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Iron and Zinc Absorption from Two Bean (*Phaseolus vulgaris* L.) Genotypes in Young Women

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Extrinsic and intrinsic iron and zinc labels were used to test iron and zinc absorption from two bean (*Phaseolus vulgaris*) genotypes, containing normal (common beans, CB) or higher (HFeZnB) iron and zinc concentrations, fed in single meals to young women with low iron reserves. The women were divided into two groups, with one receiving a CB test meal ($n = 12$) and the other, an HFeZnB test meal ($n = 11$). The beans were intrinsically labeled hydroponically with ^{55}Fe (CB and HFeZnB) and with ^{70}Zn (HFeZnB). Concentrations of zinc and iron were 98 and 65% higher, respectively, in HFeZnB as compared to CB, but phytic acid contents were similar. Extrinsic labels were ^{59}Fe (CB and HFeZnB), ^{67}Zn (CB), and ^{68}Zn (HFeZnB). Iron and zinc percent absorption levels were calculated from radio-iron activity in red blood cells and from urinary excretion of zinc isotopes. Intrinsic and extrinsic iron absorption measures were highly correlated ($R^2 = 0.986$) (average extrinsic/intrinsic ratio was 1.00). Iron absorption was low (geometric mean < 2%) in both bean types, and total iron absorbed was not different between types. Intrinsic zinc absorption from the HFeZn beans was higher than extrinsic absorption (15.2% vs 13.4%, $p < 0.05$) (average extrinsic/intrinsic was 0.90). The correlation between intrinsic and extrinsic zinc measures was not as high as that for iron ($R^2 = 0.719$). Percent zinc absorption levels were similar in both bean types, but total extrinsic zinc absorbed was 90% higher ($p < 0.05$) from the HFeZnB meal. Thus, the less expensive and time-consuming extrinsic labeling may be used to screen various varieties of beans for iron bioavailability in humans, but it underestimates zinc absorption by ∼10%. Selective breeding for high-zinc bean genotypes may improve zinc status. However, high-iron genotypes appear to have little effect on iron status when fed alone in single meals to women with low iron reserves.

KEYWORDS: Iron and zinc bioavialability; humans; plant breeding; isotope labeling

INTRODUCTION

Iron deficiency is the most prevalent nutrient deficiency worldwide (*1*). Zinc deficiency is also thought to be prevalent in developing countries (*2*). Iron and zinc deficiencies affect mainly pregnant women, infants, and children. Iron deficiency can increase maternal and infant morbidity and mortality, decrease resistance to infection, and impair mental and psychomotor development of children (*3*, *4*). Zinc deficiency can impair growth and cognitive and immune function and may contribute to complications during pregnancy and retard fetal growth (*5*). Iron and zinc are found commonly together in foods, so poor iron and zinc nutritional statuses are usually associated, particularly in developing countries where the diets are based on plant foods with low iron and zinc bioavailability and/or concentration (*6*).

Plant breeding is a tool that can be used to improve the nutritional quality of foods (*7*−*9*). An international collaborative project has been initiated to assess the feasibility of breeding for micronutrient enrichment of staple plant crops (*10*, *11*). The

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main target nutrients in plant breeding programs are iron, zinc, and vitamin A (β-carotene); cereals and beans are the primary focus.

The common bean (*Phaseolus vulgaris*) is a staple food in many countries, particularly in Latin America, and it is an important source of protein, vitamins, and minerals including iron and zinc (*12*, *13*). Measurements of the iron and zinc contents of varieties of beans show that selective breeding strategies could increase the concentration of iron by 60−80% and that of zinc by ~50% (*14*). Zinc and iron concentrations in different bean varieties are highly associated (*15*). Therefore, selecting for high-iron bean varieties will also tend to select for high-zinc varieties. Bean genotypes with higher seed iron concentrations resulted in increased amounts of bioavailable iron to rats (*15*). However, the rat is not an ideal model for determining iron (and possibly zinc) bioavailability to humans (*16*). Therefore, before the high-iron, high-zinc varieties of beans are introduced into crop-breeding programs, the bioavailability of iron and zinc from these legume seeds needs to be determined in humans.

