Bioavailability of Zinc from a Diet Based on Isolated Soy Protein: Application in Young Men of the Stable Isotope Tracer, ^{70}Zn

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ABSTRACT With the aid of the stable isotope, ^{70}Zn, as a tracer and neutron activation analysis, a combination of extrinsic labeling of meals and fecal monitoring of isotope excretion was used as a safe and noninvasive approach for assessing the effects of the vegetable (soy) and animal (milk, beef) proteins on the absorption of zinc in healthy, adult human volunteers. A known amount of ^{70}Zn was added as $ZnCl_2$, to six consecutive meals over a 2-day period during which either one of three isonitrogenous liquid formulas (skim milk; soy isolate; or a 50:50 mixture) or one of two bologna sausages (soy isolate or beef) were given. The mean absorption of ^{70}Zn from milk, soy and soy/milk was 41 ± 4, 34 ± 4, and 41 ± 7% (mean ± SEM), respectively, the presence of soy protein having no effect on absorption of the extrinsic label. For beef bologna and soy bologna, fractional absorption of the ^{70}Zn tracer was 41 ± 4 and 30 ± 3%, respectively. Beef might favor absorption of extrinsic zinc. The kinetics of isotope excretion, pooling procedures for stool samples and the utility of fecal markers were also evaluated. J. Nutr. *112*:(10): 1809–1821, 1982.

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Recently, soybeans have become an increasing source of protein in human diets (1–3). Because it has been suggested by experiments in rats (4) and men (5) that zinc may be better absorbed from foods of animal origin than from plant foods, and because soy is being used to extend or replace animal protein, such as meat and dairy products, a specific concern regarding the biological availability of dietary zinc in certain soy-based foods has been raised (6–9). A number of nonhuman species, including chicks (10–13), turkey poults (14, 15) and rats (16–22), have been used to examine the bioavailability of zinc in a variety of soy-based foods, diets and prototype human foods. In rats, progressive decreases in zinc bioavailability were observed with a freeze-dried soy beverage, full-fat flour and soy-protein isolate, respectively (17, 20). Texturized-vegetable-protein meat-extenders and meat-substitutes based on soy protein have been reported to produce significantly less growth and to impair zinc status in rats, when compared to a diet of similar zinc content based on egg albumin (18, 19). However, using the slope-ratio assay for rat femur zinc content, no differences in zinc bioavailability were found between milk

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and isolated soy protein–based infant formulas (16).

A few comparable observations have been made in human subjects. Zinc balance studies in normal infants showed a comparable retention of zinc from a soy-based formula as compared to a milk-based infant formula (23). In Jamaican preschool children recovering from severe protein-energy malnutrition, Golden and Golden (22) reported a greater fall in plasma zinc, a slower rate of weight gain, and a higher energy cost of tissue synthesis in marasmic children consuming a soy-based diet than comparable patients on a milk-based regimen. These conflicting observations in animals consuming prototype human foods or in children receiving soy-based formulas may be explained, in part, by the observations of Rackis and associates (8, 12) that zinc bioavailability in only some, but not all, soy isolates is poorer than that of soybean meal, or by those of Vohra and Kratzer (25) that presumably similar soy isolates may have markedly different effects on the zinc nutriture of chicks. Thus, it is important to determine through direct human studies, whether or not changes in the nutritional quality of the diet might result from increased substitution with soy-protein sources.

Methodological limitations have hampered the direct experimental investigation of zinc absorption from meals based on soy foods in subjects. The approaches available include balance studies and isotopic techniques. Zinc balance studies (26–28) provide only approximate data on intestinal absorption due to the large and variable contribution of endogenous zinc to the fecal zinc output. Recently, ^{65}Zn has been used in human studies as a tracer in composite meals (29, 30), but its application is limited due to the excessively long (245 day) radioactive half-life of the isotope. Although the isotope, ^{69m}Zn has been introduced into human zinc absorption research (31, 32), the short half-life of this isotope (13.9 hours) limits its use in prolonged metabolic or nutritional studies, and the radiation exposure is equivalent to that from conventional doses of ^{65}Zn.

As described earlier (33–40), stable isotopic tracers offer a viable, nonhazardous alternative to the use of radioisotopes. Their use, however, is complicated due to several factors related to their natural abundance and the methodology used for their quantitative determination (33, 34). In the present investigation, the stable isotope of zinc, ^{70}Zn, was used to label experimental diets based on cow's milk, beef or an isolated soy protein. The diets were consumed by young men under metabolic balance conditions. This approach required the development of analytical methodology for determination of ^{70}Zn in feces and an appropriate method for the estimation of the absorption of the stable isotope tracer.

MATERIALS AND METHODS

Subjects

The subjects in the experiments were young male MIT students: five participated in the first experiment, involving use of liquid formula diets; a total of 10 subjects volunteered for the second study. They were all in good health as indicated by normal findings on a medical history, physical examination and routine biochemical determinations. They agreed to participation in the study after its nature and purpose had been fully explained, and they were required to sign consent forms. The experiments were approved by the MIT Committee on the Use of Humans as Experimental Subjects and the Executive Committee of the Clinical Research Center (CRC). The subjects were ambulatory, and the studies were conducted on an outpatient basis, by using the diet kitchen of the Department and the sample-processing laboratory of the CRC. The subjects were simultaneously participating in studies of iron absorption, which used stable ^{58}Fe. The methodological issues for this phase of the study have been considered elsewhere (37) and the results for iron will be described in a separate report.

