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Consuming Iron Biofortified Beans Increases Iron Status in Rwandan Women after 128 Days in a Randomized Controlled Feeding Trial[1–3]

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Abstract

Background: Food-based strategies to reduce nutritional iron deficiency have not been universally successful. Biofortification has the potential to become a sustainable, inexpensive, and effective solution.

Objective: This randomized controlled trial was conducted to determine the efficacy of iron-biofortified beans (Fe-Beans) to improve iron status in Rwandan women.

Methods: A total of 195 women (aged 18–27 y) with serum ferritin <20 μg/L were randomly assigned to receive either Fe-Beans, with 86 mg Fe/kg, or standard unfortified beans (Control-Beans), with 50 mg Fe/kg, 2 times/d for 128 d in Huye, Rwanda. Iron status was assessed by hemoglobin, serum ferritin, soluble transferrin receptor (sTfR), and body iron (BI); inflammation was assessed by serum C-reactive protein (CRP) and serum α1-acid glycoprotein (AGP). Anthropometric measurements were performed at baseline and at end line. Random weekly serial sampling was used to collect blood during the middle 8 wk of the feeding trial. Mixed-effects regression analysis with repeated measurements was used to evaluate the effect of Fe-Beans compared with Control-Beans on iron biomarkers throughout the course of the study.

Results: At baseline, 86% of subjects were iron-deficient (serum ferritin <15 μg/L) and 37% were anemic (hemoglobin <120 g/L). Both groups consumed an average of 336 g wet beans/d. The Fe-Beans group consumed 14.5 ± 1.6 mg Fe/d from biofortified beans, whereas the Control-Beans group consumed 8.6 ± 0.8 mg Fe/d from standard beans ($P < 0.05$). Repeated-measures analyses showed significant time-by-treatment interactions for hemoglobin, log serum ferritin, and BI ($P < 0.05$). The Fe-Beans group had significantly greater increases in hemoglobin (3.8 g/L), log serum ferritin (0.1 log μg/L), and BI (0.5 mg/kg) than did controls after 128 d. For every 1 g Fe consumed from beans over the 128 study days, there was a significant 4.2-g/L increase in hemoglobin ($P < 0.05$).

Conclusion: The consumption of iron-biofortified beans significantly improved iron status in Rwandan women. This trial was registered at clinicaltrials.gov as NCT01594359. *J Nutr* 2016;146:1586–92.

Keywords: iron, beans, biofortification, women, Rwanda, ferritin, hemoglobin

Introduction

Iron deficiency is one of the most common micronutrient deficiencies in the world, affecting women, children, and infants most severely. It is especially prevalent in resource-poor settings. According to WHO 2012 mortality data, ~17,000 deaths each year in women of reproductive age worldwide are attributed to iron deficiency anemia, with >70% of those deaths occurring in Africa (1).

The most common consequence of iron deficiency is anemia, defined for women as hemoglobin <120 g/L (2). Iron deficiency with or without anemia is prevalent among women of reproductive age because of menstrual losses and the high physiologic requirement for iron. Functional consequences of iron deficiency include decreased physical performance and physical activity, decreased cognitive performance, depression, and fatigue (3, 4).

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[3] Supplemental Table 1 is available from the "Online Supporting Material" link in the online posting of the article and from the same link in the online table of contents at http://jn.nutrition.org.

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Social and economic consequences include increased maternal and perinatal mortality, low work productivity, increased energy needs, and lost disability-adjusted life-years (5).

Nutritional strategies to reduce the burden of iron deficiency include dietary diversity, supplementation, fortification, and biofortification (6). Interventions to implement these strategies have not been universally successful. Of these strategies, biofortification, or enhancing iron in staple food crops through breeding or agronomic management, is the newest and has the potential to become a sustainable, inexpensive, and effective solution at the population level (7).

A recent review by Petry et al. (8) provided convincing evidence for the use of the common bean (*Phaseolus vulgaris*) as an excellent vehicle for iron biofortification. High iron concentrations (up to 100 mg/g) have been achieved by selective breeding, and the iron has been shown to have good fractional absorption (4–7%). Beans are a primary staple food in Rwanda, where per capita consumption estimates have been reported to be among the highest in Africa (9). The high amount of bean consumption and availability of multiple experimental biofortified bean varieties with significantly greater iron content than common varieties (e.g., 86 compared with 50 mg/kg) provided an opportunity to evaluate their efficacy for improving iron status in a human population. The objective of this study was to determine the efficacy of iron-biofortified beans in improving iron status compared with control beans in an iron-depleted population.

