Molybdenum absorption and utilization in humans from soy and kale intrinsically labeled with stable isotopes of molybdenum[1,2]

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ABSTRACT

Background: Stable-isotope studies of molybdenum metabolism have been conducted in which molybdenum was added to the diet and was assumed to be absorbed and utilized similarly to the molybdenum in foods.

Objective: Our objective was to establish whether the molybdenum in foods is metabolized similarly to molybdenum added to the diet.

Design: We first studied whether sufficient amounts of molybdenum stable isotopes could be incorporated into wheat, kale, and soy for use in a human study. Enough molybdenum could be incorporated into soy and kale to study molybdenum absorption and excretion. Two studies were then conducted, one in women and one in men. In the first study, each meal contained ≈100 μg Mo from soy, kale, and extrinsic molybdenum. In the second study, soy and extrinsic molybdenum were compared; the meal contained ≈300 μg Mo.

Results: In the first study, molybdenum was absorbed equally well from kale and an extrinsic source. However, the molybdenum in soy was less well absorbed than the molybdenum in kale or that added to the diet. In the second study, absorption of molybdenum from soy was less than from the extrinsic label. Urinary excretion of soy molybdenum was also lower than urinary excretion of the extrinsic label, but excretion as a percentage of the absorbed dose was not significantly different between treatments.

Conclusions: The molybdenum in soy is less available than molybdenum added to the diet, but the molybdenum in kale is as available as molybdenum added to the diet. Once absorbed, excretion is not significantly different for soy, kale, and extrinsic molybdenum. *Am J Clin Nutr* 1999;69:1217–23.

KEY WORDS Molybdenum, absorption, utilization, humans, stable isotope, intrinsic label, soy, kale

INTRODUCTION

Isotopic tracers have been used in several experiments to study the metabolic fate of molybdenum in humans. In 1964, a study of molybdenum distribution in humans in which ^{99}Mo was injected was reported (1). Tracer data showed that the main pathway of molybdenum excretion is via the kidney, with fecal excretion being relatively low. More recently, stable isotopes of molybdenum have been used to follow molybdenum's metabolic fate. Studies were conducted using ^{95}Mo and ^{96}Mo to determine the suitability of proton nuclear activation analysis for studies in humans (2). Other work evaluated the feasibility of thermal ionization mass spectrometry with ^{95}Mo and ^{96}Mo (3) or ^{97}Mo and ^{100}Mo (4).

We conducted stable-isotope-tracer studies to measure absorption, excretion, and retention of molybdenum over a broad range of dietary intakes in young men (5, 6) and to develop a model of molybdenum metabolism (7, 8). These studies showed that molybdenum is well absorbed over a range of intakes, that it is rapidly excreted via the urine, and that total body molybdenum retention is regulated primarily via urinary excretion. With high molybdenum intake, molybdenum absorption increases and excretion is more rapid. However, these studies were all done by adding stable isotopes to the diet. It was thus unknown whether the metabolism of extrinsically labeled molybdenum reflected the metabolism of molybdenum incorporated into foods. The bioavailability of molybdenum from labeled foods had not been investigated previously in humans or animals because short half-lives of molybdenum radioisotopes (≤67 h) precluded preparation of endogenously labeled foodstuffs.

In the present study, we labeled plants with stable isotopes of molybdenum for use in human bioavailability experiments. Kale, soybeans, and wheat were selected for labeling to represent a dark-green leafy vegetable, a legume, and a cereal grain, respectively. Two human experiments were conducted to determine whether molybdenum added to the diet was absorbed and retained similarly to molybdenum in food.

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TABLE 1
Subject characteristics[1]

Study	Age	Height	Weight
	y	*cm*	*kg*
1 (*n* = 12 women)	32 ± 6 (23–42)	165 ± 7 (156–173)	73 ± 18 (51–107)
2 (*n* = 11 men)	31 ± 6 (25–41)	177 ± 8 (167–187)	72 ± 17 (58–115)

[1] $\bar{x}$ ± SD; range in parentheses.

