Stable Iron Isotope Studies in Rwandese Women Indicate That the Common Bean Has Limited Potential as a Vehicle for Iron Biofortification1,2

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Abstract

Biofortification of plants is a new approach to combat iron deficiency. Common beans (Phaseolus vulgaris) can be bred with a higher iron concentration but are rich in iron absorption inhibitors, phytic acid (PA), and polyphenols (PP). To evaluate the potential of beans to combat iron deficiency, three iron absorption studies were carried out in 61 Rwandese women with low iron status. Studies 1 and 2 compared iron absorption from high and low PP beans, similar in PA and iron, fed as bean puree in a double meal design or with rice and potatoes as multiple meals. Study 3 compared iron absorption from high and normal iron beans with similar PP levels and a PA:iron molar ratio, fed with potatoes or rice in multiple meals. Iron absorption was measured as erythrocyte incorporation of stable iron isotopes. In study 1, iron absorption from the high PP bean (3.4%) was 27% lower (P < 0.01) than from low PP bean (4.7%), but when fed in multiple meals (study 2), there was no difference (7 and 7.4%, respectively; P > 0.05). In study 3, iron absorption from the high iron bean (3.8%) was 40% lower (P < 0.001) than from the normal iron bean (6.3%), resulting in equal amounts of iron absorbed. When beans were combined with other meal components in multiple meals, high PP concentration had no negative impact on iron absorption. However, the quantity of iron absorbed from composite meals with high iron beans was no higher than with normal iron beans, indicating that efficacious iron biofortification may be difficult to achieve in beans rich in PA and PP. J. Nutr. 142: 492–497, 2012.

Introduction

Biofortification, the development of micronutrient-dense crops by traditional plant breeding or by genetic engineering, is a promising new approach to combating micronutrient deficiencies (1) and has the potential to be more sustainable and cost effective than food fortification or supplementation (2–4). By distributing seeds that efficiently accumulate soil iron, the supply of iron to low-income households in rural areas with limited access to commercial markets could be increased (5). To improve iron status, however, the iron concentration and bioavailability as well as the consumption of the biofortified staple must provide an additional intake of bioavailable iron that makes a considerable contribution to the gap between current iron intake and iron requirement. Additionally, to be accepted by the farmers, the biofortified crop must have a sufficiently high yield that is stable over different environments and climatic zones (6).

Current iron biofortification research programs focus on increasing the iron concentration of staple crops such as wheat, maize, rice, beans, and millet (1,7–10). The common bean is the major staple food for over 300 million people in Eastern Africa and Central and South America and is one of the most important legumes worldwide (11). The iron concentration of beans varies between 3.5 and 9 mg/100 g, depending on the genotype, and is relatively stable when grown under different environmental conditions (7,12). Traditional plant breeding approaches have been reported to increase the iron concentration of some bean varieties by 60–80% (7,13). Beans, however, are high in PA5, a potent inhibitor of iron absorption (14,15). Wheat and rice are also high in PA, but milling and polishing of the grains can substantially decrease its concentration in the consumed food and, with wheat flour, iron fortification compounds such as NaFeEDTA can be used to overcome the negative effect of PA on iron absorption (16). Cooked beans are neither milled nor fortified but are typically consumed whole without any separation process to decrease PA and the colored varieties are additionally rich in PP compounds, which are also strong inhibitors of iron absorption (17).

Several human studies have reported low iron absorption from beans, with published fractional iron absorption values ranging from 1.5 to 2.6% (18,20). Whereas the PA concentration of beans is always high, the PP concentration varies considerably with the bean variety and color (21,22), and it seems likely that only colored beans are sufficiently high in PP to additionally inhibit iron absorption.

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5 Abbreviations used: Hb, hemoglobin; PA, phytic acid; PP, polyphenol; PF, plasma ferritin.
What is unknown, however, is whether the inhibitory effects of PA and PP in beans are additive and to what extent the other components of a bean meal facilitate iron absorption and thus dampen the inhibitory effect of PA and PP. Several studies have reported a diminished effect of known inhibitors and enhancers when consumed with other foods in a composite meal (23,27).

The aim of the present studies was to better understand the potential of the common bean as a biofortification vehicle for iron. The 3 stable isotope studies were designed to evaluate whether the PP component of colored beans additionally inhibited iron absorption in the presence of high PA and determine whether consuming beans with other meal components in composite meals decreases the inhibitory effect of PP on iron absorption. Finally, the performance of biofortified, high iron beans was tested by evaluating whether more iron is absorbed from composite meals containing high iron beans than from the same meals containing normal iron beans.