Methods

Participants. Participants were nonpregnant women, aged 18–27 y, with low iron stores and who were otherwise healthy and who were students at the University of Rwanda at Huye. Only women who were iron-depleted with serum ferritin <20 μg/L at screening without moderate or severe anemia (i.e., hemoglobin ≥90 g/L) were invited to participate. Women with hemoglobin <90 g/L were treated with 60-mg/d ferrous sulfate capsules for 30 d and referred to the university hospital for follow-up. The exclusion criteria were as follows: use of iron supplements, any major medical conditions, use of medications that could interfere with dietary iron absorption, use of psychoactive drugs, pregnancy, lactation, and BMI (in kg/m^2) <16. Two months before the start of the feeding trial, 1000 women were screened for iron deficiency, 761 were excluded, and 239 women were enrolled in the feeding trial (**Figure 1**). After accounting for dropouts and missing blood values, data for a sample of 195 women were available for analysis. An estimated final sample size of 105 subjects/group was calculated before the study to test for an effect size of a 3.26-μg/L (0.45 SD units) difference in change in ferritin at $\alpha = 0.05$ and 90% power. We anticipated a drop-out and lost data rate of 15% but experienced an 18% loss, due, in part, to 35 subjects whose baseline ferritin values increased from their screening value of <20 μg/dL, which disqualified them because they were no longer iron-depleted.

Study design. This was a double-blind randomized efficacy trial of iron-biofortified beans (Fe-Beans)[11] and standard unfortified beans (Control-Beans), which were similar in appearance, taste, and cooking properties. Before initiating the feeding trial, the acceptability of the 2 bean varieties was tested on a sample of 20 University of Rwanda female students. Both bean varieties were scored as 100% acceptable on a 3-point hedonic scale, and no discernable difference in preference was recorded between the varieties.

A total of 239 Rwandan women with screening serum ferritin <20 μg/L were randomly assigned to 1 of 4 color groups (pink, white, green, or blue) and consumed beans for 2 meals/d every day for 128 d from 1 of 4 buffet-style serving dishes that matched the subject's color code. Two of the colors represented Fe-Beans and 2 colors represented Control-Beans. Four color groups were used to reduce the probability that subjects and staff would be able to easily determine to which of the 2 treatment groups they were assigned. Participants were served 175 g (wet wt) cooked beans/d at both the lunch and dinner meals and were encouraged to consume more if they wanted more. Plate waste was measured to determine compliance. Feeding occurred over 128 d between 7 January and 15 May 2013.

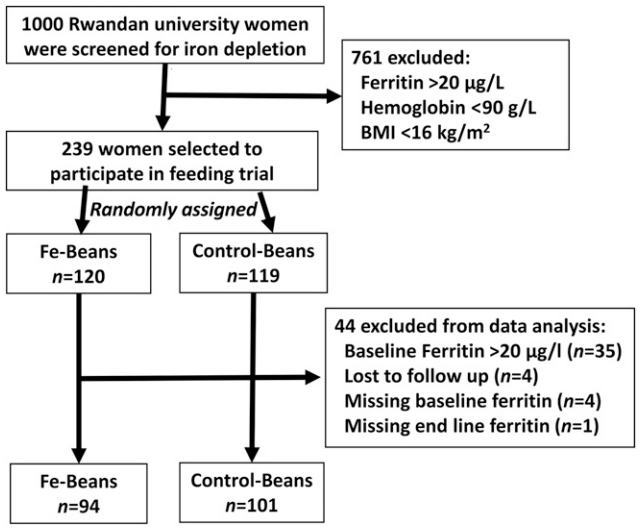

FIGURE 1 Sample selection.

A blood sample was collected by trained phlebotomists during the first week of feeding to measure baseline iron status by hemoglobin, serum ferritin, and soluble transferrin receptor (sTfR) concentrations and inflammation status according to C-reactive protein (CRP) and α1-acid glycoprotein (AGP) concentrations. Participants were initially categorized as anemic if their hemoglobin was <120 g/L, iron-depleted if they had serum ferritin <20 μg/L, and iron-deficient if their serum ferritin was <15 μg/L. Inflammation status was classified as positive with the use of previously published cutoffs (CRP: >5 mg/L; AGP: >1 g/L) (10).

Random serial sampling. For 8 consecutive weeks between 15 February and 3 April, midpoint blood data were obtained for all participants with a random serial sampling method (11), whereby during each of the 8 wk a randomly selected subset of ~12% of the study population provided a blood sample. End-line blood sampling occurred for all subjects on 13–15 May 2013.

Ethics approval. Informed consent was obtained individually from the subjects who participated in the study before the first screening blood sample was taken. The institutional review boards of Cornell University, The University of Oklahoma, and The Pennsylvania State University; the ethical committee of The Swiss Federal Institute for Technology (ETH) Zurich; and the Rwandan National Ethical Committee approved the research protocol. A permit to conduct this research was issued by the Rwanda Ministry of Education, Directorate of Science, Technology, and Research. The study was registered with clinicaltrials.gov (NCT01594359).