Experimental diets

Subjects consumed all of their meals under the supervision of a research dietitian. Total caloric intake was constant throughout the study for each individual. It was based on an estimate of customary energy intake, as derived from a dietary history. The diet was

fed as three equal meals at about 0800, 1200 and 1700 hours.

In the first experiment (experiment 1), three isonitrogenous experimental liquid formula diets, supplying 0.8 g protein per kilogram per day were employed: *1*) a milk diet (diet 1), based on a nonfat dry milk as the exclusive protein source; 2) the isolated soy protein diet (diet 2), based on Supro-620 (Ralston Purina Co., St. Louis, MO); and 3) the soy/milk diet (diet 3), consisting of equal portions of nitrogen from milk and Supro-620 (table 1). In addition to the basic liquid formula meals, energy was provided from zinc-free, protein-free supplements and included cornstarch pudding, protein-free cookies and apple juice. Apple juice was consumed at each meal and provided 75 mg of ascorbic acid three times daily. To assure adequate micronutrient intakes zinc- and iron-free multivitamin/mineral preparations were consumed in capsular form each evening (table 1). Zinc and iron intakes were adjusted to achieve a constant and known intake of about 15 mg daily (see table 6). The zinc supplied by distilled water used for drinking was not measured.

The second experiment (experiment 2) was designed to explore zinc absorption with isonitrogenous diets based on beef protein or the isolated soy protein prepared in the form of a bologna. Total protein intake was 1.0 g per kilogram per day. In addition, because the first experiment supplied a generous intake of ascorbic acid from apple juice, the effect of a relatively high dietary intake of ascorbic acid on zinc absorption was examined. During the initial phase of the experiment two diets were evaluated. The first diet based on isolated soy protein alone (diet 4) was given for 19 days. During the first 12 days four subjects received, in addition to a multivitamin capsule, a supplement of 75 mg ascorbic acid at each meal, while another four subjects received only the ascorbic acid provided by the multivitamin capsule. For the remaining 7 days this procedure was reversed for each group. The second diet was based on beef protein, obtained from a single lot of fresh lean beef (diet 5), and this was given for 12 days. The soy-based diet was supplemented with inorganic zinc, as $ZnCl_2$, to achieve a total zinc intake close to that supplied by the beef diet alone (table 2). Micronutrients, energy supplements and water were supplied as in experiment 1.

TABLE 1

Composition of formula diets, based on dried skim milk or isolated soy protein (Supro-620) as protein sources[1]

Ingredient of formula	Dietary protein source		
	Milk	Milk/ Supro-620	Supro-620
		g	
Nonfat dry milk[2]	155.8	77.9	—
Supro-620[3]	—	31.3	62.6
Lactose[4]	—	38.7	77.4
Polycose[5]	101.4	102.2	103.1
Corn oil[6]	114.6	115.0	115.4
Avicel[7]	5.0	5.0	5.0
K_2HPO_4[8]	0.4	3.2	6.1
$Ca_{10}(OH)_2(PO_4)_6$[8]	—	—	2.0
NaCl[9]	1.2	1.2	1.2
Water	450	450	450

[1] Amount of formula shown for a 70-kg subject. Additional calories were supplied by protein-free cookies; cornstarch pudding, gingerale and apple juice. Also the following supplements were supplied: One-A-Day Vitamin (Miles Laboratories, Inc., Elkhart, IN). Vitamin A, 5000 IU; riboflavin, 1.7 mg; vitamin E, 15 IU; niacin, 20 mg; vitamin C, 70 mg; pyridoxine, 2 mg; folic acid, 0.4 mg; cyanocobalamin, 6 μg; thiamin, 1.5 mg; vitamin D, 400 IU. ¼ Tablet D-pantothenic acid (12 mg) (Wm. T. Thompson Co., Carson, CA). Trace mineral capsule (0.7 g) composition as follows: MgO, supplying 398 mg Mg; $Cr_2(SO_4)_3 \cdot 4H_2O$, supplying 0.168 mg Cr; $CuCl_2 \cdot {}^2H_2O$, supplying 2 mg Cu; $MnSO_4 \cdot H_2O$ supplying 0.83 mg, Mn; Na_2SeO_3, supplying 0.037 mg Se; KI, supplying 0.29 mg I. Choline (choline bitartrate) 2 each daily supplying a total of 500 mg (Plus Products, Irvine, CA). 3 ml of an Fe and Zn solution: 1 ml added to each of the three meals to supply total daily Fe and Zn intakes of about 15 mg each. Dical - D - Wafers (Abbott Laboratories, North Chicago, IL). Each provides 232 mg CA; 180 mg P. K - Lyte Tablets (Mead-Johnson Laboratories, Evansville, IN). One tablet provides 25 mEq K (as potassium bicarbonate). The mineral and vitamin supplements were taken in the evening and without a meal. [2] Non-Fat Dry Milk; (Lot No. 786-29-3; Land O'Lakes Co.). [3] Supro-620; Ralston Purina Co., St. Louis, MO. Phytic acid content estimated to be 1.5% (provided by manufacturer). [4] Reagent grade (M.C.B., Norwood, OH). [5] Polycose (Glucose polymers; Ross Laboratories, Columbus, OH). [6] Mazola corn oil (Best Foods, CPC International, Inc., Englewood Cliffs, NJ). [7] Microcrystalline cellulose; donated by FMC Corp., Philadelphia, PA. [8] Reagent grade (J. T. Baker Chemical Co., Phillipsburg, NJ). [9] Sodium chloride crystals (A. R. Mallinckrodt, Inc., Paris, KY).