The preferred method for measuring iron and zinc bioavailability in humans requires the oral administration of isotopic tracers (*17*, *18*). Use of an extrinsic label is preferred because it is less expensive and easier to administer than an intrinsic label. However, the accuracy of extrinsic tracers for measuring iron or zinc varies with the test food (*19*−*24*). In general, extrinsic labeling for non-heme-iron bioavailability appears to be valid for most foods (*25*). Zinc extrinsic tags, however, do not appear to exchange fully with endogenous zinc in many foods (*21*). Comparison of the intrinsic and extrinsic measurements of zinc and iron bioavailability in beans needs to be determined before the less expensive extrinsic labels can be used in future field studies.

The purpose of our study was twofold: (1) to determine if extrinsic iron and zinc labels can be used to measure iron and zinc absorption from beans and (2) to compare iron and zinc absorptions from two genotypes of beans, with normal or high-iron, high-zinc concentrations but with similar amounts of phytic acid. The study was done in young women with low iron reserves.

MATERIALS AND METHODS

Subjects. Twenty-three women, 20−28 years of age, participated in the study. Seventeen of the women were Caucasians, two Asian-Americans, two Mexican-Americans, and one African-American. All of the women were nonsmokers, apparently healthy with no recent use of mineral and/or vitamin supplements, and users of oral contraceptives with plans to continue contraceptive use for at least two more years. Only women with no plans for a pregnancy during the next two years were selected for study. At entry into the study, all women had hemoglobin concentrations >110 g/L and plasma ferritin concentrations <25 μg/L. Prior to participation in the study, the subjects kept a 4-day record of all food and beverage intake. Nutrient intake was estimated using The Food Processor (ESHA Research). The study was approved by the Radiation Safety Committee and by the Committee for Protection of Human Subjects at the University of California, Berkeley. Written informed consent was obtained from each subject.

Study Design. Subjects were randomly divided in two groups. Group I ($n = 12$) received a test meal of typical common beans (CB); group II ($n = 11$) received a test meal of high iron/zinc beans (HFeZnB). Intrinsic and extrinsic iron absorptions were measured in both groups. Extrinsic zinc absorption was measured in both groups. Intrinsic zinc absorption was not measured in group I fed the CB. Intrinsic and extrinsic zinc absorptions were compared in the HFeZnB group.

The test bean meal was fed after an overnight fasting on day 1. Blood and urine samples were obtained just before ingestion of the test meal for measurement of zinc and iron status. A nonfasting blood sample was obtained on day 14 for determination of iron absorption. Spot urine samples were obtained on days 3−5 for determination of zinc absorption.

Beans and Test Meals. The beans were grown in nutrient solutions at the USDA−ARS Plants, Soils and Nutrition Laboratory greenhouse, Ithaca, NY. Two bean varieties of *Phaseolus vulgaris* from the CIAT (Centro Internacional de Agricultura Tropical) core bean collection (*15*) were grown, a common iron/zinc genotype (CIAT 8465) labeled with ^{55}Fe, and a higher iron/zinc genotype (CIAT 3096) labeled with both ^{55}Fe and ^{70}Zn. Because the ^{70}Zn isotope was available in limited amount, only the high iron/zinc genotype was intrinsically labeled with zinc. Isotope labels were supplied to the growing plant at flowering. ^{55}Fe was supplied as ^{55}Fe-labeled iron(III) *N*,*N*′-ethylenebis[2-(2-hydroxyphenyl)glycine] (Amersham), and ^{70}Zn was supplied as ^{70}ZnO, 88.5% enriched (Trace Science International Inc.). Plants of the same genotype were also grown without the isotope labels to feed with the labeled beans in the test meals. Seeds were harvested at maturity and allowed to dry at room temperature.

Dried bean seeds were soaked for 2 h in a covered glass container with two parts by weight of deionized water and sodium chloride (1 g/100 g of beans) (Baker Analyzed). Soaked beans were cooked in a microwave oven at high potency during 10 min, homogenized in a glass/stainless steel blender, and divided into portions kept at −20 °C until use in the absorption tests. Aliquots were analyzed for iron and zinc contents and specific activity of ^{55}Fe and ^{70}Zn enrichment.

