

Soy Proteins in Human Diets in Relation to Bioavailability of Iron and Zinc: A Brief Overview¹

V. R. YOUNG and M. JANGHORANI, Department of Nutrition and Food Science, Clinical Research Center and Nuclear Reactor Laboratory, Massachusetts Institute of Technology, Cambridge 02139

ABSTRACT

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Because soy proteins are finding increased use in human diets, careful consideration of the relationship between soy protein intake and trace element nutrition in human subjects is necessary. Selected aspects of dietary mineral availability in reference to soy are reviewed, with particular reference to iron and zinc. Preliminary results of an MIT experiment, in

which young men received diets containing soy protein isolate (Supro-620) and the stable isotopes ⁵⁸Fe and ⁷⁰Zn, suggest that iron in the soy protein isolate is readily available and that the protein source does not reduce the availability of dietary zinc.

Advances in food technology have produced a variety of new edible soy products and, in consequence, increased consumption of soy foods by populations of technically developed regions of the world (Gallimore 1979). Some of the nontraditional uses of soy are listed in Table I. In studies with human subjects, researchers have explored the potential of soy to contribute significantly to the protein intake and amino acid requirements of humans. These studies (Wilcke et al 1979, Young et al 1979) support the conclusion that properly processed soy protein foods are well tolerated and of good protein value for humans of all ages. Furthermore, the amino acid profile of the soy flour and isolates studied in human metabolic experiments indicates that human requirements for essential amino acids can be met with soy as the sole or unsupplemented source of dietary protein.

Hence, for protein nutrition, soy can partially or quantitatively replace food proteins of known high nutritional value, such as cow's milk, meat, and eggs, without a change or deterioration in the overall *protein* nutritive value of the diet. However, because animal protein foods make an important contribution to the total intake of many vitamins and minerals (Weir 1976, Young 1980), and in forms that are highly available for meeting human needs, a comprehensive assessment of the potential role of soy proteins in the human diet requires consideration of the impact of increased soy consumption on the *total* nutrition of human population groups. The availability of trace elements in foods of animal origin and in diets based on a high proportion of animal foods is usually thought to be higher than in plant foods and diets where the major energy and protein supply is from vegetable sources.

In this review, we will discuss some selected aspects of dietary mineral bioavailability in reference to the use of significant quantities of soy in human diets. This topic has been reviewed (Kratzer 1965, O'Dell 1979, Rackis and Anderson 1977), but the major emphasis has been on work in experimental and farm animal species. Hence, our purpose is to consider this topic in relation to human studies, although little work has been undertaken in this area to date. For the present purpose, bioavailability is defined as the fraction of the dietary intake of the element that is absorbed from the gastrointestinal tract.

TRACE MINERAL BIOAVAILABILITY

Chemical determination of the total content of an element in a food or diet does not indicate the amount of the element that is available to meet the physiological requirements of the consumer. This is because the absorption and subsequent utilization of the element is influenced by many factors, and for some elements these play an important role at the gastrointestinal stage of nutrient

¹ Based on a paper presented at a symposium, Impact of Foods on Trace Mineral Availability and Metabolism, AACC 64th Annual Meeting, Washington, DC, October 1979.

utilization.

Table II lists these factors, categorized according to the epidemiologist's triad of agent (or diet), host, and environmental factors. Clearly, the extent to which the dietary intake of a mineral is absorbed from the lumen of the intestinal tract and subsequently made available for meeting the metabolic needs of cells and organs depends upon a multiplicity of factors. Furthermore, many of these factors interact in a complex way, making prediction of the bioavailability of a metal in a food or specific diet difficult.

