Phytic Acid Concentration Influences Iron Bioavailability from Biofortified Beans in Rwandese Women with Low Iron Status¹,²

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Abstract

Background: The common bean is a staple crop in many African and Latin American countries and is the focus of biofortification initiatives. Bean iron concentration has been doubled by selective plant breeding, but the additional iron is reported to be of low bioavailability, most likely due to high phytic acid (PA) concentrations.

Objective: The present study evaluated the impact of PA on iron bioavailability from iron-biofortified beans.

Methods: Iron absorption, based on erythrocyte incorporation of stable iron isotopes, was measured in 22 Rwandese women who consumed multiple, composite bean meals with potatoes or rice in a crossover design. Iron absorption from meals containing biofortified beans (8.8 mg Fe, 1320 mg PA/100 g) and control beans (5.4 mg Fe, 980 mg PA/100 g) was measured with beans containing either their native PA concentration or with beans that were ≈50% dephytinized or >95% dephytinized.

Results: The iron concentration of the cooked composite meals with biofortified beans was 54% higher than in the same meals with control beans. With native PA concentrations, fractional iron absorption from the control bean meals was 9.2%, 30% higher than that from the biofortified bean meals (P < 0.001). The quantity of iron absorbed from the biofortified bean meals (406 µg) was 19% higher (P < 0.05) than that from the control bean meals. With ≈50% and >95% dephytinization, the quantity of iron absorbed from the biofortified bean meals increased to 599 and 746 µg, respectively, which was 37% (P < 0.005) and 51% (P < 0.0001) higher than from the control bean meals.

Conclusions: PA strongly decreases iron bioavailability from iron-biofortified beans, and a high PA concentration is an important impediment to the optimal effectiveness of bean iron biofortification. Plant breeders should focus on lowering the PA concentration of high-iron beans. This trial was registered at clinicaltrials.gov as NCT01521273. J. Nutr. 144: 1681–1687, 2014.

Introduction

Biofortification of crop plants is a multidisciplinary approach designed to combat micronutrient deficiencies by increasing the concentration and/or bioavailability of essential nutrients in plants through traditional plant breeding, genetic engineering, and/or agronomic management without affecting yield and other agronomically desirable traits (1). The main nutritional challenge of biofortification is to provide an additional amount of absorbable micronutrient that is high enough to make a useful contribution to filling the gap between current mineral intake and mineral requirements (2). Iron is a key micronutrient in biofortification programs because iron deficiency is thought to affect nearly 2 billion people worldwide, mainly women and children in developing countries (3,4). The common bean is 1 of the crops targeted for iron biofortification (1) because it is a major staple for >300 million people in Africa and Latin America, where mean per capita consumption can reach 100–180 g/d (5). The mean iron concentration of beans is 5.5 mg/100 g (5,6), and they potentially could provide an important source of iron. However, they are high in phytic acid (PA)8 (7,8), 1 of the major dietary inhibitors of iron absorption (9–12), and iron bioavailability from bean-containing meals is reported to be low (12–15).

Iron biofortification of beans via conventional plant breeding has been highly successful, and iron amounts in some bean lines have been doubled to reach 10 mg Fe/100 g (16). However, findings from a recent human absorption study with biofortified high-iron beans cast doubt on beans as a useful vehicle for iron biofortification (13). In that study, fractional iron absorption from composite meals containing the high-iron beans was

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8 Abbreviations used: CRP, C-reactive protein; lpa, low phytic acid; PA, phytic acid; PF, plasma ferritin.
significantly lower than that from the same meals containing regular beans, resulting in similar amounts of iron being absorbed from both bean meals. A higher PA concentration in the iron-biofortified beans was suggested to be responsible.

The present stable isotope studies evaluated the influence of PA on iron absorption from high-iron, carioca (pinto type) biofortified beans and control beans with a comparable genetic background. Iron absorption from typical bean meals containing high-iron-biofortified beans or control beans was measured by erythrocyte incorporation of stable isotopes in Rwandan women with low iron status. Subjects consumed multiple composite bean meals with potatoes or rice. The beans contained either their native PA concentration or were ~50% or >95% dephytinized.