TABLE 2

Composition of bologna products and diets given to subjects in experiment 2

Protein source and mineral supplement	Protein per 100 g	Moisture per 100 g	Fat per 100 g	Iron	Zinc	Daily amounts for a 70-kg subject[1]
				ppm	*ppm*	*g*
Supro-620 + zinc	13.0	56.2	21.9	25.9	25.4	538
Beef + iron	13.4	55.7	23.5	27.7	29.2	522

[1] Additional calories were supplied with protein-free cookies, cornstarch pudding, gingerale and apple juice together with vitamin and mineral supplements, as described in table 1.

Isotopic labeling of diets

Six consecutive meals served during 2 days of each dietary period were labeled extrinsically with ^{70}Zn, as $^{70}ZnCl_2$ (Oak Ridge National Laboratories, Oak Ridge, TN). Beginning on day 5 of each dietary period (experiment 1) six consecutive liquid formula meals were consumed, each containing 292 μg ^{70}Zn as the extrinsic tag. The tracer was added the day before the meal was eaten, mixed into the formula and refrigerated until shortly before the meal was taken. Thus, the daily intake of ^{70}Zn from the extrinsic tag during each day of the isotope enrichment period was 876 μg. In the bologna sausage studies (experiment 2), ^{70}Zn, as $^{70}ZnCl_2$, was added to each of the six consecutive meals by mixing the isotope 1 day before in a mince of the bologna. The total amount of extrinsic ^{70}Zn administered as tracer during each 2-day period was 1000 μg. The tracer was given on days 6 and 7 and on days 13 and 14 of the 19-day soy period and on days 6 and 7 of the 12-day beef period.

Fecal collections

Each subject in the first experiment with the liquid formula diets was studied continuously during a total of 56 days, divided into four consecutive diet periods of 14 days each. All subjects consumed each of the three experimental diets (1–3) in a randomized order (table 3). During the fourth period, the diet that had been consumed in the first period was repeated for four subjects. Each bowel movement during the entire 56-day period was collected quantitatively, identified and kept frozen until processed for determination of ^{68}Zn and ^{70}Zn. A fecal marker (carmine red) was added to the first meal and the last meal of each isotopic labeling period, and its appearance in a subsequent defecation noted and later compared to the fecal appearance of added stable isotope, as described in the results section.

With the bologna sausage regimens, the diet periods were of variable duration as described above. Not all of the 10 subjects participated in all diet periods, as indicated in

TABLE 3

Order of diet periods followed by young men studied for effects of isolated soy protein on zinc absorption experiment 1

Subject initials	Order of diet period			
	1	2	3	4
DC	Milk	Soy	Soy/milk	Milk
JK	Soy/Milk	Soy	Milk	
TS	Soy	Soy/Milk	Milk	Soy
GZ	Soy/Milk	Milk	Soy	Soy/Milk
LL	Soy	Milk	Soy/Milk	Soy

the results section. Fecal collections, without use of markers, were continued throughout this experiment.

Isotopic determinations

For fecal samples from experiment 1, the individual samples were weighed accurately to the nearest 100 mg and homogenized with an equal amount of deionized, distilled water. An aliquot of the homogenate was freeze-dried to a constant weight. A subsample of about 1 g of each freeze-dried aliquot was carefully weighed and spiked with ^{65}Zn tracer. It was then digested with concentrated nitric acid. For the second study (experiment 2), involving isotopically labeled bologna sausage, a modified approach was used. After weighing and homogenizing the stools, a constant percentage of each fecal homogenate from the first five consecutive stools produced after oral administration of ^{70}Zn was weighed accurately and combined to form a fecal pool for the respective dietary period. This pool contained all of the unabsorbed isotope as described in the results section. The fecal sample was then submitted to a series of chemical operations, previously described in detail (41), to separate zinc from other constituents in the fecal matrix. After preirradiation chemical separations, each sample was irradiated for 10 minutes at a thermal neutron flux of 5×10^{13} ncm^{-2} S^{-1} in the MITR-II. The irradiated sample was then subjected to postirradiation chemical separation and the separated zinc was counted for ^{69m}Zn [^{68}Zn (n,γ)] and ^{71m}Zn [^{70}Zn (n,γ)] by using a large volume lithium drifted germanium or Ge (Li) detector and multichannel analyzer (Canberra Industries, Model 8180, Meriden, CT).

