION**

We have observed in the present study that consumption of MFPP-fortified extruded rice kernels mixed with normal rice close to meal preparation for a period of 8 mo through MDM significantly improved body iron stores and reduced ID among primary-school children. A significant increase of similar magnitude in the mean hemoglobin concentrations with a consequent significant decrease in prevalence of anemia was observed in both the FR and UFR groups. However, despite a clear improvement in body iron stores in the FR group, any additional impact of intervention on hemoglobin status or on the prevalence of anemia observed even after exclusion of the data on children with elevated CRP concentrations.

**TABLE 2**

Nutritional status, iron status, and CRP concentrations in children in the UFR and FR groups at baseline and at 8 mo\(^{1}\)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Nutritional status</th>
<th>Iron status</th>
<th>CRP</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>UFR group (n: 65)</td>
<td>FR group (n: 63)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Baseline 8-mo</td>
<td>Baseline 8-mo</td>
<td></td>
</tr>
<tr>
<td>Nutritional status</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Height (cm)</td>
<td>121.6 ± 11.01(^{2})</td>
<td>126.3 ± 10.95</td>
<td>120.8 ± 10.04</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>19.8 ± 4.67</td>
<td>22.8 ± 5.77</td>
<td>19.8 ± 4.43</td>
</tr>
<tr>
<td>Underweight (%)</td>
<td>30.8</td>
<td>26.2</td>
<td>25.6</td>
</tr>
<tr>
<td>Stunting (%)</td>
<td>12.3</td>
<td>10.8</td>
<td>15.2</td>
</tr>
<tr>
<td>Wasting (%)</td>
<td>22.4</td>
<td>21.5</td>
<td>12.1</td>
</tr>
<tr>
<td>Hemoglobin (g/dL)</td>
<td>11.4 ± 1.00(^{a})</td>
<td>12.5 ± 1.01(^{b})</td>
<td>11.5 ± 1.09(^{a})</td>
</tr>
<tr>
<td>Ferritin (µg/L)</td>
<td>24.4 ± 15.30(^{a})</td>
<td>21.4 ± 13.8(^{a})</td>
<td>24.7 ± 15.30(^{a})</td>
</tr>
<tr>
<td>Ferritin (µg/L)(^{3})</td>
<td>20.0 (17.88, 22.13) (^{a})</td>
<td>16.3 (14.12, 18.49) (^{a})</td>
<td>24.7 (17.43, 21.73) (^{a})</td>
</tr>
<tr>
<td>CRP (mg/L)</td>
<td>0.21 ± 0.29</td>
<td>0.20 ± 0.29</td>
<td>0.24 ± 0.32</td>
</tr>
<tr>
<td>&gt;0.8 mg/L (%)</td>
<td>7.7</td>
<td>7.7</td>
<td>11.1</td>
</tr>
</tbody>
</table>

\(^{1}\) All values were comparable at baseline. CRP, C-reactive protein; FR, fortified rice (normal rice mixed with Ultra Rice); UFR, unfortified rice. Values in the same row with different superscript letters were significantly different from respective baseline values by paired \(t\) test for hemoglobin and Wilcoxon signed rank test for ferritin values: \(P < 0.001\).

\(^{2}\) Mean ± SD (all such values).

\(^{3}\) Values are geometric means; 95% CIs in parentheses.

\(^{4}\) Significantly different from the UFR group at 8 mo by Wilcoxon Mann-Whitney \(U\) test for ferritin values, \(P < 0.001\).

\(\text{FIGURE 2. Mean (±SE) changes from baseline in hemoglobin and ferritin concentrations in the FR [fortified rice (normal rice mixed with Ultra Rice)] and UFR (unfortified rice) groups after exclusion of cases with C-reactive protein ≥0.8 mg/L (}n = 52\text{ in the FR group, }n = 56\text{ in the UFR group). Bars with different superscript letters indicate significant differences between groups by the Wilcoxon Mann-Whitney }U\text{ test: }P < 0.001. \text{Hb, hemoglobin.} \)
and IDA was obscured by the improvement of these variables in the UFR group as well. These observations indicate that other factors beyond additional iron intake had a large influence on the hemoglobin concentration. Similar results were also observed in other iron intervention trials that used low-dose iron supplements in East African children (32) or iron-fortified rice in Mexican women (15) or Indian children (33). The reasons for these findings are not clear but could be the result of regular consumption of MDM, thereby improving overall food intakes in both the groups as a result of supervised meals. Although parasitic load was not measured in this study, it is likely that deworming all of the children before intervention could have contributed to the improvement in hemoglobin status and thereby the reduced anemia prevalence in both groups. Moreover, studies in rural Indian schoolchildren of similar age have indicated a high prevalence of hookworm infections (34), and a worm load of approximately ≤1000 eggs/g feces has been reported among the majority of the rural populations (35). The association between intensity of hookworm infection and anemia is well documented (36), and reducing intestinal parasitic load has been shown to be beneficial in improving iron status of populations in endemic areas (37).

