The Journal of Nutrition
Community and International Nutrition

A Randomized Trial of Iron-Biofortified Pearl Millet in School Children in India[1,2]

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Abstract

Background: Iron deficiency is the most widespread nutritional deficiency in the world.

Objective: The objective of this randomized efficacy trial was to determine the effects of iron-biofortified pearl millet (Fe-PM) on iron status compared with control pearl millet (Control-PM).

Methods: A randomized trial of biofortified pearl millet (*Pennisetum glaucum*), bred to enhance iron content, was conducted in 246 children (12–16 y) for 6 mo in Maharashtra, India. Iron status [hemoglobin, serum ferritin (SF), soluble transferrin receptor (sTfR), and total body iron (TBI)], inflammation (C-reactive protein and α-1 acid glycoprotein), and anthropometric indices were evaluated at enrollment and after 4 and 6 mo. Hodges-Lehmann-Sen 95% CIs were used to examine the effect of the Fe-PM on iron status compared with commercially available Control-PM. Linear and binomial regression models were used to evaluate the effects of Fe-PM on iron status and incidence of anemia and iron deficiency, compared with Control-PM.

Results: At baseline, 41% of children were iron deficient (SF <15 μg/L) and 28% were anemic (hemoglobin <12.0 g/dL). Fe-PM significantly increased SF concentrations and TBI after 4 mo compared with Control-PM. Among children who were iron deficient at baseline, those who received Fe-PM were 1.64 times more likely to become iron replete by 6 mo than were those receiving Control-PM (RR: 1.64, 95% CI: 1.07, 2.49, $P = 0.02$). The effects of Fe-PM on iron status were greater among children who were iron deficient at baseline than among children who were not iron deficient at baseline.

Conclusions: Fe-PM significantly improved iron status in children by 4 mo compared with Control-PM. This study demonstrated that feeding Fe-PM is an efficacious approach to improve iron status in school-age children and it should be further evaluated for effectiveness in a broader population context. This trial was registered at clinicaltrials.gov as NCT02152150. *J Nutr* 2015;145:1576–81.

Keywords: international nutrition, iron, anemia, biofortification, children, India

Introduction

An estimated 1.6 billion people worldwide are anemic (1, 2), and iron deficiency (ID)[8] is the leading cause of anemia (1, 3). Its prevalence is highest in resource-limited settings and among children and women of reproductive age (4). Even mild ID can have an adverse impact on the cognitive performance and behavior of children and physical work capacity in adults (5–7).

Established interventions to target micronutrient malnutrition such as dietary diversification, micronutrient supplementation, and food fortification have reduced ID but have not been universally successful (8, 9). Biofortification, the process of increasing the concentration and bioavailability of essential nutrients in staple crops by traditional plant breeding (and/or genetic engineering), is a promising, sustainable, and cost-effective approach to combat micronutrient deficiencies, and it was recently highlighted in the Lancet Maternal and Child Nutrition Series (10–12). Varieties of rice, maize, wheat, sweet potato, beans, pearl millet, and cassava have been the main targeted crops of biofortification.

Pearl millet (*Pennisetum glaucum*) is a staple food in India, particularly in the states of Rajasthan, Gujarat, and Maharashtra (13, 14). The high level of pearl millet consumption and the availability of a locally grown experimental pearl millet variety

[1] Supported by a grant from HarvestPlus/International Food Policy Research Institute.

[2] Author disclosures: JDH received funding as an expert consultant for HarvestPlus. JLF, SM, SAU, PSG, SVL, MJW, LEM-K, and EMP, no conflicts of interest. The funders of the study had no role in the study design, implementation, data collection, data analysis, data interpretation, or writing of the manuscript.

[7] JLF and SM contributed equally to this work.

[8] Abbreviations used: AGP, α-1 acid glycoprotein; Control-PM, control pearl millet; CRP, C-reactive protein; Fe-PM, iron-biofortified pearl millet; ID, iron deficiency; SF, serum ferritin; sTfR, soluble transferrin receptor; TBI, total body iron.

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Manuscript received December 1, 2014. Initial review completed January 5, 2015. Revision accepted April 6, 2015.
First published online May 6, 2015; doi:10.3945/jn.114.208009.

