Effects of Vegetable Proteins on Iron and Zinc Absorption and Availability in Humans

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This review discusses research on the effects of vegetable protein diets on iron and zinc absorption and availability. Constituents of vegetables can have either a positive or negative effect on iron absorption. Although the use of soy protein to extend beef patties was found to decrease nonheme iron absorption in some studies, the use of soy-extended beef on a regular basis was not found to deleteriously affect the iron status (serum ferritin levels) of adult men, menstruating females or school age subjects. Factors such as phytic acid level, dietary fiber content or protein source of vegetable protein diets have been implicated in decreasing the utilization of iron and zinc by humans. The effect of the molar ratio of phytate on the **zinc of the diet is controversial: some studies find a decrease in zinc absorption with an increase in the phytate to zinc molar ratio; other studies find zinc absorption more dependent on the total amount of zinc in the meal than on the presence of phytic acid. Since many people meet their energy and protein requirements from vegetable sources, attention should be given to the mineral availability of these diets.**

Vegetables, in particular, legumes and cereals, provide the major protein source in the average diet of people in many parts of the world. The availability to humans of the minerals in these diets must be considered as well as the adequacy of the mineral intake. A number of nutritional factors found in vegetable protein diets have been implicated in decreasing the nutritional bioavailability of iron and zinc. However, other components of vegetable diets such as ascorbic acid and other organic acids may increase the nutritional bioavailability of some minerals $(1-3)$.

Iron availability. Since nutritional iron deficiency is common in populations where cereals and legumes supply the major energy and protein requirements, iron bioavailability from vegetable proteins has been studied in some depth. Two types of iron compounds are found in the diet heme iron from hemoglobin and myoglobin, and nonheme iron mainly from legumes, cereals, and vegetables. Nonheme iron is the main source of dietary iron consumed in developing countries and its absorption can be influenced by many other constituents of the diet such as carbonates, oxalates, phosphates, phytates, fiber, tannins and other polyphenols which inhibit absorption, ascorbic acid and some organic acids such as tartaric, succinic, malic, and lactic acid which promote absorption $(1,2)$.

Nonheme iron absorption from meals has been measured by the use of an extrinsic radioactive label (labeled iron salt given with the meal). Because heme iron and nonheme iron form separate pools of iron in the gastrointestinal tract, heme iron cannot be labeled by an extrinsic

inorganic tracer. However, by the use of two different radio iron isotopes, these two pools can be independently and simultaneously labeled with an extrinsic inorganic radio iron tracer and a biosynthetically radio iron labeled hemoglobin (2).

Lynch *et al.* (4) studied the nonheme iron absorption with legumes in twenty volunteers between 18 and 23 years of age with an extrinsic label. Because the iron status of individuals influences their absorption of iron, the iron absorptive capacity of these subjects was determined by measuring the absorption of a reference dose of 3 mg of iron as ferrous ascorbate. The mean iron absorption, as a percent of a dose of these legumes served as soups, was low: black beans, 0.84; split peas, 1.09; lentils, 1.20; soybeans, 1.66; and mung beans, 1.91%. The iron absorption from these legumes was only 5 to 10% of that of a comparable dose of ferrous ascorbate.

In the 1980s, attention has been given to the effect of soy protein consumption on iron absorption. In 1981, Cook *et al.* (5) fed extrinsically labeled nonheme iron to 15 adult men and found the geometric mean iron absorption from isolated soy protein (Supro-710) to be only 0.5 compared with that of albumin and casein at 2.5 and 2.7%, respectively (Table 1). In a subsequent experiment, Cook *et al.* (5) also found this inhibition of iron absorption over a range of processed soy products. Isolated soy protein (Supro-710) gave the lowest iron absorption at 0.4% and the textured soy protein (Supro-50A) the highest at 1.9 (Table 1). Morck *et al.* (6) found a similar decrease in iron absorption in men with isolated soy protein (Supro-710) showing a 0.6% absorption. However, the addition of 100 mg of ascrobic acid to isolated soy protein increased the iron absorption to 3.2% (Table 1). In all these studies, $FeCl₃$ was used to equalize the total iron content of the protein sources (5,6).

In a 35-day study conducted at the Beltsville Human Nutrition Research Center (BHNRC) in 1979 (7) with 16 or 17 adult males, three different protein sources (textured soy, soy isolate, and animal protein) were used to make up greater than 70% of the protein intake of the diet in a crossover design. Even with an adequate iron intake (17.5-23.4 mg/day), apparent daily iron balance (mean \pm st. dev.) was negative on all three diets (textured soy, - 2.9 \pm 6.7 mg/day, -1.4 \pm 5.8; soy isolate, -8.4 \pm 5.9, -7.9 \pm 4.9; and animal protein, -0.3 ± 3.4 , -0.8 ± 1.5) between days 15-21 and days 29-35, respectively. Only the soy isolate apparent daily iron balances were significantly lower (5% level) than that of the animal protein.