SUBJECTS AND METHODS

Intrinsic labeling of foods

Labeling of soy, kale, and wheat

Soybeans (*Glycine max* var. Century) were germinated in light peat (an inert support for plants) and then transferred to 16-L buckets containing modified Hoagland-Arnon nutrient solution (9) and grown as described previously (9). There were 4 plants per container and 2 containers for each plant type and each isotope concentration. Kale (*Brassica oleracea* var. Vates Blue Curl) was germinated in horticubes (OASIS; Smithers-OASIS, Kent, OH) in water and then grown as for soybeans. Wheat (*Triticum aestivum* var. Coldwell) was germinated in soil and then transferred to 16-L buckets containing Cheny's nutrient solution (9) and grown as for soybeans and kale.

^{100}Mo was added to the hydroponic nutrient solution of soybeans and kale starting 5 wk after germination and to wheat starting 1 wk after transplantation of the seedlings. ^{100}Mo was administered for 4 consecutive weeks in doses of 17.63 or 0.88 μg per bucket weekly for total doses of 70.5 or 3.53 μg per bucket. In addition, the stem of the wheat plant was injected at the milking stage with 0.11 or 2.21 μg per head as described previously (9). The harvested wheat and soybeans from each container were ground into a powder and transferred to individual plastic bottles. The harvested kale leaves were placed in sealed plastic bags. Portions of the plant samples were weighed and ^{97}Mo was added as an isotopic diluent.

Labeling of soybeans and kale for human feeding study

Soybeans (*G. max* var. Century) were grown hydroponically in pots as described for the previous study. One week before dosing, the nutrient solution was replaced with a molybdenum-depleted solution. Twenty-five buckets of soybeans containing 4 plants each were dosed weekly for 4 wk beginning 19 d after the onset of flowering with a total of 64 μg ^{100}Mo per bucket. After dosing, the plants were returned to the normal Hoagland-Arnon nutrient solution that contained ≈8 μg Mo/L until harvest. The soybeans from each bucket were stored in separate bags. The total yield was 3303 g. Five grams from each bag was ground into a powder and ^{94}Mo was added as an isotopic diluent. The soybeans from the buckets that had incorporated the most ^{100}Mo were combined, powdered, puréed, and stored frozen.

Kale (*B. oleracea* var. Vates Blue Curl) was grown in a circulating hydroponic system containing Hoagland-Arnon nutrient solution (9); 50 plants were grown in each of 3 troughs. ^{97}Mo was added at a rate of 220 μg per trough 6 times over 5 wk. About half of the kale had to be harvested after 3 doses because of wilting. The 2 batches of kale leaves (3526 g) were blended into separate purées. A portion (5.5 g) of each batch was saved for analysis and ^{94}Mo was added. The level of label incorporation between the 2 batches was similar, so the 2 purée batches were combined into a single purée. Three portions of each of the final soy and kale purées were weighed for analysis and ^{94}Mo was added.

Subjects

Two molybdenum absorption studies were conducted. Participants were confined to a metabolic research unit for the duration of the studies. Twelve young women participated in study 1 and 11 young men in study 2. Participants in both studies had been recruited for other studies that were independent of the molybdenum absorption studies (10, 11) but that overlapped them. Characteristics of the participants in the 2 studies are shown in **Table 1**. The protocols and consent forms for both studies were reviewed and approved by the Human Subjects Review Committee of the University of California, Davis, and by the US Department of Agriculture, Agricultural Research Service, Human Studies Committee. Details of each study were explained to participants and each participant signed a consent form before the beginning of the study

Experimental design

Study 1 lasted 22 d and study 2 lasted 23 d. For all study days except day 4, participants consumed the study diet. In study 1, subjects could choose from a variety of foods and beverages and could eat ad libitum. In study 2, the study diet consisted of a 4-d rotating menu with an energy intake estimated to maintain prestudy body weight.

On day 4 only, the diets in each study contained casseroles in which the foods were labeled with stable isotopes of molybdenum as follows. In study 1, isotopically enriched soy and kale, an extrinsic label, and natural molybdenum, prepared as described below under "Isotope preparation," were included in the diets of all subjects. The isotopic labels and their amounts are shown in **Table 2**. Two sources of molybdenum were fed at lunch and the other 2 at dinner, as shown in **Table 3**. The enriched molybdenum

TABLE 2
Molybdenum sources in the isotopically enriched meals

	Isotope	Enriched Mo content
		μg
Study 1		
Soy	^{100}Mo	44.1
Kale	^{97}Mo	43.5
Extrinsic label	^{96}Mo	45.7
Natural Mo	—	49.5
Study 2		
Soy	^{100}Mo	147.2
Extrinsic label	^{96}Mo	155.8