Of particular significance in Table II are the interactions among minerals, of which a number of examples are well known, including interactions between calcium and phosphorus, iron and copper, cadmium and zinc (Davies 1979, Mills 1974). In addition, the possible impact of food processing must be recognized because this may contribute either to enhanced or reduced availability of a particular mineral in a food source, depending upon the process and/or the primary food material in question (Erdman 1979, Rackis and Anderson 1977). Hence, generalizations about the bioavailability of minerals in vegetable foods, cereals, legumes, or oilseeds can be misleading; mineral bioavailability is not the same in all cereals or cereal products, on the one hand, or in all soy-based foods, on the other hand. For example, the extent of iron absorption from a given food is affected by the presence of other foods in the diet (Layrisse et al 1968), particularly meat and fish (Björn-Rasmussen and Hallberg 1979, Cook and Mønsen 1976, Hallberg et al 1978). Also, other dietary constituents such as tea (Disler et al 1975), dietary fiber (Jenkins et al 1975), fat (Bowering et al 1977), and ascorbic acid (Björn-Rasmussen and Hallberg 1974; Cook and Mønsen 1977; Derman et al 1977; Sayers et al 1973, 1974) affect the availability of iron in the total diet. Similarly, the availability of dietary zinc may be influenced by the level of phytic acid (myo-inositol-1,2,3,4,5,6,-hexa-kis-dihydrogen phosphate) and dietary fiber (Ismail-Beigi et al 1977; Oberleas et al 1966; Reinhold et al 1973, 1975, 1976a, 1976b).

The possible implications, for mineral nutrition, of the phytic acid content of soy-based foods has attracted considerable investigation (Erdman 1979), and results obtained in animal studies have firmly established the inhibitory influence of this plant constituent on the availability of dietary minerals, especially

TABLE I
Some Nontraditional Uses of Soy in Relation to Human Nutrition^a

Use	Group
Alternative to milk-based formula	Infants
Hypoallergenic food	Infants, children
Vegetable protein mixtures	Pre-school and school-aged children
Protein-enriched drinks	Children
Food analogues, replacers, extenders	All ages

^aFrom Young et al (1979).

calcium and zinc. However, the possible practical consequences for human mineral nutrition of phytic acid ingestion are difficult to judge. This will depend upon the specific element, its level in the diet, and the presence or absence of other interacting factors. Welch and Van Campen (1975) found that iron bioavailability did not correlate with the phytic acid content of intrinsically labeled soybean seeds (Table III), but the data of Forbes et al (1979) suggest that the phytate level of soy products may play a determining role in the availability of zinc (Table IV). Davies and Olpin (1979) have also concluded from rat feeding studies that the low availability of zinc in a soybean-based, textured, vegetable protein is functionally related to its phytate content.

However, the situation is complex, as further demonstrated by the results of a recent study conducted in our laboratories (Nahapetian and Young 1980). In this series of experiments we explored the metabolism of phytic acid in rats with the aid of U-¹⁴C-phytate prepared by labeling wheat plants with ¹⁴C-myoinositol. The fecal output of radioactivity following an oral dose of ¹⁴C-phytate was affected by the level of dietary calcium (Fig. 1), and the metabolism of phytate was reduced by a high calcium intake; phytate absorption and its oxidation were almost quantitative when dietary calcium intakes were low (Fig. 2). Thus, dietary phytate coupled with a high calcium intake may reduce the availability of trace minerals, such as iron and zinc, to a greater extent than does phytate in combination with a lower dietary calcium level. Animal feeding studies give evidence for this (O'Dell 1969). Hence, the composition of the total diet must be considered in an assessment of the nutritional significance of the phytate content of foods in relation to mineral availability.

Finally, animal models do not necessarily provide precise quantitative estimates of the actual availability of minerals in dietary constituents or the total diet of healthy humans under free-living conditions. For example, zinc repletion or retention tests are used in rats to assess availability of zinc in foods, but Davies and Flett (1978) have shown that zinc-deficient diets result in reduced intestinal phytase activity (Table V). Thus, if low basal diets are used to assess the bioavailability of zinc in foods that contain phytic acid, and perhaps also supply generous levels of calcium, the approach may not be adequate for predicting the bioavailability of dietary zinc in well-nourished humans.

TABLE II

Some Factors Affecting the Bioavailability of Trace Elements in Diets

Dietary Factors	
Intake level of element	
Chemical form	
Promoters, inhibitors (eg, ascorbic acid, phytate, fiber, sugars, fats, proteins)	
Mineral-mineral interactions	
Food processing	
Host Factors	
Nutritional state	
Physiological states (growth, pregnancy, etc)	
Pathological states	
Environmental Factors	
Biological	
Infectious agents	
Social	
Dietary and personal habits	

TABLE III

Absorption of ⁵⁹Fe from ⁵⁹Fe-Labeled Soybean Seeds in Iron-Depleted Rats^a

Treatment	Phytic Acid (% dry weight)	Absorption (% of dose)
Immature soybeans	0.65	36.2
Mature soybeans	1.77	55.5
⁵⁹ FeCl ₃	...	50.7

^aSummarized from Welch and Van Campen (1975).

