Extruded rice fortified with micronized ground ferric pyrophosphate reduces iron deficiency in Indian schoolchildren: a double-blind randomized controlled trial1–3

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ABSTRACT

Background: Iron fortification of rice could be an effective strategy for reducing iron deficiency anemia in South Asia.

Objective: We aimed to determine whether extruded rice grains fortified with micronized ground ferric pyrophosphate (MGFP) would increase body iron stores in children.

Design: In a double-blind, 7-mo, school-based feeding trial in Bangalore, India, iron-depleted, 6–13-y-old children (n = 184) were randomly assigned to receive either a rice-based lunch meal fortified with 20 mg Fe as MGFP or an identical but unfortified control meal. The meals were consumed under direct supervision, and daily leftovers were weighed. All children were dewormed at baseline and at 3.5 mo. Iron status and hemoglobin were measured at baseline, 3.5 mo, and 7 mo.

Results: At baseline, the prevalences of iron deficiency and iron deficiency anemia in the total sample were 78% and 29%, respectively. After 7 mo of feeding, there was a significant increase in body iron stores in both study groups (P < 0.001), with a greater increase in the iron group than in the control group (P < 0.05). There was a significant time × treatment interaction for iron deficiency, which fell from 78% to 25% in the dewormed iron group and from 79% to 49% in the dewormed control group. Iron deficiency anemia decreased from 30% to 15% (NS) in the iron group but remained virtually unchanged in the control group (28% and 27%). In sensory tests, the MGFP-fortified rice (fortified at 3 and 5 mg Fe/100 g) was indistinguishable from natural rice, in both cooked and uncooked form.


KEY WORDS: Iron deficiency anemia, iron fortification, schoolchildren, India, micronized ground ferric pyrophosphate, feeding trial

INTRODUCTION

Iron deficiency (ID) and iron deficiency anemia (IDA) are highly prevalent among young women and children in South and Southeast Asia (1). In low socioeconomic populations in India, the prevalence of IDA may be as high as 64–68% in school-aged children and infants (2, 3). IDA impairs cognitive performance, infant and child growth, immune status, and work capacity (1). Even mild-to-moderate ID without anemia may lower work capacity and resistance to fatigue (4, 5) and impair cognition (6, 7).

Rice is a leading staple food in South Asia, and it is typically milled before consumption. Milled rice has an iron content of only ≈4–8 mg Fe/kg (8). A rice-based diet consumed with few other foods might not supply sufficient dietary iron. Although iron fortification of rice could be an effective strategy to reduce ID and IDA (1, 9), it is technically challenging. Rice is usually consumed as intact grains, and addition of highly bioavailable, water-soluble iron compounds to artificial rice grains causes adverse sensory changes, whereas less reactive, poorly soluble iron compounds are not well absorbed (10). However, increasing the amount of poorly soluble iron compounds, such as ferric pyrophosphate, can overcome their low bioavailability and may make them more useful food fortificants. Additionally, reducing the particle size might positively influence iron absorption from ferric pyrophosphate (11–14). In a recent intervention trial in Morocco, salt fortified with micronized ground ferric pyrophosphate (MGFP) was highly effective in reducing IDA in children (15).

Using an extrusion method (16, 17), we have developed artificial rice grains fortified with MGFP (mean particle size of ≈2.5 μm) (16). In both cooked and uncooked form, the texture, taste, appearance, and storage stability of the MGFP-fortified rice closely resembles that of natural unfortified rice, and losses of iron from the extruded grains during rinsing are <3% (16). The primary aim of this study, which was carried out in an urban slum...
in Bangalore, India, was to test the efficacy of the MGFP-fortified rice in iron-deficient children. We also measured rice intake, iron intake, and iron bioavailability from the local diet, and determined whether the extruded MGFP-fortified rice could be distinguished from natural rice by the local population in raw form and when cooked in local meals.