TABLE 3
Combinations of labeled foods fed on day 4 of the study

Study and subject number	Lunch	Dinner
Study 1[1]		
1–3	Kale, natural	Soy, extrinsic
4–6	Kale, extrinsic	Soy, natural
7–9	Soy, extrinsic	Kale, natural
10–12	Soy, natural	Kale, extrinsic
Study 2[2]		
1–12	Soy, extrinsic	—

[1] Each meal contained ≈100 μg Mo.
[2] The meal contained ≈310 μg Mo.

content of each meal was ≈100 μg and the total molybdenum intake for the day was 207 μg. This design allowed us to compare the absorption and excretion of molybdenum in soy with the absorption and excretion of molybdenum in kale and to determine the effect of each on the absorption and excretion of extrinsic molybdenum. In study 2, the lunch meal on day 4 contained soy with 147 μg Mo enriched in ^{100}Mo and the extrinsic label contained 156 μg ^{96}Mo, for a total of 303 μg labeled Mo. The amounts of soy and molybdenum in study 2 were increased to determine the effect of soy on absorption when the amounts of soy and molybdenum were higher. In study 2, the other meals consumed on day 4 were the usual study meals; the total molybdenum intake for the day was 442 μg.

The menus on day 4 were as follows. In study 1, breakfast consisted of scrambled eggs, sausage, milk, rusks, margarine, and applesauce; lunch and dinner consisted of either the soy or the kale casserole, a rusk, and lemon-lime soda; and the evening snack consisted of canned peaches and a rusk. In study 2, breakfast consisted of a toasted bagel with cream cheese, bacon, and low-fat milk; lunch consisted of the soy casserole, a low-protein rusk, margarine, and lemon-lime soda; dinner consisted of a beef-potato casserole, a salad, and grape juice; and the evening snack was ice cream.

The soy casserole for study 1 contained 20 g low-protein, cooked noodles, 85 g soy purée (20 g soybeans cooked in chicken bouillon), 55 g cooked egg white, and 5 g margarine. The kale casserole for study 1 contained 276 g kale purée (120 g raw kale cooked and puréed in chicken bouillon), 230 g low-protein noodles, 55 g cooked egg white, and 5 g margarine. The soy casserole for study 2 contained 230 g low-protein noodles, 15 g margarine, 240 g soy purée (60 g dry soy cooked in 180 g broth), and 55 g cooked egg white.

Isotope preparation

Molybdenum metal powders enriched in ^{94}Mo (93.77 atom%), ^{96}Mo (96.44 atom%), ^{97}Mo (94.25 atom%), and ^{100}Mo (97.42 atom%) (Oak Ridge National Laboratory, Oak Ridge, TN) and unenriched molybdenum were weighed into acid-washed polytetrafluoroethylene beakers, a 3:1 mixture of ultrapure hydrochloric acid and nitric acid (Seastar Grade; Seastar Chemicals, Sidney, Canada) was added, and the solutions were heated in a laminar flow hood until the molybdenum was dissolved. The solutions were transferred to acid-washed polypropylene bottles and diluted with deionized water. The ^{94}Mo solution was diluted to 10 μg/g for use as the isotopic diluent in the analysis. The ^{96}Mo solution was diluted to 45.7 μg/g for use as the extrinsic label and the unenriched molybdenum solution was diluted to 49.5 μg/g. One-gram aliquots of these were pipetted into acid-washed microcentrifuge tubes and frozen for later addition to the meal. The ^{97}Mo and ^{100}Mo solutions were diluted to 8 mg/L to be incorporated as intrinsic labels in the kale and soy. The molybdenum concentrations of the isotope solutions were verified by isotope dilution by using a molybdenum standard solution (Ricca Chemical Co, Arlington, TX) (4). Precautions were taken to avoid trace element contamination during all phases of isotope solution preparation, sample collection and preparation, and analysis as described previously (5).

Sample collection and preparation

Two food composites were prepared for study 1 containing all foods fed on day 4. For one composite, the lunch contained the soy casserole and the natural molybdenum solution and the dinner contained the kale casserole and the extrinsic ^{96}Mo solution. For the other composite, the lunch contained the kale casserole and the natural molybdenum solution and the dinner contained the soy casserole and the extrinsic ^{96}Mo solution. Two portions of each of the 2 daily food composites were weighed for analysis and ^{94}Mo was added. Two food composites of the lunch meal alone and 2 composites of the entire day's meals were prepared for study 2. Two aliquots of each composite were weighed for analysis and ^{94}Mo was added.

