^{54}Mn Absorption and Excretion in Rats Fed Soy Protein and Casein Diets (42852)

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Abstract. **Rats were fed diets containing either soy protein or casein and different levels of manganese, methionine, phytic acid, or arginine for 7 days and then fed test meals labeled with 2 μCi of ^{54}Mn after an overnight fast. Retention of ^{54}Mn in each rat was measured every other day for 21 days using a whole-body counter. Liver manganese was higher ($P < 0.0001$) in soy protein-fed rats (8.8 μg/g) than in casein-fed rats (5.2 μg/g); manganese superoxide dismutase activity also was higher in soy protein-fed rats than in casein-fed rats ($P < 0.01$). There was a significant interaction between manganese and protein which affected manganese absorption and biologic half-life of ^{54}Mn. In a second experiment, rats fed soy protein-test meals retained more ^{54}Mn ($P < 0.001$) than casein-fed rats. Liver manganese (8.3 μg/g) in the soy protein group was also higher than that (5.7 μg/g) in the casein group ($P < 0.0001$), but manganese superoxide dismutase activity was unaffected by protein. Supplementation with methionine increased ^{54}Mn retention from both soy and casein diets ($P < 0.06$); activity of manganese superoxide dismutase increased ($P < 0.05$) but liver manganese did not change. The addition of arginine to casein diets had little effect on manganese bioavailability. Phytic acid affected neither manganese absorption nor biologic half-life in two experiments, but it depressed liver manganese in one experiment. These results suggest that neither arginine nor phytic acid was the component in soy protein which made manganese more available from soy protein diets than casein diets.** [P.S.E.B.M. 1989, Vol 190]

Plant proteins such as soy protein are considered to be poorer sources of available minerals than animal proteins (1–5). Several investigators have reported that soy protein was a poor source of manganese for animals (2–5). Davis *et al.* (3) found that the addition of 10 mg of manganese/kg or 0.07% EDTA to soy protein diets containing 6.5 mg of manganese/kg significantly increased the growth of chicks. Recently, Halpin *et al.* (4) reported that the absorption of inorganic manganese, determined by a slope-ratio assay, was 40% greater in chicks fed casein diets than in those fed corn-soy diets. Keen *et al.* (5) observed that ^{54}Mn uptake in 14-day-old rats was lower from soy formula than from cow milk, cow milk formula, or human milk. High concentrations of phytic acid in soy products might be responsible for poor availability of manganese, because Davies and Nightingale (2) observed that 1% phytic acid added to casein diets reduced whole-body content of manganese in rats after 21 days of feeding. Differences in amino acid composition between soy and non-soy products may also affect manganese bioavailability.

Direct determinations have not been made of the effect on manganese absorption or turnover of dietary protein except for milk and infant formula (5). We have been interested in the relative effects of dietary manipulation on manganese absorption and turnover because bioavailability is the sum of these two processes; i.e., the net effect of dietary composition on manganese utilization. The initial objective of our study was to examine the effects of soy protein and casein on absorption and excretion of ^{54}Mn in growing male rats. Two initial experiments showed that manganese in soy protein diets was more available to rats than manganese in casein diets. Some of the major differences in the composition of soy protein and casein are methionine, arginine, and phytic acid concentrations. Therefore, we also investigated the effects of added methionine, arginine, and phytic acid on manganese metabolism.

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Materials and Methods

Animals and Diets. Male 21-day-old rats (Long-Evans) were either bred at our facility or purchased

from Harlan Sprague-Dawley (Indianapolis, IN).[1] The animals were housed individually in stainless steel wire cages in a temperature-controlled room with 12-hr cycles of light and dark. Animals were fed test diets *ad libitum* for the duration of each experiment. Demineralized water was available to the rats at all times. Daily food intake and weekly growth rate were monitored. The composition of the basal diet is shown in Table I (6).

Concentrations of certain minerals were higher in soy protein than in casein protein. Therefore, appropriate amounts of manganous carbonate in sucrose were added to casein diets so that casein and soy protein diets in each experiment contained the same concentration of dietary manganese. Ferric citrate, calcium carbonate, and magnesium sulfate were added to casein diets to equalize the dietary mineral content with that in soy protein diets in Experiments 1, 2, and 4. The final dietary concentrations were 41.7 mg of iron/kg, 5.4 g of calcium/kg, and 0.3 g of magnesium/kg diet. The phytic acid concentration in the soy protein used was 1.2%.

