Effects of Soya Protein on Mineral Availability

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INTRODUCTION

It is not clear whether soya protein per se directly affects the bioavailability of minerals endogenous to the soybean or of minerals from other sources in the diet. However, it has been frequently reported that for monogastric animals the bioavailability of certain minerals such as zinc is poor from most soya protein-based diets (1-12).

Rackis and coworkers (1,2) noted from surveys of the literature that for experimental animals, zinc availability from soya protein isolates was very low compared to zinc availability from soybean meal, casein or other animal protein-based diets. They attributed the differences in availability to formation of phytate-protein-mineral complexes during the processing of isolates. These complexes, according to Rackis, render the mineral poorly soluble at intestinal pH and reduce mineral absorption.

This paper will present a selective review of the literature that relates to the role of soya protein and phytic acid in the availability of minerals, especially zinc. Emphasis is given to studies involving phytic acid interactions with soya protein (amino acid side chains) and/or minerals and the possible role these interactions may have upon mineral availability. Little work has been repoted that has utilized human subjects; therefore, most results reported here will be from animal investigations.

VARIABILITY OF MINERAL BIOAVAILABILITY FROM DIFFERENT SOYA PROTEIN PRODUCTS

Lease and Williams (12,13) found that whereas one soybean meal-based ration did not require supplemental zinc for optimal growth of chicks, a second soya meal and a soya protein isolate-based diet required up to 30 ppm of added zinc to obtain optimal growth. The zinc content of the two meals showed little difference upon analysis.

O'Dell and coworkers (4) reported that soybean meal had a relative bioavailability of 67% for the chick, which was higher than that of cereal sources but was lower than that of animal sources of zinc. Forbes and Yoke (14) found that for the rat, the relative bioavailability of zinc from isolate was 44%, midrange of that for cereals and considerably lower than the zinc availability from animal sources.

More recently, the bioavailabilities of zinc and magnesium from different soy protein products were tested (Table I) (7,8,15). The response of growth (zinc studies), bone mineral accumulation (both zinc and magnesium studies) or serum magnesium to increasing mineral in the diet from the test substances was compared with the response from highly available inorganic sources of zinc or magnesium. As previously reported (16), bone zinc is a more sensitive indicator of zinc status for slope ratio-type studies than is growth.

The relative bioavailability of zinc from soya products was highly variable and usually quite low, compared to the availability of zinc from zinc carbonate. The results in Table I suggest that zinc bioavailability from soya flour and soya beverage is better than that from isolates and concentrates. Within the isolates and concentrates, zinc utilization is lower from neutralized soya concentrates and better from the acid form of isolates. When growth is used as the parameter of zinc availability, the acid forms of isolates and concentrates produce excellent bioavailbility. Discussion of a chemical basis for this variation is found in the next section of this paper.

TABLE I

Relative Bioavailability of Zinc and Magnesium from Soy Products (7,8,15)

Product	Phytate-to-zinc molar ratio	Relative bioavailability (%)[a]			
		Zinc		Magnesium	
		Weight gain	Long bone zinc	Serum	Bone
Full-fat soy flour	28	55[b]	34[b]	96	106
Freeze-dried soy beverage	26	63[b]	40[b]	102	104
Spray-dried soy concentrate (neutral form)	52	41[b]	20[b]	77[b]	80[b]
Freeze-dried soy concentrate (neutral form)	57	66[b]	29[b]	90	78
Freeze-dried soy isolate (neutral form)	34	85	46[b]	85	85
Freeze-dried soy concentrate (acid form)	66	113,93[c]	48[b]	–	–
Freeze-dried soy isolate (acid form)	35	106	64[b]	–	–

[a]Data points represent comparisons of slopes of responses of test diets (containing soy) with responses of control diets (without soy products) with added minerals as the carbonate X 100.

[b]Significantly different from control diets ($P < 0.05$).

[c]Experiment was run twice.

In agreement with Lo et al. (17), Table I shows that magnesium bioavailability from various soya products is quite good. Only in the spray-dried neutralized concentrate (which also produced the lowest relative zinc bioavailability) was there a significant reduction in available magnesium. Soya products have a high concentration of magnesium and can be considered to be good sources of this mineral.

The bioavailability of iron from soya products seems to be equivalent to that from other plant sources of the mineral. At least this is the case when researchers test for the bioavailability of endogenous iron without addition of sodium phytate (18). Steinke and Hopkins (19) compared hemoglobin repletion in anemic rats fed one of three soya protein isolates for their iron source with rats receiving ferrous sulfate. They found a mean relative iron availability of 61% for endogenous iron. Picciano et al. (20) observed that iron from two soya products (full fat soya flour and a neutralized soya concentrate) regenerated hemoglobin in anemic rats to the same degree as did ferrous sulfate, whereas iron from a freeze-dried soya beverage resulted in significantly less hemoglobin regeneration. Layrisse and coworkers (21) have observed relatively high iron utilization from soybeans for man.