The test meal consisted of 40 g (dry weight) of cooked beans providing 2 μCi (74 kBq) of ^{55}Fe as an intrinsic iron label for both bean types. The HFeZnB test meal also contained 1.0 mg of ^{70}Zn as intrinsic label. The day prior to the absorption test, individual portions of the bean meal were thawed at 4 °C and extrinsically labeled with iron and zinc. The CB meal was labeled extrinsically with 1 μCi (37 kBq) of ^{59}Fe and 1.0 mg of ^{67}Zn (group I); the HFeZnB meal was extrinsically labeled with 1 μCi (37 kBq) of ^{59}Fe and 3.0 mg of ^{68}Zn (group II). The label was allowed to equilibrate with the bean matrix overnight at 4 °C. Sources of extrinsic isotopes were $^{59}FeCl_3$ (Amersham); ^{67}ZnO, 94.60% enriched, and ^{68}ZnO, 99.42% enriched (Oak Ridge National Laboratory).

All of the materials used for the preparation and consumption of the bean meals were acid washed and rinsed with deionized water prior to use.

Preparation of Zn Isotopes for Intravenous Infusion. ^{70}ZnO, 88.03% enriched, or ^{67}ZnO, 94.60% enriched (Oak Ridge National Laboratory), was dissolved in concentrated HCl (Optima, Fisher) (3 μL of HCl/mg of ZnO). The solution was diluted with triply deionized water to a final concentration of 0.3 mg of ^{70}Zn/mL or 1.0 mg of ^{67}Zn/mL. The solution was sterilized by filtration and pyrogen tested by the School of Pharmacy, University of California, San Francisco. Doses containing 0.3 mg of ^{70}Zn, or 1.0 mg of ^{67}Zn, were stored in individually sealed, sterile vials.

Sample Collection. The subjects arrived at the metabolic unit at 7:00 a.m. after an overnight fast. Their height and weight were measured, and a spot urine sample (~40 mL) was collected into Zn-free plastic containers. A fasting blood sample (30 mL) was drawn from the antecubital vein into trace mineral-free polypropylene syringes (Sarstedt Monovette, NH_4-heparin, Sardsted Inc., Newton, NC). Blood samples were kept on ice for no more than 1 h before processing.

The test meal was fed at 7:15 a.m. and consumed in <15 min. At 7:30 a.m., a 1.0 mL solution of 0.3 mg of ^{70}Zn (group I) or 1.0 mg of ^{67}Zn (group II) was infused over a time period of 1 min into the antecubital vein, using a "butterfly" infusion set (Becton Dickinson and Company, Sandy, UT). The butterfly tubing was flushed with 5 mL of sterile saline solution (0.9 g % NaCl, Elkins-Sinn) to ensure that all of the isotope had been infused. The exact amount of the isotope solution infused was determined by weighing the syringe before and after the infusion.

Aliquots of fasting blood samples obtained on day 1 were used for the determination of hemoglobin. Plasma was obtained from centrifugation of the blood samples, transferred into polyethylene tubes, and stored at −70 °C until analysis for ferritin and zinc.

On days 3−5 following the test meal day, subjects were instructed to collect at home the first morning urine voids (40−100 mL) into Zn-free plastic containers. All urine samples were acidified with HCl (Fisher, TM grade; 8 μL/mL of urine) and stored at −20 °C until analyzed for total zinc, zinc isotopes, and creatinine.

On day 14, the subjects returned to the metabolic unit in the morning (fasting not required) for a blood sample (30 mL) by antecubital venipuncture using sodium heparin Vacutainer tubes (Becton Dickinson). Whole blood samples were kept at 4 °C until analyzed for ^{55}Fe and ^{59}Fe.

Analysis in Beans and Test Meals. The zinc and iron contents in the beans and test meals were determined by atomic absorption spectrometry (Thermo-Jarrell Ash 22, Franklin, MA). Before measurement, samples were digested with concentrated HNO_3 (Fisher Scientific, TM grade) in a microwave digestor (MDS 2000, CEM Corp.). A bovine liver standard (National Bureau of Standards) was used as an internal control. The CV for measurements of the standard was 1.6% for zinc and 2.1% for iron. The other minerals in the beans were analyzed in acid digests by ICP-AES. Phytic acid (*myo*-inositolhexaphosphoric) content was determined by ion chromatography and tannin content by a vanillin redox/precipitation assay, as previously described (*15*).

