Influence of Vegetable Protein Sources on Trace Element and Mineral Bioavailability[1]

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ABSTRACT Vegetable protein sources are often mixed with cereals for complementary feeding. Both contain high levels of phytic acid, which can inhibit trace element and mineral absorption. In adults, phytic acid has been reported to inhibit the absorption of iron, zinc, calcium and manganese but not copper. There are far fewer studies in infants. Phytic acid is a strong inhibitor of iron absorption in both infants and adults, but its influence on zinc absorption in infants seems to be modest and perhaps most important in children recovering from infection. The influence of phytic acid on calcium and magnesium absorption would seem of minor importance. Because iron and zinc deficiencies are widespread in infants and young children in developing countries, the bioavailability of iron and zinc from complementary food is a major concern. Iron absorption may be as low as 2–3% from porridge based on whole-grain cereals and legumes (phytic acid ~1 g/100 g) even in iron-deficient subjects. Decreasing phytic acid by 90% (~100 mg/100 g dried product) would be expected to increase absorption about twofold and complete degradation perhaps fivefold or more. More modest reductions in phytic acid content may not usefully improve iron absorption. Complete enzymatic degradation of phytic acid is recommended. If this is not possible, it is recommended that the molar ratio of phytic acid to iron in an iron-fortified food be <1, preferably <0.5. At these low levels of phytic acid (20–30 mg/100 g dried product), zinc absorption should be satisfactory. J. Nutr. 133: 2973S-2977S, 2003.

KEY WORDS: • *complementary food* • *phytic acid* • *iron* • *zinc* • *bioavailability*

Complementary foods are often based on mixtures of cereal grains and legume seeds. Common legumes, such as soybean, mung bean, black bean, lentils and chick pea, increase the protein content and improve the protein quality of cereal-based complementary foods. Both legumes and cereals, however, are rich in phytic acid (1), which is a potent inhibitor of mineral and trace element absorption. In isotope studies, phytic acid has been reported to inhibit the absorption by human subjects of iron (2), zinc (3), calcium (4) and manganese (5). Phytic acid does not appear to influence copper absorption (6).

Phytic acid inhibits iron absorption in infants to an extent similar to that in adults (7,8), and low iron absorption from legume and cereal-based complementary foods is a major cause of the widespread iron deficiency in infants in developing countries. Iron deficiency in infants can lead to reduced psychomotor and mental development with possible long-term negative consequences on school performance (9).

The influence of phytic acid on magnesium and calcium absorption by infants from complementary foods is less clear and appears to be of less importance. There are no published studies on infants or adults investigating the influence of phytic acid on magnesium absorption, although our own unpublished studies on adults indicate that phytic acid inhibits magnesium absorption. However, whole cereal grains are relatively high in magnesium, magnesium deficiency is uncommon in infants, and unlike iron, magnesium has a strong homeostatic control mechanism whereby a lower absorption can be counteracted by a lower urinary excretion. The high phytic acid content of cereals and legumes also appears to be of little or no importance in relation to calcium nutrition in infants. Davidsson et al. (10) reported a relatively high apparent calcium absorption (~60%) in young infants (aged 7–17 wk) fed a high phytate (0.76%) complementary food based on soy flour and whole wheat. Calcium absorption was similar from a low phytate (0.07%) porridge based on wheat flour and milk powder (11). As with magnesium, there is a strong homeostatic control of calcium via both intestinal absorption and urinary excretion, and any calcium deficiency is more likely to be due to a low intake rather than a low absorption.

Phytic acid has not been clearly demonstrated to similarly inhibit zinc absorption in healthy infants and adults. Zinc however, is an extremely important nutrient during the weaning period, and high phytate complementary foods may be involved in the etiology of zinc deficiency. In addition to decreasing the absorption of dietary zinc, phytic acid may also inhibit the reabsorption of endogenous zinc secretions that

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enter the lumen of the small intestine postprandially (11,12). Davidsson et al. (10) reported a relatively high apparent zinc absorption (~45%) by healthy young infants consuming the high phytate wheat and soy complementary food discussed above. Absorption was similar to the zinc absorption from a low phytate cereal or milk porridge. Manary et al. (13) also reported identical zinc absorption values (24%) by Malawian children aged 3–17 y who were fed corn and soy porridges with 0.12% or 0.03% phytate. In the same study a similar group of children recovering from tuberculosis increased zinc absorption from 24% with the 0.12% phytate porridge to 41% with the 0.03% phytate porridge. In neither group of children did phytic acid influence the excretion of endogenous fecal zinc.

