Genetic Reduction of Phytate in Common Bean (Phaseolus vulgaris L.) Seeds Increases Iron Absorption in Young Women1–4

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Abstract

Iron bioavailability from common beans is negatively influenced by phytic acid (PA) and polyphenols (PPs). Newly developed low-PA (lpa) beans with 90% less PA and variable PPs might improve iron bioavailability. The aim of this study was to evaluate the influence of lpa beans on iron bioavailability in women (n = 20). We compared iron absorption from 4 different beans using a paired, double meal, crossover design. Iron absorption was measured as erythrocyte incorporation of stable iron isotopes (Fe57, Fe58) from 2 lpa bean lines, one high in PPs (means ± SDs; PA = 124 ± 10 mg/100 g; PPs = 462 ± 25 mg/100 g) and one low in PPs (PA = 70 ± 10 mg/100 g; PPs = 54 ± 2 mg/100 g). The other 2 beans used were their parents with a normal PA concentration, one high in PPs (PA = 1030 ± 30 mg/100 g; PPs = 676 ± 19 mg/100 g) and one low in PPs (PA = 1360 ± 10 mg/100 g; PPs = 58 ± 1 mg/100 g). Fractional iron absorption from the lpa bean high in PPs was 6.1% (95% CI: 2.6, 14.7), which was 60 and 130% higher compared with the parent high in PPs (P < 0.001) and low in PPs (P < 0.001), respectively. The total amount of iron absorbed per test meal from the lpa bean high in PPs (372 µg; 95% CI: 160, 890) was 60 and 163% higher compared with the parent high in PPs (P < 0.001) and low in PPs (P < 0.001), respectively. Fractional iron absorption from the lpa line low in PPs (4%; 95% CI: 1.8, 8.7) was 50% higher and the total amount of iron absorbed per test meal (261 µg; 95% CI: 120, 570) was 85% higher than iron from the parent low in PPs (P < 0.001). There was no difference between the lpa beans high or low in PPs or between the parents high or low in PPs. A 90% reduction in PA leads to an increase in bioavailable iron from beans, independent of the PP concentration. The lpa mutation could be a key tool for improving iron bioavailability from beans. J. Nutr. 143: 1219–1224, 2013.

Introduction

Micronutrient deficiencies are a major cause of malnutrition and affect large proportions of the world’s population. It is estimated that 2 billion people worldwide are iron deficient. Children and women of reproductive age are the most vulnerable, although iron deficiency (ID) affects all population groups, particularly those subsisting on plant-based diets high in iron absorption inhibitors, low in animal tissue, and therefore low in bioavailable iron (1). Phytic acid (PA), which is probably the most important inhibitor of non-heme iron absorption, is present at high levels in all major cereal grains and legume seeds (2–4). Polyphenol (PP) compounds, however, can be equally potent as PA in reducing iron bioavailability and can also occur at high levels in legume seeds as well as fruits, vegetables, and beverages such as tea, coffee, and red wine (5–7).

Although PA and PPs can inhibit iron absorption due to their ability to form nonabsorbable complexes with iron in the gastrointestinal tract, they also fulfill essential biological functions in the plant. PA, stored in seeds, provides the growing seedling with phosphorous and essential minerals at the whole-plant level and appears to be involved in signaling (8) and response to plant pathogens (9). Certain PPs serve the plant as protection against pathogens or UV radiation and as antioxidants (10,11). Breeding for lower concentrations of these compounds to improve the nutritional value of plants might therefore lead to negative effects on seed and plant performance (12). Nevertheless, the genetic reduction of the PA concentration by disrupting its biosynthetic chain, resulting in selection of a low-PA (lpa) phenotype, is a possible solution to alleviate ID. Mendoza et al. (13), using radioisotopes, reported that iron absorption from meals based on lpa maize in iron-replete men was significantly higher than from meals based on wild-type maize.
In addition to maize, lpa mutations have been identified in some of the other major staple food crops such as wheat (14), rice (15), and soybeans (16). These mutants exhibit normal phosphate uptake, transport, and accumulation but reduced PA phosphate due to various mutations in PA biosynthesis or transport (17,18). So far, lpa crops are in an early stage of development and the reduction of PA is often associated with negative effects on seed physiology and plant performance (19). Campion et al. (20,21) using chemical mutagenesis have recently isolated in the common bean an lpa mutant (lpa -280–10) (20) associated with a defective gene, coding for an ATP-binding cassette transporter engaged in PA storage in protein bodies during seed maturation (21). Despite a >90% reduction in PA, this mutation results in a good grain yield and high germination rate (22). To further optimize iron bioavailability, the same authors introgressed the mutation into a white seed coat trait in the same genetic background, low in PP.