The enrichment of the HFeZnB meal with the intrinsic label ^{70}Zn was determined by ICP-MS (Sciex ELAN 500 ICOP-MS, Perkin-Elmer, Norwalk, CT). Zinc was purified from acid digests of bean meal samples by ion-exchange chromatography (type AG1X-8; 200−400 mesh, chloride form, Bio-Rad Laboratories) prior to analysis (*26*).

Measurement of the intrinsic label ^{55}Fe in the test meals was done by an adaptation of the method described for blood (*27*) using liquid scintillation counting. Iron was initially purified from acid digests of bean meal homogenates by ion-exchange chromatography (type AG1X-8; 200−400 mesh, chloride form, Bio-Rad Laboratories) and then radioassayed in a Tri-Carb liquid scintillation analyzer model 1600 TR (Packard Instrument Co., Meridan, CT). Recovery of known amounts of ^{55}Fe added to samples was 99% with a CV of 0.5%.

Blood Analyses. Blood hemoglobin was determined with HemoCue Systems (Helsingborg, Sweden). Plasma ferritin was measured by enzyme-linked immunosorbent assay using a kit (Spectro Ferritin, S-22, Ramco Laboratories Inc., Houston, TX). Zinc in plasma and urine samples was measured by atomic absorption spectrometry. Creatinine in centrifuged urine samples was measured by an automated procedure (Cobas Fara Autoanalyzer).

Measurement of Iron Absorption. Iron absorption was estimated from the incorporation of radiolabels (^{55}Fe for intrinsic and ^{59}Fe for extrinsic) into red blood cells collected 14 days after the oral administration of the radioisotope. Contents of ^{55}Fe and ^{59}Fe in the blood samples were determined after acid digestion, ion-exchange chromatography, and liquid scintillation radioassay (*27*). Recoveries of known amounts of ^{55}Fe and ^{59}Fe added to blood samples were 99.0 and 99.5%, respectively, with CVs of 0.5 and 0.9%, respectively.

The amount of iron radiolabels (^{55}Fe or ^{59}Fe) in the total blood of the subjects was calculated from the measurements in the blood samples and estimates of total blood volume (*28*). These estimates take into consideration the sex, weight, and height of the individual. Incorporation of the radiolabel into total red cell mass was estimated according to the iron status of the individual based on blood hemoglobin and plasma ferritin levels. Iron absorption was calculated as the ratio of ^{55}Fe (intrinsic) or ^{59}Fe (extrinsic) incorporated into the total red cell mass over the ingested dose of the corresponding isotope and expressed as a percentage (*27*).

Estimation of Zinc Absorption. Urinary zinc was purified by ion-exchange chromatography (*26*). Measurement of zinc isotopes in the purified samples was performed by ICP-MS using a Sciex ELAN 500 ICOP-MS (Perkin-Elmer, Norwalk, CT). The results from the three urine samples following each clinical test were averaged.

For the CB group, using two zinc isotopes (^{67}Zn as extrinsic meal label and ^{70}Zn as the intravenous tracer), extrinsic zinc absorption was estimated by the double-isotope tracer ratio method (*26*) as initially described by Friel et al. (*29*). Fractional zinc absorption (FZA) was calculated on the basis of the following equation:

$$\text{FZA} = (\text{oral tracer:tracee ratio in urine/IV tracer:tracee ratio in urine}) \times (\text{IVtracer dose in mg/oral tracer dose in mg})$$

For the HFeZnB group, using three zinc isotopes (^{70}Zn and ^{68}Zn as intrinsic and extrinsic meal labels, respectively, and ^{67}Zn as the intravenous tracer), the tracer/tracee ratios of zinc in urine were calculated using a novel algorithm for three isotopic tracers (*30*) based on the general case described by Cobelli et al. (*31*).

Statistical Analyses. Data were tested for normality and log-transformed for plasma ferritin and iron absorption prior to statistical analyses. Comparison of zinc and iron absorptions between the two bean genotypes was done by Student *t* test. Comparison of intrinsic and extrinsic measurements of zinc and iron absorptions from the same bean genotype was done by paired *t* test. The association between measurements was tested by Pearson correlation analysis. Statistical analyses were done with the SPSS program (SPSS Inc., Chicago, IL), and results were considered significant at $p < 0.05$.