Calculation of zinc absorption based on fecal monitoring of isotope ratios

For experiment 1, we explored various approaches for calculating zinc absorption. In using a method based on naturally occurring stable isotopes, it must be recognized that ^{70}Zn in the fecal pool originates from two sources: *1*) the unabsorbed fraction of the ^{70}Zn originating from all sources of fecal zinc other than the administered label, that is, dietary zinc; and *2*) endogenous zinc secretions and any source of zinc contamination. However, regardless of the source, the ^{70}Zn:^{68}Zn ratio for all *unenriched* sources of zinc contributing to the total zinc content in a fecal sample is constant and known precisely. Thus, ^{70}Zn in the fecal pool from all sources other than the administered label can be determined accurately by direct measurement in the same fecal pool of the ^{68}Zn content. Hence, the expression for calculating the fractional absorption (F) of the ^{70}Zn dose is as follows (41):

$$F = \frac{A^{\circ}_{0,^{70}Zn} - (A_{f,^{70}Zn} - R \times A_{f,^{68}Zn})}{A^{\circ}_{0,^{70}Zn}}$$

where $A^{\circ}_{0,^{70}Zn}$ = ^{70}Zn in the administered dose of tracer; $A_{f,^{70}Zn}$ = the total ^{70}Zn in the fecal pool; R is mass isotopic ratio of ^{70}Zn:^{68}Zn for natural zinc; $A_{f,^{68}Zn}$ is the quantity of ^{68}Zn in the fecal pool.

Statistical analysis

Comparisons of zinc absorption from the various diets were accomplished by using the two-sample and paired Student's t-tests (42).

RESULTS

Precision of quantitative determination of zinc isotopes in feces

The inter- and intrasubject variation in ^{70}Zn content of unenriched stool samples, which reflect variations in total fecal zinc, were found to be significantly greater than 10%. This observation is based on both the ^{70}Zn in the total stool, and the ^{70}Zn content per unit weight of freeze-dried stool sample (table 4). This variation is largely due to the variability in daily stool volume within each diet period. The ^{71m}Zn:^{69m}Zn peak area ratios, which are directly proportional to isotopic weight ratios, vary around 10% of the mean value, reflecting the true precision of isotope abundance measurements for fecal samples that are *unenriched* with respect to ^{70}Zn. As discussed previously (41), the counting statistics associated with the gamma line for ^{71m}Zn (neutron-capture product of ^{70}Zn) limit the precision of ^{70}Zn measurements in unenriched fecal samples to 5–10%. When

TABLE 4

Variation in the content of zinc isotopes in individual stool samples not enriched with ^{70}Zn experiment 1

Subject	No. of stools for analysis	Fresh weight	Dry:Wet ratio	$\mu g^{70}Zn$ per stool	$\mu g^{70}Zn$ per gram dry stool	^{70}Zn:^{68}Zn ratio
		g				
DC	52	114 ± 45[1]	0.23 ± 0.12	66 ± 29 (44)	2.75 ± 0.91 (33)	0.0100 ± 0.0013[2] (13)[3]
LL	54	98 ± 32	0.23 ± 0.10	.87 ± 36 (41)	3.63 ± 0.91 (25)	0.0098 ± 0.0012 (13)
GZ	32	119 ± 48	0.29 ± 0.16	125 ± 52 (42)	3.56 ± 0.56 (16)	0.0096 ± 0.0013 (13)
TS	37	128 ± 90	0.25 ± 0.24	111 ± 75 (68)	4.28 ± 1.37 (32)	0.0102 ± 0.0011 (10)
JK	52	102 ± 43	0.25 ± 0.14	91 ± 23 (25)	3.57 ± 0.75 (21)	0.0110 ± 0.0013 (11)

[1] Mean ± SEM. [2] These results are peak area ratios for 387 kev and 439 kev gamma lines, respectively. [3] Number in parentheses is percent relative standard deviation.

oral ^{70}Zn is administered, however, stool enrichment with the isotope improves the counting statistics to about 1%.

Kinetics of ^{70}Zn excretion in feces

Variations in the rate of isotope excretion among different subjects and among different diet periods within the same subject were evaluated. Figure 1 illustrates the change in ^{70}Zn:^{68}Zn ratio in stools after administration of isotope with the same liquid meal. Table 5 shows the cumulative excretion of isotope after consumption of the liquid formula diets containing added ^{70}Zn. We had originally