The aim of the present study was to test the potential of the lpa trait to improve iron bioavailability from beans. Iron absorption by young women was measured using stable isotopes. The women consumed 4 different bean porridges based on a white coated lpa line low in PP (lpa-W), a brown coated lpa line high in PP (lpa-B), and their parent wild-type beans with normal PA levels: one brown coated high in PP (wt-B) and one white coated low in PP (wt-W).

Methods

Subjects. Thirty-six women from the student and staff population of ETH Zurich were screened for iron status [hemoglobin (Hb), plasma ferritin (PF), and C-reactive protein (CRP)] as well as for body weight and height. Women with known metabolic, chronic, and gastrointestinal diseases, on long-term medication, or who had donated blood or experienced considerable blood loss within the 6 mo prior to the study were excluded. Twenty apparently healthy, nonpregnant, nonlactating women with PF concentrations between 119 and 146 g/L (Table 1) aged between 18 and 30 y, and <65 kg body weight and normal BMI (19–24 kg/m²) were included in the study. Intake of vitamin and mineral supplements was not allowed 2 wk prior to and during the study period.

The experimental procedures were approved by the ethical committee of ETH Zurich and written informed consent was obtained from all participants before the investigation began.

Study design. One iron absorption study was undertaken using a randomized, crossover, double meal design. The study investigated the influence of bean PA and PP on iron absorption from 2 lpa bean lines (lpa-W, lpa-B) and their parent (wt-W, wt-B).

On d 0, body weight and height were measured and the first blood sample was taken for iron status and inflammation measurements. Test meals were served during 2 pairs of consecutive days. On d 1 and 2 again on d 17 and 18, the participants received isotopically labeled test meals. To reduce the effect of the intra-subject meal-to-meal variation in iron absorption, each participant received 2 identical test meals per day, one in the morning between 0700 and 0900 h after an overnight fast and the second meal at least 3 h later. Participants were not allowed to eat or drink between the test meals and for 3 h after the second meal. Test meals plus water (0.3 L) were consumed completely in the presence of the investigators. To ensure that the whole bean porridge was ingested, the empty bowl was rinsed twice with 10 mL water and the rinse was consumed. If the participants received the lpa bean at the first meal day of the study (d 1 or 17), they received the wild-type bean with comparable PP levels on the following day (d 2 or 18) or vice versa. Participants were randomly assigned to consume the high- or low-PP beans on d 1 or 2 or on d 17 or 18.

A second and third blood sample was taken after an overnight fast for iron isotopic analysis d 14 after d 2 (d 16) and d 18 (d 32). Fractional iron absorption was calculated based on erythrocyte incorporation of iron-stable isotope labels 14 after intake of labeled test meals (23).

Test meals. The test meals were fed as sweetened, homogenized bean porridge made of 50 ± 1 g beans (dry weight), 107 ± 2 g water, and 3.0 ± 0.1 g sugar for a total 160 ± 3 g/meal (fresh weight). The bean seeds were first washed, soaked for 90 min at room temperature, boiled in water for 110 min, homogenized, and sugared. The test meal tests were prepared in batches and stored frozen at −25°C until the day of administration. Two mg 57Fe or 2 mg 58Fe as ferrous sulfate was added to each test meal shortly before administration (30–37% of total iron).