RESULTS

The chemical compositions of the two bean genotypes studied are shown in **Table 1**. The high iron−zinc bean type had almost twice (98% more) as much zinc and ∼65% more iron than the common bean type. The amounts of other minerals and of phytic acid were similar in both bean types. Although the phytate/iron and phytate/zinc molar ratios were high in both varieties of beans, the phytate/iron ratio was reduced by 38% in the HFeZnB and the phytate/zinc ratio was reduced by 46%. The amount of tannins was about one-third lower in the HFeZnB compared to the CB type.

The characteristics of the participants in the study are shown in **Table 2**. The women were 23 ± 2.2 years of age and had normal body mass indices, 22.5 ± 3.5 kg m^{-2}. They were nonanemic, but they had low iron reserves as indicated by the low plasma ferritin concentrations (*32*). Plasma zinc was, on average, in the lower range of normal values (*33*), suggesting a marginal zinc status. Urinary zinc was similar to that previously observed in healthy adult women (*34*).

There were no differences between intrinsic and extrinsic measurements of percent iron absorption from either the CB or HFeZnB meal (**Table 3**). The intrinsic and extrinsic measurements of iron absorption were strongly correlated ($R^2 = 0.986$) (**Figure 1**). The extrinsic/intrinsic iron absorption ratios were 1.029 ± 0.049 for the CB meal and 0.995 ± 0.059 for the HFeZnB meal. The percent of iron absorbed was low in both bean types (<2%), but it was ∼45% lower in the HFeZnB meal than in the CB meal. This difference did not reach statistical significance ($p < 0.10$). Iron absorption from the bean meals was inversely related to the women's plasma ferritin concentrations ($R = -0.695$, $p < 0.001$, log-transformed values) (**Figure 2**).

The test meal of HFeZnB provided, on average, 37% more iron than the test meal of CB (**Table 3**). Iron intake in the tests meals was essentially all bean iron, because a negligible amount of extrinsic radioiron was used as label for iron absorption. The total amount of iron absorbed from the test meal (either intrinsic or extrinsic) was low for both bean types (geometric means of 0.042 and 0.033 mg for CB and HFeZnB, respectively). The difference between bean types did not reach statistical significance, although it was ∼30% higher in CB.

The estimated percent zinc absorption from the intrinsic tag in the HFeZnB meal was ∼10% higher than that estimated from the extrinsic label ($p = 0.036$) (**Table 3**). The extrinsic/intrinsic zinc absorption ratio was 0.90 ± 0.14. The correlation between the intrinsic and extrinsic estimates had an $R^2 = 0.720$ (**Figure 3**). Zinc absorption was low (13−16%) in meals of the two

Table 1. Chemical Composition (Dry Weight Basis) of the Bean Genotypes

component	common bean	high Fe/Zn bean
zinc (μg g^{-1})	28.0	55.4
iron (μg g^{-1})	50.4	82.9
manganese (μg g^{-1})	17.8	16.1
copper (μg g^{-1})	8.44	8.75
molybdenum (μg g^{-1})	10.8	13.3
potassium (mg g^{-1})	16.2	17.8
calcium (mg g^{-1})	1.38	0.90
magnesium (mg g^{-1})	1.78	1.72
phosphorus (mg g^{-1})	7.77	7.76
phytic acid (μmol g^{-1})	28.2	30.2
phytate/iron molar ratio	31.2	20.3
phytate/zinc molar ratio	65.8	35.6
tannins (mg g^{-1})	1.40	0.48

Table 2. Characteristics[a] of the Participating Women

characteristic	group I (common beans)	group II (high Fe/Zn beans)
age (years)	24.0 ± 2.4	22.5 ± 2.0
body mass index (kg m^{-2})	22.4 ± 3.4	22.6 ± 3.6
blood hemoglobin (g dL^{-1})	12.5 ± 0.8	12.7 ± 1.3
plasma ferritin[b] (μg L^{-1})	12.4 (7.1–21.6)	10.5 (6.4–17.2)
plasma zinc (μmol L^{-1})	10.24 ± 1.53	10.26 ± 1.45
urinary zinc (μmol g^{-1} of creatinine)	3.71 ± 1.91	4.28 ± 1.71