(ICTP 8203-Fe) with substantially greater iron content provided an opportunity to evaluate its efficacy in improving iron status in human populations. We hypothesized that daily consumption of iron-biofortified pearl millet (Fe-PM) would improve iron status [hemoglobin, serum ferritin (SF), and total body iron (TBI)] in 6 mo. To examine this hypothesis, we conducted the first randomized efficacy trial of iron-biofortified pearl millet and iron status in secondary school children in Maharashtra, India.

Methods

Study population. This study was conducted from September 2011 to March 2012 in school-aged children (12–16 y), in Ahmednagar district, Maharashtra, India. Participants were students from economically disadvantaged families attending boarding schools in a rural community within a 2 h drive of Ahmednagar city. This population was selected based on their high risk of ID, low-iron diets, and regular consumption of pearl millet typical of rural Maharashtra. Students in these schools consume sufficiently large quantities of pearl millet (~150–350 g/d, dried) in the form of bhakri, a flat, unleavened bread, 2 times/d, and all meals are prepared in common kitchens with fixed daily menus.

Study design. Boarding schools in the Ahmednagar district were contacted and the study objectives and protocol were explained to the administration. A prescreening survey was conducted in 18 hostels within a 3 h drive of the city of Ahmednagar. It included information on school size, weekly menus, and capacity to support an efficacy trial, including 6 mo of monitored feeding. Twelve hostels were subsequently chosen for hemoglobin screening based on the numbers of students between 12 and 16 y, infrastructure to support the research, and administrative approval. All consenting eligible students (n = 594) provided a capillary blood sample for hemoglobin analysis (HemoCue). One school with 3 hostels and the highest prevalence of anemia (>25%) was selected as the final sample pool (n = 288) to be further screened for exclusion criteria.

Random assignment and masking. This was a double-blind, randomized efficacy trial of iron-biofortified pearl millet, ICTP8203, compared with a popular commercial variety of pearl millet (DG9444).

Of the 288 children initially screened, 42 were not eligible [because of severe anemia (hemoglobin <8.5 g/dL), taking iron supplements or medications that could interfere with iron absorption, chronic illnesses, or unwilling to participate in the study]. Six weeks after screening, 246 children who were eligible for study inclusion provided a baseline venous blood sample to be analyzed for iron status [hemoglobin, SF, and soluble transferrin receptor (sTfR)] and inflammation [serum C-reactive protein (CRP) and serum α-1 acid glycoprotein (AGP)] and were assigned to 1 of 2 feeding groups (**Figure 1**). All children with severe anemia were excluded and received clinical management as per standard of care (iron supplementation as 60 mg/d ferrous sulfate for 30 d with continued treatment as needed). Anthelmintic treatment (200 mg albendazole) was administered to all eligible participants 4 wk before initial baseline blood collection and at the study midpoint. Qualified children were between 12 and 16 y of age, healthy, and residing full-time in the boarding school. After stratifying subjects by hostel of residence, the children were randomly assigned to receive iron-biofortified pearl millet (Fe-PM; 86.3 ppm iron, n = 122) or control pearl millet (Control-PM; 21.8 ppm iron, n = 124). The children at one of the hostels were all male, those at a second were all female, and those at the third hostel were mixed sex. Within each hostel, individual identification numbers were randomly assigned to 1 of 2 groups, H or P, which corresponded to 1 of the 2 types of pearl millet. Children were divided into 14 feeding groups for mid-day and evening meals in order to facilitate rapid distribution of the pearl millet as bhakri and to ensure correct distribution of the Fe-PM and Control-PM bhakri to the 2 feeding groups at each meal. These feeding groups had ~15–20 children who had the same assigned intervention group (H or P), resided at the same hostel, and were of similar age. The sacks of pearl millet were labeled and delivered to the school by a warehouse coordinator who oversaw the storage, milling, packaging, labeling, and transport of the pearl millet. Study personnel and participants were blinded to which labeled sacks contained Fe-PM or Control-PM. The study pearl millet was substituted for the regularly consumed pearl millet during week 1 of baseline blood collection. Loss to follow-up (n = 26) was similar in both groups and resulted in the final sample of 220 children, with 109 children in the Fe-PM group and 111 in the Control-PM group. Two days of venous blood collection were scheduled during a 1 wk period at baseline and 4 mo (midline) and 6 mo after enrollment (endline).