Experimental Design. There were four separate experiments each arranged in a 2 × 2 factorial design. The objective of Experiment 1 was to examine the effect of types of protein and levels of dietary manganese on manganese metabolism in rats. Animals (six per group) were divided by an equal mean weight (78 ± 9 g) and fed soy protein or casein diets containing 2.5 mg or 51.7 mg of manganese/kg. Rats were fed diets for 1 week. Then, after a 20-hr fast, 2 μCi of ^{54}Mn were administered in test meals. Test meals were made of 4 g of soy protein diet or casein diet containing 2.5 mg of manganese/kg; thus, the test dose was 10 μg of manganese. Two hours after ^{54}Mn administration, ^{54}Mn retention of each rat was measured using a small animal whole-body counter equipped with a multichannel analyzer (Nuclear Data Inc., Schaumburg, IL). The measured radioactivity was corrected for background and decay. Four hours after ^{54}Mn administration, rats were allowed to resume eating their respective diets. The ^{54}Mn retention was measured three times a week for 3 weeks.

Percentage of apparent absorption of ^{54}Mn was defined as the intercept obtained by extrapolating the linear portion of a plot of ln % retention vs time to Day 0 (7). Excretion of ^{54}Mn was expressed as the biologic half-life (BH) which is calculated using the equation:

$$BH = \frac{-\ln 2}{\lambda_b}$$

where λ_b is the slope of the linear portion of a plot of ln % retention of the oral dose vs time from Days 7 to 21 after ^{54}Mn administration.

In Experiment 2, animals (n = 6/group, mean weight 63 ± 13 g) were fed soy protein or casein diets containing 4.3 mg of manganese/kg with or without methionine (Sigma Chemical Co., St. Louis, MO) supplementation (0.3%) to examine the effect of types of protein and methionine on manganese bioavailability.

Administration of ^{54}Mn was as described in Experiment 1. However, the ^{54}Mn dose for each rat was only 0.5 μCi. Because of the low dose of ^{54}Mn, absorption and excretion could not be measured as described in Experiment 1. Instead, percentage of retention of ^{54}Mn on Day 7 after ^{54}Mn administration was used as a measure of ^{54}Mn absorption.

The purpose of Experiment 3 was to evaluate the effect on manganese bioavailability of adding arginine (L-arginine, free base; Sigma Chemical Co.) and phytic acid as sodium phytate (Aldrich Chemical Co., Inc., Milwaukee, WI) to methionine-supplemented casein diets containing 7.0 mg of manganese/kg. The calculated level of dietary arginine was 2%. The analyzed level of dietary phytic acid was 1.7%. Animals were divided into groups of equal mean weight (72 ± 13 g). Because soy-based diets were used in this experiment, no calcium or magnesium beyond that provided by the mineral mix was added.

Table I. Composition of Basal Diet[a]

Ingredients	Weight %
Casein (vitamin free) or soy protein[b] (Teklad, Madison, WI)	20
Sucrose (American Crystal Sugar Co., Moorhead, MN) or Cornstarch (Best Foods, Englewood Cliffs, NJ)	65
Corn Oil[c]	5
Cellulose (Teklad, Madison, WI)	5
AIN-76 Modified Mineral Mix (Teklad, Madison, WI) (manganese free)	3.5
AIN-76 Vitamin Mix[b] (Teklad, Madison, WI)	1.0
DL-Methionine (Teklad, Madison, WI)	0.3
Choline chloride (Life Technologies, Inc., Chagrin Falls, OH)	0.2

[a] Calcium carbonate, magnesium sulfate, and ferric citrate in addition to the amounts of these minerals provided by the mineral mix were added to casein diets to provide the same concentrations of the minerals in soy protein diets in Experiments 1, 2, and 4. A powdered sucrose-manganese mixture containing 1 mg of manganese/g or 4 mg of manganese/g was used to provide appropriate levels of dietary manganese. The adjustment of the weight for the additions was made at the expense of sucrose.
[b] Soy protein contained 1.2% phytic acid and approximately 2% carbohydrate.
[c] Corn oil contains 0.02% ethoxyquin (Pfaltz and Bauer Inc., Waterburg, CT).

[1] Mention of a trademark or proprietary product does not constitute a guarantee or warranty of the product by the U.S. Department of Agriculture, and does not imply its approval to the exclusion of other products that may also be suitable.

The design of Experiment 4 was similar to that of Experiment 3, except that the diets were not supplemented with methionine. In addition, calcium, magnesium, and iron were added to the casein diets so that they would contain amounts of minerals similar to those of the soy protein diets used in Experiments 1 and 2. Animals weighed 42 ± 7 g. Diets contained 5.1 mg of manganese/kg.