Bean varieties. The International Center for Tropical Agriculture cultivar BAT881 (wt-B), the lectin-free line 586/8 (wt-W), and 2 Fe lectin-free > lpa lines [586/8 × 87 brown (lpa-B) and 586/8 × 87 white (lpa-W)] (22) were used in the experiment (Supplemental Table 1). The term “lectin-free” indicates the absence in the seed of 3 major lectin components: arcelin, phytohemagglutinin, and α-amylase inhibitor (24).

Seed production. The seeds for the human study were produced at Montanasso Lombardo (Italy). Bean cultivation was carried out in 2011 without supplying fertilizers and without applying herbicides and pesticides before and during plant growth.

Stable isotope labels. Isotopically labeled 58FeSO4 and 57FeSO4 were prepared from isotopically enriched elemental iron (57Fe-metal: 97.8% enriched; 56Fe-metal: 99.9% enriched; both Chemgex) by dissolution in 0.1 mol/L sulfuric acid and treated as previously described (25).

Food analysis. Prior to analytical measurements, the bean seeds were milled with a rotor mill (ZM1, Retsch) using a titanium sieve (0.25-mm mesh). The bean porridges were freeze-dried prior to PA and iron measurements, but PP was measured directly in the fresh samples. The total PP concentration in bean seeds and porridges was measured with a modified Folin-Ciocalteau method (26). For iron measurements, samples were mineralized by microwave digestion (MLS ETHOSplus, MLS) and then quantified by graphite furnace atomic absorption spectrophotometry (GF-AAS, AA240Z, Varian). The PA concentration in bean porridges and bean seeds was measured by a modification of the Makower method (27), in which iron was replaced by cerium in the precipitation step. Following the mineralization of the precipitates, inorganic phosphate was determined according to Van Veldhoven and Mannareth (28) and converted into PA concentrations. Wheat bran (PA assay) and milled beans (PP assay), stored under argon to avoid PP 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 the 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 Hb, PF, and CRP. Whole-blood samples were divided into aliquots for the analysis of Hb and

| TABLE 1 Anthropometric data and Hb, plasma CRP, and PF concentrations of the women studied1 |
|-----------------|-----------------|
| Variable        | Summary value   |
| Weight, kg      | 59.0 ± 3.2      |
| Height, cm      | 167 ± 6         |
| BMI, kg/m²      | 21.2 ± 1.5      |
| Hb, g/L         | 129 ± 7         |
| CRP, mg/l       | 0.9 ± 0.7       |
| PF, µg/l        | 19.1 (4.5, 67.4) |

1 Values are means ± SDs or geometric mean (95% CI), n = 20. CRP, C-reactive protein; Hb, hemoglobin; PF, plasma ferritin.
isotopic composition. Plasma was separated, aliquoted, and frozen for the later analysis of PF and CRP. Hb was measured with a Coulter Counter (Beckmann International). PF and plasma CRP were measured on an IMMULITE 2000 (Siemens, Healthcare Diagnostics).

Isotope analysis. Prior to isotopic analyses, whole-blood samples were processed according to Walczek et al. (23). All isotopic analyses 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 (23,29).

Calculation of Fe absorption. The amounts of \(^{57}\)Fe and \(^{58}\)Fe isotopic labels in blood were calculated as previously described (6). The calculations were based on the principles of isotope dilution and took into account that iron isotopic labels were not monoisotopic (23). For calculation of fractional absorption, 80% incorporation of the absorbed Fe into RBCs was assumed (30).

Statistical analysis. Analyses were conducted with SPSS statistical software (SPSS 19.0; SPSS Inc.) and Microsoft Office Excel 2010. The total amount of iron absorbed per test meal was calculated by multiplying fractional iron absorption by the iron content of the test meal, including added iron isotopes (2 mg). Fractional and total iron absorption values were log-transformed for statistical analysis and recomputed for reporting. One-way repeated-measures ANOVA followed by a post hoc Bonferroni test was used for comparisons among the 4 different bean test meals within the same study participant.

Pearson correlation regression models were used to study associations between different variables. \(P<0.05\) was considered significant. Values in the text are presented as means \(\pm\) SDs and geometric means (95% CI).