SOY PROTEINS AND MINERAL BIOAVAILABILITY IN HUMANS

These issues have been raised to emphasize the desirability of obtaining confirmatory data on dietary mineral bioavailability in direct human studies.

Van Stratum and Rudrum (1979) used the chemical balance technique, coupled with measurement of various biochemical indicators of mineral nutrition status, to determine whether inclusion of soy, mostly from concentrates and accounting for about one quarter of the total protein intake, in the diet of a large group of adult volunteers over a four-week period had any unfavorable physiological effects. A partial summary of their data (Table VI) indicates that ingestion of food grade soy protein concentrate at a level equivalent to about 23 g of protein per day did not result in any unfavorable trends in calcium, magnesium, zinc, and iron nutriture. The changes observed were small and related to differences in mineral intake from the control and soy-based diets. These findings are consistent with balance studies in adolescent girls indicating that zinc availabilities in meat and soy are similar and that zinc nutriture is maintained with a soy-based diet under practical conditions (Greger et al 1978).

Because of the limitations of the chemical balance technique for

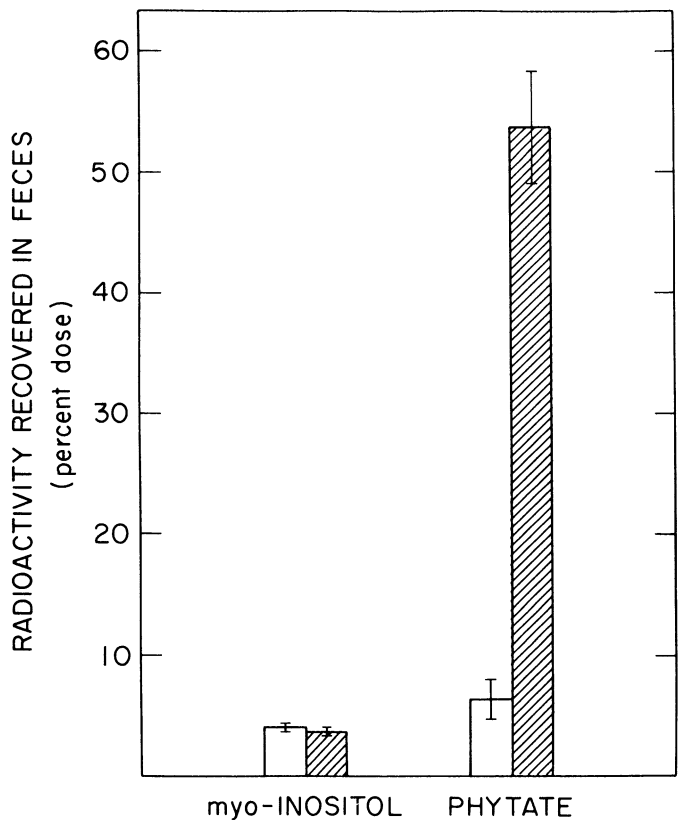


Fig. 1. Total fecal radioactivity for 48 hr following oral dose of ¹⁴C-myoinositol or ¹⁴C-phytate for the low calcium (□) and high calcium (▨) diets. Vertical lines are ± SEM.

TABLE IV

Bioavailability of Minerals in Soy Products as Evaluated in Rats^a

Product	Phytate to Zinc Molar Ratio	Relative Availability ^b		
		Zinc	Calcium	Magnesium
Full fat soy flour	28	...	94	106
Soy beverage	26	0.40	106	104
Soy concentrate	52	0.20	102	80

^aSummarized from Forbes et al (1979).