Complete fecal and urine collections were made throughout the study and pooled, beginning with an initial 3-d pool before the isotope feeding day (day 4) and followed by 4-d pools beginning on the feeding day. Urine samples were frozen until analyzed. Diet composites and fecal pools were homogenized, refrozen, lyophilized, crushed to a fine powder, and stored in desiccators as described previously (5).

For fecal analysis, 3-g portions of the dried fecal pools were weighed with 4 μg ^{94}Mo added as an isotopic diluent. For urinalysis, 100-g portions of the urine pools were weighed with 5 μg ^{94}Mo added as an isotopic diluent. The diet, fecal, and urine samples were dried and ashed in a muffle furnace. The ashed samples were dissolved in 6 mol ultrapure HCl/L and the molybdenum was separated and purified by ion-exchange chromatography as described previously (4, 5).

Sample analysis and calculations

Molybdenum isotope ratios were determined with a computer-controlled, 90° magnetic sector, thermal ionization mass spectrometer (model 261; Finnigan MAT, Bremen, Germany) as described previously (5). The same quality-control procedures were followed as described previously (4). The total molybdenum and molybdenum isotope contents of the labeled plants and of the diet, urine, and fecal samples were determined by isotope dilution. Triple-isotope dilution, with the intrinsic ^{100}Mo from soy, extrinsic ^{96}Mo solution, and ^{94}Mo isotopic diluent, was used in study 2. These calculations were described previously (4, 5). Quadruple-isotope dilution calculations, with the additional intrinsic ^{97}Mo from kale, were used in study 1 as shown in **Appendix A**.

Molybdenum absorption was calculated by subtracting the amount of each enriched source of molybdenum appearing in the feces during the 8-d period after feeding from the amount fed. This value was divided by the amount fed, multiplied by 100, and expressed as percentage absorbed. Urinary excretion of the isotope labels was determined over the same 8-d period and calculated both as a percentage of the fed dose and a percentage of the absorbed dose.

TABLE 4
Accumulation of ^{100}Mo in hydroponically grown plants

Plant	^{100}Mo dose	Dry weight	Mo Content	^{100}Mo	^{100}Mo	^{100}Mo
	μg/bucket	*μg/bucket*	*μg/bucket*	*μg/g dry wt*	*% of total Mo*	*% of dose*
Soy	70.5	10.4 ± 0.8[1]	81.4 ± 9.1	4.56 ± 0.78	57.8 ± 1.2	66.6 ± 6.2
Soy	3.53	13.9 ± 1.6	29.9 ± 11.3	0.19 ± 0.03	10.0 ± 4.0	71.7 ± 1.4
Kale	70.5	8.05 ± 1.08	24.3 ± 1.9	2.71 ± 0.62	86.8 ± 1.8	29.9 ± 3.0
Kale	3.53	4.90 ± 2.16	5.47 ± 0.16	0.31 ± 0.03	26.6 ± 9.1	41.7 ± 15.3
Wheat[2]	70.5	3.31 ± 0.70	2.72	0.287	27.6	1.06
Wheat	3.53	3.33 ± 0.17	—	—	—	—

[1] $\bar{x}$ ± SD.
[2] Because of low molybdenum content, only one wheat sample was analyzed.

Statistics

The isotope dilution calculations were carried out and summarized and statistical analysis was performed with SAS (personal computer version 6.10; 12, 13). PROC GLM was used to perform an analysis of variance (ANOVA) on the absorption and urinary excretion of molybdenum from the intrinsic and extrinsic labeled dietary sources. If a significant effect was found, the Student-Neuman-Keuls test was used to determine which means differed. For study 1, ANOVA models were used to test for any significant effect on absorption and urinary excretion of molybdenum due to the meal (lunch or dinner) in which the soy, kale, or extrinsic label was fed or to whether the extrinsic label was fed with soy or kale. When no significant difference between the 4 feeding groups was observed, the data for the groups were combined. For both studies, ANOVA models with the Student-Neuman-Keuls test were used to determine the effect of dietary molybdenum source on molybdenum absorption (% of fed dose) and on urinary molybdenum excretion (both % of fed dose and % of absorbed dose). A significance level of 0.05 was used in all statistical tests.