Beans. The beans were a carioca (cream- and brown-striped) grain type grown at the International Center for Tropical Agriculture [Centro Internacional de Agricultura Tropical (CIAT)] campus in Cali, Colombia, and shipped to Rwanda. Both Fe-Beans and Control-Beans were produced under similar conditions of soil and climate. The Fe-Beans were a mixture of high-iron sister lines that carry the CIAT SMC code indicating higher concentrations of iron, whereas the Control-Beans, G4825, were a land race obtained from the Genetic Resources Unit of the CIAT. Nineteen weekly random bean samples taken throughout the study were analyzed for mineral content by inductively coupled plasma mass spectrometry at the USDA/Agricultural Research Service Robert W Holley Center for

[11] Abbreviations used: AGP, α1 acid glycoprotein; BI, body iron; CIAT, Centro Internacional de Agricultura Tropical (International Center for Tropical Agriculture); Control-Beans, standard unfortified beans; CRP, C-reactive protein; Fe-Beans, iron-biofortified beans; sTfR, soluble transferrin receptor.

Agriculture and Health, Ithaca, NY. The Fe-Beans contained (mean ± SD) 86 ± 4.5 mg Fe/kg dry wt, whereas the Control-Beans contained 50 ± 3.9 mg Fe/kg. All beans were stored in an air-conditioned, pest-free, and secured warehouse at the Rubona research facility of the Rwanda Agriculture Board. The 2 bean types were packaged separately at CIAT with color-coded identifiers. None of the study participants, field staff, or researchers knew the code, which was only broken after the intent-to-treat analysis was complete.

Serving of beans and meals. Subjects were provided with the meals in a specially equipped cafeteria room separated from the main university dining facilities used by nonparticipating students. Lunches and dinners were served buffet-style, with each meal consisting of beans, 2 starch side dishes, a vegetable, and a tomato-based sauce. No meat was served during the feeding trial.

Menus varied daily and from lunch to dinner, with the same menus rotating over a 2-wk cycle. Starches included white potatoes, sweet potatoes, rice, pasta, cassava, maize porridge (*ugali*), and plantains. Vegetables included cabbage and cassava greens. Bottled potable water was the only liquid served at mealtimes. Ice cream and fruit were served as a dessert on Sundays. Beans were served by trained servers from color-coded, heated serving dishes. All beans were prepared under controlled conditions in a separate kitchen by cooks who were trained to keep beans separated by color code from storage to serving. Although beans were prepared by study staff, the side dishes were prepared in the general university kitchen following standard recipes.

Individual subjects' daily nutrient intakes from the whole diet were assessed during 3 d (2 weekdays, 1 weekend day) at the midpoint of the feeding trial. Individual food portions were weighed in the study cafeteria for each subject at lunch and dinner and collected by 24-h diet recall for breakfast and snacks (12). Individual nutrient intakes were calculated from food-composition tables compiled for East African diets (13).

Adherence. The preparation and intake of beans were monitored daily by field assistants specifically recruited and trained for bean consumption assessment. The weight of beans consumed (to the 0.5-g unit) by each woman was recorded for each meal. Subjects were allowed to consume side dishes ad libitum. Subjects consumed between 150 and 175 g cooked beans/meal (75.0–87.5 g dry wt).

Laboratory methods. Whole-blood samples were collected by venipuncture by trained phlebotomists and placed in EDTA-coated tubes at the prescreening, baseline, random midpoint, and end line. Whole blood was analyzed within 6 h of collection by the Kanombe Military Hospital in Kigali. Blood was analyzed with a Sysmex Automated Hematology Analyzer (model XS-1000i) for complete blood count, including hemoglobin. Plasma was separated and stored at −20°C before shipping by air on dry ice to the VitMin Laboratory in Willstaett, Germany, where it was analyzed for serum ferritin, sTfR, CRP, and AGP following a sandwich ELISA procedure (14). Laboratory samples were tested in batches by a senior technician, and instruments were calibrated daily on the basis of standardized procedures. Total body iron (BI) was estimated as the ratio of sTfR and serum ferritin according to Cook's formula (15).

Statistical analysis. This study was designed to investigate, as the primary objective, the efficacy of Fe-Beans compared with Control-Beans by using mixed regression models with repeated measurements. This analysis was based on data from 195 subjects who were iron-depleted at baseline (serum ferritin <20 μg/L) and who had baseline and end-line biomarker data.

Individual subjects' serum ferritin values were evaluated for bias due to inflammation (10) by using AGP and CRP as markers of inflammation. Analyses for group differences in serum ferritin, log serum ferritin, and BI were conducted with values that were adjusted by the Thurnham multipliers (10) as well as with unadjusted values. Thurnham corrections were applied to a maximum of 3.6% of subjects at baseline, 4.6% at midpoint, and 8.2% at end line. There were no group differences in AGP or CRP at any blood sampling time during the study. Because the statistical analysis of Thurnham-adjusted values did not differ from the unadjusted values, we did not adjust the individual ferritin values in the subsequent analysis, although AGP and CRP were included as covariates in multiple regression analyses.