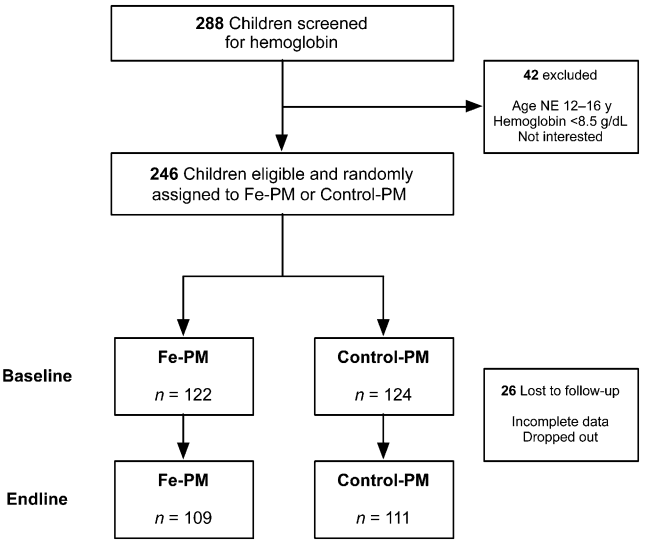

FIGURE 1 Sample selection. Control-PM, control pearl millet; Fe-PM, iron-biofortified pearl millet; NE, not equal to.

Ethics approval. Informed written consent was obtained from each participant, as well as their guardians and institution heads at screening and again at baseline. The Intersystem Biomedical Ethics Committee, Mumbai, India, and the institutional review boards of Cornell University, The University of Oklahoma, and The Pennsylvania State University approved the research protocol.

Intervention. All pearl millet was supplied by HarvestPlus and stored in a properly ventilated and secure warehouse facility until milled. The 2 pearl millet varieties were repackaged into 50 kg knitted burlap sacks with letter-coded identifiers to distinguish the Fe-PM and Control-PM. The code was not known to any of the field staff or participants, or those involved in data collection and analysis. In the warehouse, the Fe-PM was stored at 10–15°C, and the Control-PM was stored separately at ambient temperature. Both grain types were milled separately with the use of different milling machines in small batches. Approximately 50 kg flour was packed and sealed in individual polythene bags, marked with either the letter H or P, and supplied to each hostel every 2–3 d. The Fe-PM and Control-PM were similar in color, taste, and phytochemical composition and content. All children received ~200–300 g (dry) pearl millet/d in the form of bhakri during lunch and dinner. Bhakri was prepared 2 times/d by 7 cooks who used only one type of pearl millet flour and followed a protocol to standardize bhakri diameter, weight, and consistency.

After 4 mo of the trial, the pearl millet variety in the control group was switched from DG9444 (21.8 ppm iron) to JKBH778 (52.1 ppm iron). Consumption of pearl millet was greater than anticipated; as a result, the supply of Control-PM ran out by 4 mo and was replaced by a similar pearl millet (JKBH778) available on the market at that time. Biochemical analysis of this replacement Control-PM was conducted after completion of the study when it was determined that the iron content was substantially higher than observed in the original Control-PM (52.1 ppm vs. 21.8 ppm), but still lower than the Fe-PM, which was consumed throughout the 6 mo of the feeding trial. Also, both intervention and control groups started receiving shev, a savory snack

consumed throughout the day, which was prepared with the use of the same pearl millet flour varieties as the bhakri.