Iron absorption was also studied when soy protein was used as a ground beef extender. When Cook *et al.* (5) substituted soy protein for beef in a 3:1 and a 2:1 ratio of meat to unhydrated textured soy flour, iron absorption decreased by 61 and 53%, respectively (Table 2). This study has been criticized for improperly rehydrating the textured soy flour used. However, because of the nonheme iron content of the soy flour, the total iron absorbed (mg) from the all beef patty and the soy-extended patties

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TABLE 1

Effect of Soy Protein on Nonheme Iron Absorption

 a Cooketal. (5).

*b*Morck et al. (6) .

TABLE 2

Soy Protein-Extended Beef and Iron Absorption

aCook *et al.* (5).

bCalculated by assuming that 25% of the heme iron in meat was absorbed.

cMorck et *al.* (6).

dHallberg and Rossander (8).

eLynch *et al.* (9).

was not greatly different (Table 2) (1). When Morck *et al.* (6) feed 100 g beef with soy isolate, percentage of iron absorption was 1.44%, similar to Cook's 1.51% (Table 2).

In other investigations of iron absorption, Hallberg and Rossander (8) found that decreasing the meat content of a hamburger by 50% reduced the percent of nonheme iron absorption from 11.2 to 8.4% (Table 2). Substituting textured soy or defatted soy flour for beef in a 1:1 ratio further decreased nonheme iron absorption to 7.2 and 5.6%, respectively. Only the percentage of nonheme iron absorption from the defatted soy flour was statistically different (p<0.02) from that of the 50% hamburger patty. However, the added iron in the soy flour made the total amount of nonheme iron absorbed from the soyextended patties greater than the amount absorbed from the 50% hamburger patty. The removal of phytate from the soy flour did not increase the nonheme iron absorption.

Lynch *et al.* (9) published the results of a dual radio iron absorption study where both heme and nonheme iron absorption were measured. Nonheme iron absorption decreased from 24.8 to 0.87% when soy was substituted in a beef patty (1:1), but the heme iron absorption increased from 17.0 to 27.1% (Table 2). They demonstrated "that the deleterious effects on iron nutrition of substituting soy protein for beef are partially offset by improved availability of the remaining heme iron as well as an increase in the nonheme iron content of the meal." (9) This improvement in heme iron absorption was shown in a number of beef-to-soy-flour ratios.

The Department of Agriculture conducted a study at the Beltsville Human Nutrition Research Center (BHNRC) (I0,11) to determine under practical conditions the effect of consumed beef extended with soy protein on the iron status, as measured by serum ferritin levels, of adult men, menstruating women and school age children. Two hundred and twenty-seven subjects participated in this study (50 adult men, 41 menstruating adult females, and 115 school age subjects) by consuming either all beef or soy-extended beef for 180 days. A "time and variability" control group of subjects (n=62) consuming their normal diets was also included.

TABLE 3

Changes in Serum Ferritin Levels (Geometric) in Subjects Consuming Soy-extended Beef Patties. a

% Change in Ferritin Levels ^b		
Adult males (18 yr or older)	Menstruating females (18 yr or older)	School age participants $(6-18 \,\text{yr})$
4.8	17.5	14.9
12.2 9.6 -8.7	0.0 25.6 -13.2	23.5 0.0 17.9
-4.5 4.8 44.9	14.5 38.2 -17.3	12.0 20.0 7.0
-4.6	-2.0	0.0

aBodwell *et al.* (11)

bFinal Ferritin - Initial Ferritin

Initial Ferritin

TABLE 4

Iron Absorption from Simple Latin American-type Meals a

aHallberg and Rossander **(13).**

bBasal meal of maize tortillas, boiled polished rice, and black beans cooked in water and ground to a paste.

TABLE 5

Absorption of Nonheme Iron from Western-type Lunch and Dinner Meals a

aHallberg and Rossander (14).

bCorrected to 40% reference dose absorption.

eLow ascorbic acid vegetable meal consisted of navy beans, brown rice, bread of *corn*

flour and unfortified wheat flour, apples, walnuts, almonds and yogurt.

dHigh ascorbic acid vegetable meal consisted of red kidney beans mixed with tomato sauce, bread made of unfortified wheat flour, cauliflower, cottage cheese, canned pineapple **and banana.**

e(Amount of fortified iron).