**SUBJECTS AND METHODS**

**Study site**

Bangalore is situated on the Deccan plateau in South India (≈900 m above sea level), and has a population of ≈4.3 million (2001 census, 18). This region is not endemic for malaria, and it is estimated that incidence of the disease is <2% (19). The study site was the Franciscan School, a primary school serving the population of the Rock-Colony neighborhood, a crowded urban slum. The school has ≈970 students aged 4–14 y. A subsidized lunch feeding program is in place that provides the students with a 200–300-g meal of cooked rice daily. Informed, written consent was obtained from the parents of the children, and oral consent was obtained from the children. The protocol of the study was approved by the ethical committee at St John’s National Academy of Health Sciences, Bangalore, and by the ethical committee of the Swiss Federal Institute of Technology, Zurich, Switzerland. The study period was August 2004 to April 2005.

**Estimation of rice and iron intakes and iron bioavailability from the local diet**

Food intake was assessed by use of 3-d weighed-food records in 20 randomly selected families living in the Rock Colony neighborhood adjacent to the school. During record keeping, the households were asked to maintain their usual food habits. Edible portions of all foods were weighed during preparation and at the time of consumption by using food scales with a precision of ±1 g (Soene-Waagen GmbH, Murrhardt, Germany). Foods consumed outside the home were reported and quantities estimated by the study participants. Consumption data were entered into a dietary survey program (Ebispro-NutriSurvey, 2004; University of Hohenheim, Stuttgart, Germany) into which food-composition data from the Indian food-composition database (20) were integrated. Dietary intakes of heme iron, nonheme iron, and vitamin C were calculated. The phytate content of local staple foods (rice, millet, and wheat flour) and of locally consumed pulses (black gram dahl, tur dahl, Bengal gram dahl, moong dahl, and beans) was directly analyzed (21). The percentage of heme iron in animal foods was estimated to be as follows: chicken, 30%; mutton, 70%; and fish, 25% (22, 23). Dietary iron bioavailability was then estimated by using published algorithms to estimate iron absorption (24–27) and was adjusted for body iron stores (28). Iron intake and bioavailability were calculated on an individual basis, and averages were calculated for different age groups.

**Sensory tests**

Triangle tests (29) were performed to determine whether local women could distinguish the iron-fortified rice from unfortified rice. Four local recipes were tested along with cooked and uncooked rice. The panel was composed of 24 middle-class Indian women. The subjects were blinded and were informed about the procedures of the test only after completion of the entire study. Samples were presented in a randomized block design, and no more than 3 consecutive tests per session were performed. Tests were done in a private setting under uniform lighting conditions. Both raw rice and cooked rice were served on coded, rectangular polyethylene cups (dimensions: base, 60 × 70 mm; height, 30 mm). Each portion of raw rice contained 30 g fortified or unfortified Sona Masuri rice (Sona Masuri; Bangalore Rice Traders, Bangalore, India). The cooked rice portions were simultaneously prepared by using pressure cookers equipped with a pressure valve. Rice was prepared by using a standardized procedure similar to the traditional preparation in South Indian households. Rice was washed in preparation for cooking. Rice portions were cooked with seasoning ingredients in household pressure cookers for 8 min after reaching peak pressure, after which pressure was released. Test servings contained ≈35 g cooked rice.

**School-based intervention study**

At the Franciscan school, serum transferrin receptor (TfR), serum ferritin (SF), and hemoglobin were measured in all consenting children (n = 554). All children who met the inclusion criteria of iron deficiency or low iron stores (defined as SF < 20 µg/L or TfR > 7.2 mg/L; n = 184) were randomly assigned to receive either the iron-fortified daily lunch meal or an unfortified control (see details below). The study was double-blind. The sample size was estimated at 70 children per group to be able to detect a difference of 30% in the geometric mean SF concentration from a mean baseline of 15 µg/L, assuming a significance level of 0.05 (2-tailed) and a power of 90%. Anticipating a drop-out rate of 20%, ≈90 children were recruited per group.

At baseline, height and weight were measured in the participating children, and 5 mL blood was collected by venipuncture into EDTA-containing tubes for the measurement of hemoglobin, SF, TfR, and C-reactive protein (CRP). Measures were repeated after 3.5 mo (midpoint) and after 7 mo (endpoint). At baseline and at the midpoint of the study, all participants were dewormed with 400 mg albendazole (Low-Cost Pharmaceuticals, Bangalore, India). As part of the current Indian national supplementation campaign, children in the study were treated with vitamin A supplements (200 000 IU) 4 mo before the start of the study and near the study midpoint. Subjects who remained anemic after completion of the trial received supervised treatment with oral iron tablets [60 mg Fe (as ferrous sulfate) 4 d wk for 12 wk].