The phytic acid content of the corn and soy porridges used by Manary et al. (13) was much lower than could be expected in other cereal legume blends; phytic acid degradation was not well controlled and the study groups were small (4–6 children). Infants in developing countries commonly have infections and inflammatory disorders (14) and their zinc requirement could increase during the recovery period. Any influence of high phytate complementary foods on zinc absorption therefore could help precipitate zinc deficiency. Such deficiency contributes to impaired growth and reduced immune function, which in turn can lead to an increased severity and incidence of diarrheal, malarial and respiratory infections (15).

Based on the above discussion, the remainder of this review will discuss the role of phytic acid in relation to iron and zinc absorption by human subjects from complementary foods based on cereals and legumes.

Iron absorption from legume protein sources

Iron absorption in adult human subjects from legume soups made from soybeans, black beans, lentils, mung beans or split peas is low. Absorption ranged from 2 to 4% even after the absorption values had been adjusted upwards to represent iron absorption from iron-deficient individuals (40% reference dose absorption) (16). Similarly, after adjusting iron absorption values to represent absorption by individuals with no iron stores (serum ferritin 12 μg/L) (17), absorption from ferrous fumarate–fortified porridges based on wheat and soy, wheat or quinoa ranged from 2 to 7% (18). Absorption in iron-replete adults was 0.7–2%. Iron absorption of complementary foods based on mixtures of cereals and legumes is thus very low even in iron-deficient subjects with a high demand for iron. The low iron absorption is mainly due to the presence of phytic acid although some legume proteins per se, such as soy, are also inhibitors of iron absorption.

Improving iron absorption from soy and pea protein isolates by phytate degradation. Phytic acid removal or degradation significantly improved iron absorption from soy protein and pea protein isolates. Phytic acid is a potent inhibitor of absorption, and phytic acid in soy protein isolate had to be decreased by >90% to give a twofold increase in iron absorption and had to be completely degraded to increase absorption three- to fourfold (**Fig. 1**) (19). In these studies, phytic acid was partly removed by dialysis after acid and salt treatment of the isolate or was completely degraded by addition of a commercial phytase enzyme extracted from *Aspergillus niger*. The isolates (~30 g) were fed to adult human subjects as part of a liquid formula meal also containing maltodextrin, corn oil and water and fortified with ferrous sulfate (~6 mg iron/meal). Iron absorption was measured relative to a formula containing egg white protein fed to the same subjects. Mean iron absorption from the egg white control protein in different studies ranged from 5 to 9% and was designated a relative absorption

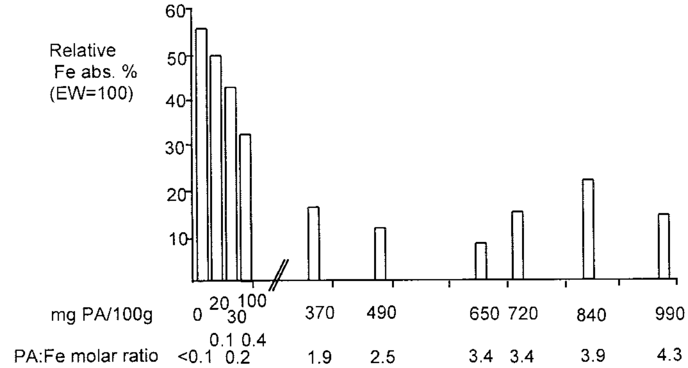

FIGURE 1 Effect of phytic acid removal or degradation on Fe absorption by adults from soy protein isolates fed in a liquid formula meal (19).

of 100. Decreasing the phytic acid content of the isolates from 990 to 370 mg/100 g, representing a decrease in the molar ratio of phytic acid to iron from 4.3 to 1.9, did not improve iron absorption, which remained at 10–24% relative to egg white (Fig. 1) (19). Decreasing the phytic acid to 100 mg/100 g isolate (molar ratio of phytic acid to iron of 0.4) increased absorption approximately twofold to 33% relative to egg white, and decreasing the phytic acid to 30 mg/100 g isolate and below (molar ratio of phytic acid to iron <0.2) increased absorption three- to fourfold to 43–56% relative to egg white. Thus even after complete degradation of phytic acid, soybean protein still inhibits iron absorption.