^bAvailability of zinc in soy products relative to ZnCO₃, MgCO₃, or CaCO₃, using femur zinc, tibial magnesium, femur calcium.

measurement of trace element absorption (Hallberg 1974), as well as the safety and ethical issues related to use of radiotracers, we have begun to explore the potential of stable isotope techniques for use in human studies (Janghorbani and Young 1980a). In a recent review we discussed extensively the experimental aspects of stable isotope methodology in relation to human studies of dietary mineral bioavailability (Janghorbani and Young 1980b). We have also discussed the accuracy of the fecal monitoring method (isotope balance) in relation to quantitative estimations of trace element absorption (Janghorbani and Young, 1980b) and have described (Janghorbani et al 1980b, 1980c) data for patterns of excretion of

the ^{70}Zn and ^{58}Fe stable isotopes in feces following oral administration of these tracers given to young adult men consuming diets based on a well-processed soy isolate (Supro-620, Ralston-Purina Company, St. Louis, MO).

In applying the nonradioactive isotopes of zinc and iron, the approach that we have followed is that of the extrinsic tag technique. The principle of this approach is illustrated schematically in Fig. 3 and is based on the assumption that the dietary (intrinsic) metal completely exchanges with a label or extrinsic tag and that absorption of the element occurs from a common pool containing both the unlabeled dietary metal and the