Results

Participant characteristics. Seven (35%) of the 20 study participants had a PF concentration <15 \(\mu\)g/L, which denotes ID (31), and one participant had an Hb <120 g/L, which denotes anemia (31). All women had a CRP concentration <3 mg/L, indicating no inflammation. All end BMI of all women were in the normal range (19–24 kg/m\(^2\)) (Table 1).

Bean seed and bean meal composition. The iron concentration (7.9–9.6 mg/100 g) of the beans used in the study (Table 2) was in the upper range of what has been reported in bean seeds [5.5 mg/100 g (3.4; 8.9)] (32). The iron concentrations in the seeds of wt-B, lpa-B, and lpa-W were similar, whereas the concentration in the seeds of wt-W was 18% lower.

The PA concentration (70 and 124 mg/100 g) in the beans of the 2 lpa lines was ~90% lower than that of their wild-type parents; the PA concentration of the porridge containing lpa-B beans was ~45% lower compared with the meal containing lpa-W.

The PP concentration of wt-B (676 mg/100 g) was ~30% higher than that of lpa-B. The PP concentrations of the brown beans were ~7–11 times higher compared with the white beans.

Meal preparation reduced the PP concentration of the wt-B and lpa-B by 48 and 40%, respectively, whereas no reduction was observed in the wt-W and lpa-W beans. The final amount of PP in the brown bean porridges was ~4–5 times higher than in the white bean meals (Table 2).

Iron absorption. The mean fractional iron absorption and total amount of iron absorbed from the lpa bean porridges were significantly greater than from the bean porridges made with the parent beans (Table 3). Mean fractional iron absorption from lpa-B bean (61.4%) was 60% higher than from the wt-B parent (\(P<0.001\)) and 130% higher than from the wt-W bean (\(P<0.001\)). Iron absorption from lpa-W was ~50% higher than that from wt-W parents (\(P<0.001\)). The observed difference between the lpa beans and their parents was even greater when comparing the total amount of iron absorbed, which from lpa-B bean porridge (372 \(\mu\)g) was 60% higher than from the wt-B (\(P<0.001\)) and 163% higher than from the wt-W bean porridge (\(P<0.0001\)). Total iron absorbed from the lpa-W bean porridge was 85% higher than from wt-W bean porridge (\(P<0.001\)).

Despite the large difference in PP content, no differences were detected in fractional iron absorption (\(P=0.09\)) or the total iron absorbed (\(P=0.26\)) between the lpa-B and lpa-W bean porridges. Furthermore, iron absorption did not differ between wt-B and lpa-W bean porridges (\(P=1.00\)).

Correlations between different study variables. No correlation was observed between iron absorption from the 4 different bean meals and PF (\(P=0.69\)).

Discussion

The study conducted in common bean demonstrated that reducing PA by >90% (from >1000 to 70 and 124 mg/100 g, respectively) significantly increased iron absorption from 60 to 163%, whereas the PP concentration, in the presence of PA, did not influence iron bioavailability.

The total amount of iron absorbed from the lpa bean lines was up to 163% higher than from their parents with native phytate content. Comparing iron absorption between all 4 tested beans showed that the lpa-B bean was the best iron source with a mean fractional iron absorption of 6.14%, equivalent to 372 \(\mu\)g of total iron absorbed per test meal. This was unexpected, as iron absorption from plant foods and beverages in the absence of PA has been reported to depend on total PP content (5,33) and,

<table>
<thead>
<tr>
<th>TABLE 2</th>
<th>Total PPs, PA, and iron in bean seeds and bean meals as fed to study participants(^1)</th>
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</thead>
<tbody>
<tr>
<td>Bean</td>
<td>Bean seed concentration</td>
</tr>
<tr>
<td>variety</td>
<td>PP</td>
</tr>
<tr>
<td>wt-B</td>
<td>676 ± 19</td>
</tr>
<tr>
<td>lpa-B</td>
<td>462 ± 25</td>
</tr>
<tr>
<td>wt-W</td>
<td>58 ± 1</td>
</tr>
<tr>
<td>lpa-W</td>
<td>54 ± 2</td>
</tr>
</tbody>
</table>

\(^1\) Values are means \(\pm\) SDs, \(n=3\) independent analyses. lpa-B, low-phytic acid bean brown; lpa-W, low-phytic acid bean white; PA, phytic acid; PP, polyphenol; wt-B, wild-type brown; wt-W, wild-type white.

