Women with low iron stores absorb iron from soybeans[1–4]

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ABSTRACT

Background: Worldwide, 30% of the population, a greater proportion of whom are women and children, is iron deficient. Soybeans are a major source of nonheme iron in many human diets, but information on iron bioavailability is still conflicting. Because much of soybean iron is in ferritin [distinct from the poorly bioavailable iron in cereals resulting from interactions between calcium, Fe(III), phytate, and proteins in the meal], soybeans provide a target for manipulating seed iron composition to achieve increased iron bioavailability.

Objective: The aim was to reevaluate soybean iron bioavailability.

Design: Eighteen women, most with marginal iron deficiency, consumed meals with intrinsically labeled (^{55}Fe) soybeans (hydroponically grown and nonnodulating) as soup ($n = 11$) or muffins ($n = 7$) and a reference dose of ^{59}Fe as ferrous sulfate in ascorbate solution. The radioactivity in red cells was measured 14 and 28 d later.

Results: The mean ^{55}Fe absorption from either soup or muffins was 27% and that from the reference dose was 61%. ^{55}Fe was distributed approximately equally between protein ($49.3 \pm 3.0\%$) and phytate, a contrast with nodulating soybeans likely caused by a high phosphate content in the growth medium. There was an expected inverse correlation ($r = -0.793$, $P < 0.001$) between red cell radioactivity and serum ferritin concentration.

Conclusions: These results show that soybeans appear to be a good source of nutritional iron in marginally iron-deficient individuals. More study is needed on the effect of plant nodulation on the form of soybean iron, aimed at enhancing bioavailability to combat iron deficiency in at-risk populations.   *Am J Clin Nutr* 2003;77:180–4.

INTRODUCTION

Soybeans or soybean products are dietary components for a large fraction of the human population and are a potential source of iron. Legumes in general and soybeans in particular have a high iron and ferritin content (1, 2); soybean cultivars vary in iron content over a significant range (3). Previous dietary studies of the bioavailability of iron in soybeans yielded conflicting results (4–6) and used varied populations, iron status, sex, and labeling method. In seeds from nodulating soybeans, much of the iron is in ferritin ($\approx$90%; 7), which contrasts with wheat grain, in which most of the iron is complexed to phytate as monoferricphytate and is relatively unavailable (8).

We previously showed that iron in soybeans and purified ferritin, when provided in amounts of iron equal to that in ferrous sulfate, was equivalent to ferrous sulfate in reversing iron deficiency in rats (1). These results indicated that earlier studies in humans may have suffered from a methodologic bias that was not understood at the time. An example of such a bias was the use of labeled ferritin iron produced during inflammation, a condition now known to change the dynamics of iron loading into the core of the ferritin molecules (3). Thus, it is likely that the intrinsically labeled ferritin iron did not fully represent the ferritin core iron in the absorption trials.

Iron minerals in ferritin from animals and plants have different forms. It is now known that animal ferritin is more ordered and has less phosphate than plant ferritin (9, 10). Such variations could influence radioactive labeling of the ferritin iron mineral and account for some of the different results that have been published (4, 6). When animal ferritin mineral (purified horse spleen ferritin) and plant ferritin mineral (soybean meal) were both used in the same study, they contributed equally to the erythron iron in rats (3). Possible effects of the iron mineral structural differences in plants and meat have not been examined systematically in humans.

Increases in soybean consumption, awareness of the different forms of iron in soybeans (ferritin, ferric phytate; 7, 11), and the bioavailability of soybean and ferritin iron in the rat model (3) all suggested that reinvestigation of soybean iron bioavailability in humans, with the use of carefully controlled conditions and subjects with minimal iron stores, was warranted at this time.

SUBJECTS AND METHODS

Recruitment and screening

Potential subjects were identified by advertisement in student newspapers and on bulletin boards at The Pennsylania State University. Interested individuals were given a full explanation of the study and scheduled for screening. Written informed consent was obtained from each subject. Screening involved the assessment of iron status via blood obtained from an antecubital vein, administration of a health questionnaire to determine whether the subjects had a history of gastrointestinal or hematologic disorders, a brief physical exam, and a pregnancy test. Subjects were excluded from the study if they were pregnant, had a history of gastrointestinal or hematologic disorders, or were taking medications that could interfere with hematopoiesis or absorption. Eighteen women participated in the study. The procedures followed were in accord with the ethical standards of the Institutional Review Board of The Pennsylvania State University. All procedures were reviewed and approved by the board.