Descriptive statistics are reported as medians (IQRs) and means ± SDs (or 95% CIs). Group differences in means at baseline for continuous variables were tested by using Student's *t* test. Group differences at baseline for categorical binary variables were tested by using Pearson's chi-square test.

Intent-to-treat analyses are reported for all iron status markers, and group differences were assessed by 2 methods: *1*) mean difference in change in values at end line minus baseline (difference-in-difference) by Student's *t* test, which does not adjust for group differences at baseline and any covariates, and *2*) change from baseline to end line by using mixed-effects regression models with repeated measures that account for all sampling time points. Mixed-effects models estimate the overall mean population response over time and take both population variability (fixed effects) and individual variability (random effects) into account in the analyses (11, 16, 17). Because blood data were collected for each subject at 3 time points during the feeding trial, mixed-effects models for repeated measurements were derived separately for each outcome variable (hemoglobin, serum ferritin, log serum ferritin, sTfR, and BI) with time (week of measurement) and treatment group (Fe-Beans or Control-Beans) and interactions as fixed effects for each treatment group. The random effects (individual variations) were introduced for the intercept and slope of each subject's set of repeated measurements. All of the data were analyzed by using SAS version 9.4 (SAS Institute). When appropriate, potential confounding due to the amount of beans consumed, subject's age, menstrual status, CRP, AGP, weight, and height was analyzed in the multivariate regression modeling, with $P < 0.10$ required to accept the covariate. Significance was set at $P < 0.05$ for primary objectives and $P < 0.10$ for secondary or plausibility analyses. Biologically implausible values were excluded from the analyses. A secondary objective was a test of internal validity or plausibility of the intent-to-treat results by ordinary least squares regression analysis for the relation between change in hemoglobin and the total iron consumed from beans over the study period.

Results

After screening, 239 iron-depleted (serum ferritin <20 μg/L) Rwandan women were randomly assigned to receive either Fe-Beans ($n = 120$) or Control-Beans ($n = 119$) (Figure 1). During baseline assessment, which occurred 2 mo after screening, 35 subjects had serum ferritin >20 μg/L and were eliminated from this analysis. Incomplete data ($n = 5$) and loss to follow-up ($n = 4$) resulted in a final sample of 195 subjects. At baseline, 37% of these women were anemic (hemoglobin <120 g/L), 86% were iron-deficient (serum ferritin <15 μg/L), 35% were iron-deficient anemic, 35% had elevated sTfR (>8.3 mg/L), and 55% had negative total BI (<0 mg/kg). Inflammation (either CRP >5 mg/L or AGP >1 g/L) was present in 4% of the women. There were no differences between treatment groups for any of the baseline measures (**Table 1**).

During the 128-d feeding trial, subjects in both groups consumed a mean of 218 meals and 43.0 kg cooked beans (21.5 kg dry wt) from the study cafeteria (**Table 2**). The Fe-Beans group ingested a total of 1.9 ± 0.2 g Fe from the Fe-Beans, and the Control-Beans group consumed 1.1 ± 0.1 g Fe from Control-Beans. Daily consumption of iron from beans was 14.5 mg in the Fe-Beans group compared with 8.6 mg in the Control-Beans group ($P < 0.05$). On the basis of the estimated fractional absorption of iron of 7.3% and 9.2% for the Fe-Beans and Control-Beans, respectively, reported by Petry et al. (18) for the same beans used in this study, the daily amount of absorbed iron from beans was 1.06 mg/d and 0.79 mg/d, respectively. This represents 75% and 56% of the daily iron requirement (Estimated

TABLE 1 Baseline characteristics of iron-depleted Rwandan women after random assignment to either the Fe- or Control-Beans intervention groups[1]

	Fe-Beans (n = 94)	Control-Beans (n = 101)
Demographic characteristics		
Age, y	22 (21, 23)	22 (21, 24)
Anthropometric characteristics		
Height, cm	158 (155,163)	158 (154,164)
Weight, kg	56.0 (51, 61)	56.0 (51, 60)
BMI, kg/m^2	22.4 (22.5, 24.4)	22.4 (20.4, 24.1)
Prevalence, %		
Anemia[2] (hemoglobin <120 g/L)	40	34
Iron deficiency[2,3] (serum ferritin <15 μg/L)	84	88
Iron deficiency anemia[2,3]	37	33
Elevated sTfR[4] (>8.3 mg/kg)	32	38
Total BI[3,5] (BI <0.0 mg/kg)	55	56
CRP[6] (serum CRP >5 mg/L)	4	0
AGP[6] (serum AGP >1 g/L)	2	5

[1] Values are medians (25th, 75th percentiles) unless otherwise indicated. There were no differences ($P > 0.05$) between groups for any of these measures at baseline. AGP, α1 acid glycoprotein; BI, body iron; Control-Beans, standard unfortified beans; CRP, C-reactive protein; Fe-Beans, iron-biofortified beans; sTfR, soluble transferrin receptor.
[2] Cutoff values according to WHO (2).
[3] No corrections were made for inflammation.
[4–6] Cutoff values according to [4]Erhardt et al. (14), [5]Cook et al. (15), and [6]Thurnham et al. (10).