RESULTS

In the labeling study, the yields and ^{100}Mo accumulation in soybeans, kale, and wheat (**Table 4**) were low because the plants were grown in a greenhouse during the winter months. The fresh yield per bucket averaged 13 g for soybeans, 73 g for kale, and 4 g for wheat. Accumulation of ^{100}Mo was high for soybeans, moderate for kale, and low for wheat. Stem injection of wheat resulted in a high rate of isotope incorporation, but the amount of molybdenum that could be incorporated per stem was low. The total concentration of ^{100}Mo incorporated in soybeans and kale from the higher dose (4.56 and 2.71 μg/g dry wt, respectively) was sufficient for a human feeding study. The low accumulation of ^{100}Mo by wheat (<0.75 μg per bucket) suggested that intrinsic labeling of this food would not be feasible.

The plants grown for the human feeding studies resulted in higher yields. The fresh yield of soybeans averaged 130 g per bucket. The total molybdenum concentration was 2.7 μg/g fresh wt. Accumulation of ^{100}Mo averaged 73% of the dose. Yields for kale were 1324 g for the batch harvested early and 2191 g for the batch harvested later. Molybdenum concentrations of the 2 batches were 0.040 and 0.045 μg/g fresh wt and the ^{97}Mo concentrations were 0.34 and 0.37 μg/g fresh wt. The 2 batches were combined, resulting in a total molybdenum concentration of 0.43 μg/g fresh wt and a ^{97}Mo concentration of 0.36 μg/g fresh wt.

Absorption

The highest fraction of the enriched isotopes appeared in the first 4-d fecal pool; the fraction in the second 4-d pool was ≈20% of that in the first. By the third 4-d pool, from 0.1% to 0.5% of the dose appeared in the feces per day, with less appearing after that. The 8-d collection period was therefore used to calculate absorption.

In study 1, there were no significant differences in absorption due to the order in which the labeled meals were fed. Therefore, data were combined for statistical tests. The mean (±SE) absorption of molybdenum was 87.5 ± 1.5% from the extrinsic molybdenum, 86.1 ± 1.5% from kale, and 56.7 ± 1.5% from soy (**Figure 1**). There was no significant difference in absorption between kale molybdenum and the extrinsic molybdenum, but absorption of molybdenum from soy was significantly less than absorption of extrinsic molybdenum and kale molybdenum. Absorption of the extrinsic molybdenum was not significantly different when it was combined with soy or kale. In study 2, in which more soy and molybdenum were fed, absorption was again significantly less from soy (58.3 ± 1.2%) than from the extrinsic molybdenum (92.8 ± 1.2%) (**Figure 2**).

Urinary excretion

Urinary excretion of molybdenum in the 8 d after feeding in study 1 was 53.5 ± 2.3% of the dose fed for the extrinsic molybdenum, 48.7 ± 2.3% for the kale molybdenum, and 36.3 ± 2.3% for the soy molybdenum (Figure 1). Excretion was not significantly different between the extrinsic molybdenum and the kale molybdenum, but was significantly lower for the soy molybdenum than for the extrinsic molybdenum and the kale molybdenum. However, when calculated as a percentage of the absorbed dose excreted in the urine, there were no significant differences between the sources of molybdenum. Mean excretion, as a percentage of the absorbed dose, was 60.8 ± 3.3% for extrinsic molybdenum, 56.6 ± 3.3% for kale, and 63.9 ± 3.3% for soy. In study 2, results were similar to study 1. Excretion of molybdenum in the urine was significantly lower for soy (39.6 ± 0.8%) than for the extrinsic molybdenum (62.6 ± 0.8%) (Figure 2). However, the percentage of the absorbed dose excreted in the urine was not significantly different between treatments: 67.8 ± 1.3% from the extrinsic molybdenum and 68.8 ± 1.3% from the soy.