Iron sources

Soybeans (Tokyo cultivar) were cultivated hydroponically in a high-nitrogen medium (nonnodulating plants) as previously described (11) in a growth chamber with a 24 °C–18 °C day-night temperature cycle. The growth chamber was initially set at 14-h days during vegetative growth for 2.5 mo. The day regimen was changed to 8-h days to induce flowering for 3 wk. After flowering the chamber was set to 16-h days until harvest (11). The growth chamber was equipped with artificial lights that supplied 2000 μE[[TS]$\cdot$ m$^{-1}\cdot$ s^{-1} at plant height. Nutrient solutions were changed periodically to resupply depleted nutrients (monthly to weekly depending on the growth stage of the plants). Radiolabeled iron [^{55}Fe(III)-ethylenediaminedi(o-hydroxyphenylacetic) acid with a specific activity of 6.167×10^3 kBq/mmol Fe] was added with each change of nutrient solution from the time of flowering until harvest at maturity.

^{55}Fe distribution was determined in soybean soluble extracts that were prepared as previously described (9). Beans (0.75 g) were ground with 1.0 mL extraction buffer [20 mmol Tris/L, pH 7.5; 100 mmol NaCl/L; 1 mmol MgCl$_2$/L; 20 mmol EDTA/L; 1% β_mercaptoethanol, and Protease-Arrest (Geno Technology, Inc, St Louis)], followed by centrifugation at $10\,000 \times g$ for 30 min at 4 °C. The clarified extracts were divided into trichloroacetic acid (TCA)–insoluble (protein) and –soluble fractions (ferric phytate and other low-molecular-weight forms of iron). Radioactivity was measured by liquid scintillation counting for the 10% cold, TCA-soluble (phytic acid) and TCA-precipitable (protein-ferritin) fractions, after digestion in a Beckman Tissue Solubilizer 450 (Beckman Instruments, Fullerton, CA). The percent of ^{55}Fe in the protein (ferritin) fraction averaged $49.3 \pm 3.0\%$ ($\overline{x} \pm$ SD), with the remainder being acid soluble (monoferricphytate and other soluble low-molecular-weight forms of iron). Iron for the reference meal was prepared with ascorbate to ensure the reduction of the ^{59}ferric to ferrous. Three milligrams iron as FeSO$_4$(7H$_2$O) tagged with ^{59}FeCl$_3$ (1 μCi) and 18.9 mg ascorbic acid/50 mL water were mixed immediately before administration.

Phytate content was determined with an ion chromatography method as follows. Mature soybeans were ground in a coffee mill and a 0.200- to 0.300-g subsample of the ground material was vortexed and shaken horizontally for 2 h in 10 mL of 0.5 M HCl in capped 15-mL polypropylene centrifuge tubes and then centrifuged at $1800 \times g$ for 10 min at 22 °C. A 1.0-mL aliquot of the supernatant fluid was diluted with 9.0 mL deionized H$_2$O. A 0.50-mL aliquot of the diluted extract was placed in an autosampler vial and analyzed for phytic acid via ion chromatography as follows. The ion chromatography system included a Dionex liquid chromatography module (model CHB; Dionex Corp, Sunnyvale, CA) with an advanced gradient pump, computer interface, conductivity detector, and autosampler. The Dionex columns used in series included an NG1 (a nonpolar organic adsorption precolumn), an AG11 guard column, and an AS11 analytic column (anion-exchange column). The injected sample (50 μL) was eluted with the use of a tertiary gradient composed of the following reagents: 200 mmol NaOH/L, 50% MeOH in water, and deionized H$_2$O. The elution gradient varied from an initial condition of 84:12:5 (by vol) NaOH:MeOH:H$_2$O to a final condition of 35:60:5 NaOH:MeOH:H$_2$O over an 11-min period. Appropriate phytate standards (from 0 to 60 mmole phytate/L) and blanks were also analyzed and used to quantify the amount of phytate in the soybean extracts.