Morbidity in both study groups was assessed weekly. Subjects were asked to identify diseases or complaints from a list and to quantify their severity by estimating the number of days they were affected.

**Preparation and feeding of the lunch meals**

The premix of extruded iron-fortified rice was produced as previously reported (13). The extruded rice grains contained 10 mg Fe/g as MGFP. The MGFP was produced by conventional grinding and had a mean particle size of ≈2.5 µm (Paul Lohmann AG, Emmerthal, Germany). The premix was mixed with local rice (Sona Masuri; Bangalore Rice Traders) in 50-kg batches at a 1:50 ratio to result in a fortification level of 200 mg Fe/kg rice. Rice was mixed monthly by using barrel mixers of 15 kg and 50 kg capacity. First, 1 kg premix was mixed with 14 kg natural rice for 15 min. Then, the 15-kg blend was mixed for 30 min with 35 kg natural rice. The iron-fortified and unfortified rice were packaged in color-coded 10-kg polyethylene bags.
The separate lunch meals containing the 2 types of rice were prepared daily under supervision in the kitchen of the Division of Nutrition at St John’s National Academy of Health Sciences. A dedicated technician was responsible for preparing the ingredients according to the recipes and for supervising the cooking. The rice meals were packed into color-coded plastic lunch boxes; the daily portion of rice per lunch was 100 g dry rice. This provided 20 mg Fe as MGFP in the iron-fortified meals.

At the school, the group assignment of the participating children was identified by using a color-coded personal badge. Lunch was served 6 d/wk (except for school holidays). Three local recipes of rice cooked with different seasoning ingredients were presented in repeating sequence to maintain interest. The main seasoning ingredients of the 3 recipes were as follows: for tomato rice, onions and tomatoes; for lemon rice, groundnuts, roasted lentils, and lemon juice; and for vegetable pulao, French beans, beetroot, cauliflower, carrots, and onions. The children ate their lunch in a large hall under the direct supervision of the study team. After the children finished their lunch, boxes with visible leftovers were individually weighed and the data recorded.

Laboratory analyses

The iron content of the iron-fortified and control meals was analyzed biweekly (fortified meal: n = 17; control meal: n = 16). The test meals were lyophilized and successively mineralized by using an HNO3/H2O2 mixture and microwave digestion. The iron content of the solution was determined by graphite-oven-atomic absorption spectrometry with a commercial iron standard for calibration (Titrisol; Merck, Darmstadt, Germany). The vitamin C content of the menus was measured by HPLC with a reversed-phase column and photometric detection (30), and the phytate content was measured by using a modification of the Makover method (21) in which Ce replaced Fe in the precipitation step.

Hemoglobin was measured with an AcT8 Counter (Beckman Coulter, Krefeld, Germany) on the day of the blood collection. Serum samples were divided into aliquots and frozen at −80 °C until analyzed. SF and TfR were measured by using commercial immunoassays and control materials (TfR: Ramco, Houston, TX; SF: Immulite 1000, DPC, Los Angeles, CA). CRP was measured by nephelometry (Turbox; Orion Diagnostica, Espoo, Finland). Analytic sensitivity was 1.5 μg/L for SF, 0.6 mg/L for TfR, and 5 mg/L for CRP. The CVs (intraassay) for the assays were as follows: SF, 7.9–9.7%; TfR, 4.4–5.0%; and CRP, 5.6–7.6%. Reference values were as follows: SF, 15–300 μg/L; TfR, 2.9–7.6 mg/L (30); and CRP, <10 μg/L. Iron deficiency was defined as either SF < 15 μg/L or TfR > 7.6 mg/L (31–33). Anemia was defined as hemoglobin < 115 g/L in children aged 5–11 y (1). Serum ferritin values from subjects with elevated CRP (CRP > 10 mg/L) were excluded from analysis. Total body iron was calculated from the ratio of TfR to SF by using the method of Cook et al (34, 35). Iron absorption from the extruded rice was calculated by comparing the change in body iron in the treatment and control groups and by estimating the total iron dose given during the study.