Baseline and follow-up procedures. A physician conducted a clinical examination of each child before the study to assess general health status. Individual children within a school hostel were assessed for dietary iron intake (described below), blood variables, anthropometry, cognitive development, and physical performance. Anthropometric measurements were obtained by trained research assistants using standardized procedures and calibrated instruments at baseline, midline, and endline. As per WHO guidelines (15), stunting was defined as height-for-age *z* score less than −2. Dietary intake was evaluated by trained research assistants at baseline, midline, and endline, using a 24 h diet recall administered to each child (with the use of food models, weights, and standard measuring equipment). The food composition database for nutrient intake was developed as follows: During the screening of hostels at the school, detailed information was collected on the menus regularly used at the different hostels. The ingredients used and method of preparation and the yield with the use of a weighing method were recorded daily for a period of 1 wk. These preparations were standardized in the Nutrition Laboratory at S.N.D.T. Women's University, and these standard recipes were then included in the food composition database for analysis. Participant food and nutrient intake data were analyzed by investigators using CS Dietary System software (CS Dietary System, version 1.1). Throughout the study, a record of menstrual history, morbidity, and medications was updated on a fortnightly basis.

Preparation and intake of bhakri were monitored daily by field research assistants specifically recruited and trained for bhakri assessment. Trained research assistants recorded the number of bhakri consumed (to the 0.25 unit) by each child, reasons for partial consumption, and nonconsumption. Trained field assistants also periodically weighed and recorded the flour used for bhakri preparation and 3 randomly selected bhakri were weighed each day in order to estimate actual intakes by the children throughout the study.

Laboratory methods. Whole blood samples were collected from participants for analysis of iron status by a trained phlebotomist at prescreening, baseline, midline, and endline. Whole blood was analyzed within 6 h of collection for hemoglobin (HemoCue) by Sanjeevani Laboratory in Ahmednagar. Whole blood collected in a second tube with heparin was analyzed for complete blood count, including hematocrit, mean cell volume, and red cell distribution width. Serum was separated by centrifugation, divided into aliquots, and stored at −20°C. The samples were picked up by Metropolis Labs within 72 h, transported on ice to Mumbai within 6 h, and stored at −80°C. Serum concentrations of SF, CRP, sTfR, and AGP were analyzed by Metropolis HealthCare Laboratory in Mumbai. Laboratory samples were tested in batch, and instruments were calibrated daily based on standardized procedures.

Iron status. SF concentrations were measured by the 2-site sandwich immunoassay with the use of the fully automated ADVIA Centaur immunoassay system, and sTfR concentrations were assessed by ELISA. TBI was calculated as the ratio of sTfR and SF according to Cook's formula (16), as described below. Because this formula used sTfR determined by the Ramco ELISA kit, we converted the Metropolis-derived sTfR to Ramco-adjusted sTfR with the prediction equation derived from 35 random duplicate samples as follows:

$$\mathrm{sTfR_{Ramco}} = (4.17 \times \mathrm{sTfR_{Metropolis}}) - 0.081 \quad (r^2 = 0.89) \qquad (1)$$

Serum CRP was assessed by a turbidimetric quantitative method with the use of a quantitative turbidimetric immunoassay (Tulip Diagnostics (P) Ltd). Serum AGP was assessed by radial immunodiffusion procedure (Kent Labs) with the use of commercially available reagents. Inflammation was defined as CRP >5 mg/L and AGP >1 g/L.

Definitions of outcomes. Primary outcomes were hemoglobin and SF concentrations, TBI, anemia, and ID. Anemia was defined as hemoglobin <12.0 g/dL, and severe anemia was defined as hemoglobin <8.5 g/dL, in accordance with WHO criteria and clinical guidelines in India. TBI was estimated with the approach originally proposed by Cook (16):

$$\mathrm{TBI(mg/kg)} = -\{\log_{10}[\mathrm{sTfR(mg/L)} \times 1000/\mathrm{SF}(\mu\mathrm{g/L})] - 2.8229\}/0.1207 \qquad (2)$$

ID was defined as SF <15μg/L for the primary analyses and as TBI <0 mg/kg or sTfR >8.3 mg/L in additional analyses.

Statistical analysis. Descriptive statistics were expressed as medians and IQRs and a nonparametric Wilcoxon test was used for comparative statistics. Hodges-Lehmann-Sen 95% CIs were used to express the effect size of the difference between the 2 groups. We compared measurements of iron status at enrollment in the Fe-PM and Control-PM groups to ensure the success of random assignment. We also examined the effects of consuming Fe-PM in children who were iron deficient at baseline (SF <15 μg/L), and by sex, compared with the Control-PM. Tests of interaction were based on a comparison of the nonparametric estimate of the treatment effect (17) between strata, with the use of their SEs (18).