After 28 days of dietary treatment, rats in each experiment were fasted for 20 hr (overnight) prior to being killed. They were anesthetized with pentobarbital before blood was removed by heart puncture. Liver was collected, freeze dried, and ashed with nitric acid/hydrogen peroxide (8). Manganese was determined with an inductively coupled argon plasma emission spectrophotometer model 1140; (Jarrel-Ash, Waltham, MA). Heart was also collected to determine the activity of manganese superoxide dismutase (SOD) in Experiments 1 and 2 (9, 10).

Measurement of Dietary Phytic Acid. Dietary phytic acid was extracted with 1.2% hydrocyhloric acid that contained 10% sodium sulfate. Phytate was then precipitated with 0.4% ferric chloride and washed with 0.5 *M* hydrochloric acid as described by De Boland *et al.* (11) and Ellis *et al.* (12). Washed samples were mixed with 3 ml of nitric acid and 1 ml of sulfuric acid and ashed in a Kjeldahl flask. Phosphorous was determined by plasma emission spectroscopy. Phytic acid concentration was calculated on the basis of phosphorous concentration; we assumed that 1 mole of phytate contained 6 moles of phosphorous.

Data Analysis. All data were subjected to analysis of variance (13).

Results

Experiment 1. Because dietary treatment did not affect either feed intake or weight gain, they are not reported. There was no effect of protein source on manganese absorption or BH, but dietary manganese affected ^{54}Mn absorption and BH (Table II). A significant interaction between dietary manganese and protein affected both absorption and BH; the manganese effect on apparent absorption was more marked in rats fed soy protein than casein diets, and the manganese effect on BH was more pronounced in rats fed casein than soy protein diets. Soy protein-fed rats had more manganese in liver and activity of manganese SOD in heart than did casein-fed rats (Table II). Increasing dietary manganese was more effective in increasing liver manganese in casein-fed than soy protein-fed rats.

Experiment 2. The type of protein did not affect either feed intake or weight gain (Table III) but methionine supplementation increased feed intake and weight gain. The effect of methionine was more marked in rats fed soy protein diets than in those fed casein diets. There were significant effects of both protein and methionine on percentage of ^{54}Mn retention at Day 7; rats fed soy protein retained more ^{54}Mn than rats fed casein and methionine-supplemented rats retained more ^{54}Mn than unsupplemented rats. Soy protein-fed rats had significantly higher concentrations of liver manganese than casein-fed rats (Table III). Supplementation of methionine significantly increased manganese SOD activity in heart (Table III).

Experiment 3. Neither added arginine nor phytic acid affected daily feed intake and weight gain. The BH of ^{54}Mn in rats fed diets with added arginine at 2% was significantly longer than the BH in rats fed diets without added arginine (Table IV). Absorption of ^{54}Mn and concentrations of liver manganese were not affected by either added arginine or phytic acid (Table IV).

Experiment 4. Daily feed intake among rats of different groups was similar, but daily weight gain of rats fed added arginine was greater than that of rats fed diets containing no arginine (Table V). Dietary arginine or phytic acid did not affect absorption or BH of ^{54}Mn

Table II. Percentage of Apparent Absorption, BH of ^{54}Mn, Concentration of Manganese in Liver, and Activity of Manganese SOD in Heart of Rats Fed Different Types of Protein and Different Amounts of Dietary Manganese (Experiment 1)[a]

Diets	Manganese (mg/kg)	Apparent absorption (%)	BH (days)	Liver manganese dry wt (μg/g)	Manganese SOD[b] wet wt (units/g)
Soy protein	2.8	3.8	24	7.9	249
Soy protein	52.8	1.0	9	9.7	371
Casein	2.2	2.6	26	3.6	187
Casein	50.6	2.3	6	6.8	245
Root mean square error		0.8	2	0.8	52
Analysis of variance				*P*	
Protein		NS	NS	0.0001	0.0004
Manganese		0.003	0.0001	0.0001	0.0006
Protein × manganese		0.002	0.02	0.05	NS

[a] Means of six rats.
[b] One unit of activity reduces the auto-oxidation of pyrogallol (0.2 m*M*) by 50%.