Soybean protein can be separated by ultrafiltration into four major fractions: 2S, 7S, 11S and 15S; although the 7S and 11S fractions are by far the major constituents of soy protein isolates. The 7S fraction is composed of 7S globulin (conglycinin) whereas the 11S fraction is composed of 11S globulin (glycinin). Using an identical protocol to the study described above, Lynch et al. (20) reported that completely dephytinized glycinin had an iron absorption of 124% relative to egg white whereas completely dephytinized conglycinin had a relative absorption of only 44%. The conglycinin fraction of soy protein isolate is thus an inhibitor of iron absorption.

The dephytinized soy isolates were subsequently incorporated into commercial soy infant formula and iron absorption was measured in infants using a stable isotope technique (7). The soy formulas were fortified with ferrous sulfate at a 2:1 mol/L ratio of ascorbic acid to iron. Degrading the phytic acid in the isolate by 83% gave a soy formula with 77 mg phytic acid/L compared with the normal, ~400 mg/L. The molar ratio of phytic acid to iron decreased from 2.1 to 0.4 and iron absorption increased significantly from 5.5 to 6.8% ($P < 0.05$). A more substantial increase in absorption (3.9 to 8.7%) was achieved when phytic acid was completely degraded.

Recently Davidsson et al. (21) also reported an improvement in iron absorption after complete degradation of phytic acid in pea protein isolates added to an experimental infant formula. The pea formula had a composition similar to that of commercial soy formula and contained 13 mg iron/L as ferrous sulfate and a 2:1 mol/L ratio of ascorbic acid to iron. Iron absorption in young women with a low iron status (mean serum ferritin 13 μg/L) increased from 21 to 33% on dephytinization. In comparison, iron absorption from soy formula fed to iron-replete adults increased from 2.4 to 6% on dephytinization (6.6 to 16.6% on adjusting the absorption to a serum ferritin concentration of 13 μg/L). The much higher fractional iron absorption from pea isolate compared with soy isolate is not due to a difference in iron concentration because the pea formula had a concentration of 13 mg/L compared with 15 mg/L in the soy formula. Pea isolate does not contain the inhibitory 7S conglycinin but contains vicilin and convicilin in the 7S fraction and legumin in the 11S fraction (22).

How much phytate should be removed? There are only two studies in the literature that investigated a range of phytic acid levels, including zero, on iron absorption in humans. The soy isolate study is shown in Figure 1 (19). This study demonstrated that phytic acid is a potent inhibitor of iron absorption from iron-fortified formulas at low concentrations (~100 mg phytic acid/100 g isolate) and at molar ratios of phytic acid to iron of >0.4:1. This finding is supported by the bread roll study of Hallberg et al. (2), who added decreasing levels of free phytic acid to phytate-free wheat bread rolls and found that phytate levels as low as 10 mg/100 g decreased iron absorption by 20% and 20 mg/100 g by 40% (**Fig. 2**). At the usual level of phytic acid in white wheat flour (~100 mg/100 g), iron absorption was decreased by 60%. In both the soy isolate and the bread studies, decreasing phytic acid from ~1 to 0.1% improved iron absorption twofold compared with a three- to fivefold increase with zero phytate.

In contrast to the soy studies, in which there was no increase in iron absorption on decreasing phytic acid from 990 to 370 mg/100 g, the bread studies indicated that iron absorption increased progressively as phytic acid was decreased, although it took a 90% decrease (from 1080 to 108 mg/100 g) to double iron absorption. From the studies of Hallberg et al. (2) and Hurrell et al. (19), it can be concluded that to achieve maximum benefit to iron absorption (three- to fivefold increase) from iron-fortified foods, phytic acid should be completely removed or degraded. Because this might not always be possible, it is suggested that a useful increase in absorption (approximately twofold) can be achieved if the molar ratio of phytic acid to iron is decreased to below at least 1:1 and preferably below 0.4:1. This represents phytic acid levels of 30–40 mg/100 g in soy isolate or wheat flour.