DISCUSSION

Soybeans and kale, but not wheat, were successfully intrinsically labeled with stable isotopes of molybdenum. The efficiency

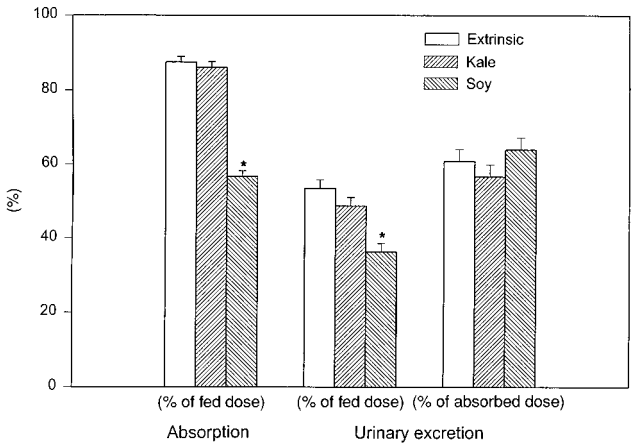

FIGURE 1. Mean (±SE) molybdenum absorption and urinary excretion in study 1 from an extrinsic label of ^{96}Mo, kale labeled with ^{97}Mo, and soy labeled with ^{100}Mo. Each labeled item contained 44–46 μg Mo. Excretion is presented both as a percentage of the dose of molybdenum administered and as a percentage of the absorbed dose. n = 12 women. *Significantly different from extrinsic molybdenum and kale molybdenum, $P < 0.05$.

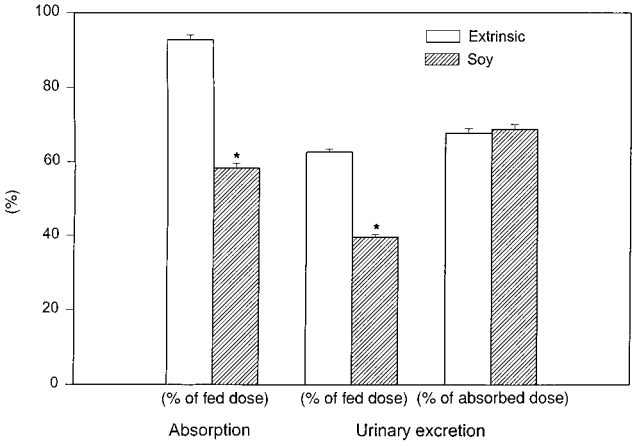

FIGURE 2. Mean (±SE) molybdenum absorption and urinary excretion in study 2 from an extrinsic label of ^{96}Mo and soy labeled with ^{100}Mo. Labeled items contained 156 and 147 μg Mo, respectively. Excretion is presented both as a percentage of the dose of molybdenum administered and as a percentage of the absorbed dose. n = 11 men. *Significantly different from extrinsic molybdenum, $P < 0.05$.

of uptake of molybdenum was greatest for the legume, intermediate for the green leafy vegetable, and very low for the cereal grain. Information on the efficiency of uptake of molybdenum by plants throughout the growth cycle was previously unavailable because of the short half-lives of radioisotopes of molybdenum (≤67 h). Very short-term studies showed rapid accumulation of molybdenum by tomato roots and subsequent rapid translocation to upper plant parts, but no accumulation of molybdenum in stem tissue (14).

Soybeans have also been shown to accumulate high quantities of zinc (27.5% of dose) and iron (12.4% of dose), although uptake was less efficient than for molybdenum (15). As with molybdenum, uptake of iron into the edible seeds was greater for soybeans than for wheat (5.5% of dose), but uptake of zinc by wheat (25.5% of dose) was not significantly different from uptake by soybeans. In contrast with molybdenum, calcium accumulation by leaves is much greater than for any seeds (15).

In calculating absorption, we assumed that the isotope appearing in the stools >8 d after the feeding reflected excretion of the absorbed isotope. This conclusion was based on an earlier study in which a stable isotope of molybdenum was infused. In that study, we found that only 2–4% of the infused dose appeared in the feces in the first 6 d after infusion (0.3–0.7%/d) and that excretion was gradually less after this time (5, 6). This is consistent with the excretion of 0.1–0.5%/d in the feces during the 8 d after isotope feeding in the present study. The results show that molybdenum absorption from soy, but not from kale, was less efficient than absorption of molybdenum added to the diet. Additionally, molybdenum absorption from soy was less efficient than that from the extrinsic label at both intakes, suggesting that the effect was not dose dependent. Once molybdenum is absorbed, it appears to be handled similarly regardless of the source, as shown by urinary excretion after correction for absorption. However, the presence of soy in the meal does not alter the absorption of other molybdenum in the meal. The absorption of extrinsic molybdenum was the same whether it was combined with kale or with soy. The lower absorption of molybdenum from soy has also been observed for other minerals. Soy protein, even after phytate has been removed, inhibits iron absorption (16). The bioavailability of zinc is also less from soy food products than from animal-derived foods (17).