The bioavailability of other minerals native to soya products such as phosphorus, copper and manganese remains to be clarified. Although a number of studies have shown that phosphorus from isolated phytic acid (usually the sodium salt) is readily absorbed by rats (22) and by man (23), Nelson et al. (24) demonstrated that chicks did not utilize phytate phosphorus from soybean meal. Nelson and coworkers further showed that if the phytic acid was hydrolyzed by mold phytase, then the hydrolyzed phytate phosphorus was utilized as efficiently by the chick as was inorganic phosphorus.

One must also consider the effect that soya protein may have upon the bioavailability of minerals from the rest of the diet. Table II lists some of the published results from our laboratories using the rat model. The results in this table demonstrate that the presence of soybean products in rat diets has little detrimental effect upon the bioavailability of zinc or of calcium added as the carbonate. Hardie-Muncy and Rasmussen (10) and O'Dell et al. (4) also found that inorganic zinc added to soya isolate (washed with EDTA) was better utilized by rats than was the zinc inherent to a soya protein isolate.

Steinke and Hopkins (19) reported that inorganic iron added to diets containing isolated soybean protein was not as available to rats as was inorganic iron when added to casein-based diets. However, the bioavailability of the iron added to isolated soya protein had approximately the same availability as that of the endogenous soya iron. These results support a common dietary iron pool hypothesis. Unpublished results from our laboratory (20) would support this finding.

PROTEIN (AMINO ACID SIDE-CHAIN)-PHYTIC ACID-MINERAL COMPLEXES

Cheryan (9) has published an excellent review of the chemistry of the binding of protein, especially soya protein, to phytic acid and/or minerals. The reader is directed to that paper for more detailed discussion than will be found in this paper.

Formation or hydrolysis of protein-phytic acid-mineral complexes during various food processing steps involved in soybean product processing may explain the variability of bioavailability of minerals from different soya products. In intact soybeans, phytic acid is distributed throughout the cotyledon located within subcellular inclusions called protein bodies. Although soybean phytic acid is concentrated within protein bodies, it appears to have no specific localization within the protein body (25). Upon disruption of the soybean cotyledon cell, phytic acid can react strongly with glycinin, the major protein in the protein body (25). Depending upon pH, ionic strength and other conditions, phytic acid could presumably interact chemically with other components of the soybean. The phytic acid chelation with protein, minerals and other molecules in situ is poorly understood and needs further study.

Fontaine and coworkers (26) investigated the protein-phytic acid relationships of peanut, cottonseed and soybean flour slurries over a range of pH. They noted that, in contrast to peanut and cottonseed, in soybean flour there was little decrease in solubility of phosphorus compounds in relation to nitrogen compounds above pH 7. This phenomenon strongly suggests an interaction and complexing of soya protein and phytic acid in the neutral pH region.

TABLE II

Effect of Soy Protein upon Bioavailability, Zinc and Calcium Added to Soya Protein Diets As Carbonates (7,8,15)

Product	Phytate-to-zinc molar ratio	Relative bioavailability (%)[a]		
		Zinc		Calcium
		Weight gain	Long bone zinc	Bone
Full-fat soy flour	28	94	94	94
Freeze-dried soy beverage	26	—[b]	—[b]	106
Spray-dried soy concentrate (neutral form)	52	77[c]	70[c]	102
Freeze-dried soy concentrate (neutral form)	57	84	78	—[b]
Freeze-dried soy concentrate (acid form)	66	65	83	—[b]

[a]Data points represent comparisons of slopes of responses of test diets (containing soy) with responses of control diets (without soy products) with added minerals as their carbonate X 100.

[b]Test not run.

[c]Significantly different from control diets ($P < 0.05$).

More recently, de Rham and Jost (27) reinvestigated phytate-protein interactions in soybean extracts and also found similar solubility of phytate and nitrogen (about 80% soluble) in the neutral pH region. In addition, they reported that 40% of the phytate was protein-bound (nondialyzable) at pH 7.5. Calcium (and/or magnesium) is probably involved as a salt bridge between phytic acid (negatively charged) and the protein (also negatively charged at neutral pH) (27).