[a] Values given as mean ± SD except for plasma ferritin. [b] Values given as geometric mean and ± 1 SD range in parentheses.

bean genotypes (**Table 3**). Although percent zinc absorption was ~17% lower in the HFeZnB meal compared to the CB meal, the difference was not statistically significant ($p > 0.20$). There was no significant correlation between percent zinc absorption and the women's plasma zinc concentration.

The test meal of HFeZnB provided, on average, 128% more zinc than the test meal of CB (**Table 3**). In contrast to iron, about half of the zinc in the test bean meals was in the form of the extrinsic zinc label (**Table 3**), due to the use of stable isotope labels, which requires larger amounts than radioisotopes. In the HFeZnB meal, the estimate of zinc absorption from the intrinsic label was 13% higher than that estimated from the extrinsic label ($P = 0.033$). On the basis of the extrinsic label, the total amounts of zinc absorbed were, on average, 0.36 and 0.68 mg for the CB and HFeZnB meals, respectively, that is, 90% higher ($p < 0.001$) for the HFeZnB meal (**Table 3**). On the basis of the zinc intake from the beans alone (i.e., subtracting the added isotopic zinc tracer), the estimated total amounts of zinc absorbed from bean zinc were 0.20 ± 0.11 mg for CB and 0.28 ± 0.07 mg for HFeZnB, on average 40% higher for HFeZnB ($p = 0.04$).

DISCUSSION

Measurements of iron absorption using intrinsic and extrinsic tracers agree well for a number of foods including maize, wheat, polished rice, soybeans, and eggs. This suggests that there is complete isotopic exchange between the extrinsic tracer and the native iron in the common pool of non-heme iron (*35*). Exceptions are unpolished rice, due to impaired extrinsic iron diffusion through the outer layer of the seed, and ferritin and hemosiderin food iron, which do not fully exchange with extrinsic iron (*25*). Ferritin is an important source of storage iron in legumes, and the ferritin content of legume seeds may vary with genotype (*36*). Our results showed almost identical results of iron absorption with intrinsic and extrinsic tags for the two bean varieties tested, suggeting that when beans are processed as we did and extrinsic iron was allowed to equilibrate overnight, the less expensive and time-consuming extrinsic labeling can be used to measure iron absorption from different bean varieties.

Zinc absorption estimated with intrinsic and extrinsic tags agreed well for milk-based foods (*20*, *22*,) and eggs (*24*) but not for a variety of other foods, such as peas, chicken meat, and yeast (*19*, *21*). Thus, the exchange of an extrinsic zinc tag with the endogenous zinc pool seems to depend on the food matrix. For example, in legume seeds, extrinsic zinc may be more readily bound to phytate than intrinsic zinc and less bioavailable for intestinal absorption. On the other hand, a protein-bound Zn seed may be better absorbed than "free Zn" that may exchange with extrinsic Zn. Exploration of this possibility may open new possibilities in seed improvement for Zn absorption. In our study, the estimate of zinc absorption from the extrinsic tag in the high-iron, high-zinc bean was significantly lower than the intrinsic measurement. However, the average difference between intrinsic and extrinsic measurements was only ~10%. Because zinc absorption varies between and within individuals (*37*), the small difference between the intrinsic and extrinsic zinc measurements may not be biologically relevant. Although there was a slightly lower estimate of zinc absorption from beans using the extrinsic tag, this method may still be useful for screening the availability of zinc from bean varieties.

The bioavailabilities of iron and zinc from the beans tested were low, about 1.5 and 15%, respectively. Previous human studies have also shown low efficiency of iron and zinc absorptions from legume seeds. Iron absorptions of intrinsically labeled black beans (geometric mean) measured in men were found to be 1.5% (*38*) and 2.6% (*39*). A similar value (2.6%) has been found for iron absorption of soybeans in healthy adults (*35*). Zinc absorption of soybean products ranged between 14 and 20.9% (*40*), whereas zinc absorption from meals of cooked white beans combined with white bread and tomato sauce ranged between 19.3 and 26.2% (*41*).