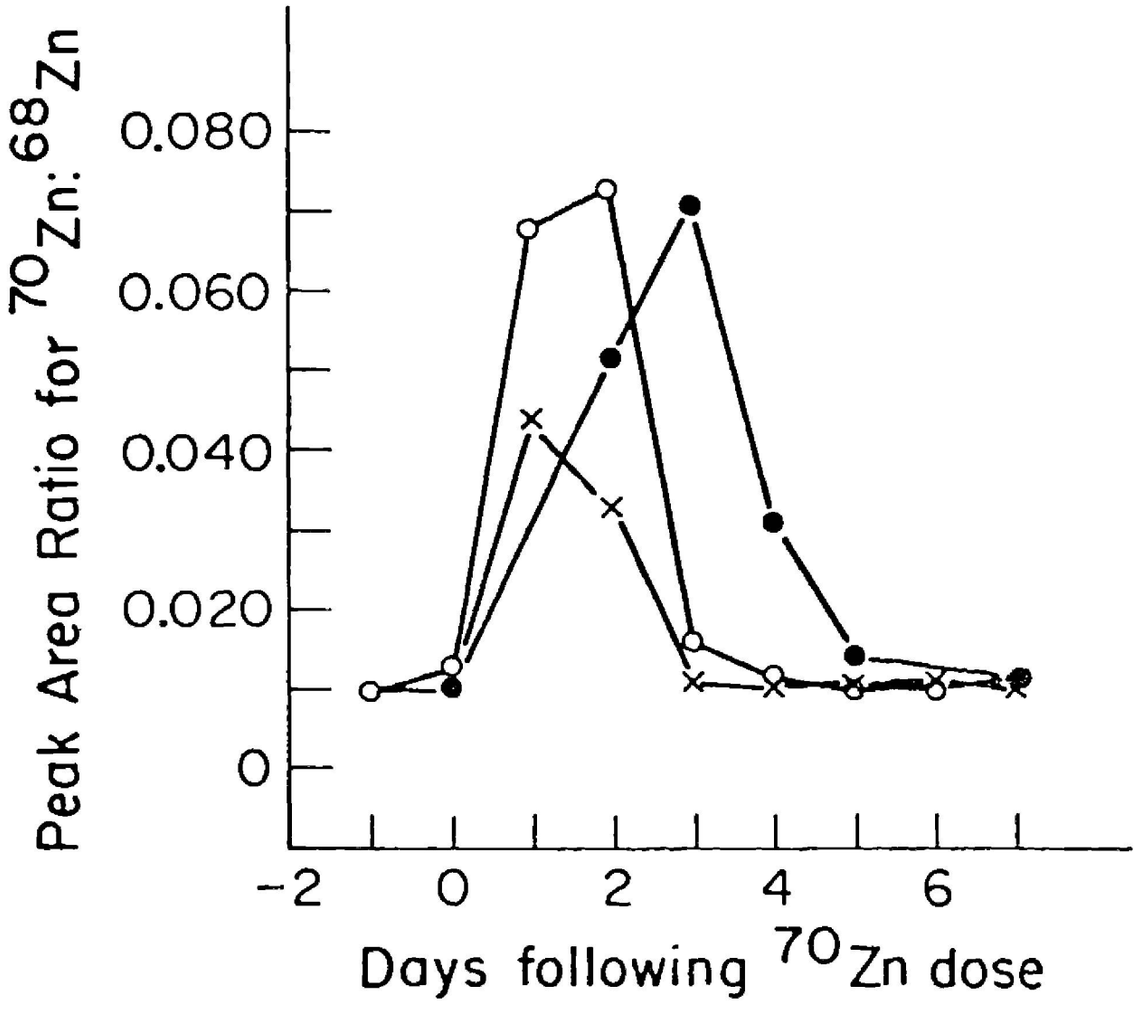

Fig. 1 Examples of change in enrichment of stools, as measured by peak area ratio of ^{70}Zn to ^{68}Zn in two subjects following oral administration of ^{70}Zn tracer (experiment 1). The curves refer to data for subject LL (○) during diet period 1, and for subject TS during periods 1 (×) and 4 (●). The individual points represent analysis of each consecutive stool sample obtained from each subject beginning just prior to isotope administration

TABLE 5

Rate of excretion in feces of unabsorbed dose of oral ^{70}Zn experiment 1

Subject	Diet period	Consecutive fecal sample no after ^{70}Zn dose				
		1	2	3	4	5
DC	1	2[1]	51	46	1	
	2	•29	63	8		
	3	•75	25			
	4	•69	31			
LL	1	4	35	47	6	8
	2	5	33	57	4	
	3	•55	44	1		
	4	•31	10	58		
GZ	1	• 9	62	28	1	
	2	•10	86	4		
	3	• 2	61	36		
	4	4	65	23	3	6
TS	1	•69	31			
	2	51	49			
	3	•17	77	6		
	4	•29	17	37	16	
JK	1	•51	41	7		
	2	•60	31	1	8	
	3	•46	39	14		

[1] Value is percent of total unabsorbed dose of ^{70}Zn recovered after administration of ^{70}Zn. • Indicates that a stool was obtained between isotope administration and the first stool containing a detectable increase in the ^{70}Zn:^{68}Zn isotope ratio.

thought that fecal markers would aid in monitoring the fecal output of stable zinc. A generally good correspondence between first appearance of enrichment levels of ^{70}Zn and of carmine red in the stools was observed in the first experiment, but in three instances the unabsorbed isotope preceded the marker. In these subjects, use of the marker to aid preparation of a pooled fecal sample would have resulted in about a 9% error in calculating fecal isotope recovery.

Usually, one defecation occurred between the first meal of the isotope enrichment cycle and the first stool to contain a detectable increment in the ^{70}Zn:^{68}Zn ratio. Moreover, most of the unabsorbed isotope was excreted in three consecutive defecations, although complete collection of as many as five stool samples appeared to be necessary for some subjects (table 5). From these findings, we conclude that, for routine purposes in adults, five stools should be collected, starting immediately after isotope administration, in order to quantitatively recover the unabsorbed isotope. Any effect of overpooling on the precision of isotope analysis, moreover, would be negligible. Hence, the pooling of five consecutive stools was used for purposes of isotope analysis in the second study.