Other studies support this conclusion. Siegenberg et al. (23) varied phytic acid in iron-fortified wheat bread rolls by adding different levels of phytate-containing and phytate-free maize bran. They reported that reducing phytic acid from 250 to 40 mg/100 g (decreasing the molar ratio of phytic acid to iron from 3.1 to 0.5) increased iron absorption twofold. Larsson et al. (24) reported that using the malting process to decrease phytic acid in oats from 1310 to 440 mg/100 g (molar ratio of phytic acid to iron decreased from 9.5 to 3.1) did not improve iron absorption in adult subjects, although a further reduction to 240 mg/100 g (molar ratio of phytic acid to iron of 1.7) led to a small increase (36%) in absorption.

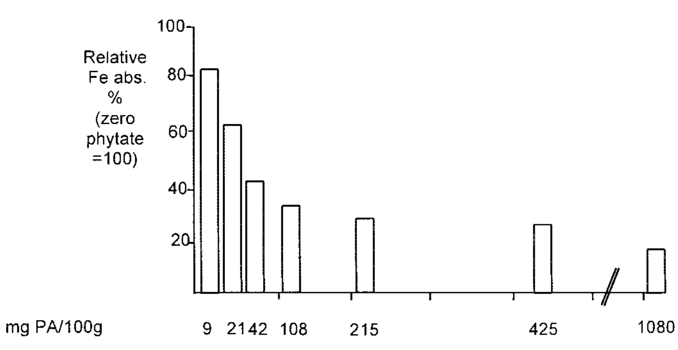

FIGURE 2 Dose-dependent inhibition of phytic acid added to phytate-free wheat bread rolls [calculated from Hallberg et al. (2)].

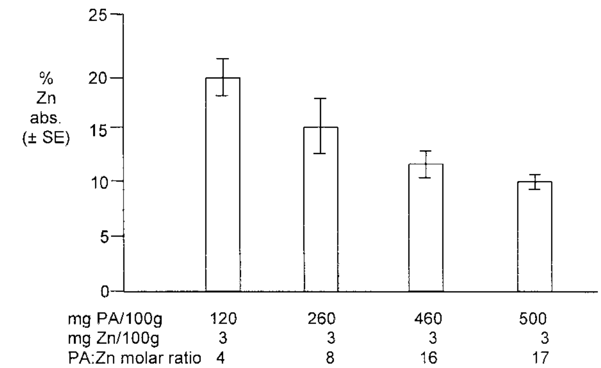

FIGURE 3 Zinc absorption in adults from wheat bran rolls with different phytate levels [calculated from Nävert et al. (3)].

Influence of phytic acid on zinc absorption

Although the influence of phytic acid on zinc absorption in infants is uncertain, phytic acid does inhibit zinc absorption in adults in a remarkably similar way to its influence on iron absorption (**Fig. 3**) (3). In studies by Nävert et al. (3), adult subjects were fed wheat bread rolls containing bran (20%) that had been fermented for different lengths of time to degrade progressively more phytic acid. Zinc absorption was measured using ^{65}Zn as an extrinsic tag followed by whole body counting. As the phytic acid content of the bran rolls was decreased from 0.66 to 0.15% (molar ratio of phytic acid to zinc decreased from 17 to 4), zinc absorption increased from 9.6 to 19.8%. Iron absorption was also reported to double in the same phytic acid range (2,19). In the zinc studies phytic acid was not degraded to zero, although a further increase in zinc absorption up to ~40% absorption could be expected.

Larsson et al. (24), in their studies on malted oats, also measured zinc absorption. They reported a 1.5-fold increase in zinc absorption (11.8 to 18.3%), using the same extrinsic tag whole-body counter technique, when phytic acid was decreased from 0.94% to 0.24% (molar ratio decreased from 30 to 8). This is in line with the dose-response curve of Nävert et al. (3). More recently Egli (25), using a fecal-monitoring stable isotope technique, reported that complete dephytinization of a wheat and soy complementary food containing phytic acid at 400 mg/100 g increased zinc absorption 1.5-fold (23 to 35%, $P < 0.01$) but had no influence on copper absorption (20 compared with 24%).

From these few studies, it is evident that phytic acid degradation in cereal- and legume-based complementary foods will increase zinc absorption in adults. Reducing the phytic acid level of whole-grain legumes or cereals from ~1 to 0.1% would approximately double zinc absorption whereas complete degradation could result in a further twofold increase. Because zinc absorption from high phytate foods (~10%) is far higher than iron absorption (2–3%), a doubling of zinc absorption could be of great benefit to zinc nutrition. It is more difficult to define a molar ratio of phytic acid to zinc that would give a useful zinc absorption. In nonzinc-fortified foods, however, a molar ratio of 4–8 should result in a zinc absorption of ~20% in adults. Zinc absorption in infants appears to be much less sensitive to phytic acid, and relatively high zinc absorption values (~40%) have been reported from high phytate wheat and soy complementary foods (10).