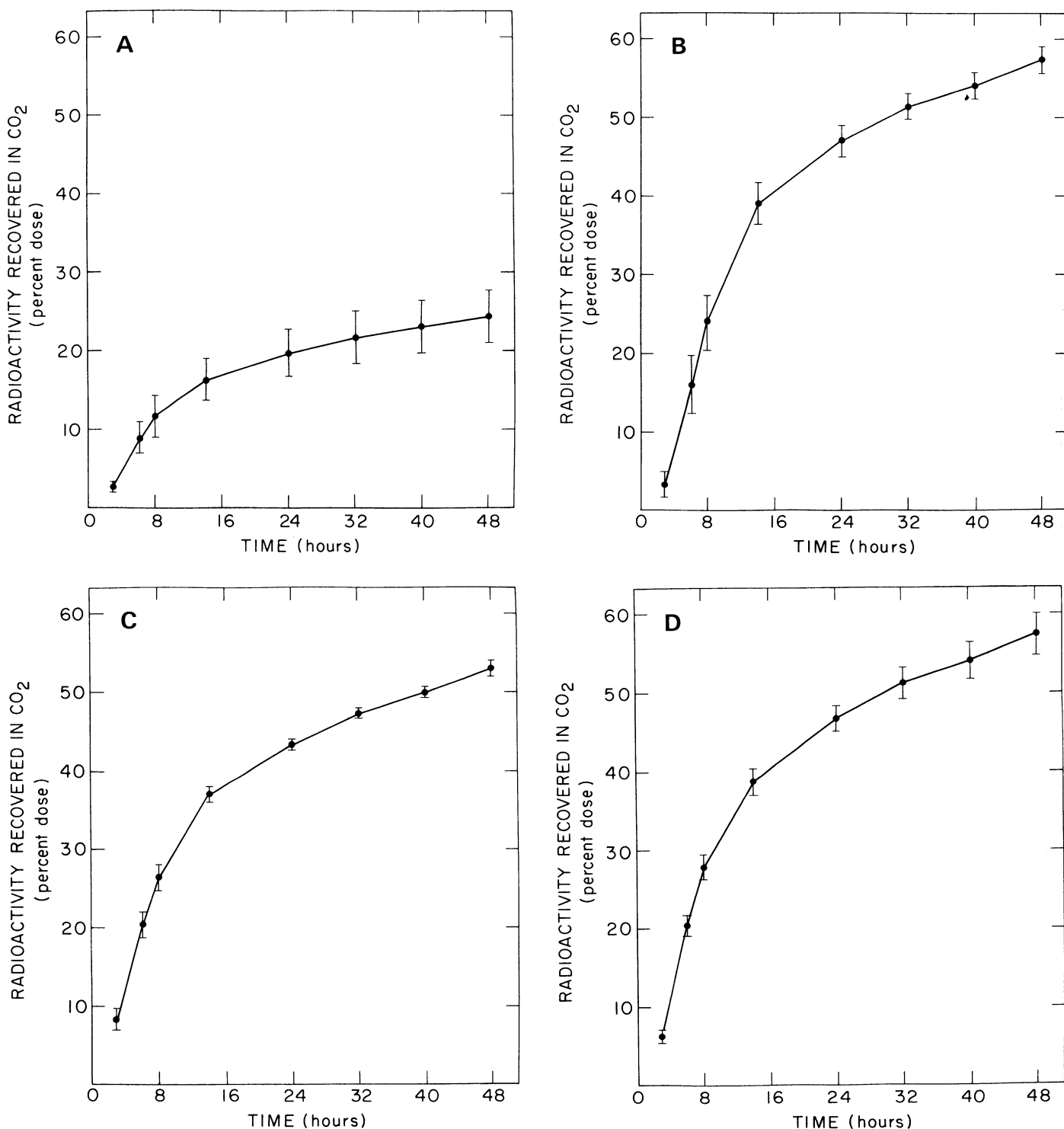


Fig. 2. Appearance of ^{14}C in expired air following an oral dose of $\text{U-}^{14}\text{C}$ -myoinositol or $\text{U-}^{14}\text{C}$ -phytate in rats receiving either low calcium or high calcium diets. Adapted from Nahapetian and Young (1980). **A**, High calcium, phytate oxidation; **B**, low calcium, phytate oxidation; **C**, high calcium, myoinositol oxidation; **D**, low calcium, myoinositol oxidation. Vertical lines are \pm SEM.

extrinsic tag. Thus, the appearance of the tracer in body compartments, such as blood and urine, or its disappearance from the intestine, as monitored by the appearance of an oral dose of tracer in the fecal pool, is taken to provide a quantitative index of the degree of absorption of the element present in the diet or test meal. This technique has been validated for estimation of absorption of nonheme iron (Björn-Rasmussen 1973; Björn-Rasmussen et al 1972; Cook et al 1972; Monsen 1974) and zinc (Evans and Johnson 1977) under specific experimental and dietary conditions. However, isotopic exchangeability and availability of dietary iron or zinc may not always be equivalent because the composition of the test meal may affect the properties of the pool of isotopic exchange (Hallberg 1974). Indeed, intrinsically labeled zinc in corn and rye forage (Neathery et al 1975) and intrinsically labeled zinc in spinach (Welch et al 1977) were of higher availability than were inorganic forms of zinc added to these foods.

Nevertheless, the extrinsic tag approach has been used extensively in the study of dietary iron availability in human subjects, and we have chosen this technique to explore the absorption of iron and zinc in healthy adults receiving soy-based diets. The design of one of our studies is summarized in Table VII. It involved five healthy young male volunteers who received each of three isocaloric, isonitrogenous diets with two protein sources that provided the intake of dietary protein. Protein intake was at a level (0.8 g of protein per kilogram per day) sufficient to meet the long-term protein needs of nearly all healthy subjects in this age group, and the diets supplied a constant, known intake of about 15 mg of Fe and 15 mg of Zn per day.

At intervals during each of the three two-week diet periods, the subjects received doses of ^{58}Fe and ^{70}Zn , the stable isotope forms of these elements, divided among six consecutive meals, and a determination was made of the bioavailability of iron and zinc for each diet, based on the fecal monitoring method (Janghorbani et al 1980b, 1980c).

In Fig. 4, the appearance of ^{58}Fe in feces after the tracer dose was given during each diet period is shown for one example subject; these results indicate a relatively rapid appearance of the label in the fecal pool followed by an equally rapid return to the basal levels of ^{58}Fe output from ^{58}Fe supplied by the natural dietary iron. A similar pattern of isotope excretion was also observed for ^{70}Zn (Fig. 5) when this isotope was given simultaneously with the tracer dose of ^{58}Fe .

Based on the measured output of ^{58}Fe and ^{70}Zn in the feces following the ingestion of the tracer, determination of the absorption of dietary nonheme iron and zinc is possible. The results for the three diets examined in our experiments are summarized in