We found previously that molybdenum absorption was high over a broad range of intakes and that absorption was higher with the highest intakes (6). When daily molybdenum intake ranged from 25 to 122 μg/d, absorption averaged 89%, but when it ranged from 466 to 1488 μg/d, absorption was significantly higher, averaging 93%.

There were several differences between the 2 studies reported here. Study 1 was conducted in women and study 2 was conducted in men and participants lived in the metabolic unit at different times. The labeled meal in study 2 contained ≈3 times as much molybdenum as the meal in study 1 and absorption tended to be higher in study 2. The meals contained amounts in the low (study 1) and high (study 2) ranges of our earlier work and results are consistent with those results. Average absorption in study 2, when the molybdenum content of the meal was higher, tended to be higher than in study 1: 92.8 ± 1.2% compared with 87.5 ± 1.5% for the extrinsic molybdenum and 58.3 ± 1.2% compared with 56.7 ± 1.5% for the soy molybdenum.

Molybdenum turnover in our earlier study was faster with higher dietary molybdenum intakes (6). This was reflected by urinary excretion of labeled molybdenum, both from infusions and feedings. The results of the current study support this interpretation of higher turnover when molybdenum intake is higher. More of the absorbed molybdenum tended to be excreted in the urine in study 2 (when molybdenum intake was higher) than in study 1: 67.8 ± 1.3% compared with 60.8 ± 3.3% of the absorbed extrinsic label and 68.8 ± 1.3% compared with 63.9 ± 3.3% of the soy molybdenum.

The current dietary recommendations for molybdenum are from 75 to 250 μg/d (18). We estimated the minimum requirement to be ≈25 μg/d for men on the basis of studies with an extrinsic stable-isotope label (5, 6). The dietary intake of the US population was estimated to average 180 μg/d in 1980 (19) and

76 μg/d for women and 109 μg/d for men in 1987 (20). Thus, even if the molybdenum in some foods, such as soy, is not as available as an extrinsic label, it appears that risk of inadequate dietary molybdenum is unlikely.

In conclusion, as shown by the intrinsically labeled soy, not all food sources of molybdenum are absorbed as well as molybdenum added to the diet. However, the intrinsically labeled kale showed that the molybdenum in some foods is as available as extrinsically added molybdenum. Despite this difference, molybdenum is better absorbed than many minerals and inadequate dietary molybdenum is unlikely.

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APPENDIX A

Mass balance equation for R^t_{ij}:

$$R^t_{ij} = \frac{f(A^n_i M^n/W^n + A^e_i M^e/W^e + A^k_i M^k/W^k + A^s_i M^s/W^s) + A^d_i M^d/W^d}{f(A^n_j M^n/W^n + A^e_j M^e/W^e + A^k_j M^k/W^k + A^s_j M^s/W^s) + A^d_j M^d/W^d} \quad (A1)$$

Equations for isotope dilution calculations:

$$\begin{aligned}
&M^n(A^n_j/W^n)(R^t_{gj} - R^n_{gj}) + M^e(A^e_j/W^e)(R^t_{gj} - R^e_{gj}) + M^k(A^k_j/W^k)(R^t_{gj} - R^k_{gj}) + M^s(A^s_j/W^s)(R^t_{gj} - R^s_{gj}) = (M^d/f)(A^d_j/W^d)(R^d_{gj} - R^t_{gj})\\
&M^n(A^n_j/W^n)(R^t_{hj} - R^n_{hj}) + M^e(A^e_j/W^e)(R^t_{hj} - R^e_{hj}) + M^k(A^k_j/W^k)(R^t_{hj} - R^k_{hj}) + M^s(A^s_j/W^s)(R^t_{hj} - R^s_{hj}) = (M^d/f)(A^d_j/W^d)(R^d_{hj} - R^t_{hj})\\
&M^n(A^n_j/W^n)(R^t_{ij} - R^n_{ij}) + M^e(A^e_j/W^e)(R^t_{ij} - R^e_{ij}) + M^k(A^k_j/W^k)(R^t_{ij} - R^k_{ij}) + M^s(A^s_j/W^s)(R^t_{ij} - R^s_{ij}) = (M^d/f)(A^d_j/W^d)(R^d_{ij} - R^t_{ij})\\
&M^n(A^n_j/W^n)(R^t_{mj} - R^n_{mj}) + M^e(A^e_j/W^e)(R^t_{mj} - R^e_{mj}) + M^k(A^k_j/W^k)(R^t_{mj} - R^k_{mj}) + M^s(A^s_j/W^s)(R^t_{mj} - R^s_{mj}) = (M^d/f)(A^d_j/W^d)(R^d_{mj} - R^t_{mj})
\end{aligned} \quad (A2)$$