\(^2\) Values include native iron from beans and 2 mg Fe added as Fe\(^{57}\) or Fe\(^{58}\).
in the present research, the total PP concentration in the lpa-B bean meal was 140 mg compared with only 32 mg in the lpa-W bean meal. We previously demonstrated that red bean PPs are inhibitory to iron absorption of a phytate-free meal and that removing PPs from dephytinized red beans doubled the iron absorption (6). In the same study, we found that 50 and 200 mg of bean PPs from red bean hulls compared with a bread meal to which no PP was added decreased iron absorption by 14 and 45%, respectively. Although numerous factors influence iron absorption, one reason for the unexpected finding in the present study could be that PA is a much stronger inhibitor than the brown bean PP. The PA concentration in the lpa-B bean was only about one-half than that of the lpa-W bean and the higher molar ratio of PA:Fe of 1.2:1 in the lpa-W bean compared with 0.6:1 in the lpa-B bean could explain the difference in iron absorption (2) even in the presence of the higher PP content.

Even small amounts of PA have been reported to be inhibitory (34). Hallberg et al. (3) added increasing amounts of PA to a noninhibitory, PA-free, wheat-based test meal and reported an 18% reduction in iron absorption with only 2 mg PA and a 64% lower absorption with 25 mg. It has been reported that iron absorption rises strongly at molar ratios of PA:Fe <1 and preferably <0.4 (2). In our present study, the PA:iron molar ratio was 11:1 in wt-W beans and 8:1 in wt-B beans and would be expected to override any effect of PPs. However, the mutation reduced the ratio to 1:2:1 in the lpa-W beans and 0:6:1 in the lpa-B beans and the final PA:iron molar ratios in the porridges were 0.4:1 (lpa-B) and 0.6:1 (lpa-W), which is within the suggested range to significantly improve iron bioavailability (2).

The weaker inhibition of brown bean PP compared with PP from certain vegetables (33) or beverages (5) is presumably due to their structure. PP compounds in plant foods have a wide variety of monomeric and polymeric structures with a variety of sugar and organic acid side chains (10). The ability of PPs to form nonabsorbable complexes with iron in the intestinal tract as well as the strength and nature of bonding depends to a large extent on the PP structure (5,35). Monomeric and polymeric flavonoids, which bind iron through 2 or more sites, can be powerful ligands (36) provided the hydroxyl groups are free to react. It has been suggested that at least 2 hydroxyl groups in the ortho-position are necessary for PPs to effectively bind iron (6). Black tea PPs rich in gallic acid with 3 hydroxyl groups are reported to have the strongest inhibitory action (5). The lower affinity of bean PPs in general to iron can be explained by the unique composition of the bean proanthocyanidins. Approximately 15% of the proanthocyanidins in beans are reported to be (epi) azelechin (37), a flavanol with only one hydroxyl group at the b-ring (38), and are thus not expected to inhibit iron absorption. The differences in PP structures between beans of different colors (39) further complicate the estimation of their impact on iron absorption.

In addition to the overriding effect of PA and the weak iron binding of some bean PPs, the relatively good iron absorption from the brown beans compared with the white may be due to other iron-binding compounds in beans that could increase or decrease absorption. In an earlier study, we reported that removal of the red bean hulls containing most of the PPs led to a decrease in iron absorption, suggesting that bean hulls contained an absorption enhancer, possibly a structural carbohydrate (6). This would appear contrary to the finding from the same study (reported above) that, in the absence of phytate, red bean PPs inhibit iron absorption (6). However, removal of the hulls did not substantially decrease the PA, as PA is mainly in the cotyledon. It is therefore possible that, in the presence of phytate, the structural carbohydrate enhanced iron absorption, whereas the red bean PPs had no effect. In such a situation, removal of the hulls would decrease iron absorption. Another potential inhibitor in legumes is the protein fraction (40) and lectins from soybean specifically have shown an inhibitory nature in rat studies (41). However, as the lowest iron absorption in the present study was from the lectin-free wt-W bean, a major inhibitory effect of bean lectins seems unlikely.