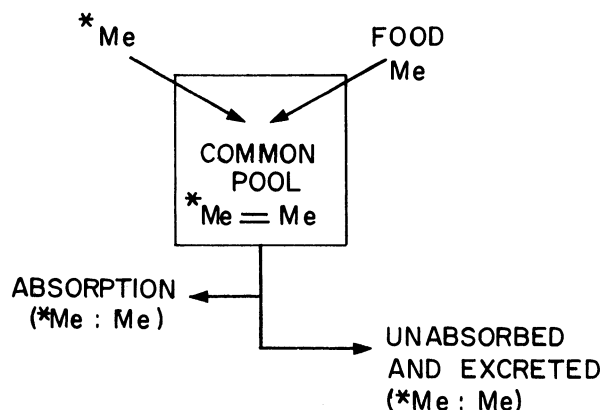


Fig. 3. Schematic outline of the extrinsic tag approach for determination of the bioavailability of dietary minerals. Isotopic exchange of the dissolved and dissociated mineral (eg, nonheme iron or zinc) in a meal and the added inorganic isotope tracer is assumed to occur. Absorption of the test mineral thus occurs from a common pool of the mixture of intrinsic (natural) mineral and extrinsically labeled mineral. **Me** = trace metal, ***Me** = isotopic tag.

Tables VIII and IX. Mean absorption for all diets approximates 20% of iron ingested with no significant differences in iron absorption between the diets (Table VIII). Each diet provided 15 mg of iron per day, with two thirds from the protein isolate in the soy-based diet and almost all from the inorganic iron supplement (FeCl_3 together with ascorbate in each diet) in the milk diet. Similarly, the absorption of zinc from the total diet averaged 44% of zinc ingested and did not differ between the soy-isolate and milk-based diets (Table IX).

Based on this initial study, therefore, we concluded that iron availability in the soy diet is high. However, the conditions of our experiment were designed to incorporate conditions that favor iron availability; inhibitory factors such as dietary fibers, tea, and high calcium were not present and so could not limit iron absorption from the experimental diets. The present results must be viewed against these conditions. In addition, a generous intake of ascorbic acid, as provided by our experimental diets, significantly enhances the availability of iron from rice-based and cereal-based diets (Derman et al 1977, Sayers et al 1974).

From the present study, mean iron absorption approximates 3 mg per day, a value similar to that reported by Crosby et al (1963) and by Pritchard and Mason (1964) for adult humans.

TABLE V
Effect of Zinc Deficiency on Alkaline Phosphatase and Phytase Activity^a of Rat Duodenal Muscular Homogenates^b

Group	Alkaline Phosphatase	Phytate
Zn deficiency		
Six days	6.6	0.8
Fourteen days	3.6	0.4
Zn supplement		
Six days	15.5	1.3
Fourteen days	12.8	1.5

^a μmol phosphate liberated per milligram of protein per hour.

^b Summarized from Davies and Flett (1978).

TABLE VI
Effect of a High-Soy Protein Diet on Mineral Metabolism in Adult Subjects^a

	Soy Diet	Control
Calcium ^b		
Urine	4.9	5.2
Feces	23.4	23.5
Magnesium ^b		
Urine	5.24	5.27
Feces	11.7	10.1 ^c
Zinc		
Serum ^d	17	16
Feces ^b	192	216 ^c
Iron-serum ^d	17.9	20.0
Iron-binding capacity	62.3	61.8

^a Summarized and adapted from Van Stratum and Rudrum (1979).

^b $\text{mmol}/24\text{ hr}$.

^c Significant at $P = 0.02$.

^d $\mu\text{mol}/\text{l}$.

TABLE VII
Design of Study to Assess Iron and Zinc Bioavailability in Diets Based on a Soy Isolate^a

Subjects	Six young adult men
Diets	1. Soy isolate 2. Soy isolate/milk (50:50) 3. Milk
Periods	14 days
Approach	^{58}Fe and ^{70}Zn given as an oral dose on days 6 and 7 of each period
Assessment	By fecal isotope monitoring

^a Supro-620 (Ralston-Purina Company, St. Louis, MO). Given as 0.8 g of protein per kilogram per day. Unpublished M.I.T. study.

Furthermore, our results are compatible with the rat-feeding studies by Steinke and Hopkins (1978), who concluded that soybean proteins are a good source of dietary iron, and with the interpretation of Beaton and Patwardhan (1976) that the iron in a variety of soy protein foods is highly available for meeting human needs (Table X). In addition, Rios et al (1975) found that the bioavailability of the intrinsic iron of soybean was essentially the same as that obtained for ferrous sulphate used as an inorganic iron supplement in a soy-based infant formula (intrinsic iron/supplemental iron, 1:14). These various observations are consistent with our findings and with the interpretation that iron in the soy protein isolate tested is readily available and did not reduce the overall availability of total dietary iron.