Table III. Feed Intake, Weight Gain, Concentration of Manganese in Liver and Femur, Activity of Manganese SOD in Heart, and Percentage of Retention of ^{54}Mn in Rats Fed Diets Containing 4.3 mg of Manganese/kg and Different Types of Protein with and without Methionine Supplementation (Experiment 2)[a]

Diets	Feed[b] intake (g/day)	Weight[b] gain (g/day)	Liver manganese dry wt (μg/g)	Manganese SOD[c] wet wt (units/g)	$\%R_7$[d]
Soy protein/no methionine	15.2	5.7	8.5	299	3.4
Soy protein/with methionine	18.0	7.4	8.1	400	4.7
Casein/no methionine	18.0	7.1	5.5	263	2.6
Casein/with methionine	18.7	6.9	5.8	332	2.7
Root mean square error	1.9	0.8	0.6	97	0.9
Analysis of variance			*P*		
Protein	NS	NS	0.0001	NS	0.001
Methionine	0.03	0.06	NS	0.05	0.06
Protein × methionine	0.03	0.01	NS	NS	NS

[a] Means of six rats.
[b] Values measured between Days 14 and 28.
[c] One unit of activity reduces the auto-oxidation of pyrogallol (0.2 m*M*) by 50%.
[d] Percentage of retention 7 days after oral ^{54}Mn administration.

Table IV. Concentrations of Liver Manganese, Percentage of Apparent Absorption, and BH of ^{54}Mn in Rats Fed 7.0 mg of Manganese/kg with Different Amounts of Phytic Acid and Arginine in Methionine-Supplemented Casein-Based Diets (Experiment 3)[a]

	Liver manganese dry wt (μg/g)	Apparent absorption (%)	BH (days)
No phytic acid, no arginine	6.6	8.1	22.2
Phytic acid (1.7%), no arginine	7.1	8.7	21.5
No phytic acid, arginine (2%)	6.5	9.7	23.7
Phytic acid (1.7%), arginine (2%)	6.6	8.3	24.0
Root mean square error	0.7	3.3	2.0
Analysis of variance			
Phytic acid	NS	NS	NS
Arginine	NS	NS	0.02
Phytic acid × arginine	NS	NS	NS

[a] Means of six rats.

(Table V). However, addition of phytic acid to the diets significantly reduced liver manganese (Table V).

Discussion

Previous studies using rats (2, 5) and chicks (3, 4) demonstrated that manganese in soy protein diets was less available than manganese in casein diets. We found that liver manganese and heart manganese-SOD activity were increased by feeding soy protein. These increases in liver manganese and heart manganese SOD activity suggest an enhanced absorption of manganese by rats when fed soy protein diets. In Experiment 2, percentage of retention at Day 7 of ^{54}Mn was significantly higher in soy protein-fed rats than in casein-fed rats. When diets containing approximately 50 mg of manganese/kg were fed in Experiment 1, manganese absorption was so low that it was of the same magnitude as the root mean square error for the absorption measurements. This may explain why a significant effect of protein on manganese absorption was not seen in Experiment 1. When dietary manganese was less, rats fed soy protein absorbed more ^{54}Mn than those fed the casein diets. We used the low amounts of dietary manganese to reduce variance in the estimation of ^{54}Mn absorption in the remaining studies.

A significant increase in feed intake and weight gain in rats fed soy protein diets supplemented with methionine indicated that these diets were low in methionine. However, an effect of methionine on the concentration of liver manganese was absent. The effect of methionine supplementation on percentage of retention on Day 7 was not as pronounced as the effect of the type of protein. Supplemental methionine significantly increased the activity of heart manganese SOD. The biologic significance of the apparent selective effect of methionine is not clear. These findings suggest that dietary methionine is not the dietary factor responsible for the increased availability of manganese in soy protein diets.

The effect of 1.7% phytic acid supplement on manganese metabolism was not consistent. Added phytic acid did not affect absorption or BH of ^{54}Mn in Experiments 3 and 4. Although phytic acid did not have any effect on absorption or BH of ^{54}Mn, it signif-

Table V. Feed Intake, Weight Gain, Concentrations of Liver Manganese, Percentage of Apparent Absorption, and BH of ^{54}Mn in Rats Fed 5.1 mg of Manganese/kg with Different Amounts of Phytic Acid and Arginine in Casein-Based Diets without Added Methionine (Experiment 4)[a]

	Feed[b] intake (g/day)	Weight[b] gain (g/day)	Liver manganese dry wt (μg/g)	Apparent absorption (%)	BH (days)
No phytic acid, no arginine	18.1	6.3	6.5	5.1	17
Phytic acid (1.7%), no arginine	18.9	6.4	5.1	3.8	17
No phytic acid, arginine (2%)	19.9	7.8	6.3	4.1	19
Phytic acid (1.7%), arginine (2%)	18.7	7.0	4.7	4.7	17
Root mean square error	1.8	0.8	0.9	1.9	5.9
Analysis of variance					
Phytic acid	NS	NS	0.0001	NS	NS
Arginine	NS	0.001	NS	NS	NS
Phytic acid × Arginine	NS	NS	NS	NS	NS

[a] Means of eight rats.
[b] Values measured between Days 14 and 28.

icantly decreased the concentration of liver manganese in Experiment 4, which suggests that added phytic acid impairs manganese bioavailability as has been reported by other investigators (2–5).