Average Requirement) estimated from basal and menstrual losses of 1.41 mg/d for young women (19). Body weight significantly increased by 1.7 ± 0.2 kg in both groups over the course of the study, and the amount of beans consumed significantly correlated with change in body weight (r = 0.25, P < 0.01). Other components of the diet were estimated from 3 d of individual food weighing and 24-h recalls at the study midpoint. Subjects consumed a mean of 2174 ± 535 kcal/d (9.10 ± 2.24 MJ/d) and 7.9 mg Fe/d from nonbean sources, with no differences between feeding groups. When considering iron from all dietary sources the Fe-Beans group ingested 22.1 ± 3.1 mg/d and the Control-Beans group ingested 16.3 ± 3.1 mg/d.

Difference-in-difference analysis. The effect of the intervention on iron status is presented in **Table 3**, which reports mean values for change in each iron status indicator for the Fe-Beans and Control-Beans groups. There were no differences between Fe-Beans and Control-Beans groups at baseline for any of the iron status measures ($P > 0.05$). During the intervention there was a significant change in hemoglobin, log serum ferritin, and BI ($P < 0.05$). The mean change in hemoglobin was 3.0 g/L in the Fe-Beans group and −1.2 g/L in the Control-Beans group ($P < 0.001$). The mean change in log-transformed serum ferritin was 0.4 log μg/L in the Fe-Beans group compared with 0.3 log μg/L in the Control-Beans group (P = 0.03). The mean change in BI was 1.5 mg/kg in the Fe-Beans group compared with 1.0 mg/kg in the Control-Beans group (P = 0.03). There was no significant effect of the intervention on change in untransformed serum ferritin and sTfR concentrations. These results were confirmed by additional statistical analyses. **Supplemental Table 1** presents the confirmation of results on the basis of ordinary least squares regression analysis, which tests for change in iron status after controlling for variation in baseline values.

Because the difference-in-difference analysis does not account for variation in baseline measures and the midpoint measures taken on random subsamples measured between 5 and 12 wk, the more rigorous repeated-measures analysis was performed on all iron status measures. The results are shown in **Table 4**. There were significant time-by-treatment group interactions for hemoglobin, log serum ferritin, and BI. There was no significant effect of the intervention on untransformed serum ferritin and sTfR concentrations. The predicted values for change in iron indicators from the repeated-measures analysis are consistent with the values reported in Table 3, in which midpoint values were not included in the analysis.

Plausibility. A secondary objective tested for internal consistency and plausibility of the results from the primary analysis, which tested for the treatment effects. The amount of iron consumed from beans predicted the change from baseline to end line in hemoglobin ($P < 0.05$) (**Table 5**). **Figure 2** shows that, for every 1.0 g Fe consumed from beans over the course of the study, there was a significant 4.2-g/L increase in hemoglobin. Likewise, iron consumed from beans significantly predicted the change in log serum ferritin (0.1-log-μg/L increase/g Fe consumed; $P < 0.05$). There was a marginally significant effect of a 1.4-μg/L increase in serum ferritin and a 0.5-mg/kg increase in BI for each gram of iron consumed from beans ($P < 0.10$) and no significant relation was seen for sTfR.

Discussion

As a randomized, double-blinded, controlled feeding trial, the results of this study support an inference of causality with respect to the efficacy of biofortification as a dietary iron intervention. The strong study design allowed for rigorous primary and secondary analyses.

In this study, iron-biofortified beans significantly improved iron status in Rwandan university women after 128 d of consuming Fe-Beans. This intervention significantly increased hemoglobin, serum ferritin concentrations (as log serum ferritin), and BI. Total iron intake from beans was associated with increased hemoglobin and BI.