The preliminary findings for zinc absorption showed that the soy isolate did not have any significant effects on the bioavailability of

total dietary zinc. These observations are supported by those of Van Stratum and Rudrum (1979) and Greger et al (1978), based on chemical balance methods. Clearly, further studies will be necessary to firmly establish the relationships between ingestion of soy protein sources and the bioavailability of iron, zinc, and other trace elements in humans of various ages. We have explored only one soy product, and others will require study before any generalizations can be made about the many different soy products available on the market or being developed for use in the human food supply.

RESEARCH DIRECTIONS

A number of areas of research deserve particular attention (Table XI); continued extensive work in animal models is expected to improve knowledge of the availability of trace elements in foods and diets, but greater emphasis must be given to the quantitative and practical relevance for human nutrition of work based on these models. As indicated in Table XI and discussed in more detail elsewhere (Janghorbani and Young 1980a, 1980b), additional analytical techniques need to be developed before the full potential of stable isotopes as a tool for exploring issues of practical human mineral nutrition will be realized. The experimental protocol chosen for our initial study of iron and zinc availability in soy-based diets is not necessarily optimal for investigating all of the important questions related to dietary trace metal bioavailability.

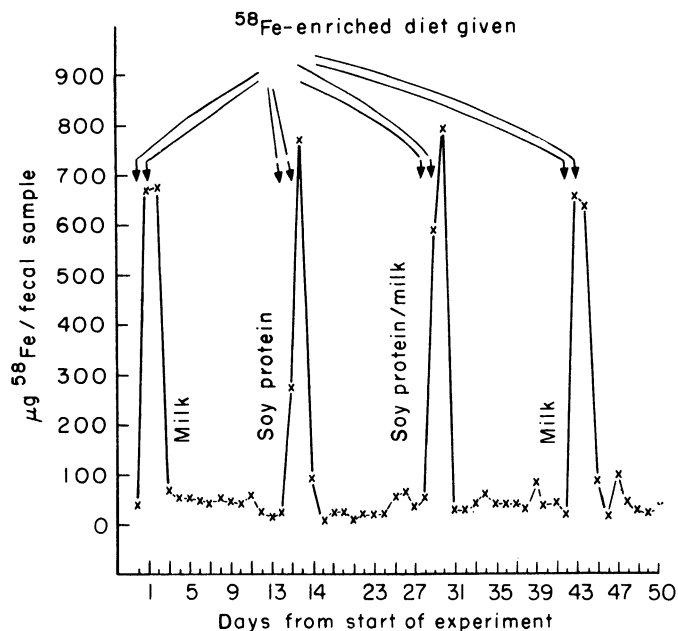


Fig. 4. Fecal output of ^{58}Fe in an adult male volunteer following oral administration of ^{58}Fe during each of the four experimental diet periods. The first and fourth diet periods were repeated.

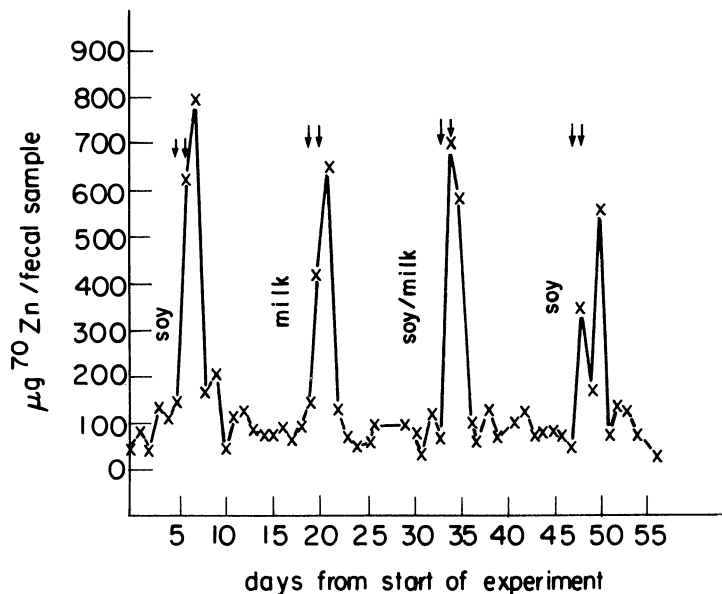


Fig. 5. Fecal output of ^{70}Zn in an adult male volunteer following an oral administration of ^{70}Zn during each of four experimental diet periods. The first and last diet periods were repeated.