Statistical methods

Data processing and statistical analysis were performed with SPSS (version 13.0, 2004; SPSS Inc, Chicago, IL) and with Microsoft EXCEL (2002; Microsoft Corporation, Redmond, WA). EPINFO was used for anthropometry calculations (version 3.3.2; CDC, Boston, MA). The normality of the data was checked before analysis with the Kolmogoroff-Smirnoff test and graphically by evaluating Q-Q plots. Results were analyzed with a univariate general linear model using the subject variable as a random factor to correct for repeated measures. Effect of treatment and time were evaluated for all outcome variables: hemoglobin, SF, TfR, and CRP. If a significant time × treatment interaction was found, the data were further analyzed by using post hoc Tukey’s tests. The normality of the residual distributions was checked graphically for the univariate model. To analyze the morbidity data, a GLM model was used with the sum of days of sickness as the dependent variable and treatment and type of disease as the fixed factors. Results of the sensory study were evaluated with the binomial distribution. Significance was set at P = 0.05.

RESULTS

Assessment of iron bioavailability from the diet

Iron intake and bioavailability from the local diet and factors used in the estimation are shown in Table 1. Mean (±SD) daily iron intakes in 6–13-y-old children were 5.0 ± 2.2 mg (boys) and 4.7 ± 2.2 mg (girls). Only ≈6% was heme iron. Estimated dietary iron bioavailability ranged between 4.5 ± 2.3% and 6.5 ± 2.9% (boys) and between 4.6 ± 2.0% and 7.1 ± 8.2% (girls) depending on the model used to assess iron bioavailability.

Organoleptic tests

The results of the sensory study are shown in Table 2. At both 3 and 5 mg Fe/100 g rice, fortified and unfortified uncooked rice were indistinguishable. Similarly, in the all of the cooked recipes—plain white rice, lemon rice, vegetable rice, tomato rice, and tamarind rice—the meals containing rice fortified at 3 mg Fe/100 g were indistinguishable from the meals containing unfortified rice.

<table>
<thead>
<tr>
<th>Boys (n = 16)</th>
<th>Girls (n = 20)</th>
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<tbody>
<tr>
<td>Rice (g)</td>
<td>165.3 ± 73</td>
</tr>
<tr>
<td>Fe (mg)</td>
<td>5.0 ± 2.2</td>
</tr>
<tr>
<td>Heme Fe (mg)</td>
<td>0.33 ± 0.45</td>
</tr>
<tr>
<td>Vitamin C (mg)</td>
<td>33 ± 20</td>
</tr>
<tr>
<td>Phytate (mg)</td>
<td>928 ± 423</td>
</tr>
<tr>
<td>Meat, fish, and poultry (g)</td>
<td>38.5 ± 16.8</td>
</tr>
<tr>
<td>Fe bioavailability</td>
<td>4.5 ± 2.3</td>
</tr>
<tr>
<td>Fe bioavailability,</td>
<td>5.5 ± 2.8</td>
</tr>
<tr>
<td>Fe bioavailability,</td>
<td>6.5 ± 2.9</td>
</tr>
<tr>
<td>Fe bioavailability,</td>
<td>6.4 ± 9.6</td>
</tr>
</tbody>
</table>

1 All values are x ± SD. Iron bioavailability was calculated by using algorithms assuming low iron stores (serum ferritin = 20 μg/L).
2 Algorithm from Tseng et al (24).
3 Algorithm from Reddy et al (25).
4 Algorithm from Bhargava et al (26).
5 Algorithm from Hallberg et al (27).
The children in the iron-fortified group consumed a mean of 16.1 mg Fe/d from their meals, whereas children in the control group consumed a mean of 1.07 mg Fe/d from the control meal. However, considering absenteeism, when averaged over all of the feeding days in the study, the daily mean (±SD) iron intake per subject in the iron group was 13 ± 2.4 mg, and that in the control group was 0.99 ± 0.44. The school had a 3-wk vacation in October, which interrupted the feeding program during this period.