The reduced bioavailability of zinc in neutralized as compared to acid-precipitated products (see Table I) may be a result of the formation of stable protein-phytic acid-zinc complexes in the dried neutral product. Since protein-phytic acid-mineral associations occur in solution at a neutral pH (27), these associations may well form more tightly-bound complexes during the drying of the soya protein. The exclusion of water from the protein-phytic acid-mineral system could lead to complexes that are much more thermodynamically stable that the associations occurring in solution. In the digestive tract, these stable complexes may inhibit the complete digestion of protein to free amino acids. Short peptides or amino acid residues bound to zinc and phytic acid may result. These would be resistant to digestion and, consequently, inefficient absorption of zinc would occur (8).

Cheryan (9) suggested that the imidazole group of histidine is a probable binding site for phytic acid through a mineral (II) bridge. Other amino acids likely for such involvement are glutamate and aspartate. These are found in large quantities in soya protein and, at neutral pH, their free carboxyl groups could easily complex with phytic acid through a Zn (II) bridge to form a cochelate. Alternatively, it is possible that basic amino acids at neutral pH might bind directly with a free phosphate group of a phytic acid-zinc chelate. Then, upon drying, a conformational change of protein could trap the zinc and reduce its availability (8).

Further evidence for complexation of phytic acid and protein at neutral pH has come from several investigations of phytate removed from soya protein. Okubo et al. (28) used a large molar excess of EDTA per mole of calcium plus magnesium to remove these ions, thus disrupting the protein-phytate binding and eliminating phytate by ultrafiltration. Omosaiye and Cheryan (29) reported reduced phytate separation from full-fat soya protein via ultrafiltration as the pH was raised from 6.7 to 8 and to 10. They suggested that these results could be explained by an increasing strength of salt linkage (higher association constant) between phytate and protein with increasing pH.

Likuski and Forbes (30) pointed out that the presence of intact protein in animal diets containing phytate is not essential for phytate to decrease zinc utilization. They found that phytic acid decreased the bioavailability of zinc to chicks as effectively when amino acids served as the nitrogen source as when casein was the protein source. The mechanism that reduces zinc utilization in neutralized products might involve the binding of small peptides and/or amino acids to a phytic acid-mineral complex. Therefore, it is plausible that poorly absorbable complexes could form with or without intact protein in the diet. Perhaps it is incorrect to refer to these chelates as protein-phytate acid-mineral complexes (except in intact protein systems); instead, more accurate terminology might be amino acid side-chain-phytic acid-mineral complexes (8).

PHYTATE-TO-MINERAL MOLAR RATIO

Oberleas (31) first suggested that the molar ratio of phytate to zinc might predict the zinc bioavailability from phytate-rich foods. Molar ratios of greater than 20:1 seem to be indicative of poorly available zinc. O'Dell (11) added that the calcium-to-phytate molar ratio should also be considered. Increased dietary calcium is known to cause reduced zinc bioavailability in the presence of phytate (6,11). Davies and Olpin (32) presented strong support for use of the phytate-to-zinc ratio as an indicator of zinc availability from high-phytate foods. They found that ratios as low as 10:1 and 15:1 induced marginal zinc deficiency in rats, as shown by significantly reduced plasma zinc, and that values of 15:1 and greater caused a reduction in growth rates in diets containing 1.2% calcium. They determined that when diets contain 0.6% dietary calcium, 25:1 or larger phytate-to-zinc molar ratios were necessary to demonstrate reduced zinc bioavailability. They also determined that the reduced availability of zinc present in textured soya protein based diets could be accounted for entirely by their phytate contents.

Most recently, Morris and Ellis (33) reported that for rats receiving approximately their dietary requirement for zinc (10-12 ppm), growth was affected by phytate-to-zinc molar ratios greater than 12:1 if the level of dietary calcium was 0.75%, but was depressed at ratios greater than 6:1 if the level of calcium was 1.75%.

From the results in Table I, it is apparent that one cannot strictly rely upon the phytate-to-zinc molar ratio to predict zinc availability. The molar ratios of soya isolates, 35, and concentrates, 62, were quite high (calcium levels of all diets were about 0.6%), yet the growth of rats fed the acid forms of the protein was excellent.

Molar ratios of phytate-to-zinc are a good indicator of zinc availability under controlled conditions. However, differentially processed soya foods may disrupt the efficacy of molar predictions. No published work is available that has investigated molar relationships of phytate with other minerals as they relate to mineral availability.

UNIT PROCESSING OF SOYBEAN FOODS AND MINERAL BIOAVAILABILITY

Those individual food processing steps that have been shown to alter mineral availability in soya food seem to cause changes in phytic acid chelation to minerals and protein.