Comparative zinc absorption with liquid formula diets

The fractional absorption of zinc for five subjects consuming the liquid formula diets (diets 1–3) is presented in table 6. Only the data from the first three consecutive diet periods have been used to determine the group means. The fractional absorption of tracer ^{70}Zn was 34 ± 4% (mean ± SEM) for the isolated soy protein diet (diet 2), 41 ± 4% for the whole cows' milk diet (diet 1) and 41 ± 7% for the soy/milk combination formula (diet 3). No statistical differences by paired *t*-test were observed between any two treatments ($P > 0.1$). For the four subjects who completed a fourth diet period, replicating that which had followed during the first diet period, the mean values for zinc absorption

TABLE 6

Absorption of ^{70}Zn from the soy (Supro-620) and milk protein diets (experiment 1)[1]

Subject	Soy	Milk	Soy/Milk
		%	
DC	31	59	72
LL	14	39	25
GZ	44	35	32
TS	24	42	50
JK	36	31	39
Mean	34	41	41
SEM	4	4	7

[1] For a 70-kg subject the daily zinc intake supplied by the soy protein diet was 3.3 mg from soy and 12 mg from zinc chloride; for the milk diet the distribution of zinc intake was 6.5 mg from the protein and 9.0 from the inorganic supplement; for the soy/milk diet, zinc from the proteins was 4.9 mg and from the supplement 10.5 mg.

were 32 ± 9 and 38 ± 4% for the first and final diet periods, respectively.

Zinc absorption from soy and beef bologna diets

Zinc absorption was not significantly affected by the generous intake of ascorbic acid, being 24 ± 5 and 36 ± 6 (mean ± SEM) in the absence and presence of supplemental ascorbic acid, respectively, during the soy-based-diet period (table 7). The mean value for fractional absorption of zinc during the two phases of the diet in which the isolated soy provided the sole source of protein was 30 ± 3. We have accepted this value as the best estimate of zinc absorption for the group of subjects receiving the soy isolate as the sole source of dietary protein (table 2). The fractional absorption of isotopic zinc tracer in the beef diet (diet 5) was 41 ± 4% in seven individuals (table 7). The difference between the diets based on the soy and beef products reached statistical significance ($P < 0.05$) when evaluated by two-sample Student's *t*-test. If the data for the eight studies in which the soy bologna was fed without added ascorbic acid are used for comparison, however, (table 7, column one) there is an apparent 41% reduction in tracer absorption as compared to the all-beef diet ($P < 0.01$).

DISCUSSION

The isolated soy protein (Supro-620) used in the present study has been found to be of high protein quality for both children (43) and adults (44). However, since concerns about a possible adverse impact of various soy-based foods on zinc bioavailability have arisen from observations in animal species, we were prompted to evaluate the effects of this soy product on zinc absorption in human

TABLE 7

Fractional absorption of extrinsic zinc from diets based on isolated soy protein and beef protein given to subjects in experiment 2

Subject	Fractional absorption			
	Isolated soy protein	Isolated soy protein plus ascorbate	Isolated soy protein (mean score)	Beef
		%		
MU	22	8	15	—
HG	8	51	29	33
WS	17	54	35	30
GS	30	21	25	59
JM	30	28	29	36
SW	3	59	31	41
MR	46	39	42	—
CR	34	29	35	—
MD	—	—	—	53
DC	—	—	—	35
Mean ± SEM	24±5	36±6	30±3	41±4

subjects. Hence, we developed and evaluated methodology for the safe and reliable determination of zinc absorption using stable isotope tracers, fecal monitoring, and analytical procedures for chemical separation and determination of ^{70}Zn and ^{68}Zn by neutron activation analysis (41).

Lutwak (45) has discussed the principles of using fecal monitoring of isotopic tracers to determine intestinal absorption in human subjects. Although his data were drawn from extensive experience with radiocalcium, the principles can be generalized for all mineral isotopes, including stable isotopes. It is evident that two important aspects of the normal physiological metabolism of a given mineral nutrient will influence the design and interpretation of fecal monitoring experiments: *1*) the degree of uptake of the dietary mineral from the lumen that is trapped in the mucosal cells, not transferred to the body, and subsequently excreted in the feces after sloughing of the mucosal cells (mucosal trapping); *2*) the contribution of endogenous minerals from the total-body pool to the daily fecal excretion (reentry). For zinc, the endogenous contribution of total fecal output is considerable (46), and it has been suggested that mucosal trapping may be involved in the normal intestinal regulation of zinc absorption (47). Thus, to avoid overestimation of the net absorption of tracer, complete collection of fecal samples containing *all* unabsorbed isotope must be accomplished. Because it was not possible to determine a priori the appropriate interval for fecal collection, we established, for normal young adults under the conditions of our first experiment, that five consecutive stools passed after the oral administration of a dose of stable isotopic zinc contained all of the unabsorbed label, within the detection limits of our analytical methodology. Furthermore, based on the extrapolation from the data of Aamodt et al. (31) on the reentry of ^{69m}Zn tracer (a radioisotope) during a 5-day period, we can be confident that the changes in ^{70}Zn:^{68}Zn ratio of fecal samples due to reentry of absorbed ^{70}Zn tracer would not be detectable.