Randomization was effective in accounting for potential group differences in measured baseline characteristics. The Fe-Beans and Control-Beans groups were similar at baseline for all important measured confounders. We assume that randomization also resulted in the consumption of similar diet components, such

TABLE 2 Bean consumption and iron intake of iron-depleted Rwandan women who consumed Control- and Fe-Beans over 128 d[1]

Variable	Fe-Beans (n = 94)	Control-Beans (n = 101)
Total meals consumed, n/128 d	217 ± 26	218 ± 23
Total cooked beans consumed, kg/128 d	43.3 ± 4.2	43.0 ± 4.8
Iron intake from beans, mg/d	14.5 ± 1.6[2]*	8.6 ± 0.8[3]
Total iron intake from beans, g/128 d	1.86 ± 0.2*	1.10 ± 0.1
Total iron absorbed from beans, mg/128 d	135.6 ± 15.2[4]*	101.5 ± 9.8[5]
Iron absorbed from beans, mg/d	1.06 ± 0.12*	0.79 ± 0.08

[1] Values are means ± SDs. *Different from Control-Beans by t test, $P < 0.001$. Control-Beans, standard unfortified beans; Fe-Beans, iron-biofortified beans.
[2] Based on an iron concentration of 43 mg/kg, wet wt.
[3] Based on an iron concentration of 25 mg/kg, wet wt.
[4] Based on fractional absorption of 7.3% (18).
[5] Based on fractional absorption of 9.2% (18).

TABLE 3 Effect of the biofortified bean intervention on change in iron status over 128 d from baseline to end line in iron-depleted Rwandan women: difference-in-difference analysis[1]

	Fe-Beans (n = 94)		Control-Beans (n = 101)		
	Mean ± SD	95% CIs	Mean ± SD	95% CIs	P (t test)
Hemoglobin, g/L					
Baseline	121 ± 13.9	118, 124	123 ± 13.7	120, 125	0.40
End line	124 ± 13.8	121, 127	122 ± 12.9	119, 124	0.17
Change[2]	3.0 ± 6.6	2.0, 4.0	−1.2 ± 8.0	−2.8, 0.4	<0.001
Ferritin, μg/L					
Baseline	10.0 ± 4.2	9.1, 10.8	10.0 ± 3.9	9.2, 10.7	0.90
End line	15.4 ± 9.0	13.6, 17.3	13.6 ± 7.9	12.2, 15.1	0.13
Change[2]	5.5 ± 7.5	3.9, 7.0	3.7 ± 6.0	2.5, 4.9	0.07
Log ferritin, ln μg/L					
Baseline	2.2 ± 0.5	2.1, 2.3	2.2 ± 0.4	2.1, 2.3	0.77
End line	2.6 ± 0.5	2.5, 2.7	2.5 ± 0.5	2.4, 2.6	0.16
Change[2]	0.4 ± 0.4	0.3, 0.5	0.3 ± 0.4	0.2, 0.3	0.03
sTfR, mg/L					
Baseline	8.0 ± 4.1	7.2, 8.9	8.0 ± 3.5	7.3, 8.7	0.99
End line	7.8 ± 3.6	7.0, 8.5	8.0 ± 3.2	7.3, 8.6	0.73
Change[2]	−0.2 ± 2.5	−0.8, 0.3	−0.1 ± 2.3	−0.5, 0.4	0.64
Body iron, mg/kg					
Baseline	−0.7 ± 2.8	−1.3, −0.1	−0.7 ± 2.5	−1.2, −0.2	0.97
End line	0.8 ± 2.8	0.2, 1.3	0.3 ± 2.7	−0.3, 0.8	0.22
Change[2]	1.5 ± 1.6	1.1, 1.8	1.0 ± 1.6	0.6, 1.3	0.03

[1] Control-Beans, standard unfortified beans; Fe-Beans, iron-biofortified beans; sTfR, soluble transferrin receptor.
[2] Change over 128 d.

as ascorbic acid and polyphenolics that affect iron absorption. Except for beans, all of the subjects consumed the same side dishes from the limited buffet line. The quantity of bean consumption was similar for both groups, and the length of the intervention trial was sufficient to detect changes in iron status.

Intention-to-treat analyses were performed by using a repeated-measures analysis, which takes full advantage of the random serial sampling that was part of the original study design. Plausibility analyses confirmed the results of the intention-to-treat analyses and indicated that the consumption of iron from beans was the source of the changes in iron status. Detailed bean consumption data were collected, including grams of beans consumed per meal per day. The availability of published data (18, 20) on fractional absorption of iron from the identical study beans and similar subjects allows more accurate estimates of individual absorbed iron to compare with individual changes in iron status.

The sample size was adequate to test for significant effects of consuming the Fe-Beans compared with the Control-Beans with 90% power, and other than the 35 subjects whose ferritin increased from screening to baseline there were few dropouts or subjects with incomplete data (4.5%). The sample was selected to be iron-depleted at baseline and thus likely to benefit from increased iron in the diet. There was a significant difference in iron content of the 2 varieties of beans (86 compared with 50 mg/kg, dry wt). Bean consumption was high and well-monitored, with 43 kg (21.5 kg dry wt) consumed over the study period or 168 g wet wt/meal. This allowed for a substantial amount of iron to be ingested from cooked beans (14.5 compared with 8.6 mg/d), and after accounting for fractional absorption of iron the difference in absorbed iron was 34 mg greater in the Fe-Beans group over the 128 feeding days of the study. The fact that the Control-Beans group consumed a substantial amount of absorbable iron from Control-Beans accounts for their significant improvement in ferritin and total body iron. Although these gains do not approach the gains observed for the Fe-Beans group, it does suggest the importance of nonbiofortified beans as a potential source of iron in the diet in those who rely heavily on beans as a staple food. The 34-mg group difference in total absorbed iron, if transferred in its entirety to BI, would account for a 0.53-mg/kg greater increase in the Fe-Beans group. The estimated BI increase is 0.50-mg/kg greater in the Fe-Beans group than in the controls, suggesting that there was sufficient absorbed-iron differential between the 2 bean varieties to result in a plausible increase in iron status over the period of this study.