For human or animal consumption, raw soybeans or meal must receive heat treatment. Heating soya protein products in ranges reduces trypsin inhibitors, optimizes protein digestibility, provides palatable food and probably improves mineral bioavailability. However, depending upon the pH, heat during drying may reduce zinc utilization from the dried product.

Phytate from soya is quite stable to heat; de Boland et al. (34) showed that 60 min of autoclaving reduced the phytate content of soybean protein by less than 15%.

Tempering or soaking soybeans is unlikely to reduce phytate appreciably, due to rather low endogenous phytase contents of the oilseed. However, yeast will cleave phosphates from phytic acid during fermentation of legumes. Ranhotra et al. (35) demonstrated that all of the phytate in wheat bread and more than three-fourths of that in soya-fortified wheat flours (10% soy, 90% wheat) was hydrolyzed during the process of breadmaking, apparently due to phytases in the wheat or yeast.

Several groups of workers have separated phytate from the soybean, using the differential solubilities of phytate and soybean protein. The use of ultra- and diafiltration under various conditions has been discussed in other reviews (6,9).

Both the action of phytase or the removal of phytic acid

is presumed to increase bioavailability of minerals from soya products. Working with low-phytate wheat brans produced by enzymatic hydrolysis or by extraction, Morris and Ellis (36) demonstrated that phytate reduction significantly improved zinc but not iron bioavailability from the bran for rats. Nelson and coworkers (24) found increased phosphorus utilization for chicks fed soybean meal treated with a mold phytase.

Martinez (37) has reviewed the food processing effects upon phytate destruction or removal from plant foods. She points out that the manner in which oilseeds are fractionated and processed directly affects the disruption of membranes surrounding aleurone protein bodies and membranes surrounding inclusions containing phytic acid. Formation of insoluble phytate complexes with protein or other food components during food processing depends upon the extraction pH, and the presence of minerals or other chelators.

The basic understanding of the effects of individual unit food processes upon the interaction of soya protein with phytic acid and minerals is meager at best. More work must be done to understand the interactions of the components of food before systems can be recommended to adequately improve mineral availability.

HUMAN STUDIES ON MINERAL BIOAVAILABILITY FROM SOYA PROTEIN

Few studies of humans have been reported that have examined the bioavailability of minerals from soya protein products. One study (38) involved feeding human volunteers diets with or without 25% of their protein coming from soybean foods in a 4 + 4 week crossover design. One hundred biochemical parameters were compared from the two feeding periods and no statistically significant alterations were noted in overall mineral metabolism or digestion. This study, although reassuring, was too broad in design to adequately test for a single parameter, such as zinc bioavailability. To do that, zinc must either be limiting or just at requirement levels in the diet.

Greger and coworkers (39) determined zinc and nitrogen balance in adolescent females fed with or without substitution of soya (rehydrated vegetable protein [TVP], ADM, Decatur, IL) for 30% of the meat in luncheon menus. They reported that the substitution for soya did not significantly alter zinc balance. In this study, 45% of the zinc for the two groups was added to the diets as a zinc solution dispersed in the lemonade served at lunch. Thus, Greger's study reaffirmed the animal studies (7,10,15) that showed that the presence of soy protein in the diet does not significantly reduce zinc bioavailability from the rest of the diet.

Sandström and Cederblad (40) extrinsically labeled meals with ^{65}Zn and monitored absorption of the label by whole-body retention of the isotope about 2 weeks after ingestion of a single meal. They found that when defatted soy flour was substituted for 25% of the animal protein (chicken or beef) in the meal, zinc absorption was not seriously influenced. They 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 or calcium.

The use of the extrinsic tag method with ^{65}Zn (40) or stable isotopes as proposed by Janghorbani and Young (41) is a valuable tool for testing the effects of the presence of components on the diets upon exogenous minerals. This method will not test for the bioavailability of endogenous minerals unless there is a complete mixing of the mineral pool. For iron, there seems to be a mixing of the extrinsic and intrinsic pools, but for zinc there is no good evidence that zinc from an extrinsic label exchanges with zinc in soya or protein-phytic acid-mineral complexes. In fact, the results in Table I and II strongly suggest that endogenous zinc is poorly available to the rat as compared to zinc added to soya protein-based diets.

CONCLUSIONS

There is no evidence that soy protein per se directly affects the bioavailability of minerals. Most researchers believe that soybean protein in soybean products plays a casual role in reduced bioavailability of minerals from soya.