Interestingly, dephytinized soy protein in adults is modestly inhibitory to zinc absorption. Davidsson et al. (26) added different protein sources to a liquid formula meal containing maltodextrin, corn oil and water and measured zinc absorption using the extrinsic tag technique with ^{65}Zn. Zinc absorption from the formula containing dephytinized soy protein was 75% relative to the formula with no added protein (zinc absorption 45–49%). Bovine casein, bovine whey and egg albumin had no influence on zinc absorption, although bovine serum albumin reduced absorption by 20%.

Phytic acid degradation in cereal and legume complementary foods

There are three ways to decrease the inhibitory effect of phytic acid on mineral absorption from complementary foods based on cereal and legume mixtures. The first method is to remove the phytic acid, the second method is to degrade it enzymatically and the third method is to add compounds to the food that prevent phytate-mineral binding. Such compounds include ascorbic acid (7) and sodium EDTA (18), which improve iron absorption. Milling of cereals can decrease phytic acid by 90% as it is removed with the bran. Because phytic acid in legume seeds is contained in the protein bodies of the cotyledon and not the aleurone layer, the manufacture of protein isolates or concentrates tends to increase the phytic acid level. Phytic acid in legume protein isolates can similarly be decreased by ~90% by dialysis or ultrafiltration after treatment with acid or alkali to overcome the phytate protein binding (19). Aqueous extraction with dilute acids gives a similar reduction (27).

The most effective way to decrease phytic acid is by enzymatic degradation. Commercial phytases can completely degrade phytic acid in ~2 h when added to an aqueous slurry of the complementary food held at optimum pH for phytase activity (28). Traditional food preparation processes such as soaking, germination and fermentation can also activate native phytases and substantially degrade phytic acid. Marero et al. (29) reported that germinating rice for 3 d and mung bean for 2 d reduced phytic acid by 44 and 41%, respectively. Subsequent drying, dehulling and milling of the germinated grains gave a complementary food containing 60 mg phytic acid/100 g instead of 780 mg/100 g in the corresponding whole-grain mixture. By including an additional fermentation step after soaking and germination, Sharma and Kapoor (30) reduced the phytic acid of pearl millet from 800 mg/100 g to zero. Germination unfortunately activates proteases and amylases as well as phytases and often results in an unacceptable texture of the porridge.

More recently Barclay et al. (31) and Egli (25) used whole wheat, whole rye or whole buckwheat as sources of phytase to degrade phytic acid in complementary food mixtures based on cereals and legumes. By adding 10% whole wheat, whole rye or whole buckwheat to complementary foods (e.g., wheat and soy, millet and cowpea, and rice and chickpea foods) it was possible to degrade phytic acid completely in 1–2 h by holding the mixture in aqueous solution at the optimum pH of the phytase (25).

Conclusions

When legume and cereal mixtures are used as complementary foods, the high phytate content of the porridge is a major concern in relation to iron and possibly zinc nutrition of infants and young children. There is less concern in relation to calcium and magnesium nutrition and no concern in relation to copper. Phytic acid is a potent inhibitor of iron absorption in infants as well as in adults and absorption may be as low as 2–5% even in iron-deficient subjects. Complete degradation of phytic acid can increase absorption up to fivefold whereas 90% degradation (1% to 0.1%) can increase absorption approximately twofold. Although it is clearly recommended to completely degrade phytic acid, this might not always be possible, and to achieve a twofold increase in iron absorption from an iron-fortified complementary food, the molar ratio of phytic acid to iron should be reduced from its native level to <1 or preferably <0.5.

Although phytic acid inhibits zinc absorption in adults, the situation in infants and children is unclear and young infants have shown relatively high zinc absorption from high phytate cereal and legume mixtures. Nevertheless, decreasing phytic acid by 90%, from 1% to 0.1%, would also be expected to double zinc absorption in adults, and complete degradation could increase zinc absorption further. If the molar ratio of phytic acid to iron in an iron-fortified food were reduced to <0.5, zinc absorption in adults would be expected to increase markedly. The influence of phytic acid reduction on zinc absorption in infants and young children appears to be modest but needs further evaluation.

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