Experimental data on zinc absorption from the human intestine are limited. An expert committee of the World Health Organization estimated that the biological availability of zinc in regional diets throughout the world would vary from 10–40% (49). For comparative purposes, a listing of results from human zinc absorption studies employing isotopic tracers is presented in table 8. A range of 36–60% for zinc absorption from aqueous

TABLE 8

Comparison of zinc absorption data from isotopic studies in human subjects

No. of subjects	Isotope	Vehicle	Zinc absorption		
			Mean	Range	Reference
			%		
5	^{65}Zn	Water	36	27–44	(49)
5	^{65}Zn	Water	66	58–77	(50)
13	^{65}Zn	Water	54	19–99	(51)
5	^{69m}Zn	Water	61	41–79	(32)
22	^{70}Zn	Meal	38	n.a.[1]	(35)
11	^{65}Zn	Bread	25	12–32	(52)
5	^{65}Zn	Meal	32	24–40	(52)
8	^{65}Zn	White bread	38	30–52	(29)
8	^{65}Zn	Wholemeal bread	17	9–23	(29)
13	^{65}Zn	Wholemeal bread, cheese, milk	14	9–22	(29)
11	^{65}Zn	Chicken	36	25–47	(30)
6	^{65}Zn	Soybeans	20	13–26	(30)
5	^{70}Zn	Liquid (soy) formula	34	14–44	Present study
10	^{70}Zn	Bologna (soy)	30	15–42	Present study
7	^{70}Zn	Bologna (beef)	41	30–59	Present study

[1] n.a., not available.

solutions (32, 49–51) and of 14–38% in the presence of various foods (29, 30, 35, 52, 59) has been determined. Thus, the mean absorption from our liquid formula, in the range of 34–41%, and from the beef- and soy-bologna diets, 41 and 30%, respectively, appear to be reasonable in the context of the limited data for zinc absorption in human subjects.

Observations in a variety of experimental animal models prompted concern for the adequacy of zinc availability from soy-based diets, due to a variable phytate content in different soy-protein foods (19). Phytic acid has been implicated directly as the factor responsible for the reduced biological availability of zinc from various soy products (19, 20). However, in human studies Sandström and Cederblad (30) found no change in zinc absorption when chicken meat was partially replaced by defatted soy flour. Greger et al. (28) also found no effect of substituting 30% of a lunchtime meat with a texturized-soy-protein on the apparent absorption of intrinsic zinc, nor did it affect the absorption of 6 mg of elemental zinc added to the lemonade consumed with the test meals. Our experiments with liquid formulas and with bologna products in adults also failed to reveal any major effects on fractional absorption of a zinc tracer after substitution of conventional protein sources with an isolated soy protein. The fractional absorption of zinc from the beef bologna diet was slightly higher than that from the zinc-supplemented soy-based diet (experiment 2), but the nutritional significance of the difference is difficult to judge, for reasons discussed below.

An accurate nutritional interpretation of our studies depends on the validity of the "extrinsic-labeling" approach for zinc. Evans and Johnson (54) compared the absorption of ^{65}Zn from corn and liver fed to rats. In half of the experiments, hydroponically labeled corn and bioorganically labeled liver were used as sources of intrinsic radiozinc; in the remaining animals, exogenous, carrier-free ^{65}Zn was added to the meals as an extrinsic tag. An approximately 1:1 correspondence between the results obtained with intrinsically labeled and extrinsically labeled foods was observed. On the other hand, Forbes and associates (18, 21) in a series of nonisotopic studies compared the bioavailability of zinc, as determined by the slope-ratio method for tibia or femur zinc in rats, from full-fat soy flour, freeze-dried soy beverage, soy-concentrate and inorganic zinc carbonate each as sole sources of dietary zinc. The relative bioavailability for the respective soy products tested in their studies was 34, 40 and 20% of the availability of zinc from zinc carbonate. However, when zinc carbonate was added in the presence of the various soy products its bioavailability was about 77–94% of that found in the presence of a soy-free diet based on egg albumin. They have interpreted these results to indicate that intrinsic zinc in soy products and zinc in the remainder of the diet do not form a common absorptive pool, and have been careful to use the terminology "biological availability of zinc in and as influenced by various soy products" (21).

The issues of extrinsic labeling of diets with isotopic zinc have now been raised in human studies. Sandström and co-workers (29, 30) have extrinsically labeled a series of meals with trace doses of ^{65}Zn and have employed a value for the estimate of total zinc absorption derived from multiplying the total zinc in a test meal by the fractional absorption of the tracer. This would imply that the extrinsic tag had equilibrated with a common zinc pool, but, despite the findings of Evans and Johnson (54), the validity of this expression for total zinc absorption in human studies cannot be accepted unequivocally. It is known that chemical differences create two distinct pools of iron: a nonheme (inorganic) pool and a heme (organic) pool (55). We have insufficient knowledge of the mechanisms concerned with the intestinal handling of dietary zinc to preclude the possibility that zinc has more than one absorptive pool or that, consequently, a $^{70}ZnCl_2$ tracer might reflect only *part* of the total zinc absorbed from a meal. To estimate accurately the disposition of all of the zinc in a meal, the fraction of zinc in food that is freely exchangeable with the stable isotope tracer must be established.