Participants and Methods

Participants. The study participants were selected from a group of 100 women from student and staff of the National University of Rwanda who were screened for abnormal hemoglobin, plasma ferritin (PF), and C-reactive protein (CRP) values. Women with known metabolic, chronic, and gastrointestinal diseases; who were taking long-term medication; or who had donated blood or experienced heavy blood loss within the 6-mo period before the study were excluded. Twenty-five apparently healthy, nonpregnant, nonlactating women with low iron status (PF ≤20 μg/L, aged between 18 and 30 y, between 42 and 65 kg body weight, and with normal BMI (19–24 kg/m2) were included in the study. The intake of vitamin and mineral supplements was not allowed from 2 wk before and during the study. The experimental procedures were approved by the National Ethics Committee of Rwanda and the ethical committee of ETH Zurich, Switzerland. Written informed consent was obtained from all study participants.

Iron stable isotope study. A randomized crossover design was used in which each subject acted as her own control. The study was divided into three 2-wk feeding periods, which were separated by 2-wk rest periods. In each feeding period 2 different bean meals were served (in total, 6 different bean meals; Fig. 1). Three test bean meals were prepared from control cream-stripped (carioca) beans and 3 test meals were prepared from cream-stripped (carioca) biofortified beans. The test meals contained either beans with their native PA concentration or beans that were ~50% or >95% dephytinized.

The beans were grown from certified seeds at the International Center for Tropical Agriculture (CIAT) campus in Cali, Colombia, following best agronomic practices and minimizing soil and dust contamination from harvesting and postharvest processing, and shipped to Rwanda. The biofortified beans were a mixture of high-iron sister lines that carry the SMC code, whereas the low-iron bean, G4825, was a land race obtained from the Genetic Resources Unit of CIAT. The beans were treated the same way without adding the enzyme. The thick bean slurry after its temperature had fallen below 55°C was washed and potatoes were peeled, washed, and cut into pieces before cooking. Rice and potatoes were prepared daily before feeding. Soy oil (1.6 g) and 0.6 g noniodized salt were added to each portion. The iron stable isotopes (0.4 mg 57Fe or 0.4 mg 58Fe as ferrous sulfate) were added to each test meal in solution shortly before test meal administration.

Feeding protocols. On day 0, body weight and height were measured and the first blood sample was taken for iron status and inflammation measurements. The subjects were randomly assigned to start in week 1 of each feeding period with 1 of the 6 different bean meals. Each bean meal was served for 5 consecutive days, twice per day, in the morning after an overnight fast between 0700 and 0900 and for lunch between 1100 and 1300 from Monday to Friday (10 labeled meals per bean meal). Per period, each subject consumed a biofortified bean meal and a control bean meal with the same level of PA (native phytate, ~50% dephytinized, or >95% dephytinized). For example, if a subject consumed a >95% dephytinized biofortified bean meal in week 1 of the period, she was allocated the >95% dephytinized control bean meal in week 2. The test meals were labeled with either 57Fe or 58Fe. Fourteen days after the consumption of the last test meal of each period a blood sample was taken for isotopic measurements (end of week 4 of each period; Fig. 1).

The participants consumed the test meals (including water) completely in the presence of the investigators and were not allowed to eat or drink between the test meals and for 3 h after the second meal. Rice or potatoes were randomly served with the beans. If rice was served with beans in the morning, potatoes were served for lunch and vice versa. Iron absorption was calculated on the basis of erythrocyte incorporation of iron stable isotope labels 14 d after intake of the last labeled test meal (17).

Stable isotope labels. Isotopically labeled 58FeSO4 and 57FeSO4 were prepared from isotopically enriched elemental iron (17Fe-metal: 97.8% enriched; 18Fe-metal: 99.5% enriched; both from Chemgas) by dissolution in 0.1 mol/L sulfuric acid and treated as described earlier (18).

Food analysis. Before analytical measurements, the beans were milled (ZM1; Retsch) by using a titanium sieve (0.25-mm mesh). The bean slurries were freeze-dried before PA and iron measurements. Total polynucleotid ion concentration (expressed in gallic acid equivalents) in bean seeds and slurries was measured with a modified Folin–Ciocalteau method (19). For iron measurements, samples were mineralized by microwave digestion (ETHOSplus, MLS) and then quantified by graphite furnace atomic absorption spectrophotometry (GF-AAS, AA240Z; Varian). The PA concentration in the slurries and bean seeds was measured by a modification of the Makower method (20), in which iron was replaced by cerium in the precipitation step. After the mineralization of the precipitates, inorganic phosphate was determined according to Van Veldhoven and Mannaeerts (21) and converted into PA concentrations. Wheat bean (PA assay) and milled beans (polyphenol assay), stored under argon to avoid polyphenol oxidation, were analyzed together with each series of samples and were used as in-house quality control material to monitor reproducibility. A rice flour reference sample (standard reference material 1568a; National Institute of Standards and Technology) was measured together with each series of samples to monitor accuracy of the iron estimation with atomic absorption spectrophotometry.