Study protocol

One of 2 test meals was given to each subject, followed by administration of a reference meal. Both test meals contained soybeans intrinsically labeled with ^{55}Fe, and the reference meal contained ferrous sulfate radiolabeled with ^{59}Fe as a reference dose. All meals were consumed between 07:30 and 09:30 after a 12-h overnight fast. Only water was allowed during the subsequent 3 h.

On the morning of the first day, the subjects arrived at the General Clinical Research Center (GCRC) in a fasted state. Immediately before administration of the test meal, a venous blood sample was obtained from each subject and a second pregnancy test was administered. The subjects were then fed a hot meal consisting of either soybean soup ($n = 11$) or muffins ($n = 7$), each made with ^{55}Fe-labeled beans, and water as a beverage. The soup contained 46.5 g soybeans (containing 2 μCi ^{55}Fe) that were baked for 1 h at 149 °C, a chicken bouillon cube, and 100 g water. The muffins contained 23.25 g soybeans (containing 1 μCi ^{55}Fe) that were baked for 1 h at 149 °C and then ground into flour with a food processor, high-extraction flour, baking powder, sugar, cream of tartar, salt, shortening, and milk. The muffins were baked in a toaster oven for 15 min at 177 °C. Both the soup and the muffins were prepared the day before, frozen, and then reheated in a microwave before consumption by the subject. The soup meal was found to contain 4.5 mg Fe, and the muffin meal contained 3 mg Fe. Lunch and dinner were prepared for each subject by a registered dietician at the GCRC, and each subject was instructed to eat and drink only what was prepared for her (with the exception of water). All meals prepared by the GCRC staff consisted of a balanced diet that provided all of the macro- and micronutrients recommended by the National Academy of Science Recommended Dietary Allowance Committee and provided each subject with 15 mg Fe/d. The amounts of phytates and ascorbic acid in the meals provided during the days of test meal or reference dose administration were kept constant.

On the morning of the second day, the subjects once again arrived at the GCRC in a fasted state. They were given the reference meal, which consisted of 3 mg Fe as FeSO$_4$(7H$_2$O) radiolabeled with 1 μCi ^{59}FeCl$_3$ and 18.9 mg ascorbic acid in 50 mg water. They were then given their lunch and dinner and again instructed to eat and drink only what was prepared for them. Fourteen and 28 d after consumption of the soybean meal, the subjects

TABLE 1
Iron absorption from soybean soup or muffins (^{55}Fe) and from a reference dose (^{59}Fe–ferrous sulfate)

Test meal and subject no. and age	Hemoglobin	Hematocrit	Transferrin saturation	Ferritin	Iron absorption			
					Test meal day 14	Test meal day 28	Reference meal day 14	Reference meal day 28
	g/L	%	%	μg/L	% of dose			
Soup								
1, 23 y	127.5	38.0	14.0	7.3	23	23	53	55
2, 22 y	142.5	44.0	32.0	45	9	10	26	27
3, 20 y	128.0	41.0	16.0	3.1	32	37	84	86
4, 21 y	134.0	42.0	22.0	12.5	18	21	70	75
5, 21 y	122.0	36.0	17.0	9.5	27	29	56	57
6, 21 y	147.5	45.0	31.0	10.2	22	23	45	48
7, 19 y	133.5	40.0	51.0	15.4	32	38	51	58
8, 20 y	127.5	37.0	33.0	6	30	28	57	59
9, 20 y	139.0	41.0	33.0	20.1	14	15	52	57
10, 21 y	133.0	41.0	12.0	10.6	36	39	59	57
11, 22 y	123.0	39.0	39.0	7.4	22	24	47	49
Muffin								
12, 22 y	138.5	43.0	33.0	4.4	35	31	68	72
13, 21 y	124.0	38.0	33.0	10.4	25	45	71	74
14, 19 y	127.5	38.0	15.0	6.5	36	40	55	61
15, 19 y	118.0	36.0	37.0	7	25	25	58	64
16, 22 y	124.5	39.0	37.0	4.4	34	37	68	76
17, 21 y	133.5	41.0	31.0	12.5	20	22	72	74
18, 21 y	129.0	39.0	22.0	9.4	27	30	68	75
Mean	131.0	40.0	28.0	11.2	25.9	28.7	58.9	62.4
Geometric mean	130.0	40.0	26.0	9.1	24.5	27.0	57.3	60.7

returned for a fasting venous blood draw to ensure detection of maximum iron incorporation.