The question of complete and uniform miscibility of the isotopic tracer with the remainder of the zinc in the meals is also important in light of the observations by Sandström and co-workers (29, 30) that the *total*

zinc in the diet has a marked effect on the fractional absorption. These investigators found that the absorption of radiozinc tracer from white bread, with a total intrinsic zinc content of 0.4 mg, was 38.2 as compared to 13.2% when the total zinc content of the meal was raised to 3.6 mg by addition of zinc chloride supplement. In the present studies, we adjusted the *total* zinc intake of our various regimens to achieve a constant daily amount. In the liquid formulas, the intrinsic zinc of the milk and the isolated soy protein/milk diets was approximately equivalent, and supplemental $ZnCl_2$ represented 60–70% of total zinc intake. With the bologna products, however, the mean intake of *intrinsic* zinc from the beef (diet 5) was 15.7 mg, whereas that from an isonitrogenous intake of the isolated soy protein (diet 4) was 2.5 mg and total zinc intake adjusted by addition of a supplement of $ZnCl_2$ to the soy-bologna diet. If the isotopic tracer reflects more closely the inorganic zinc in the diet, the dilution factor of the supplemental zinc may entirely explain the lower fractional absorption of the ^{70}Zn tracer with the soy bologna as compared with the beef bologna.

Another important consideration regarding the finality of the conclusions about the efficiency of zinc absorption from the respective foods is the inherent intraindividual variability in the method and the small number of subjects enrolled in this our preliminary inquiry into bioavailability using the stable isotope tracer method. With the number of observations and the variance in the liquid formula experiments, no significant difference was found among treatments. However, if the 20% lower absorption of zinc from the soy formula were found to be consistent in a sufficiently large sample of subjects, it would have implications for the formulation of such products as the sole source of nutrition in the infant. Similarly, when we pool our data on soy bologna to give us the best statistical approximation, the 35% reduction in zinc absorption from the soy-based product as compared to beef is not statistically significant. With a larger sample size, however, the same considerations that apply to the observations with liquid formulas would be applicable here as well. If just the data from the soy-bologna diet without added ascorbic acid are used for comparison, a statistically significant reduction of zinc absorption is observed, suggesting that beef favors the absorption of extrinsically labeled zinc.

The preceding discussion raises several significant issues regarding the interpretation of experiments that use an extrinsic stable isotopic tracer of zinc. First, a sufficiently large sample size must be used to improve the certainty with which the statistical conclusions can be asserted. Second it must be recognized that the relatively larger amounts of extrinsic ^{70}Zn tracers (as compared to a radiozinc tracer) may not have been freely exchangeable with the zinc bound to organic molecules in the soy-based diets. Thus, the most prudent interpretation of our data would be that within the limits of variance, soy-isolate and milk have equivalent effects on the absorption of *extrinsic* zinc. Because commercial infant foods are likely to be adjusted to a given zinc content with a zinc salt, our findings are relevant to practical nutritional concerns. With the bologna sausage preparation, the issues are more complex. Our data suggest the possibility that beef might favor the absorption of the extrinsic zinc in a meal. Whether this, if true, would be significant to zinc nutriture by virtue of the effect meat might have on the zinc in the remainder of a given meal, is unclear at present. In agreement with Sandström and Cederblad (30), however, we conclude that the relatively low content of zinc in soy protein isolates as compared with beef must be considered in the overall evaluation of soy-substituted meat products in human diets. Given the small sample sizes in our treatment groups, the dietary conclusions are still tentative at the present time.

In conclusion, refinements in the application of stable isotope tracer methodology in the present experiments improve the sensitivity and versatility of the approach reported earlier by King and associates (35). These have been achieved through a combination of: improved neutron activation procedures; multi-step, pre- and postirradiation radiochemical separations; and continuous fecal collections during prolonged balance periods. The clinical procedures and analytical method provide an urgently needed, nonhazardous and noninvasive approach for

the direct examination of zinc absorption by using an isotopic tracer in adult subjects, and in young children as well. Questions still remain about the chemistry of extrinsic labeling of meals with inorganic zinc. These have been highlighted by the present findings. Intrinsic incorporation of stable isotopes into foods by hydroponic or bioorganic labeling must be compared with extrinsic labeling at the level of total dietary zinc used in our studies before all of the interpretative issues involved in the application of this technique for evaluation of soy products and zinc absorption can be fully resolved. We have recently initiated studies with chicken meat biologically labeled with ^{68}Zn to examine the exchangeability of intrinsic and extrinsic stable isotope tracers for measurement of zinc absorption (56). Furthermore, in view of the role that soy can play as a substitute or extender of animal products, evaluation of the bioavailability of zinc to humans from other types of soy-based foods and the relationship between the level of zinc intake and major sources of food protein are areas that deserve investigation.

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