We offer two possible explanations for the inconsistent effect of phytic acid on manganese metabolism in our studies. The obvious difference between Experiments 3 and 4 was the addition of calcium (0.27 g/kg diet) and magnesium (0.07 g/kg diet) to the casein diets and the omission of methionine in Experiment 4 to mimic the soy protein diet without methionine in Experiment 2. Byrd and Matrone (14) and Oberleas *et al.* (15) demonstrated that the addition of excess calcium *in vitro* or *in vivo* rendered the phytate-zinc complex more insoluble than before the addition of calcium. The effects of dietary calcium on zinc status are most pronounced when calcium is well in excess of the requirement (>1%) (16–18). However, the amount of calcium added to the casein diets in our study (0.03%) would not be expected to exert as great as influence on manganese in liver as found in Experiment 4. Another explanation could be that the effect of phytic acid on manganese bioavailability is subtle compared with its effect on zinc bioavailability (2, 3) and is difficult to reproduce.

Two studies suggested that phytic acid decreases manganese bioavailability in rats (2) and chicks (4) fed soy protein-based diets, but neither study had phytic acid as the only variable. Halpin *et al.* (4) estimated that absorption of crop-intubated manganese was 40% greater in chickens fed phytic acid and fiber-free casein-based diets than in those fed corn-soy diets. However, the manganese concentrations of these diets were 1.4 mg/kg and 30 mg/kg, respectively. Thus, the increased absorption of manganese by chickens fed the casein diet may have been caused by its lower manganese content rather than the lack of phytate. Differences in fiber content of the two diets also may have affected manganese absorption. Halpin and Baker (19) later reported that neutral detergent fiber isolated from corn-soya diets depressed manganese uptake by bones of chicks. Davies and Nightingale (2) examined the effect of feeding 1% phytic acid diets on whole-body retention of zinc, copper, iron, and in manganese zinc-deficient and control rats. Zinc-adequate rats fed phytic acid accumulated 35.9 μg of manganese/g body wt which was significantly lower than the 150.4 μg of manganese/g body wt accumulated by similar rats that were not fed phytic acid (2). However, zinc-deficient rats that did not receive phytic acid in their diets accumulated 62.8 μg of manganese/g body wt which was also lower than that in zinc-adequate rats not fed phytic acid. Therefore, the reported reduction in whole-body manganese retention could have been a secondary effect caused by a relative zinc deficiency induced by the feeding of phytate.

Keen *et al.* (5) reported that in 14-day-old rats ^{54}Mn retention from soy formula diet was 64.5% and from human milk was 81.5%. The lower ^{54}Mn retention from soy formula could have been the result of the 30-fold higher concentration of manganese in soy formula (0.3 μg/ml) than in human milk (0.01 μg/ml). However, Keen *et al.* (5) argued that the high manganese concentration in soy formula diets was not the factor responsible for the low ^{54}Mn retention because they found a 100-fold increase in manganese dose was necessary to reduce ^{54}Mn retention. Craig (20) reported no differences in plasma manganese of infants fed human milk, cow milk formula, or soy formula, regardless of the manganese concentration of the product, which suggests that feeding soy to human infants did not affect manganese nutriture. Gibson *et al.* (21) found that

manganese status, as measured by hair analysis, was significantly higher in vegetarians than nonvegetarians. The vegetarians received 21% of dietary manganese from legumes, nuts, and soy products, compared with 4.5% for the nonvegetarians; manganese intake was also higher for vegetarians than nonvegetarians.

The addition of arginine to casein-based diets failed to cause an increase in ^{54}Mn absorption or liver manganese and affected BH inconsistently. These findings show that dietary arginine was also not the dietary agent responsible for the increased manganese availability from soy protein diets. Therefore, the reason for the enhanced availability of manganese in soy protein diets is still unclear.

When availability of manganese in soy protein and casein diets was compared by measuring ^{54}Mn absorption, concentration of liver manganese, and activity of heart manganese SOD, manganese in soy protein diets was more available to rats than manganese in casein diets. The increased bioavailability of manganese in soy protein may have been caused by increased absorption. Differences in the concentrations of methionine, arginine, or phytic acid between soy protein and casein did not explain the enhanced manganese utilization by soy protein-fed rats. Added dietary phytic acid decreased liver manganese, but its effect was not consistent.

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