It is also clear from this study that consumption of MFPP-fortified extruded rice kernels in a rice-based meal was more efficacious in improving body iron stores and ID and less effective in reducing IDA or improving hemoglobin status. This could be because of poor upregulation of the absorption of the small-particle-size MFPP as indicated by Moretti et al (38). The other reasons could be that only a small percentage of the participating children had IDA; concurrent inadequacies of other micronutrients existed, particularly vitamin C, vitamin A, riboflavin, folic acid, and vitamin B-12, limiting the absorption and utilization of iron for hemoglobin synthesis (9–12); inadequate duration of intervention; or a combination of these factors. Long-term follow-up studies would be required to evaluate the effects of the fortification of rice with iron on hemoglobin status, IDA among children, or both. Regular consumption of MDM resulting from supervised meals, thereby improving overall food intake or deworming all the children before intervention are plausible possible factors contributing to the improvement in hemoglobin status and thereby reduced anemia prevalence in the UFR group and to that of a similar magnitude in the FR group.

The presence of subclinical infection, acute or chronic inflammation, or both has been associated with increased serum ferritin concentrations (39), impaired iron utilization (40), or both. This may not be the cause in the present study because, even after excluding the data of children with higher-than-normal CRP values (an indicator of subclinical infection/inflammation), the effect of intervention remained significant on ferritin concentration and ID. These results suggest the plausibility of the observed impact to be attributed to the consumption of rice containing MFPP-fortified extruded rice kernels.

We have shown in this study that MFPP-fortified extruded rice kernels mixed with normal rice has indistinguishable sensory qualities compared with unfortified rice in the cooked form as evaluated among schoolchildren participating in the MDM program. We have observed that the relative in vitro availability of iron from FR-MDM was 3 times lower than from UFR-MDM. However, translating this result into available iron would mean that children in the FR group would have absorbed ~0.27 mg iron/d, whereas those in UFR group would have obtained only ~0.07 mg iron/d through MDM. Therefore, despite lower bioavailability, providing 21 mg iron in FR through a single MDM is expected to meet approximately one-third (0.273 mg) of the daily requirements of 0.8 mg for 5–11-y-old children and has
been efficacious in significantly improving their body iron stores and reducing ID. Similar observations related to iron absorption were elucidated in recent studies that used rice fortified with ferric pyrophosphate (15, 16, 33). Our study is limited in that the total dietary intake of iron from other sources apart from MDM was not determined during the intervention period. In view of these results, further studies are needed to show the technical feasibility of this approach as a food-fortification strategy on a large scale. Also, community-based intervention studies are needed to assess the impact among other vulnerable population groups such as preschool children, adolescent girls, pregnant women, and lactating mothers. Use of this technology through targeted programs such as supplementary feeding programs and other food security programs of national governments could be considered to test the effectiveness of this strategy. Large-scale, community-based effectiveness trials are warranted to confirm the findings of the present efficacy trial and the potential effectiveness of this technology with multiple micronutrients. Simultaneously, there is a need to develop an appropriate technology for mixing or blending the MFNP-fortified extruded rice kernels with normal rice and distributing the mixture through fair price shops in India.

We gratefully acknowledge the financial assistance provided by Department of Biotechnology (DBT), Government of India. We also thank PATH USA for providing Ultra Rice premix for the use in the study. We also gratefully acknowledge the help and cooperation extended by PATH India. We are grateful to K Venkiah for helping in coding the rice samples, enabling fortification process and study group randomization; B Pothuraju, C Saibabu, SPV Prasad, JS Acharya, B Arumugham, M Srinivas, A Rajesh Naik, D Balamani, D Sarala, and N Anil Kumar for technical assistance; and P Swathi Chitra, K Nalini, P Lakshmi Kalyani, V Chandra Babu, and M Goud for assistance in the field. We also thank the principal teachers, and cooks of the primary school and the participating children and their parents who invested their valuable time and effort in the study.

The authors’ responsibilities were as follows—GNVB and KMN: conceived, supervised, and provided overall guidance for the study; MSR, RHK, and CGR: participated in and supervised the field implementation and coordinated with the collaborating division/department; MMVR: carried out the statistical analysis; PR: carried out all the laboratory support; MSR: wrote the manuscript; and GNVB and KMN: provided critical revision. All of the authors contributed to the study design, implementation, data analysis, and interpretation of results and to the editing and approval of the final manuscript. None of the authors had a potential conflict of interest related to this study.

REFERENCES
27. Human CRP ELISA Kit, Catalogue No. EL 10022. Needham, MA: Abazyme, LLC.