Analyses of the biological plausibility of the treatment effects included assessment of potential to benefit over the continuous range of iron status measures. Participants with more severe ID were expected to show a

TABLE 1 Characteristics of children randomly assigned in the trial (n = 246)[1]

	Fe-PM (n = 122)	Control-PM (n = 124)
Baseline characteristics		
Female	38.5	39.5
Age, y	13.9 (12.9, 14.7)	13.9 (13.1, 14.6)
Hemoglobin, g/dL	12.5 (11.9, 13.2)	12.5 (11.8, 13.1)
<12.0	28.2	28.2
SF, μg/L	16.3 (10.8, 24.7)	16.4 (10.6, 24.4)
<15	45.3	41.0
Serum sTfR, mg/L	1.5 (1.3, 1.8)	1.5 (1.3, 1.7)
>8.3	11.1	9.4
TBI, mg/kg	2.1 (0.8, 3.8)	2.1 (0.4, 3.6)
<0	21.4	21.4
Serum CRP >5 mg/L	3.4	0.0
Serum AGP >1 g/L	6.8	3.4
Weight, kg	35.7 (29.8, 40.8)	34.3 (31.1, 39.7)
Height, cm	148.0 (141.5, 154.0)	148.0 (143.0, 153.5)
HAZ < −2	40.0	38.1
BMIZ < −2	40.0	41.0
Bhakri and iron consumption during the study		
Bhakri consumed, *n*		
Total	127 (104, 149)	127 (108, 160)
Baseline–4 mo	84.5 (68.4, 100)	81.0 (70.5, 96.9)
4–6 mo	44.1 (35.5, 54.8)	48.3 (36.8, 61.3)
Iron from bhakri and shev, total mg		
Total	2440 (2090, 2880)	1030 (850, 1280)
Baseline–4 mo	1400 (1140, 1750)	380 (320, 460)
4–6 mo	1040 (920, 1260)	690 (540, 820)
Iron from bhakri and shev, mg/d		
Total	22.0 (18.4, 25.2)	9.1 (7.7, 10.3)
Baseline–4 mo	19.6 (16.0, 24.3)	5.2 (4.4, 6.1)
4–6 mo	24.7 (22.2, 27.3)	15.4 (13.2, 18.0)

[1] Values are percentages or medians (IQRs). sTfR adjusted to Ramco values and used to calculate body iron. WHO HAZ scores were computed from age- and sex-specific reference (WHO) (15). AGP, α-1 acid glycoprotein; BMIZ, body mass index *z* score; Control-PM, control pearl millet; CRP, C-reactive protein; Fe-PM, iron-biofortified pearl millet; HAZ, height-for-age *z* score; SF, serum ferritin; sTfR, soluble transferrin receptor; TBI, total body iron.

greater change in iron status indicators than participants with less severe or no ID. We repeated the aforementioned analyses in individuals who were iron deficient at baseline (SF <15 μg/L or TBI <0 mg/kg). A second plausibility analysis assessed the potential nonlinear association between mean daily iron consumed and relative risk of anemia and ID nonparametrically with restricted cubic splines (19). We also investigated the effects of the intervention on the "resolution" of hematologic endpoints during the trial; resolution was defined as an individual having the outcome of interest at baseline and no longer having that outcome at the end of the time period (e.g., iron-deficient at baseline and iron-replete at endline). We conducted analyses in individuals with the outcome of interest at baseline to determine if these cases resolved during the trial. These methodologic approaches have been described in detail elsewhere (20). Tests for nonlinearity used the likelihood ratio test, comparing the model with only the linear term to the model with the linear and the cubic spline terms. SAS version 9.4 was used for all of the analyses. This trial was registered at ClinicalTrials.gov as NCT02152150.

Results

The baseline characteristics of the study participants included in these analyses are presented in **Table 1**. After screening, 246 children were randomly assigned to receive either Fe-PM (n = 122) or Control-PM (n = 124) (Figure 1). Median age was 14 y and median hemoglobin was 12.5 g/dL. Median serum CRP and AGP concentrations were 0.1 mg/L and 0.6 g/L, respectively. At baseline, 28% of children were anemic (hemoglobin <12.0 g/dL), 41% were iron deficient (SF <15 μg/L), and 21% had a negative TBI. Approximately 40% of the children were stunted at baseline. One-third of the female subjects had achieved menarche before the study. There were no differences between treatment groups for any baseline measures.