The patties fed were 80% ground beef and 20% reconstituted soy protein. Three soy protein sources were used: soy isolate (PP-220), soy concentrate (Procon 2060) and textured soy flour. Seven patty products were fed: all beef, beef extended with each of the three soy proteins and beef extended with each of the three soy proteins fortified with iron (60 mg/100 g soy protein) as ferrous fumarate and zinc (25 mg/100 g soy protein) as zinc oxide. The nutrient composition of these patties has been published (12). Each of the seven beef products was provided to a group of families with children as their principal source of protein for one meal a day (children 6-18 years of age) or one or two meals a day (11 meals a week, adult men and women). At interim times (0, 41, 90, 132 and 181 days) blood samples were collected for serum ferritin analysis. Clinical parameters of iron status (hematocrit, hemoglobin, serum iron, total iron binding capacity, transferrin saturation, and free erythrocyte protoporphyrins) were measured at the beginning and the end of the study.

No deleterious effects were found on the clincial parameters of iron status when subjects consumed beef extended with soy protein for 180 days (11). By linear regression analysis, none of the dietary treatment groups showed a significant decrease in serum ferritin levels over time. The percentage change in ferritin levels over the 180-day study is shown in Table 3. We found no evidence that consumption of soy protein-extended beef at the levels used in this study deleteriously affected the iron status of adult men, menstruating females and school age subjects (11).

Hallberg and Rossander (13) have completed a number of studies on improving the nonheme iron absorption from vegetable proteins with iron absorption promoters (Table 4). Their basal meal made up of vegetable protein had a nonheme iron absorption of 0.17 mg or 3.6%. The addition of 75 g of ground beef increased the absorption of nonheme iron about 2.5 times from 0.17 to 0.45 mg. The addition of the same amount of protein as defatted soy flour which was mixed into the dough of the maize tortillas increased the absorption from 0.17 to 0.51 mg. One reason for this increase is the additional iron (6 mg) in this meal derived from the soy flour. Cooked cauliflower (125 g) providing 65 mg ascorbic acid increased the absorption to 0.58 mg, while 50 mg of ascrobic acid increased the nonheme iron absorption of the basal meal to only 0.41 mg. Citric acid decreased nonheme iron absorption.

Hallberg and Rossander (14) also looked at the nonheme iron absorption from Western-type lunch and dinner meals (Table 5). The absorption of nonheme iron varied from 0.13 on the low ascorbic acid vegetarian meal to 0.98 on the high ascorbic acid meal: a seven-fold increase. Adding cauliflower to the vegetarian meal with the low initial iron absorption increased absorption 2.5 fold from 0.13 to 0.32. However, when cauliflower was excluded from the high ascorbic acid vegetarian meal, a decrease in iron absorption from 0.98 to 0.32 mg was noted. There must be some factor other than the difference in content of ascorbic acid explaining about half of the observed difference in absorption between the two meals: maybe differences in the phytate and fiber content of the diet. Adding meat to the two diets also increased the absorption of iron.

Since infants receive the majority of their nutrition from formula, the effect of soy-based formulas on iron absorption has been studied. Rios *et al.* (15) examined the absorption of iron from soy-based and cow-milk formulas in healthy infants four to seven months of age. In a group of 13 infants, the percentage of iron absorbed from soy-based infant formula (5.4%) was actually higher than the values for two iron-fortified cow-milk fomulas (3.4 and 3.9%). In this study, all the formulas were supplemented with ferrous sulfate and the total iron content of the soy formula was 17 mg/l and of cow-milk formula 12 mg/l . They concluded that a soy formula with this iron content would be adequate for infants under one year of

age as the major source of iron. However, Hertrampf et al. (16) questioned the interpretation of this study because of the low iron absorption of the reference dose of iron. When a soy formula (Prosobee-PP 710, 53 mg/1 ascorbic acid), containing 14.5 mg Fe/1 was fed to 16 adult women, the geometric mean absorption of iron as measured by an extrinsic radioactive tag was only 1.7%, but in infants this formula was as effective as iron-fortified cow's milk in preventing iron deficiency (16). Similarly Gillooly *et al.* (17) found the geometric mean iron absorption of a soybased infant milk formula (corrected for a reference absorption of 40%) to be dependent on the ascorbic acid (AA) content of the formula $(0,40,80,160 \text{ mg }AA/100g$ gave 1.8,3.3,6.9 and 7.7% iron absorption, respectively). when these same investigators compared soy and cow's milk formula, they found the iron absorption to be 3.3 and 10.5% at 40 mg AA/100g and 6.9 and 24.2% at 80 mg AA/ 100g, respectively. They suggested that soy formulas should contain 12 mg Fe/100g with ascorbic acid in a molar ratio of 4:1 for adequate iron nutrition. Derman *et al.* (18) also found the absorption of iron from a basal milk formula to be greater than from a soya-bean formula, and both ascorbic acid and citric acid enhanced the iron absorption of both formula, but to a larger degree in the milk-based formula.