Iron status measurements. Venous blood samples were drawn in EDTA-treated tubes for the determination of hemoglobin, PF, and CRP and isotopic composition and kept cool until processing. Within the next 2 h, plasma was separated, portioned into aliquots, and frozen for the later analysis of PF and CRP (IMMULITE 2000; Siemens Healthcare

![FIGURE 1](https://academic.oup.com/jn/article-abstract/144/11/1681/4590064/fig1) Study design.
Diagnostics). Hemoglobin was measured by using HemoCue (HemoCue AB) and was corrected for altitude (1750 m) according to the method of Dallman et al. (22), which applies a 4% increase in hemoglobin concentration per 1000 m of increase in altitude.

**Isotope analysis.** Before isotopic analyses, whole-blood samples were processed according to Walczyk et al. (17). Isotopic analyses were performed by negative thermal ionization MS by using a magnetic sector field mass spectrometer (MAT 262; Finnigan MAT) equipped with a multicollector system for simultaneous ion beam detection (17,23).

**Calculation of iron absorption.** The amounts of $^{57}$Fe and $^{59}$Fe isotopic labels in blood were calculated as previously described (18). Calculations were based on the principles of isotope dilution and took into account that iron isotopic labels were not monoisotopic (17). For calculation of fractional absorption, 80% incorporation of the absorbed iron into RBCs was assumed (24).

**Statistical analysis.** Analyses were conducted with SPSS statistical software and Microsoft Office Excel 2010. Results for iron absorption, food analysis, anthropometric measurements, hemoglobin, PF, and CRP are presented as means ± SDs if normally distributed. If not normally distributed, data were log-transformed for analysis, converted for reporting and are presented as geometric mean values (95% CIs). To analyze the impact of PA and bean variety on iron bioavailability, a 2-factor repeated-measures ANOVA was used for comparison of iron absorption from the same bean with native PA was 30% higher than the absorption from the control bean with >95% dephytinization (17). For calculation of fractional absorption, 80% incorporation of the absorbed iron into RBCs was assumed (24).

**Results**

**Subject characteristics.** Three subjects dropped out of the study due to health issues unrelated to the study, and 22 subjects completed the study. Mean PF concentration increased to 11.0 (6.2, 19.5) µg/L at the end of the 12-wk study period from a baseline of 8.9 (4.9, 16.2) µg/L ($P < 0.005$). Five subjects were anemic (hemoglobin <120 g/L), and none of the women had an elevated CRP concentration (>3 mg/L) at baseline. Hemoglobin, CRP, and BMI did not change during the study (Table 1).

**Bean grain and bean meal composition.** Iron concentration in the biofortified beans was 63% higher than in the control beans and the mean amount of iron in the cooked bean meals (including rice, potatoes, and isolates) was 54% higher in the biofortified than in the control beans (Table 2). Native PA concentrations in the biofortified bean seeds and biofortified bean meals were 35% higher than in the control bean seeds and the control bean meals (Table 2). Dephytinization reduced PA concentration in the biofortified beans and the control beans by >95%. The PA:iron molar ratios were almost similar in all of the control and biofortified bean meals. The polyphenol concentration of the biofortified beans was 27% higher than in the control beans and decreased considerably (>70%) during meal preparation (Table 2).

**Iron absorption from bean meals.** Two-factor repeated-measures ANOVA showed that both bean variety ($P < 0.001$) as well as PA ($P < 0.001$) had an impact on iron bioavailability. Mean fractional iron absorption from the control bean meals with native PA was 30% higher than the absorption from the native PA biofortified bean meals ($P < 0.001$) (Table 3, Fig. 2). Although lower than expected from a 54% greater iron content, the quantity of iron absorbed from the biofortified bean meals was still 19% higher ($P < 0.05$) than from the control bean meals. Dephytinization had a proportionally greater effect on iron absorption from the biofortified bean meals. Partial (>50%) and >95% dephytinization increased the amount of iron absorbed from the biofortified bean meals by 47% ($P < 0.005$) and 84% ($P < 0.0001$), respectively, compared with 45% from the control bean meal with >95% dephytinization ($P < 0.0001$). There was no significant increase in iron absorption from the control meal after a 50% reduction in PA. With <50% less PA, the amount of iron absorbed from the biofortified bean meals was 37% higher than from the control bean meals ($P < 0.005$), and with >95% dephytinization the amount of iron absorbed from the biofortified bean meals was 51% higher than from the control bean meals ($P < 0.0001$), corresponding well to their 54% higher iron content.