The effect of the intervention on iron status is presented in **Table 2**. There were no significant effects from Fe-PM on changes in hemoglobin, SF, TBI, or sTfR concentrations from baseline to the end of the trial compared with Control-PM. However, when only the first 4 mo of the trial was examined (September–January, before the control group was switched to a pearl millet variety with a higher content of iron), Fe-PM had a significant effect on changes in SF and TBI compared with Control-PM. The median change in SF in the first 4 mo was 5.7 μg/L in the Fe-PM group vs. 1.2 μg/L in the Control-PM group (P < 0.05). TBI increased by 0.8 mg/kg in the Fe-PM group vs. no changes in the Control-PM group during the same time period (P = 0.02). In analyses of participants in the Control-PM group, the increase in both SF and TBI from midline to endline was significantly greater than the increase in SF and TBI from baseline to midline within the control group (P = 0.03 and < 0.001, respectively).

In plausibility analyses in children who were iron deficient (SF <15 μg/L), the intervention Fe-PM was associated with significant increases in SF from baseline to midline (P < 0.01) and baseline to endline (P = 0.03) compared with the same changes in the Control-PM. Similarly, in analyses in children who were iron deficient (SF <15 μg/L) at baseline, the association between iron intake from pearl millet and the likelihood of being iron replete at 6 mo was significant (P = 0.03) (**Figure 2**), with no evidence of nonlinearity.

There were no significant effects from Fe-PM on the incidence of ID or anemia (new cases among individuals who were at risk of the outcome). In analyses in children who were iron deficient at baseline (**Table 3**), children in the Fe-PM group were 1.64 times more likely to be iron replete by 6 mo compared with those in the Control-PM group (RR: 1.64; 95% CI: 1.07, 2.49, P = 0.02). In other words, among children who were iron deficient at baseline, 64% of children in the Fe-PM group were iron replete by 6 mo vs. 40% of children in the Control-PM group. Findings were similar across each of the indicators of ID (SF <15 μg/L or sTfR >8.3 mg/L or TBI <0 mg/kg; RR: 1.92; 95% CI: 1.17, 3.14, P < 0.01).

Discussion

In this study, iron-biofortified pearl millet significantly improved iron status in secondary school children after 4 mo compared with control pearl millet. This intervention significantly increased SF concentrations and TBI from baseline to midline, and among children who were iron deficient at baseline, compared with control pearl millet. Iron intake from pearl millet was associated with significantly increased SF and TBI concentrations. The

TABLE 2 Effect of Fe-PM on changes in iron status in children compared with control-PM[1]

Outcome	n	Fe-PM, median (IQR)	Control-PM, median (IQR)	Hodges-Lehmann-Sen (95% CI)	P
Hemoglobin, g/dL					
Total	212	0.1 (−0.3, 0.5)	0.1 (−0.3, 0.4)	(−1.0, 2.0)	0.41
Baseline–4 mo	211	1.3 (0.2, 2.4)	0.9 (0.2, 2.5)	(−2.0, 5.0)	0.46
4–6 mo	202	−1.1 (−2.4, −0.2)	−1.1 (−2.3, −0.3)	(−4.0, 4.0)	0.93
SF, μg/L					
Total	216	7.4 (2.5, 16.6)	6.5 (1.7, 20.5)	(−2.6, 3.8)	0.74
Baseline–4 mo	193	5.7 (−2.4, 13.7)	1.2 (−5.0, 10.9)	(−7.8, 0.0)	<0.05
4–6 mo	187	3.0 (−3.9, 14.9)	5.1 (−1.3, 15.7)	(−2.1, 6.3)	0.32
Serum sTfR, mg/L					
Total	216	0.16 (−0.03, 0.39)	0.17 (0.05, 0.35)	(−0.13, 0.04)	0.23
Baseline–4 mo	193	0.21 (−0.12, 0.43)	0.33 (0.01, 0.57)	(−0.01, 0.26)	0.08
4–6 mo	187	−0.09 (−0.31, 0.33)	−0.10 (−0.37, 0.24)	(−0.16, 0.20)	0.78
TBI, mg/kg					
Total	216	1.3 (0.1, 2.7)	1.0 (−0.3, 2.6)	(−0.3, 0.8)	0.42
Baseline–4 mo	193	0.8 (−0.8, 1.9)	0.0 (−1.8, 1.4)	(0.1, 1.4)	0.02
4–6 mo	187	0.5 (−0.8, 2.2)	1.0 (−0.2, 1.8)	(−1.1, 0.1)	0.09