**Methods**

**Participants**

The study participants were selected from an initial screening of 230 women from the student and staff population of the National University of Rwanda who gave a blood sample. Women with known metabolic, chronic, and gastrointestinal diseases, on long-term medication, or who had donated blood or experienced remarkable blood loss within the 6 mo prior to the study were excluded. Sixty-one apparently healthy, nonpregnant, non-lactating women with marginal iron stores (PF <25 μg/L), aged between 18 and 30 y and <65 kg body weight were recruited. The women were randomly allocated to 1 of 3 iron stable isotope absorption studies, each with 20 or 21 participants. Intake of vitamin and mineral supplements was not allowed during the studies and for the 2 wk before the studies. The experimental procedures were approved by the National Ethics Committee of Rwanda and the ethical committee of ETH Zurich. Written informed consent was obtained from all study participants.

**Iron stable isotope studies**

Three separate iron absorption studies were made. Study 1 investigated the influence of bean PP on iron absorption from a high PA bean puree fed in a double meal design; study 2 investigated the influence of bean PP on iron absorption from a composite meal with beans, rice, or potatoes fed in a multiple-meal design; study 3. The composition of the high and normal iron beans is shown in Table 1. The black high iron bean (MB 465; planted and harvested by the International Center for Tropical Agriculture, Colombia) contained 17.9 mg iron/100 g compared to 5.2 mg/100 g in the normal iron bean (SER 16).

The composition of the bean varieties tested in study 1 is shown in Table 1. The beans (FEB 226, red; and MIB 497, white; planted and harvested by the International Center for Tropical Agriculture, Colombia) were similar in iron and PA concentration but differed strongly in PP concentration. The test meals were fed as salted, homogenized bean porridge containing 75 g beans (dry weight). The beans were first washed, soaked for 30 min at room temperature, boiled in water for 75 min, and homogenized. One gram of salt was added per test meal. The bean test meals were prepared in batches and stored frozen until the day of feeding. Polished rice was washed and potatoes were peeled, washed, and cut into pieces before cooking. Soy oil (1.6 g) and 0.6 g salt were added to each portion. Rice and potatoes were prepared daily before feeding. The iron stable isotopes (0.4 mg 56Fe or 0.4 mg 57Fe) were added to each test meal in solution shortly before test meal administration. As the iron concentrations of SER 16 and MIB 497 differed by −2 mg iron/100 g dry weight (Table 1), the iron concentration in the pureed meals was equilibrated by adding 0.5 mL of a ferrous sulfate solution (1.5 g Fe/L) just prior to serving. As in study 1, 200 g water was served with each test meal.

**Feeding protocols**

The 61 participants were assigned to studies 1, 2, or 3 in groups of 20 (study 1 and 2) or 21 (study 3) women. A randomized crossover design was used and within each study each woman acted as her own control. On d 0, body weight and height were measured and the first blood sample was taken for iron status and inflammation measurements. The labeled meals were served in the morning between 0700 and 0900 h after an overnight fast and a second meal 3 h later. 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.

In study 1, each woman received two test meals on two consecutive days (d 1 and 2), each labeled with either 57Fe or 58Fe. Fourteen days after the last test meal (d 16), a second blood sample was taken after an overnight fast for iron isotopic analysis. In studies 2 and 3, each woman received two series of 10 test meals (study 2: high and low PP bean; study 3: high and normal iron bean). Test meals were served in the morning and for lunch from Monday to Friday for two consecutive weeks (d 1–5 and d 8–12) labeled with either 57Fe or 58Fe. Bean consumption was randomized. Participants who consumed the high PP Bean (study 2) or the high iron bean (study 3) in wk 1 consumed the low PP bean (study 2) or the normal iron bean (study 3) in wk 2 and vice versa. Rice or potatoes were randomly served with the beans. If rice was served with beans in the morning, the women received beans with potatoes for lunch and vice versa. Fourteen days after the last test meal of wk 1 (d 19), and 14 d after the last test meal of wk 2 (d 26), second and third blood samples for iron isotopic analysis were taken after an overnight fast.

Iron absorption was calculated based on erythrocyte incorporation of iron stable isotope labels 14 d after intake of the last labeled test meals (28). Participants of all studies were randomly allocated to either start with test meal A or test meal B.