**Discussion**

Plant breeders have successfully biofortified beans with iron, increasing the iron amounts in beans from ~5 mg/100 g to >10 mg/100 g (16). Unfortunately, the high-iron beans also contain higher amounts of PA, which appeared to inhibit the absorption of the additional biofortification iron (13). The present study confirms the previous studies indicating that the amount of iron absorbed from high-iron, high-phytate biofortified bean meals is only slightly higher than the amount of iron absorbed from control bean meals (13,15). In the present study, fractional iron absorption from meals containing the biofortified beans with an iron concentration of 8.8 mg/100 g

### Table 1. Anthropometric measurements and concentrations of hemoglobin, plasma CRP, and PF in Rwandese women with low iron status

<table>
<thead>
<tr>
<th>Variable</th>
<th>Summary value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight, kg</td>
<td>54 ± 7.2</td>
</tr>
<tr>
<td>Height, cm</td>
<td>157 ± 6</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>21.1 ± 2.1</td>
</tr>
<tr>
<td>Hemoglobin, g/L</td>
<td>130 ± 17</td>
</tr>
<tr>
<td>Plasma CRP, mg/L</td>
<td>0.3 ± 0.3</td>
</tr>
<tr>
<td>PF, µg/L</td>
<td>8.9 (4.9, 16.2)</td>
</tr>
</tbody>
</table>

1 Values are means ± SDs or geometric mean (95% CI) at baseline, n = 22. CRP, C-reactive protein; Hb, hemoglobin; PF, plasma ferritin.
2 Corrected for altitude ($\text{Hb}_\text{altitude} = \text{Hb}_\text{measured} - 6.4\%$) according to Dallman et al. (22).

Phytate and iron absorption from biofortified beans 1683
was 30% lower than from meals containing the control beans with an iron concentration of 5.4 mg/100 g, resulting in 19% more iron being absorbed from the biofortified bean meals (Table 3). Although this finding was disappointing because the bean meals contained >50% more iron, a daily increase of 60 μg absorbed iron/d could be a biologically important improvement over time and help improve iron status. The reason for the decreased fractional absorption, as predicted, was the higher PA concentration in the biofortified beans compared with the control beans (1320 vs. 980 mg/100 g; Table 2), and after >95% dephytinization, the fractional absorption values were almost identical (13.1% vs. 13.4%; Table 3).

This current finding is similar to results from our previous study with different biofortified and control bean varieties during which we provided similar composite, multiple bean meals to Rwandese women of low iron status (13). In that study, fractional iron absorption from meals containing the biofortified beans (9.2 mg iron/100 g, 1390 mg PA/100 g) was 3.8%, which was 40% lower than that from the control beans (5.2 mg iron/100 g, 850 mg PA/100 g). The lower iron bioavailability from the biofortified beans led to equal amounts of iron absorbed per meal, which was even less favorable than the results of the present study and might be attributed to differences in the beans, or to differences in the iron status or in other physiologic factors between the participants of the 2 studies (25,26).

In both studies, despite the increased iron content in the biofortified beans, the PA:iron molar ratios in the biofortified and control beans were similar. Although fractional iron absorption was reported to decrease with increased iron intake (27), it was expected that the PA:iron molar ratio rather than the total amount of PA would dictate iron absorption. This does not appear to be the case in beans, which may be related to the speciation of iron in the beans. Up to 30% of bean iron is contained within the ferritin molecule (28). Although ferritin iron is readily released during in vitro digestion (29), it may be absorbed by a separate mechanism (30) or it may be released differently to the nonferritin iron and react with PA in a different way in the stomach or small intestine. With increasing iron concentration, however, the percentage of iron bound to ferritin decreases, resulting in a higher proportion of non–ferritin-bound iron, probably linked to PA molecules (28) and therefore of lower bioavailability.

On the basis of the current study and our previous study (13), fractional iron absorption from multiple composite bean-containing meals would appear to be modest and in the region of 6–9%. Coupled with the high iron content of regular beans (~5 mg/100 g) and the relatively high bean consumption in some populations [100–180 g/d (5)], meals containing regular beans could provide women of child-bearing age with 25–50% of their daily RDA [18 mg (31)] and the absorbable iron requirement of 1400 μg/d (32). Although the current biofortified beans have up to 2-fold more iron and thus provide up to 100% of the RDA, they are high in PA and would be expected to add only a modest amount of additional absorbable iron, which may over the long term effectively improve iron status.