TABLE VIII
Bioavailability of Iron in Soy Isolate,^a Soy Isolate/Milk, and Milk-Based Diets Given to Young Men, as Determined with ^{58}Fe and Fecal Monitoring Method^b

	Diet		
	Supro-620	Supro-620/Milk	Milk
Total iron intake, mg/day	15.0	15.3	15.5
Source of iron			
Protein	9	4.8	0.5
FeCl ₃	6	10.5	15.0
Iron bioavailability, ^c %	21 ± 6	17 ± 7	22 ± 6

^aSupro-620 (Ralston-Purina Company, St. Louis, MO).

^bUnpublished M.I.T. data. Mean ± SEM for five subjects.

^cNo significant differences ($P > 0.05$) among diets.

TABLE IX
Bioavailability of Zinc in Soy Isolate,^a Soy Isolate/Milk and Milk-Based Diets Given to Young Men, as Determined with ^{70}Zn and Fecal Monitoring Method^b

	Diet		
	Supro-620	Supro-620/Milk	Milk
Total zinc intake, mg/day	15.3	15.5	15.5
Source of zinc			
Protein	3.3	4.9	6.5
ZnCl ₂	12	10.5	9
Zinc bioavailability, ^c %	37 ± 4	47 ± 4	51 ± 6

^aSupro-620 (Ralston-Purina Company, St. Louis, MO).

^bUnpublished M.I.T. data. Mean ± SEM for five subjects.

^cNo significant difference ($P > 0.05$) among diets.

TABLE X
Absorption of Iron from Different Types of Diets^a

Percent of Energy in Diet Provided by Foods of Animal Origin or by Soybeans ^b	Assumed Upper Limit for Absorption by Normal Individuals (%)
10	10
10-25	15
25	20

^aBeaton and Patwardhan (1976), p. 467.

^bSoybean is grouped with foods of animal origin because of the high bioavailability of the iron content.

TABLE XI
Some Areas of Research Needed to Improve Knowledge of Trace Element Bioavailability in Human Diets

1. Further evaluation of human nutritional significance of animal models
2. Greater exploration of potential of stable isotopes.
 - a. Analytical: neutron activation and mass spectrometry
 - b. Design and conduct of human experiments
 - i. Controlled metabolic-tracer studies
 Appearance and disappearance methods (blood, urine, feces)
 - ii. Application of approaches in a "field" setting
3. Improved knowledge of trace element metabolism and requirements in human subjects

Thus, detailed investigation of the problem of experimental design is worthwhile in order to develop approaches that may provide reliable quantitative data of dietary mineral bioavailability under the more complex conditions of free-choice dietary situations, that is, in the "field-setting." Finally, a continued need exists for improved understanding of the basic aspects of trace element metabolism and utilization in animals and in human subjects. This should generate more reliable data on the physiological requirements for these essential nutrients and the quantitative significance of dietary and host factors that affect them. The measurement of stable isotopes in blood and urine must be actively pursued in order to refine methods for determination of trace element bioavailability in human subjects. This is an area of active investigation (Janghorbani et al 1980a, Miller and Van Campen 1979).

SUMMARY

In this brief overview, we have examined some issues of trace element bioavailability in relation to the use of soy flours, concentrates, and isolates and have emphasized the importance of exploring these problems directly in human studies. We have presented our preliminary data, based on the use of safe nonradioactive isotopes of iron and zinc with healthy subjects. Use of stable isotopes of trace minerals offers an exciting, timely, and quantitatively useful approach for investigating many unanswered but important questions concerning plant food sources in relation to human mineral nutrition and metabolism.

On the basis of our initial findings with a specific soy protein isolate (Supro-620) given to healthy young men, soybean proteins appear to be a potentially good source of available iron for meeting the needs of human nutrition without impairing the availability of dietary zinc.

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