**Stable isotope labels**

Isotopically labeled 56FeSO4 and 57FeSO4 were prepared from isotopically enriched elemental iron [57Fe-metal: 97.8% enriched; 58Fe-metal: 99.4% (study 1), 99.5% (study 2 and 3) enriched; both Chemgas]. Iron tracer solutions were analyzed for iron isotopic composition and tracer iron concentration by reversed isotope dilution MS.

**Food analysis**

Prior to measurements, beans were milled with a centrifugal mill (Retsch) and bean meal components were freeze-dried. The total PP concentration in beans and bean meal components (expressed in gallic acid equivalents) was measured with a modified Folin-Ciocalteau method as suggested by Singleton (29). Iron in beans and bean meals was analyzed by graphite furnace atomic absorption spectrophotometry (AA240Z, Varian) after the mineralization by microwave digestion (MLS ETHOSplus). The PA concentration was measured by a modified method.
The amounts of 57Fe and 58Fe isotopic labels in blood 14 d after absorption from a collector system for simultaneous ion beam detection (28,33) field mass spectrometer (MAT 262; Finnigan MAT) equipped with a multi-collector system for simultaneous ion beam detection (28,33). All isotopic analysis were performed by negative thermal ionization MS using a magnetic sector mass spectrometer. The blood matrix according to Walczyk et al. (28). All isotopic analysis and microwave digestion followed by separation of the sample iron from monoisotopic (28). For calculation of fractional absorption, 80% incorporation of the iron estimation with atomic absorption spectrophotometry.

Iron status measurements

Venous blood samples were drawn in EDTA-treated tubes for the determination of Hb, Pf, and CRP. Whole blood samples were divided into aliquots for the analysis of Hb and isotopic composition. Plasma was separated, aliquoted, and frozen for the later analysis of Fe. Hb was measured with a Coulter Counter (Beckmann International). PF and CRP were measured on an IMMULITE automatic system (DPC Buhlmann). Hb was corrected for altitude (1500 m) after the method of Dallman, which applies a 4% increase in Hb concentration/1000 m of rise in altitude (32).

Isotope analysis

Whole blood samples were mineralized using an HNO3/H2O2 mixture and microwave digestion followed by separation of the sample iron from the blood matrix according to Walczyk et al. (28). All isotopic analysis were performed by negative thermal ionization MS using a magnetic sector field mass spectrometer (MAT 262; Finnigan MAT) equipped with a multi-collector system for simultaneous ion beam detection (28,33).

Calculation of iron absorption

The amounts of 57Fe and 58Fe isotopic labels in blood 14 d after administration of the last test meals were calculated based on the shift in iron isotope ratios and the estimated amount of iron circulating in the body. Circulating iron was calculated based on the blood volume estimated from height and weight according to Brown et al. (34) and measured Hb concentration. The calculations were based on the principles of isotope dilution and took into account that iron isotopic labels were not monoisotopic (28). For calculation of fractional absorption, 80% incorporation of the absorbed iron into RBC was assumed (34).

Statistical analysis

Analyses were conducted with SPSS statistical software (SPSS 16.0) and Microsoft Office Excel 2003. Iron absorption values were converted to logarithms for statistical analysis (paired Student’s t test, 1-way ANOVA) and reconverted for reporting. Iron absorption from different test meals within the same participant was compared by paired Student’s t test. A 1-way ANOVA was used for comparisons between studies and a post hoc Bonferroni test was used for multiple comparisons. Results are presented as geometric means (range). Between study comparisons of participants (anthropometry, Hb, CRP) as well as of beans and bean meals (PF, PA, iron) were done by 1-way ANOVA followed by a Bonferroni test. Results are presented as means ± SD. Differences were considered significant at P < 0.05. A sample size of 16 women per group is sufficient to detect an intra-subject difference of 30% in iron absorption with a α level of 0.05. To compensate for possible dropouts, ~20 women were recruited for each study.

Results

Participant characteristics. All women had a low iron status (PF < 25 μg/L) (Table 2). Thirty-nine (64%) of the 61 study participants had a PF concentration <15 μg/L and 17 had an Hb concentration <120 g/L. One woman showed a slightly elevated CRP concentration of >3 mg/L, but none had a CRP concentration >10 mg/L. Mean BMI was in the normal range (19–24 kg/m2). None of the variables in Table 2 except BMI differed significantly between study groups.