Blood samples

Blood samples were used to obtain the following measurements: complete blood count including hemoglobin and hematocrit, plasma iron, and total-iron-binding capacity (12) and serum ferritin (Diagnostic Products Corporation, Los Angeles). Blood samples from day 1 were also used to assess each individual's background blood radioactivity (13). Those from days 14 and 28 were used for the above measurements as well as the measurement of incorporated red cell radioactivity. Absorption was calculated from the blood volume derived from height and weight measurements assuming that 80% of the absorbed iron was incorporated into circulating erythrocytes (14).

Statistics

All data were analyzed with SAS software for the personal computer (version 8.0; SAS Institute, Inc, Cary, NC). Replicates were averaged and the mean values used. All radioactivity counting was conducted to achieve a 2σ of $<1\%$. The within-run CV in serum ferritin measurements is $<4.5\%$ in our laboratory and $<2.5\%$ for hemoglobin and hematocrit.

RESULTS

Iron absorption was high among the subjects in the study, with a geometric mean absorption of 24.5% (range: 9–36%) of the soybean dose and 57.3% (range: 26–84%) of the reference dose at day 14 (**Table 1**). Absorption values measured at 14 d did not differ significantly from those measured at 28 d ($P > 0.1$), nor was there

a significant difference between absorption of the soup or muffins. Most of the 18 subjects displayed marginal iron deficiency (hemoglobin = 122.0–147.5 g/L, serum ferritin = 3.1–15.4 μg/L; Table 1). One subject was anemic (hemoglobin = 118.0 g/L, serum ferritin = 7 μg/L), and 2 subjects were iron replete (hemoglobin = 139.0 and 142.5 g/L, serum ferritin = 20 and 45 μg/L; Table 1). When the levels of iron stores (serum ferritin concentration) were plotted in comparison with iron absorption, a significant ($r = -0.793$, $P < 0.001$) inverse correlation was observed between serum ferritin concentration and iron absorption.

The distribution in the soybeans of the radioactive label between TCA-insoluble (protein) and TCA-soluble fractions (ferric phytate and other soluble iron forms) was $49.3 \pm 3.0\%$. This contrasts with earlier studies that used Mossbauer spectroscopy, gel filtration, or both (7; EC Theil and J Burton, unpublished observations), where 70–90% of the soybean iron was in protein (ferritin). In nodulating plants some of the soybean iron comes from the iron that accumulated in the nodule early in plant development (11). In this experiment, the addition of label late in plant development (flowering) and the use of nonnodulating plants may explain the lower percentage of the ^{55}Fe in the ferritin fraction of the soybeans. The beans were found to contain 31.77 μmol/g phytic acid, compared with nodulating field-grown soybeans, which have 15.2–21.21 μmol/g.

DISCUSSION

Dietary iron deficiency and anemia afflict 1.5 billion people worldwide (15). The major strategies currently used to combat dietary iron deficiency include supplementation and fortification. However, these strategies are relatively expensive, noncompliance

TABLE 2
Comparison of soy iron availability in humans in 3 studies

	1973 Sayers et al (4)	1984 Lynch et al (5)	2003 Present study
No. of subjects	5	10	18
Sex	Female	Male	Female
Labeling	Instrinsic	Extrinsic	Intrinsic
Food iron form	Ferritin	$FeCl_3$	Ferritin[1]
Test iron form	$FeSO_4$	$FeSO_4$	$FeSO_4$
Form of meal	Biscuit	Soup	Soup or muffin
Percentage uptake (soy meal)	20	2.8	25.9[2]
Percentage uptake (reference dose)	72.8	16	58.9[2]
Percentage uptake (meal, reference dose)	27.5	17.5	44.0[2]
Hematocrit[3]	—	0.47	0.40[2]
Hemoglobin (g/L)[3]	108	—	131[2]
Serum ferritin (μg/L)[3]	—	61	11.2[2]

[1] 50% of the iron in the soybeans was in the ferritin form.