The results of the present study also contradict a recent in vitro study (22) and highlight the risks of using the in vitro CaCo-2 model to predict iron bioavailability in humans. By applying this technique, Campion et al. (22) tested iron bioavailability from identical bean lines as tested in the present study (although in that work, seed material was produced in 2008, 3 y before that tested here) and found that PPs were the major inhibitor of iron absorption, much more so than PA. CaCo-2 cells seem to be especially sensitive to PPs and a previous study with the in vitro Caco-2 methodology also failed to accurately predict human iron absorption from beans (42).

Although mean fractional iron absorption from the lpa-B bean was still relatively modest at 6.1%, it was higher than the 1–4.7% iron absorption reported from beans tested in other human studies also using stable iron isolate single/double meal designs (6,42–45) and in the same range as iron absorption from beans fed in a stable iron isotope study using a multiple meal design (45). The lpa-B bean also had an iron content of 9.4 mg/100 g, which is in the upper range of what has been reported in beans (46). The mean total amount of iron absorbed from the lpa-W and lpa-B bean meal in our study was 261 and 372 µg, respectively. For the interpretation of our results, however, it has to be taken into consideration that ~30% of the absorbed iron is from the extrinsic tag and the total amount of iron absorbed from a bean with a similar iron concentration might therefore be lower than that reported in our study. Nevertheless, although single/double meal bean studies seem to overestimate the effect of inhibitors on fractional iron absorption, if such lpa beans were consumed in countries like Rwanda, Kenya, and Uganda with a reported mean per capita daily consumption of up to 180 g beans (47), they should provide a large part of the 1.4-mg/d estimated requirement for absorbed iron for a women of child-bearing age (48). This would improve iron status and reduce the prevalence of ID in this at-risk population group.

This study indicates that iron absorption from lpa beans is significantly higher than from their parents with normal PA levels. It also seems that the PP content of the bean seeds has little influence on iron absorption when the PA levels are between 70 and 1360 mg/100 g. This suggests that the lpa mutation could be

### Table 3

<table>
<thead>
<tr>
<th>Bean meal</th>
<th>n</th>
<th>Fractional iron absorption</th>
<th>Total iron absorbed per meal</th>
</tr>
</thead>
<tbody>
<tr>
<td>wt-B</td>
<td>20</td>
<td>3.84 (1.76, 8.38)abc</td>
<td>235 (108, 513)abc</td>
</tr>
<tr>
<td>lpa-B</td>
<td>20</td>
<td>6.14 (2.57, 14.65)a</td>
<td>372 (155, 888)a</td>
</tr>
<tr>
<td>wt-W</td>
<td>20</td>
<td>2.68 (1.26, 5.69)b</td>
<td>141 (67, 300)b</td>
</tr>
<tr>
<td>lpa-W</td>
<td>20</td>
<td>3.99 (1.83, 8.71)ab</td>
<td>261 (120, 569)b</td>
</tr>
</tbody>
</table>

1 Values are geometric means (%CI). Means in a column with superscripts without common letter differ, P < 0.05 (repeated measures ANOVA, on logarithmically transformed data; Bonferroni). All meals contained 2 mg Fe372 or 2 mg Fe589. lpa-B, low-phytic acid bean brown; lpa-W, low-phytic acid bean white; wt-B, wild-type brown; wt-W, wild-type white.
introduced into both colored and white bean varieties with a similar improvement in iron absorption. The lpa beans in this study also had high iron concentrations and although such high concentrations can be reached by traditional breeding, high iron is usually accompanied by high phytate and low absorption (45). The iron of the lpa bean seed is relatively well absorbed and, if the agronomic performance continues to be good, the development of lpa bean varieties might be a promising approach to improve iron nutrition in developing countries where beans are a major staple. However, to support the present findings and to get a better approximation of iron absorption from lpa bean seeds as part of the diet, the way forward would be a multiple meal study in a bean-consuming population where beans are administered as part of composite meals.

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Literature Cited