Changes in hemoglobin, iron status, iron deficiency, and iron deficiency anemia

As shown in Table 4, the baseline characteristics of the 2 groups did not differ significantly after the randomization step. Of the 184 subjects enrolled, 170 completed the study. Twelve of the 14 subjects who discontinued the study were in the iron fortification group and 2 were in control group. The main reasons for dropping out were leaving school (n = 9) and loss of interest in the study (n = 5). Three subjects who dropped out because of lack of interest were in the iron group and 2 were in the control group.

The results of the fortification trial are shown in Table 5. Time × treatment interactions were significant for hemoglobin (P < 0.05), SF (P < 0.05), TfIR (P < 0.05), and body iron stores (P < 0.001). CRP did not show a significant time × treatment interaction. Body iron stores were greater in the iron-fortified group after 7 mo (P < 0.01); the increase in iron stores from baseline to 7 mo was 2.7 mg/kg body wt in the iron group and 1.2 mg/kg body wt in the dewormed control group (P < 0.001). Hemoglobin concentrations were not significantly different between groups after 7 mo (P = 0.096). There was no significant change in hemoglobin in the iron group, whereas in the control group, hemoglobin decreased significantly (P < 0.001).

Changes in the prevalences of ID and IDA in the treatment and control groups are shown in Figure 1. Over the 7-mo study, the prevalence of ID decreased from 78% to 25% in the iron group and from 79% to 49% in the control group. By logistic regression, there was a significant time × treatment interaction for ID, whereas IDA was not significantly affected by treatment (P = 0.16) or time (P = 0.453). However, the prevalence of IDA
decreased from 30% to 15% in the iron group and remained virtually unchanged in the control group (28% and 27%).

There was no significant difference in mean CRP or the prevalence of elevated CRP values between the 2 groups at any point in the study. However, there was an increase in the prevalence of elevated CRP values in both groups from baseline to the midpoint of the study ($P < 0.001$), with the prevalence increasing from 7.6% to 20% in the iron group and from 9% to 17% in the control group.

### Morbidity and anthropometry

There was no significant evidence of an effect of treatment on the frequency or severity of infectious disease, as measured by the questionnaire ($P = 0.380$; Table 6). At baseline, mean $z$-scores in the entire sample were as follows: height-for-age, $z = -1.5$.

### Table 6

<table>
<thead>
<tr>
<th></th>
<th>Iron-fortification group</th>
<th>Control group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fatigue</td>
<td>263</td>
<td>210</td>
</tr>
<tr>
<td>Cold</td>
<td>1167</td>
<td>1175</td>
</tr>
<tr>
<td>Fever</td>
<td>680</td>
<td>734</td>
</tr>
<tr>
<td>Diarrhea</td>
<td>94</td>
<td>184</td>
</tr>
<tr>
<td>Vomiting</td>
<td>147</td>
<td>194</td>
</tr>
<tr>
<td>Stomach pain</td>
<td>601</td>
<td>692</td>
</tr>
<tr>
<td>Ear pain</td>
<td>165</td>
<td>207</td>
</tr>
<tr>
<td>Skin problem</td>
<td>19</td>
<td>47</td>
</tr>
<tr>
<td>Eye infection</td>
<td>79</td>
<td>178</td>
</tr>
<tr>
<td>Measles</td>
<td>24</td>
<td>9</td>
</tr>
<tr>
<td>Throat pain</td>
<td>171</td>
<td>107</td>
</tr>
<tr>
<td>Other</td>
<td>214</td>
<td>254</td>
</tr>
<tr>
<td>Total</td>
<td>3624</td>
<td>3991</td>
</tr>
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</table>

The numbers represent the sum of days on which the subjects were affected by the specific disease or illness. Morbidity was assessed weekly by questionnaire. In a univariate general linear model, there was no significant evidence that iron had an effect on the prevalence of infectious disease ($P = 0.380$).
In addition, compliance and rice consumption in the fortified rice (5 mg Fe/100 g) and in 5 local recipes fortified at 3 mg Fe/100 g. Rice was indistinguishable from natural rice in raw form (at 3 and 5 mg Fe/100 g) and in 5 local recipes fortified at 3 mg Fe/100 g. In addition, compliance and rice consumption in the fortified rice group was not significantly different from that in the control group.