Bean and meal composition. In study 1, the PA and iron concentrations of FEB 226 and MIB 497 did not differ significantly between the two bean varieties or between the cooked, homogenized bean meal tests (Table 1). The PP concentration in the high PA bean meals was ~6.5 times higher than in the low PP bean meal (P < 0.0001). Processing reduced the PP concentration of the high PP bean by ~50%, resulting in a three times higher PP concentration in the high PP bean meals compared to the low PP bean meal (P < 0.001) (Table 1).

The high PP bean fed in study 2 (SER 16) also had a PP concentration ~7-fold higher than in the low PP bean MIB 497 (P < 0.001). As in study 1, the difference in PP concentration between beans was reduced to ~4-fold on cooking and homogenization (P < 0.001). The iron concentration in the beans differed (P < 0.001) but was adjusted in the test meals as described above. PA concentrations

<table>
<thead>
<tr>
<th>Variable</th>
<th>Study 1</th>
<th>Study 2</th>
<th>Study 3</th>
</tr>
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<tbody>
<tr>
<td>n</td>
<td>19</td>
<td>21</td>
<td>21</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>49.9 ± 4.3</td>
<td>52.5 ± 5.6</td>
<td>53.1 ± 6.7</td>
</tr>
<tr>
<td>Height, cm</td>
<td>159 ± 5</td>
<td>157 ± 6</td>
<td>157 ± 6</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>19.6 ± 1.2</td>
<td>21.5 ± 2.4</td>
<td>21.5 ± 2.1</td>
</tr>
<tr>
<td>Blood Hb, g/L</td>
<td>127 ± 15</td>
<td>131 ± 12</td>
<td>126 ± 11</td>
</tr>
<tr>
<td>Plasma CRP, mg/L</td>
<td>0.7 ± 0.9</td>
<td>0.5 ± 0.7</td>
<td>0.7 ± 0.9</td>
</tr>
<tr>
<td>PF, μg/L</td>
<td>14.4 (8.7–24.0)</td>
<td>9.3 (4.2–20.6)</td>
<td>10.3 (5.4–19.6)</td>
</tr>
</tbody>
</table>

1 Values are mean ± SD or geometric mean (range). Hb, hemoglobin; PF, plasma ferritin.
2 Values corrected for altitude.
in both beans and bean meals were not significantly different (Table 1).

In study 3, the iron concentration of the high iron bean was 75% higher than that of the normal iron bean ($P < 0.001$) and final iron concentration in the test meals including the added isotopes differed by $-40\%$ ($P < 0.001$) (Table 1). The beans had similar PP concentrations; cooking reduced PP in both beans by $\sim 50\%$. However, the PA concentration of the beans differed ($P < 0.001$), with the high iron bean also having a high PA concentration, resulting in a similar PA:iron molar ratio (9:1) in both bean meals.

Rice served in studies 2 and 3 had an iron concentration of 0.2 mg/100 g and a PA concentration of 10 mg/100 g dry weight. The iron concentration of Irish potatoes consumed in study 2 and 3 was 0.4 mg/100 g as consumed.

**Iron absorption measurements.** In study 1, mean fractional iron absorption from the high PP bean was 27% lower than that from the low PP bean ($P < 0.01$), resulting in a significantly lower amount of absorbed iron. However, this decrease in iron absorption with increased PP concentration did not occur in study 2 when the beans were fed combined with rice or potatoes twice per day over 5 d as opposed to a single day in study 1. In study 2, iron absorption did not differ between the low and high polyphenol bean meal. In study 3, mean fractional iron absorption from the normal iron bean was higher ($P < 0.001$) than from the high iron bean, resulting in no difference ($P = 0.42$) in the amount of iron absorbed per meal (Table 3). For between-study comparisons, iron absorption values were adjusted to a PF concentration of 15 $\mu$g/L as the cutoff level for iron deficiency (35) using experimentally derived algorithms (36). After ferritin adjustment, the fractional iron absorption from the high PP bean meal fed in study 1 (FEB 226) was lower than iron absorption from the high PP bean meal (SER 16; $P < 0.05$) and the low PP bean meal (MB 497; $P < 0.05$) fed in study 2. Iron absorption from SER 16 bean meals, fed within the multiple meal design of studies 2 and study 3, did not differ significantly. Iron absorption from the MIB 497 low PP bean meals (studies 1 and 2) also did not differ significantly.