The low iron and zinc absorption from beans may be the result of their naturally high phytic acid concentration (*12*). The purpose of our study was to determine if the bioavailability of iron and zinc could be enhanced merely by selectively breeding for high-mineral genotypes, so no attempt was made to modify the affect of the phytic acid in beans. The percent iron and zinc absorptions did not differ significantly between bean genotypes, probably because the phytate/iron and phytate/zinc molar ratios were high in both varieties of beans. Moreover, because the test meal contained only beans, the impact of phytic acid on iron and zinc absorption may have been greater than from a complete diet (*42*).

Because the women in the study had low iron stores and low plasma zinc concentrations, higher rates of iron and zinc absorption should have occurred if these minerals were available in the beans. Percent iron absorption generally increases with the decrease in iron stores as indicated by serum ferritin concentration (*43*, *44*). In our study, iron absorption from the beans was negatively related to the iron stores of the women as expected, indicating that physiological adaptation of intestinal iron absorption to poor iron status is detectable even at low levels of iron absorption. Although the efficiency of zinc absorption appears to increase with a marginal zinc intake (*45*, *46*), we did not observe any relationship between zinc absorption and plasma zinc in the women studied.

Table 3. Intrinsic and Extrinsic Iron and Zinc Absorption from the Test Meal of Common Bean or High Iron/Zinc Bean in the Young Women

	common bean ($n = 12$)		high Fe/Zn bean ($n = 11$)	
	intrinsic	extrinsic	intrinsic	extrinsic
Iron				
total intake in meal (mg)	2.33 ± 0.02		3.20 ± 0.22	
absorption[a,b] (%)	1.83 (0.72–4.70)	1.86 (0.71–4.86)	1.03 (0.87–1.65)	1.01 (0.66–1.55)
total absorbed from meal[a] (mg)	0.041 (0.016–0.110)	0.042 (0.015–0.114)	0.033 (0.021–0.052)	0.032 (0.021–0.050)
Zinc				
total intake in meal[c] (mg)	2.21 ± 0.03		5.08 ± 0.14	
absorption[d] (%)	nd	16.1 ± 8.3	15.2 ± 4.6	13.4 ± 3.4
total absorbed from meal[e] (mg)	nd	0.36 ± 0.19	0.77 ± 0.21	0.68 ± 0.16

[a] Values are geometric mean and ± 1 SD range in parentheses. [b] There was no significant difference between intrinsic and extrinsic measurements for each bean type (paired *t* test of natural log-transformed values) nor between bean types measured with intrinsic ($p = 0.097$) or extrinsic ($p = 0.079$) labels (*t* test of natural log-transformed values). [c] Zinc intake from the extrinsic zinc label is included: 1.00 mg (^{67}Zn) in the common bean meal and 3.00 mg (^{68}Zn) in the high iron/zinc bean meal. [d] Values are given as mean ± SD; nd = not determined. Significant difference between intrinsic and extrinsic measurements in the high iron/zinc bean ($p = 0.036$, paired *t* test). There was no significant difference between bean types in zinc absorption measured with extrinsic labels ($p > 0.20$, *t* test). [e] Significant difference between intrinsic and extrinsic measurements in the high iron/zinc bean ($p = 0.033$, paired *t* test). Significant difference between bean types measured with extrinsic labels ($p < 0.001$, *t* test).

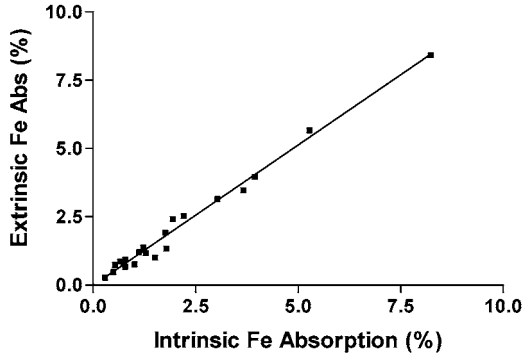

Figure 1. Regression of intrinsic and extrinsic measurements of iron absorption from the beans in the young women. Regression equation: extrinsic Fe absorption (%) = –0.015 + 1.029(intrinsic Fe absorption, %). $R^2 = 0.986$ ($p < 0.001$), where *R* is the correlation coefficient.