The bioavailability of mineral, particularly zinc, may decline during the processing of soya products by the formation of protein-phytic acid-mineral complexes. These complexes seem to form more readily at pH from 7 up to 8 or 10, especially in the presence of high amounts of dietary calcium. Neutralized soya protein concentrates and isolates have been shown to have relatively low zinc bioavailability for rats as compared to other soy products.

Experimental evidence based upon work with the rat and chick demonstrate a highly variable bioavailability of minerals, especially zinc. Unit food processing procedures can greatly modify zinc utilization for these experimental animals. These results do not necessarily predict how man will utilize minerals from soya products. Thus far, a few published studies of humans raise hopes that man can utilize soy products in mixed diets without alterations in mineral metabolism.

Considerably more basic research is needed to determine the bioavailability of minerals from soya for man and, if necessary, to develop economically feasible processing procedures that optimize recovery and functionality of soya protein, yet reduce the amount or effect of phytic acid on mineral bioavailability. The alternative action is prudent fortification of soya protein products. Fortification should be undertaken when the product has the potential or making a significant contribution to the human diet. Indiscriminate fortification is to be avoided; this could lead to induction of alternate mineral deficiencies. For example, calcium addition to diets containing phytate reduces zinc utilization, whereas zinc addition may reduce copper utilization.

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Phytic Acid in Soybeans

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ABSTRACT

Phytic acid, the hexaphosphate of myo-inositol, is the most important phosphate reserve compound in many plant seeds, but many of its salts are poorly digested by animals. It can form complexes with seed proteins, some of which sequester metal ions, making them unavailable for the animal organism. Soya protein isolates may be higher in phytate content than the soya flour from which they are obtained. Zinc is the mineral of most concern because its bioavailability from some soya products is quite low and because of its marginal levels in some human diets. The availability of iron from soya flour and soya isolates is higher than that from some other plant foods with lower phytate contents. Processes for removing the larger part of the tightly bound phytates from soya protein isolates are described.

INTRODUCTION

Many plants contain chemical compounds with antinutritional properties. Some are probably produced as defense mechanisms against animal predators. Others are constituents of some physiological or mechanical importance for the plant, but are indigestible and even harmful for some animal species. Undoubtedly, there has been a long coevolution between plants and animals, during which the former developed enough defense mechanisms to be able to survive and the latter adapted their digestive and detoxification abilities to find enough food. It is therefore not surprising that most animal species are able to forage only on a limited number of plant foods (1).

Humans can consume a relatively wide range of vegetable materials. Nevertheless it has been estimated that even primitive tribes of hunter-gatherers exploit only a small part of the plant material available in their habitat, probably no more than 1-2%.

Even the vegetable foods we currently consume contain numerous ingredients that are not digested and absorbed or that are detoxified and excreted without direct physiological benefit for the eater. The final balance is important—whether one benefits more from ingesting certain foodstuffs or suffers more harm from it. Human intelligence, moreover, in many instances permits the elimination of the major harmful ingredients, thus making food available which would otherwise be inedible.

In the human diet, plant seeds play a dominant role. This is not surprising because they are the storage organs that provide nourishment for the developing young plant, while it is still incapable of photosynthesis and is therefore more similar in its physiological needs to an animal organism. These seeds quite often contain some defense constituent that may be toxic to animals. Their reserve materials are readily digested by their own enzymes during germination, but they are not always suitable substrates for the animal digestive enzymes. This is exactly the case of phytic acid, a reserve material in many plant seeds, which is not readily hydrolyzed in the digestive tract. Several excellent review articles have been published recently on the nutritional implications of phytates (2-5).

CHEMISTRY AND FUNCTION OF PHYTIC ACID

Phytic acid is the hexa-phosphate of myo-inositol and has three strongly bound water molecules. It can form a number of insoluble salts with different metal ions and can sequester several metals by chelate formation. Two or more cations, when present simultaneously, may have a synergistic effect and can act together to increase the quantity of metallic phytate precipitated.

Phytic acid is found mainly in plant seeds, where it functions as a reserve material for phosphorus. It is hydrolyzed during germination, when the phytase activity increases very rapidly. In cereals phytic acid is found mainly in the germ, which may contain 4-6% of the acid. Defatted soya meal contains about 1.5% phytic acid. Different fractions of oilseed meals may differ widely in their phytate content. During processing of soya isolates, various phytate-protein complexes are formed. At acid pH, phytic acid reacts with proteins, and upon neutralization, insoluble complexes are precipitated. These are still able to bind metal ions.

Mono-, di-, and tri-phosphoric esters of inositol exist in some phospholipids of animal tissues, but the hexaphosphate has been found only in plants.