Iron bioavailability can be affected by many dietary constituents. Although the use of soy protein to extend beef patties was found to decrease nonheme iron absorption in some studies, the use of soy-extended beef on a regular basis was not found to deleteriously affect the iron status (serum ferritin levels) of adult men, menstruating females or school age subjects. Constituents of vegetables can have either a positive or negative effect on iron absorption, and substances added to vegetarian diets such as ascorbic acid and meat can enhance the iron availability of these vegetarian diets. Soy-based formulas of adequate iron and ascorbic acid content can meet the iron requirements of infants.

Zinc availability. Attention has also been given to the bioavailability of zinc in diets containing cereals and legumes because these foodstuffs contain phytates and fiber which are believed to reduce zinc availability. In 1982, Solomons (19) reviewed the fractional absorption of zinc from isotopic studies in humans and found that

zinc absorption varied from 14-41% depending on the type of diet consumed. Zinc absorption was lower from wholemeal bread (17%) than from white bread (38%) and was reduced even further when cheese and milk were added to the wholemeal bread (14%). Soybeans (20%) gave a lower zinc absorption than chicken (36%).

Studies on the apparent daily zinc balance of subjects consuming soy-based diets have given conflicting results. Cossack and Prasad (20) fed healthy males an animal protein diet containing 12.3 mg Zn/day for three months followed by a soy-protein based diet containing 14.3 mg Zn/day for the following three months. They found a significant difference ($p<0.05$) in the apparent zinc balance of -2.39 mg on the soy-based diet and +3.25 mg on the animal protein diet, and a significant depression in plasma and neutrophil zinc concentrations was observed at the end of the three months when the subjects consumed the soy diet compared to the meat diet (Table 6). However, this study has been questioned since the soyproteins used had been washed with ethylenediaminetetraacetate (EDTA) which could have affected the zinc absorption of the diet.

In a 35-day cross-over study, Bodwell (7,21) fed adult men three different diets in which 70% or more of the protein was provided by either animal protein, textured soy or soy isolate. Negative zinc balance was not observed with consumption of any of these diets even though the molar phytate to zinc ratio of the soy diets was high (Table 6).

When Sandstrom *et al.* (22) studied the apparent absorption of minerals in ileostomy patients, a 25% replacement of the protein of a meat, rice and bread diet with soy protein, as either soy flour, soy concentrate, or soy isolate had no obvious effect on the apparent mineral absorption. However, when two subjects consumed a diet containing 53 g of protein as soy isolate instead of meat and flour protein, the zinc content of the diet was reduced by about 50% and negative apparent zinc absorption resulted in both subjects.

Greger *et al.* (23) substituted rehydrated textured vegetable protein for 30% of the meat in the lunches of 14 adolescent girls and found no effect on urinary or fecal losses of zinc. Total protein intake per day met the Recommended Dietary Allowance, but no indication was given

TABLE 6

Zinc Availability to Adult Males from Soy Protein and Animal Protein Diets

aBodwell *et al.* (7) and Bodwell (21).

bCossack and Prasad (20).

Changes in Serum Zinc Levels in Subjects Consuming Soy-extended Beef Patties a

aMiles *et al.* **(I0).**

bFinal serum zinc - Initial serum zinc

Initial Serum Zinc.

TABLE 8

Absorption of Zinc from Composite Meals with Varying Main Protein Sources a

^aSandström and Cederblad (24).

bBasic components of all meals: 150 g of boiled potatoes, 50 g of tomato juice and 9 g of white bread.

TABLE 9

Absorption of Zinc from Meat and Bread Meals with or Without Soy Protein a

^aSandström et al. (25).

bValues are range for soy flour, soy concentrate and soy isolate.

as to how much protein and soy protein was provided in the lunch meal.

In the BHNRC study (10,11) on the effect of soyextended beef consumption, serum zinc levels were also determined at the interim times (0, 41, 90, 132 and 180 days). By linear regression analysis, the only groups to show a significant decrease **in** serum zinc levels on the 180-day study were the controls and those consuming allbeef patties. None of the groups consuming soy-extended beef patties showed a significant decrease (p <0.05) in

serum zinc levels over the 180 days of the study (Table 7).