[1] Baseline measurement was at enrollment. sTfR adjusted to Ramco values and used to calculate body iron. Control-PM, control pearl millet; Fe-PM, iron-biofortified pearl millet; SF, serum ferritin; sTfR, soluble transferrin receptor; TBI, total body iron.

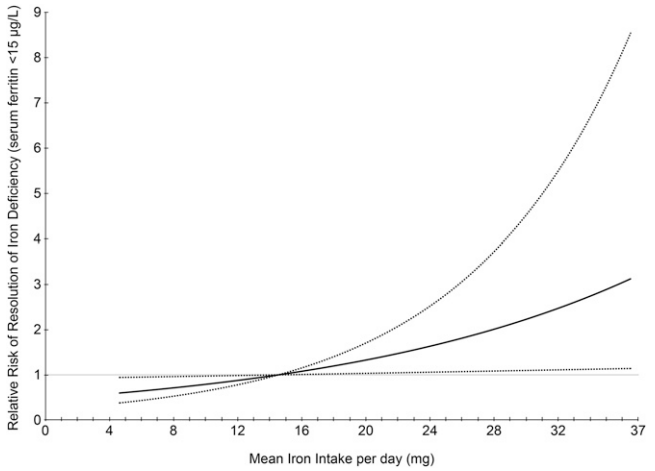

FIGURE 2 Association between mean daily iron consumed from pearl millet and likelihood of being iron replete among children who were iron deficient at baseline (n = 94). The figure is a restricted cubic spline.

intervention increased the likelihood that children would be iron replete by the end of the trial. Additionally, switching the control group to a pearl millet variety with a higher iron content and adding a pearl millet–based snack improved their iron status between months 4 and 6 during the trial.

All of the advantages of the randomized controlled trial were confirmed. Random assignment was effective in equalizing baseline characteristics; the Fe-PM and Control-PM groups were similar at baseline for iron status, inflammation, and all measured potential confounding factors. The consumption of bhakri and pearl millet were comparable in the 2 groups, and the randomized trial was of sufficient length to detect changes in iron status.

Of note were the high rates of ID (43%) and stunting (40%) among secondary school–aged children in this study. Although there are limited data in the literature on iron status of this age group, findings are consistent with previous studies and surveillance data in India (National Family Health Survey-3) from other age groups (21).

Confirmation of plausibility and internal validity was demonstrated in this study. Detailed consumption data were also collected prospectively, including bhakri consumed per day, flour and bhakri weight, and food disappearance data. Plausibility analyses confirmed findings from intention-to-treat analyses, and indicated that the strongest effects of the intervention were in children who were iron deficient at baseline.

To our knowledge, this is the first randomized trial of iron-biofortified pearl millet in this age group; there are limited data from iron-biofortification interventions to date to which results are directly comparable. However, results are consistent with findings from our previous efficacy trial of iron-biofortified rice in 192 religious sisters in Manila, Philippines (22). Iron-biofortified rice resulted in a modest increase in SF (P = 0.10) and TBI (P = 0.06), but not hemoglobin (P = 0.59), with significant improvements in SF and TBI observed only among women who were nonanemic at baseline and those who consumed the most iron from rice. Based on a reported fractional absorption of iron of 7.3% from pearl millet in young children in Karnataka, India, and the amount of iron consumed by the current study participants, sufficient iron was absorbed to meet physiologic requirements (23). Further, the quantity of iron consumed by children in the intervention group throughout the course of the trial and by the children in the control group after midline far exceeds the estimated average requirements of iron for this age group (24). In fact, the dose of iron delivered (20 mg/d) is close to that of an iron supplement, making study findings comparable to supplementation trials.