However, to optimize iron bioavailability from beans in general and from biofortified beans in particular, a way forward would be for the plant breeding efforts to include...
lower PA concentrations as an additional trait to target. Our results with the 50% dephytinized biofortified bean indicate that if breeders could decrease the PA in the biofortified beans to <700 mg/100 g then iron absorption per meal would increase substantially from 400 to 600 µg, almost double that from the control beans (340 µg; Table 3), and provide a major contribution to the daily iron needs of women of child-bearing age [1.4 mg/d (32)]. Breeding simultaneously for high iron and low PA might be possible because Blair et al. (33) reported that most phytate- and phosphorous-related quantitative trait loci might be independent of iron quantitative trait loci. There is also encouraging data from studies with beans (34), cowpeas (35), and wheat (36), which indicate that PA concentration is not associated with yield or plant health.

An alternative approach used by Campion et al. (34) is to use mutagenesis to develop a low PA (lpa) bean with normal phosphorus but only 10% of the original PA concentration. The chemically induced mutation was associated with a defective gene, coding for an ATP-binding cassette transporter taking part in PA storage in protein bodies during seed maturation (37). Despite the large reduction in PA, this mutation retained a good grain yield and a high germination rate (34), in contrast to many other previously developed lpa crops (38). Free or weakly bound iron was increased by 7-fold in the lpa beans (34), and a stable iron isotope absorption study in Swiss women found a markedly improved iron bioavailability of 6.1% from the lpa beans compared with 3.8% from the parent beans (39). The reduction of PA, by disrupting its biosynthetic chain, might be an alternative solution to alleviate the nutritional issues associated with high PA concentrations, although such low PA amounts might be subject to discussion because PA may have health-promoting properties, particularly in the prevention of certain cancers (40,41).

The fractional iron absorption and total amount of iron absorbed from both bean meals administered in the present study were somewhat higher than reported from other bean studies (12–15,42). The differences could be due to study design, bean meal composition, bean variety factors, iron status (25,43), and other subject factors (26). The present study was a multiple-meal study with potatoes and rice. The published data from single/double meal studies, sometimes without additional meal components, reported lower absorption and may have overestimated the impact of PA (44–46).

In the present study, we used the extrinsic tag technique with human subjects to measure the absorption of native bean iron. This technique was validated in human radioisotope studies (47–49) and has been widely used to identify inhibitors and enhancers of iron absorption. Human radioisotope studies comparing extrinsic with intrinsic radioisotope labeling with a variety of foods reported good agreement between the techniques, including studies with the common bean (15,47,50). For our study, we used stable isotope labels instead of radioactive labels, which necessitates providing 4 mg stable isotope iron per subject instead of the few micrograms needed with radioisotopes. In our study, 400 µg Fe as ferrous sulfate was added to each of the 10 feedings of each meal. This represented 5–11% of total meal iron and was assumed to readily enter the common iron pool, exchange with the native food iron, and be suitable for measuring native food iron absorption using erythrocyte incorporation. Although we believe this to be the case, further validation of the extrinsic tag technique in foods high in inhibitors or enhancers was suggested in a review by Consaul and Lee (51). A useful additional measure might be to monitor the completeness of the stable isotope exchange with native food iron in simulated in vitro digestions of different test meals.

Our results clearly indicate the importance of PA for bean iron bioavailability. They indicate that beans with a regular iron concentration of 5 mg/100 g when added to composite meals are a useful source of iron, but that a high PA concentration in beans is an impediment to the biofortification strategy, and the introduction of the current biofortified beans with high iron and high PA amounts will likely improve iron nutrition only marginally. A decrease in PA of ~50% to <700 mg/100 g biofortified beans would greatly improve fractional iron absorption, and the additional amount of iron absorbed would usefully contribute to improved iron nutrition. Such a decrease might be possible by plant breeding, and decreasing PA to ~100 mg/100 g by chemical mutagenesis would further increase iron absorption. However, it remains to be clarified if iron absorption could be further increased by the reduction in other potential inhibitors present in beans such as polyphenols (12) or lectins (52).

In conclusion, iron absorption from biofortified beans is strongly dependent on PA concentration. High PA is an impediment to the bean iron biofortification strategy, and plant breeders engaged in biofortification for nutritional purposes are encouraged to develop high-iron beans with lower PA concentrations so as to optimize iron absorption.
Acknowledgments

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References