$$\begin{aligned}
a_{11}\,M^n + a_{12}\,M^e + a_{13}\,M^k + a_{14}\,M^s &= b_1\\
a_{21}\,M^n + a_{22}\,M^e + a_{23}\,M^k + a_{24}\,M^s &= b_2\\
a_{31}\,M^n + a_{32}\,M^e + a_{33}\,M^k + a_{34}\,M^s &= b_3\\
a_{41}\,M^n + a_{42}\,M^e + a_{43}\,M^k + a_{44}\,M^s &= b_4
\end{aligned} \quad (A3)$$

$$M^n = \frac{\begin{vmatrix} b_1 & a_{12} & a_{13} & a_{14} \\ b_2 & a_{22} & a_{23} & a_{24} \\ b_3 & a_{32} & a_{33} & a_{34} \\ b_4 & a_{42} & a_{43} & a_{44} \end{vmatrix}}{\begin{vmatrix} a_{11} & a_{12} & a_{13} & a_{14} \\ a_{21} & a_{22} & a_{23} & a_{24} \\ a_{31} & a_{32} & a_{33} & a_{34} \\ a_{41} & a_{42} & a_{43} & a_{44} \end{vmatrix}} \quad M^e = \frac{\begin{vmatrix} a_{11} & b_1 & a_{13} & a_{14} \\ a_{21} & b_2 & a_{23} & a_{24} \\ a_{31} & b_3 & a_{33} & a_{34} \\ a_{41} & b_4 & a_{43} & a_{44} \end{vmatrix}}{\begin{vmatrix} a_{11} & a_{12} & a_{13} & a_{14} \\ a_{21} & a_{22} & a_{23} & a_{24} \\ a_{31} & a_{32} & a_{33} & a_{34} \\ a_{41} & a_{42} & a_{43} & a_{44} \end{vmatrix}} \quad M^k = \frac{\begin{vmatrix} a_{11} & a_{12} & b_1 & a_{14} \\ a_{21} & a_{22} & b_2 & a_{24} \\ a_{31} & a_{32} & b_3 & a_{34} \\ a_{41} & a_{42} & b_4 & a_{44} \end{vmatrix}}{\begin{vmatrix} a_{11} & a_{12} & a_{13} & a_{14} \\ a_{21} & a_{22} & a_{23} & a_{24} \\ a_{31} & a_{32} & a_{33} & a_{34} \\ a_{41} & a_{42} & a_{43} & a_{44} \end{vmatrix}} \quad M^s = \frac{\begin{vmatrix} a_{11} & a_{12} & a_{13} & b_1 \\ a_{21} & a_{22} & a_{23} & b_2 \\ a_{31} & a_{32} & a_{33} & b_3 \\ a_{41} & a_{42} & a_{43} & b_4 \end{vmatrix}}{\begin{vmatrix} a_{11} & a_{12} & a_{13} & a_{14} \\ a_{21} & a_{22} & a_{23} & a_{24} \\ a_{31} & a_{32} & a_{33} & a_{34} \\ a_{41} & a_{42} & a_{43} & a_{44} \end{vmatrix}} \tag{A4}$$

$$M^t = M^n + M^e + M^k + M^s \tag{A5}$$

where R is the isotopic ratio, A is the isotopic abundance, M is the mass of total molybdenum, W is the atomic mass, f is the fraction of sample weighed for analysis, t is the total molybdenum in the sample, n is the natural molybdenum (unenriched), e is the ^{96}Mo-enriched extrinsic label, k is the ^{97}Mo-enriched intrinsic label from kale, s is the ^{100}Mo-enriched intrinsic label from soy, d is the ^{94}Mo-enriched molybdenum added as an isotopic diluent, g is the ^{94}Mo enriched in the isotopic diluent, h is the ^{96}Mo enriched in the extrinsic label, i is the ^{97}Mo enriched in the kale, j is the ^{98}Mo reference isotope, and m is the ^{100}Mo enriched in the soy.