### Discussion

This study has two major findings. The first is that the inhibitory nature of bean PP on iron absorption (17) did not further increase the inhibitory effect of beans per se when the beans were consumed in composite meals with potatoes or rice over a period of 5 d. This would indicate that white beans and colored beans would provide similar amounts of bioavailable iron when consumed in traditional meals. The low iron absorption from white beans and white bean meals can be explained by the high phytate concentration of beans (22,37) and the inhibitory nature of legume proteins (38,39).

However, when the beans were consumed alone (study 1) (Table 3) over two meals on a single day, iron absorption from the high PP colored bean was 27% lower than for the white bean. In this study, in which the meals contained only beans and were fed twice on a single day, the inhibitory effect of the PP was additional to the inhibitory effect of phytate and legume proteins. Finding no additional inhibition of bean PP in the composite meals (study 2) is perhaps not surprising, as other food components in a meal have been reported to decrease the inhibitory effect of PP (27). It is possible that other food components in potatoes or rice such as ascorbic acid or proteins facilitated iron absorption from the bean meals due to their reducing or chelating effects or simply diluted the effect of the inhibitors. In addition, single meal radioisotope studies have been reported to exaggerate the inhibitory effect of food components compared to multiple meal studies (36,40) and the different study duration could offer a further explanation for the apparent discrepancy between studies 1 and 2. Multiple meal studies are preferred when stable isotopes are used to study the influence of food components on iron absorption. This is because the isotope dose can be added over many meals and thus has only a modest influence on the molar ratio of food component:iron. It should be noted that different high PP bean varieties were fed in study 1 and 2 and it is possible that different individual PP compounds in these 2 varieties had different inhibitory potentials.

The second finding, that the high iron bean did not provide a greater amount of absorbed iron when fed in multiple composite meals over 5 d, questions the usefulness of beans as a vehicle for biofortified iron. This concern was already raised by Donangelo and King (19) after similar results to our own. They compared iron absorption from two bean varieties, one being 65% higher in iron and lower in PP concentration and the PA:iron molar ratio (20:1 vs. 30:1). Iron absorption from both beans was low and the total amount of iron absorbed from the two bean types did not differ.

Beans and other cereal staples are high in phytate. The inhibitory effect of phytate in cereals can partially overcome by milling or polishing and, where cereal flours are centrally processed, they can be fortified with iron compounds such as NaFeEDTA, which overcome phytate inhibition. Bean phytate is within the protein bodies of the cotyledon (37) and is not possible to remove by milling or polishing and, because beans are not centrally processed, providing iron-fortified bean flour is not an easy option. Biofortification might thus be the best solution.

Our results, however, indicate that in the presence of high levels of phytate, PP, and maybe also inhibitory proteins, high

<p>| TABLE 3 | Fractional iron absorption and total absorbed iron per test meal of women participating in studies 1–3 |
| --- | --- | --- | --- |</p>
<table>
<thead>
<tr>
<th>Study</th>
<th>$n$</th>
<th>Bean meal</th>
<th>Total absorbed iron per meal</th>
<th>Fractional iron absorption</th>
<th>Absorption ratio (meal A:meal B)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1&lt;sup&gt;2&lt;/sup&gt;</td>
<td>19</td>
<td>Meal A (high PP bean)</td>
<td>235 (115–482)</td>
<td>3.4 (1.6–7.0)</td>
<td>0.73</td>
</tr>
<tr>
<td>1&lt;sup&gt;2&lt;/sup&gt;</td>
<td>23</td>
<td>Meal A (low PP bean)</td>
<td>341 (187–622)&lt;sup&gt;**&lt;/sup&gt;</td>
<td>4.7 (2.6–8.5)&lt;sup&gt;**&lt;/sup&gt;</td>
<td>0.73</td>
</tr>
<tr>
<td>2&lt;sup&gt;3&lt;/sup&gt;</td>
<td>21</td>
<td>Meal A (high PP bean+ rice/potato)</td>
<td>317 (161–622)</td>
<td>7.0 (4.0–14.1)</td>
<td>0.95</td>
</tr>
<tr>
<td>2&lt;sup&gt;3&lt;/sup&gt;</td>
<td>21</td>
<td>Meal B (low PP bean+ rice/potato)</td>
<td>340 (174–664)</td>
<td>7.4 (4.0–15.2)</td>
<td>0.95</td>
</tr>
<tr>
<td>3&lt;sup&gt;3&lt;/sup&gt;</td>
<td>21</td>
<td>Meal A (high iron bean+ rice/potato)</td>
<td>234 (85–538)</td>
<td>3.8 (1.5–9.4)</td>
<td>0.60</td>
</tr>
<tr>
<td>3&lt;sup&gt;3&lt;/sup&gt;</td>
<td>21</td>
<td>Meal B (normal iron bean+ rice/potato)</td>
<td>225 (111–483)</td>
<td>6.3 (3.0–13.3)&lt;sup&gt;**&lt;/sup&gt;</td>
<td>0.60</td>
</tr>
</tbody>
</table>