Further confirmation of the interacting effects of iron status and total amount of iron ingested from beans was shown by the significant improvement in hemoglobin and log serum ferritin per gram of iron consumed from beans over the course of the study (Figure 2, Table 5). On the basis of the regression coefficient of a 4.2-g/L increase in hemoglobin per gram increase in iron consumed from beans, the 0.76-g difference in iron intake over 128 d for the Fe-Beans group translates to a predicted 3.2-g/L increase in hemoglobin compared with the Control-Beans group. This approximates the 3.8-g/L observed difference in the change in hemoglobin between the 2 groups (Table 4).

These findings should be evaluated relative to several factors that may bias results of long-term feeding studies. Because serum ferritin is an acute-phase protein, it and its calculated derivative, total BI, may be influenced by inflammation, resulting in elevated values of serum ferritin and BI. In this sample of university women the prevalence of inflammation as assessed by AGP and CRP was a maximum of 8.2% at any time point during the study. Because

TABLE 4 Results of repeated-measures analysis of iron status markers by intervention group and time, controlling for beans consumed, height, date of last menses, and CRP in iron-depleted Rwandan women[1]

	Observed baseline		Predicted end line		Predicted change[2]		P for effect of		
	Fe-Beans	Control-Beans	Fe-Beans	Control-Beans	Fe-Beans	Control-Beans	Group	Week	Group by Week
Hemoglobin, g/L	121	123	124	122	2.8	−1.0	NS	NS	<0.001
Ferritin, μg/L	10.0	10.0	15.5	13.0	5.5	3.6	NS	<0.001	NS
Log ferritin, ln μg/L	2.2	2.2	2.6	2.5	0.4	0.3	NS	<0.001	<0.05
sTfR, mg/L	8.0	8.0	7.7	7.9	−0.2	−0.1	NS	<0.001	NS
Body iron, mg/kg	−0.7	−0.7	0.8	0.3	1.4	0.9	NS	<0.001	<0.05

[1] Values are observed (baseline) and predicted (end line and change) means, n = 195. All covariates are significant ($P < 0.05$) in all models. CRP is included as a covariate for models that included ferritin and body iron. NS, $P > 0.05$. Control-Beans, standard unfortified beans; CRP, C-reactive protein; Fe-Beans, iron-biofortified beans; sTfR, soluble transferrin receptor.
[2] Change over 128 d.

TABLE 5 Change in iron status predicted from the amount of iron consumed from beans over 128 d by iron-depleted Rwandan women[1]

	Change per 128 d in				
	Hemoglobin, g/L	Serum ferritin, μg/L	Log serum ferritum, log μg/L	sTfR, mg/L	BI, mg/kg
Intercept	5.2 (–3.7, 14.0)	1.7 (–1.2, 4.6)	0.5 (0.2, 0.8)**	2.1 (1.1, 3.1)**	0.4 (–0.4, 1.2)
Baseline value	−0.1 (–0.1, 0)**	0 (–0.2, 0.1)	−0.2 (–0.3, –0.1)**	−0.2 (–0.3, –0.2)**	−0.1 (–-0.2, 0)**
Iron consumed, g	4.2 (2.1, 6.3)**	1.4 (–0.1, 3.0)*	0.1 (0, 0.2)**	−0.2 (–0.8, 0.3)	0.5 (0, 1.0)*

[1] Values are ordinary least squares regression coefficients (95% CIs) for changes in 5 iron status indicators from baseline to end line or every 1-g increase in iron consumed from beans over 128 study days; n = 195. *$P < 0.10$, **$P < 0.05$. BI, body iron; sTfR, soluble transferrin receptor.

there was no significant relation between these inflammation markers and serum ferritin at any time during the study, no serum ferritin values were excluded or adjusted due to elevated CRP or AGP. Moreover, when CRP was included as a covariate in repeated-measures analysis, the size and significance of treatment effects were not affected.

A strength of this study is the ability to assess iron status at 3 time points for each subject. Although blood sampling occurred over 2 d at both baseline and end line, the midpoint samples were collected over an 8-wk period, with ~12% of subjects chosen at random each week to provide blood samples. This random serial sampling protocol allowed for a statistically robust 3-time-point repeated-measures analysis to test for treatment effects that also controlled for variable sampling points. The random serial sampling also allowed an evaluation of the time course of the hemoglobin response, which suggests that the effect of consuming Fe-Beans seemed to emerge at ~12 wk into the study (data not shown).