[2] $\overline{x}$.

[3] Values on day of soybean meal consumption.

can be high, and interactions between supplements and endogenous food components are complex (16, 17).

Ferritin is the major source of iron in the early development of animals (18, 19) and plants (20). Cellular concentrations of iron equivalent to $> 10^{11}$ times the solubility of the free Fe(III) can be achieved by ferritin.

Soybeans, in contrast to some other seeds, contain a large amount of their seed iron in the form of ferritin (7, 11). Nodulating field-grown beans usually have 70–90% of their soluble iron asssociated with the ferritin protein fraction, whereas the beans used in the present study have a lower percentage (49 ±_3%) of their iron in this fraction. Moreover, when the radioactive label is added throughout growth to nodulating soybean plants, more than half of the iron in the seed can be shown to come from the senescing nodule (11). Possible explanations for the larger fraction of ^{55}Fe in ferric phytate in the present study, in which the beans were grown hydroponically in a medium rich in nitrogen and phosphorous, are the higher amount of phytate in the seeds and the shorter time of exposure to the radiolabel, added only at flowering. Clearly, a systematic study is needed to resolve the role of nodulation in the iron distribution of soybeans. Because a large fraction of the ^{55}Fe in the seeds is taken up into the erythron, and because ferric phytate is not an available source of iron, the protein fraction of the seeds was the main source of iron. Interestingly, iron in radiolabeled ferritin is readily taken up by Caco-2 cells in culture (SL Kelleher, BL Lonnerdal, and EC Theil, unpublished observations, 2002), and soybean seeds are relatively rich in both iron and ferritin, making it likely that the radiolabel in the red cells of the subjects in the present study came from the soybean ferritin and not from the ferric phytate in the seeds.

Soybeans are consumed as an important source of high-quality vegetable protein in many parts of the world, especially in places where iron deficiency is prevalent. Therefore, the present study was conducted to reevaluate the bioavailability of iron found in soybeans. The complexity of absorption of iron in humans has only been revealed recently with the identification of new genes related to uptake of iron in different forms and for iron efflux (21, 22). Different forms of iron may contribute differently to bioavailable iron pools. Currently 22 genes are known to be involved in iron uptake in bacteria and appear to be matched to the various forms of iron. It is quite possible that the growing number of iron-uptake genes being identified in humans will approach that in microorganisms and will involve variations in mechanisms of iron uptake from different foods.

Previous studies measuring soybean iron bioavailability appear to be conflicting (**Table 2**). For example, Lynch et al (5) report a very small absorption of iron from the soy meal, and Sayers et al (4) show a much larger percentage of absorption, comparable to the results obtained in the present study. Among the possible sources of the apparent discrepancy are the food iron form, the method of isotope labeling, the sex of the participants, and the iron status of the participants (Table 2). In a study with ferric ammonium citrate as an extrinsic isotope label, parallel to the soybean bioavailability study with an intrinsic iron label, Sayers et al (4), found no differences related to the type of labeling used. However, because the chemistry of the ferric chloride used by Lynch et al (5) and the ferric ammonium citrate used by Sayers et al (4) differ significantly, the chemistry may contribute to the differences observed with different extrinsic isotope labels, given the complexity of nonheme iron uptake now known. Lynch et al (5) used extrinsically labeled soybeans, with $FeCl_3$ as the food iron form, and the participants were males whose iron status was sufficient. The present study more closely resembles that of Sayers et al (4), who used intrinsic isotope labeling, ferritin as the food iron form, and females as the study participants. Overall, the women in the Sayers et al study had a lower iron status than did the women did in the present study. The results of the present study agree with those of Sayers et al (4) and show that iron in soybeans is in fact a bioavailable source of iron for iron-deficient humans. The data support the viability of approaches aimed at amplifying the natural iron stores of plants as a novel way to combat iron deficiency worldwide. ❧

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