1<sup>1</sup> Values are geometric mean (range). Asterisks indicate different from meal A: *$P < 0.01$; **$P < 0.001$.
2<sup>2</sup> All meals contained 2 mg Fe$^{57}$ or 2 mg Fe$^{58}$.
3<sup>3</sup> All meals contained 0.4 mg Fe$^{57}$ or 0.4 mg Fe$^{58}$.  

The potential of common beans for iron biofortification 495
iron beans may not provide additionally useful amounts of bioavailable iron. In our studies, the beans were fed twice per day over a 5-d period and therefore provide stronger information than single meal studies. However, it is possible that PP, PA, and proteins do not maintain their strong negative impact on iron absorption over the long term and people can upregulate iron absorption over time to meet their iron requirements. A recent evaluation of national wheat flour fortification programs (41) has recommended an additional 6 mg fortification of iron per day as ferrous sulfate to usefully improve iron status. Although doubling the iron concentration of beans and consuming ≥100 g/d of beans would approach this value, the more inhibitory nature of beans compared to low extraction wheat flour may make it more difficult to absorb sufficient quantities of biofortified iron. The mean fractional iron absorption values of around 7% from composite bean meals by the women with low iron status, however, offer some encouragement.

It was somewhat surprising that more iron was not absorbed from the high iron bean by the participants with low iron status. If more iron was in a potentially absorbable form in the high iron bean, it would be expected that more iron would be absorbed. However, ~200 μg iron/meal was absorbed irrespective of the bean. Previous studies have reported that fractional iron absorption decreases as the quantity of iron ingested increases but that more iron is absorbed. Cook et al. (42) added 1, 3, and 5 mg labeled ferrous sulfate to a bread roll meal. Fractional iron absorption decreased, but the total amount of iron absorbed increased. Additionally, it is always assumed that increasing the amount of fortification iron increases its ability to improve iron status (41).

The most logical explanation for our finding is that the strong inhibitory nature of phytate in the high iron bean resulted in a similar amount of bioavailable iron being released during digestion in the gastrointestinal tract as is released from the normal iron bean. Whereas the phytate level usually increases with iron level, the phytate:iron molar ratio usually decreases (Matthias Hoppler, ETH; personal communication). In the high iron bean fed in our study, the phytate level was high and the phytate:iron molar ratio was similar to the normal iron bean. Because of the latter, we would have predicted a similar fractional iron absorption in the normal and high iron beans. However, if iron and phytate in the 2 bean varieties are in different compartments or are released into the stomach at different times, it is possible that the higher phytate concentration of the high iron bean had a greater inhibitory effect on iron absorption. Although iron speciation in beans has not been extensively studied, recent results from our laboratory (Matthias Hoppler, ETH; personal communication) with beans of varying iron concentrations indicate that ferritin iron is present at a similar concentration in all beans and that bean iron is increased by an increase in nonferritin iron. The nonferritin iron is possibly linked to PA and may behave differently from ferritin iron. It is also possible, although unlikely, that the high concentration of the galloyl group containing anthocyanidin delphinidin 3- glycoside, which can be found in black beans (43), was the reason for the small amount of bioavailable iron in the biofortified bean.

The way forward, however, would seem to be to select high iron beans with low PA and PP concentrations. In conclusion, these studies show that iron absorption from beans in a bean-consuming population is, with ~4-7%, higher than previously reported. Furthermore, the studies indicate that when beans are consumed as part of composite, traditional African meals, the inhibitory nature of bean PP does not further add to the negative impact of PA on iron absorption. They also suggest that increasing the bioavailable iron concentration in high phytate, high PP beans may prove difficult and that the way forward for iron biofortified beans is to select for high iron, low phytate, and low PP concentrations.

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**Literature Cited**