Our analysis is distinct from previous studies because of the population and age group, and comprehensive assessment of iron status prospectively. Of note is the assessment of both serum CRP and AGP prospectively, similar distributions of inflammation in the treatment groups, and the low rates of inflammation (<5%) in this population, which suggest limited impact from the acute phase response for the interpretation of SF.

This study has some limitations. The low prevalence of anemia (<30%), exclusion of severe anemia at baseline, and the boarding school setting may limit the generalizability of findings to other populations. Also, the greater impact of the intervention on iron status among children who were iron deficient at baseline suggests that the effects of the intervention may vary according to baseline iron status; however, the trial was not designed to examine the effects of the intervention by ID status at baseline or potential to benefit in intention-to-treat analyses. The lack of assessment of hepcidin and other micronutrients (e.g., vitamin B-12 and folate) that influence hematologic status are limitations that should be assessed in future studies.

In conclusion, consuming iron-biofortified pearl millet significantly improved iron status in children by 4 mo. Findings suggest that feeding iron-biofortified pearl millet is an efficacious approach to improve iron status in secondary school–age children. The observed effects of iron-biofortified pearl millet on iron status among children who were iron deficient at baseline suggests additional potential to benefit, particularly among populations with the highest prevalence of ID. Assessment of functional outcomes and consideration of other high-risk populations such as

TABLE 3 Effect of Fe-PM intervention on resolution of anemia and iron deficiency in children compared with Control-PM[1]

Outcome	n/n[2]	RR (95% CI)	P
Anemia			
Hemoglobin <12.0 g/dL			
Total	17/63	1.78 (0.75, 4.21)	0.19
Baseline–4 mo	35/63	0.91 (0.59, 1.42)	0.69
4–6 mo	15/43	1.20 (0.53, 2.72)	0.67
Iron deficiency			
SF <15.0 µg/L			
Total	50/94	1.64 (1.07, 2.49)	0.02
Baseline–4 mo	43/82	1.32 (0.86, 2.03)	0.21
4–6 mo	31/66	0.98 (0.58, 1.65)	0.94
TBI <0 mg/kg			
Total	28/47	1.28 (0.79, 2.07)	0.32
Baseline–4 mo	22/41	1.52 (0.84, 2.73)	0.17
4–6 mo	12/31	0.42 (0.11, 1.57)	0.20
Any iron deficiency[3]			
Total	45/99	1.92 (1.17, 3.14)	< 0.01
Baseline–4 mo	27/86	1.48 (0.77, 2.85)	0.24
4–6 mo	47/96	0.95 (0.63, 1.44)	0.82

[1] "Resolution" of hematological outcomes was defined as having the outcome of interest at the first time point and no longer having the outcome of interest at the second time point (e.g., iron deficient at baseline and iron replete at endline) (20). Control-PM, control pearl millet; Fe-PM, iron-biofortified pearl millet; SF, serum ferritin; sTfR, soluble transferrin receptor; TBI, total body iron.

[2] Number of cases out of number at risk of the outcome of interest.

[3] SF <15.0 µg/L, sTfR >8.3 mg/L, or TBI <0 mg/kg.

young children are warranted to elucidate the impact of iron interventions on human health. Additional studies should be conducted to evaluate the effectiveness of these interventions in a broader population context, particularly in those who consume pearl millet as a staple, as in western and central India.

Acknowledgments

We thank Mr. GD Khandeshe and the Ahmednagar Zilla Maratha Vidya Samaj Trust for their permission to conduct this research in their schools. We thank Anil Udawant, the research field supervisor, for his daily diligence and dedication during the 7 mo of data collection. JDH designed the research, supervised the data collection, and contributed to writing the manuscript; JLF and SM conducted the data analysis and wrote the initial draft of the manuscript; SAU, PSG, MJW, and LEM-K contributed to the study design; and SAU, PG, SVL, and EMP supervised the study implementation and data collection in the field. JDH had primary responsibility for the final content. All authors contributed to the development of this manuscript and read and approved the final version.

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