To our knowledge, this is the first efficacy trial that reports the effect of iron-biofortified beans on iron status in an at-risk population. The results reported here are consistent with previously published human studies that showed a significant effect on iron status from consumption of iron-biofortified rice in iron-deficient Filipino women (21) and iron-biofortified pearl millet in iron-deficient Indian schoolchildren (22). The size of the effect on serum ferritin observed in the current study is most similar to the pearl millet study, in part because of the large amount of iron that could be delivered over a similar 4-mo time period and the large difference in the total amount of iron between high-iron and control groups of 760 mg for Fe-Beans and 1020 mg for iron-biofortified pearl millet over 4 mo of the feeding trial. The current study and the pearl millet study showed a greater effect on iron status than was seen in the iron-biofortified rice study due primarily to the relatively low dose of iron (1.42 mg/d above control rice) and only a 381-mg difference in iron intake between feeding groups over 9 mo of the rice feeding trial.

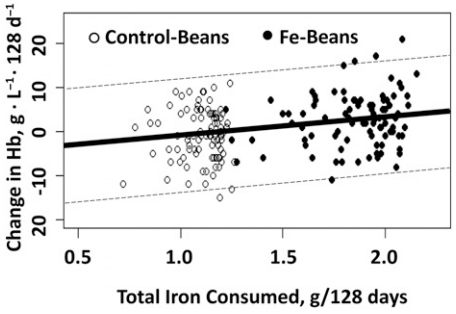

FIGURE 2 Hemoglobin change in iron-depleted Rwandan women by amount of iron consumed from Control- and Fe-Beans over 128 study days. For every 1.0 g Fe consumed over 128 study days from beans there was a significant 4.2-g/L increase in Hb ($P < 0.05$) on the basis of linear regression analysis (see Table 5). Best fit line (solid line): change in Hb (g · L^{-1} · 128 d^{-1}) = 5.15–0.01 (baseline hemoglobin) + 4.2 (iron from beans). The dashed lines represent 95% prediction limits. Control-Beans, standard unfortified beans; Fe-Beans, iron-biofortified beans; Hb, hemoglobin.

The current study is the only one of the 3 iron-biofortification trials to observe a significant effect on hemoglobin. This is due in part to the relatively high prevalence of anemia at baseline (37%) and that most of the anemia (95%) was associated with iron deficiency. The lack of a significant hemoglobin response in the pearl millet and rice feeding trials was also attributed to the high prevalence of vitamin B-12 and folate deficiencies and a relatively low prevalence of iron deficiency anemia. We did not assess vitamin B-12 and folate status in the current study. However, the strong hemoglobin response suggests that other causes of anemia did not substantially affect our ability to improve hemoglobin through the consumption of Fe-Beans. Although the populations sampled in these 3 feeding trials were very different and the total amount of additional iron consumed from iron-biofortified compared with control staple foods differed considerably, the results are consistent in that the consumption of biofortified staple foods is efficacious when subjects are iron-depleted at baseline and the duration of the feeding trial is sufficient to compensate for the study differences in iron content of the biofortified staple food.

This study benefited from a priori consideration of several design factors that improved the likelihood of observing significant effects. These included the following: a staple food that had sufficiently higher iron content than the control food, knowledge of favorable fractional absorption of iron, the sufficient amount of beans consumed as part of a typical Rwandan diet, sufficient feeding time to allow for differences in iron status to emerge, a population who has a high potential to benefit from the consumption of additional dietary iron, and inclusion of multiple iron status indicators to assess response. These conditions, some of which have been described as a rationale for beans as a vehicle for biofortification (8), need to be considered when conducting future feeding trials to test the efficacy of biofortification.

We caution that the interpretation of the size and time course of the iron response observed in this efficacy study does not directly apply to expectations for the introduction of iron-biofortified beans into the Rwandan food system. This study was conducted under controlled experimental conditions to test for the potential for a biological effect under ideal feeding conditions. The results of this study are sufficient to justify follow-up studies to test the effectiveness of introducing high-iron or biofortified beans into the Rwandan food system, with appropriate assessments along the impact pathway, from seed production

and distribution to population-level adoption of iron-biofortified beans and changes in iron status resulting in the reduction in iron deficiency. The results of this study also suggest that the consumption of iron-biofortified beans by women has the potential to improve certain functional consequences of iron deficiency, such as physical work capacity, physical activity, cognitive performance, and behavior (3). The impact of consuming iron-biofortified beans and its effects on iron status and functional correlates of iron deficiency should also be studied in other subgroups of the population, such as pregnant and lactating women and children, who are most vulnerable to iron deficiency and thus likely to benefit from increased iron in the diet.

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