Sandström and Cederblad (24) compared the absorption of zinc from composite meals by measurements of the whole-body retention of extrinsically labeled zinc about two weeks after ingestion of the labeled meal. The total zinc content of the meal was the most important factor influencing the amount of zinc absorbed. The addition of soy flour (25%) to chicken meat did not signficantly influence its zinc content or absorption (Table 8). The zinc content and absorption from the beef and soy flour (25%) meal was lower than $(p<0.05)$ that of the all-beef meal. The zinc absorption from the all-soybean meal did not differ significantly from the chicken meal, but was significantly lower than from the beef meal which had a higher zinc content. Addition of calcium in the form of milk (125 ml) to the soybean meal had a negative effect on zinc absorption. This study shows that when soy protein is present in the form of defatted flour and is used as a partial substitute for animal protein in a mixed diet, zinc absorption is not seriously influenced. However, the lower zinc content of the soy four in comparison with meat should be considered and may be of importance in people eating poor diets.

The absorption of zinc by extrinsic labeling with $65Zn$ and whole body counting was studied in human subjects consuming three types of soy protein (flour, concentrate, and isolate) in meals of rice and meat sauce or white or whole-meal bread (25). When 30% of the protein of the meat sauce was replaced with soy protein (molar ratio between phytic acid and zinc was 6-9), a marginal effect on the percentage absorption of zinc was found, but the absolute amount of zinc absorbed was lower because of the lower zinc content of the soy products replacing the meats (Table 9). When soy protein was used to totally replace the meat protein (molar ratio between phytic acid and zinc was 16-34), a significantly ($p\leq 0.05$) lower percentage and absolute absorption of zinc was observed. Solomons *et al.* (26) also found the fractional absorption of zinc from an isolated soy protein diet (30%) to be significantly different ($p<0.05$) from the beef diet (41%) when he used a stable zinc isotope for fecal monitoring. In this study, Solomons *et al.* used ZnCl₂ as a supplement so the zinc intake from the soy diet would be close to that supplied by the beef diet. This supplementation also lowered the phytic acid zinc molar ratio of the diet. When soy protein was added to white bread, zinc absorption was lowered, but absorption of zinc from the whole-meal bread was low and 50% replacement with soy protein did not change it (25). Sandstrom *et al.* (25) concluded that soy protein's effect "on zinc absorption depends on the degree of replacement, the phytic acid and zinc content of the soy product and the protein content of the meal."

Other studies have also examined the effect of the phytic acid content of vegetable protein sources on the availability of zinc. Sandström and Cederblad (24) concluded that "zinc absorption from protein-rich meals seems to depend more on the total amount of zinc in the meal than on the presence of phytic acid." Morris et *al.* (27) measured the apparent absorption of zinc as well as calcium, iron and manganese from whole-bran muffins and from dephytinized-bran muffins. The apparent absorption of these minerals, with the exception of manganese, was not improved by removal of the phytate. This led the authors to suggest "that the phytate in 36 g of wheat bran each day did not exert a detrimental effect on trace element nutriture in this study." (27) When Turnland et *al.* (28) fed a liquid formula diet supplemented with 2.34 g of phytate as sodium phytate to young men, zinc absorption fell to 17.5% (34.0% zinc absorption on the basal diet), and zinc balance was negative. As these authors state, "the phytate used in this experiment was in a purified form and it is not known if the effect would be the same if the same level of phytate were consumed in a natural food diet."

The effect of soy-milk formulas on zinc availability has been studied since infants use these formulas as their major food source. In a rat model (29) using extrinsic 65Zn, zinc availability was only 10% from soy formula compared to 15% from cow's milk and 28% from human milk. When Golden and Golden (30) fed malnourished children either cow's milk or a soya-protein based formula with 25% less zinc than the cow's milk and 1.33 mmol/1 phytic acid, the plasma zinc concentration was lower and the rate of weight gain was less on the soya diet. Using a radioisotope technique, (31,32) zinc bioavailability was lower from cow's milk than from human milk, and much lower from soy formula in human adults. Phytate was found to have a strong inhibitory effect on zinc absorption (32). These investigators suggest that the reduction in the level of phytate content of soy formulas may be more effective in improving zinc utilization from these forumlas than zinc supplementation.

Although much research has been done on the bioavailability of trace minerals in various diets, the inhibition or promotion of mineral availability by various dietary factors can be difficult to predict. Since many people meet their energy and protein requirements from vegetable sources, attention should be directed to the mineral availability of these diets.

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