Considering the moderate bioavailability of MGFP (10, 13, 14), we chose to fortify the extruded rice grains with 10 mg Fe/g as MGFP to provide ≈20 mg Fe/d. In the iron group, the mean (±SD) cumulative iron dose was 2.06 ± 0.4 g over the 7-mo study. Based on the mean difference in body iron stores between the iron and control groups after 7 mo (1.5 mg Fe/kg body wt), the calculated mean iron absorption from the fortified rice grains was ≈2.1%. This absorption value is similar to that reported in a recent study of iron-fortified salt, where iron absorption from MGFP over a 10-mo feeding trial was ≈2% (15). However, despite the low rate of absorption, the MGFP was efficacious: it significantly increased iron stores and reduced the prevalence of ID (Figure 1). One potential limitation to estimating body iron stores from the TIR-to-SF ratio in our subjects (schoolchildren) is that the equation used (35) was derived from an adult phlebotomy study and has not been validated in children.

In the present study, the MGFP was given at a high daily dose to show proof-of-concept. In a long-term fortification program, however, the iron fortification level could be set lower. On the basis of our measures of local rice consumption (Table 1), a hypothetical fortification program in Bangalore using MGFP-fortified extruded rice at a concentration of 5 mg Fe/100 mg rice would provide 10 mg Fe to women and 6.4 mg Fe to 3–8-y-old children (≈40% of the level used in the present study). Our local food record data indicate that iron intakes in these 2 target groups are only ≈40% of recommended dietary allowances (46), and provision of an extra 6–10 mg Fe/d would allow many to achieve adequate iron intakes. We found iron absorption in our feeding trial of 2% and estimated dietary iron bioavailability to be 4–7% in school-aged children. Because iron absorption from ferric pyrophosphate in rice is lower than that from the nonheme iron pool (10, 13, 14), we have assumed an absorption of 2–3% for a hypothetical fortification program in Bangalore. Such a program would supply an additional 0.2–0.3 mg of absorbed iron daily to women and 0.1–0.2 mg to young children.

In industrialized countries, rice is often enriched with iron to restore the iron content found in the unmilled grains (47). Several methods, including coating or extrusion of a grain premix, have been reported (48). Cold extrusion has been reported for the production of vitamin A–fortified rice (49, 50), and PATH (Program for Appropriate Technology in Health, Seattle, WA) recently promoted the use of extruded rice grains fortified with iron (51). In the Philippines, a large-scale rice fortification program has been reported using ferrous sulfate in coated rice kernels (52). Efforts are also underway to biofortify rice with iron by selective breeding. A high-iron rice has been reported to improve iron status in iron-deplete nonanemic women in the Philippines (53). Finally, a genetically modified rice containing a ferritin gene has been developed (54). Although biofortification and genetic engineering are promising approaches, extruded iron-fortified rice grains would offer greater flexibility in fixing the level of iron fortification and would avoid concerns about the genetic modification of rice.

Iron supplementation trials in anemic children have reported positive effects on growth and conflicting effects on morbidity (55–57). In our study, there was no significant effect of iron fortification on growth and morbidity. Scores for weight-for-age and height-for-age increased significantly in both groups, but no additional effect of iron could be detected. Our inability to
detect a growth effect may have been due to the improvement in iron status in the control group as the result of deworming, the short duration of the study, or the fact that the participating children were mostly iron deficient but not anemic (58).

Our findings indicate that providing iron-fortified extruded rice grains in a school feeding program is an effective iron fortification strategy. Whether applied more generally or targeted to school-feeding programs, extruded iron-fortified rice could help to reduce the large burden of ID and IDA in the rapidly growing urban populations of South and Southeast Asia.

We thank Tony Raj, John Vincent, Kiran D, Kalappa K, Lena Sebastian, Mari Venkatachari, Pushpaveni, Vani Amalrajan, and the staff of St Johns’ Medical College for technical assistance during the fortification trial. We also thank the teachers and the principal of the Franciscan School in Koramangala, Bangalore, and the children and their parents who participated in the study.

All authors contributed to the study design and to the data and statistical analysis. SM, DM, MBZ, and PT supervised and carried out the field work. DM wrote the first draft of the paper, and all authors contributed to its editing. None of the authors had a conflict of interest to report.

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