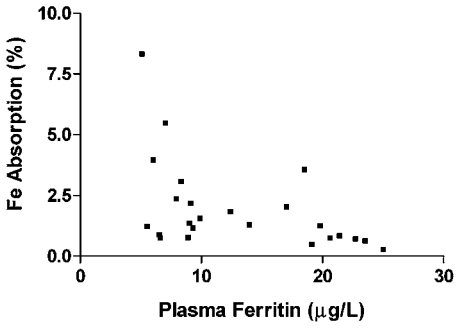

Figure 2. Relationship between percent iron absorption from the beans and plasma ferritin levels of the young women ($R = –0.706$, $p < 0.001$, for log-transformed values).

The total amount of iron absorbed from the test meal was very low for both bean types (0.03−0.04 mg). Although the high-iron, high-zinc bean meal contained ∼40% more iron than the common bean meal, percent iron absorption was ∼45% lower, resulting in similar amounts of total iron absorbed from both bean meals. The addition of 3 mg of ^{68}Zn as the extrinsic tracer to the HFeZnB variety reduced the Fe/Zn ratio to 0.6 in comparison to a ratio of 1 in the common bean. There is no evidence, however, that a modest shift in the Fe/Zn ratio due to the addition of exogenous zinc would reduce the absorption of iron. Thus, consumption of the high-iron bean variety may not improve iron status when consumed alone. Because percent iron absorption appears to be similarly low for both bean types tested, use of bean varieties with much higher iron concentration,

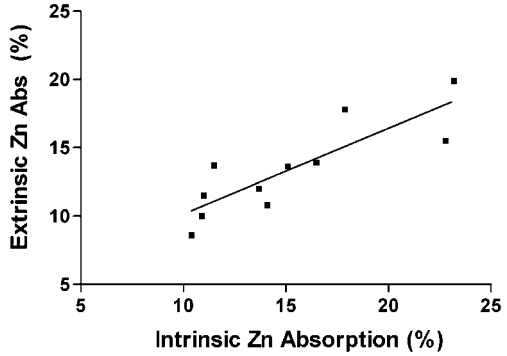

Figure 3. Regression of intrinsic and extrinsic measurements of zinc absorption from the beans in the young women. Regression equation: extrinsic Zn absorption (%) = 3.857 + 0.6276(intrinsic Zn absorption, %). $R^2 = 0.720$, $p < 0.001$, where *R* is the correlation coefficient.

with high phytase activity, or with lower phytate content may be required to significantly increase total iron absorption. Recently, bean varieties averaging >100 μg of iron g^{-1} have been identified in Peru (*47*). Alternatively, promoter enriched (e.g., high cysteine; *9*) or low-phytate variants of beans could be developed (*48*), possibly resulting in increased percent iron absorption. The fractional absorption of iron from genetically modified maize tortillas increased by ∼50% when the phytate content was reduced to one-third the amount contained in wild-type maize (*49*). However, caution should be used in decreasing phytate levels in beans because phytate is known to have some positive health benefits for humans even though it is an antinutrient with respect to iron and zinc bioavailability (*9*).

Feeding the high-zinc bean (i.e., HFeZnB), containing almost twice the zinc concentration of the common bean, resulted in 40% more total zinc absorbed by the women. Zinc fractional absorption did not decline with intake of the HFeZnB bean. This indicates that breeding for high-zinc bean varieties may be a possible way to improve total zinc absorption and possibly the zinc status of zinc-depleted individuals.

In summary, our results indicate that the less expensive and time-consuming extrinsic labeling may be used for screening bean varieties for iron bioavailability in humans but it underestimates zinc absorption. Our results suggest that selective breeding for high-zinc bean varieties may improve the zinc status in populations who normally consume these foods. However, high-iron varieties may not improve iron status. Further studies are needed to determine if the utilization of iron from these

high-iron genotypes is improved if they are consumed with complementary foods containing substances (e.g., cysteine and ascorbate) that promote iron bioavailability. More studies are needed on seed iron and zinc compartmentalization.

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