Molybdenum absorption, excretion, and retention studied with stable isotopes in young men during depletion and repletion[1–4]

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ABSTRACT A study of molybdenum absorption, excretion, and balance was conducted in four young men fed a low-molybdenum diet (22 μg/d) for 102 d followed by 18 d of the same diet supplemented to contain 467 μg/d. The study was conducted to determine the minimum dietary molybdenum requirement of healthy young men. Stable isotopes of molybdenum were used as tracers. ^{100}Mo was fed four times during the study, ^{97}Mo was infused twice, and ^{94}Mo was used as an isotopic diluent to quantify the molybdenum isotopes and total molybdenum in complete urine and fecal collections and in the diets. The study demonstrated that subjects could not consistently attain balance with the low-molybdenum diet, but balance improved with time, and no signs of molybdenum deficiency were observed. Molybdenum was very efficiently absorbed at both intakes of dietary molybdenum and urinary excretion increased as dietary molybdenum increased. Molybdenum turnover was significantly slower when dietary molybdenum was low. We estimate from these results that the minimum dietary molybdenum requirement is ≈25 μg/d or possibly less. This suggests that the lower end of the recommended range could be less than the current recommended amount of 75 μg/d. *Am J Clin Nutr* 1995;61:1102–9.

KEY WORDS Molybdenum, Mo, absorption, dietary requirement, depletion, stable isotopes

Introduction

Molybdenum is considered an essential trace element for human beings because it is required for the function of three enzymes found in humans: sulfite oxidase, xanthine oxidase, and aldehyde oxidase (1). Sulfite oxidase catalyzes the oxidation of sulfite to sulfate, a reaction that is necessary for the metabolism of the sulfur amino acids. Xanthine oxidase catalyzes the oxidative hydroxylation of purines and pyrimidines, including the conversion of hypoxanthine to xanthine and xanthine to uric acid. Aldehyde oxidase acts on many compounds. It oxidizes purines, pyrimidines, and pteridines, and may be involved in nicotinic acid metabolism. Of these enzymes, only sulfite oxidase is known to be indispensable for humans (2). Few cases of molybdenum deficiency have been reported to date and those were associated with disease conditions. A rare metabolic disorder, molybdenum cofactor (molybdopterin) deficiency, is characterized by absence of the three molybdoenzymes found in humans (3, 4). The absence of sulfite oxidase in this disorder leads to severe neurological symptoms and usually results in early death. One case of nutritional molybdenum deficiency has been reported (5, 6). A man with Crohn's disease and short bowel syndrome who was receiving permanent parenteral nutrition demonstrated symptoms of molybdenum deficiency. Excretion of sulfur metabolites was abnormal, urinary and serum uric acid concentrations were low, and hypoxanthine and xanthine excretion were excessive. Molybdenum supplementation reversed the abnormalities.

Deficiency symptoms after long-term parenteral nutrition have been reported only in the case described above. The molybdenum content of total parenteral nutrition (TPN) solutions is not well established, but one 5-d study of balance for 20 minerals found that the molybdenum content of TPN solutions was only 10 μg and all patients were in negative balance (7). The 1989 report of the Nutritional Advisory Group of the American Medical Association's Department of Foods and Nutrition recommends ≥ 100–200 μg molybdenum for adults receiving TPN (8), which would include contaminants as well as additives, but provides no basis for the recommendation. We measured the molybdenum content of infant TPN solutions from a local hospital and discovered they would provide 39–250 μg/d to the infants with no added molybdenum (9). This suggests that TPN solutions may often contain adequate molybdenum without additives.

The minimum dietary requirement for molybdenum is not known and it is toxic at high amounts (1). An estimated safe and adequate daily dietary intake (ESADDI) of 0.15–0.5 mg/d for adults was introduced in the 9th edition of the recommended dietary allowances (10). This recommendation was

[1] From the USDA/ARS/Western Human Nutrition Research Center, Presidio of San Francisco, CA, and the School of Public Health, University of California, Los Angeles.

[2] Reported in part at the Eighth International Symposium on Trace Elements in Man and Animals, Dresden, Germany, May 1993.

[3] Reference to a company or product name does not imply approval or recommendation of the product by the US Department of Agriculture to the exclusion of others that may be suitable.

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based on limited data from balance studies and estimates of normal intake. Three studies demonstrated molybdenum retention with intakes of 0.1–0.15 mg/d (11). Others observed negative balances in some subjects after 0.1 mg/d (12,13). Usual dietary intake was estimated in 1970 to be 0.36 mg/d based on dietary molybdenum analysis, with a range of 0.1–0.46 mg/d (14). A 1980 report estimated that the usual intake was considerably lower, an average of 0.18 mg/d (15). A study reported in 1987 gave an even lower estimate, an intake of 0.076 mg for females and 0.109 mg for males (16). The ESADDI was revised downward to 0.075–0.250 mg/d in the 10th edition of the recommended dietary allowances (17) on the basis of these newer reports of usual dietary intake.

Few studies of the molybdenum requirement of humans or metabolism of molybdenum in humans have been reported. In addition to the balance studies discussed above, a tracer study was conducted in 1964 using a single intravenous injection of the radioisotope ^{99}Mo in four terminal cancer patients to determine the fate of molybdenum in humans (18). It provided evidence that the main pathway of molybdenum excretion in humans is via the kidney, as it is in most animal species.

The study described in this report was designed to begin to define the minimum dietary molybdenum requirement and to obtain data on its metabolism and excretory patterns. The basis of the requirement would be obligatory urinary and fecal losses from a diet as low in molybdenumj as possible, as well as data on molybdenum absorption, excretion, balance, and turnover.

Subjects and methods

Subjects

Six young men were selected to participate in the study. They were recruited by newspaper advertisement. Screening procedures included medical history; blood, urine, and fecal examinations; electrocardiogram; and psychological examination. Two of the six were dismissed from the study, one on day 34 and the other on day 42, for noncompliance with the protocol and their data are not included. Profiles of the subjects are given in **Table 1.** The experimental protocol was reviewed and approved by the Letterman Army Medical Center Committee Institutional Review Board and the United States Department of Agriculture Human Studies Review Committee.

TABLE 1
Subject profiles

Subject	Age	Height	Weight	
			Day 1	Day 120
	y	*cm*	*kg*	
7	28	178.5	93.0	92.9
10	22	179.0	77.0	77.1
11	29	172.7	73.2	71.3
12	24	173.0	64.0	62.9
$\bar{x}$ ± SEM	26 ± 2	175.8 ± 1.7	76.8 ± 6.0	76.1 ± 6.3

Experimental design

The young men were confined to the metabolic research unit at the Western Human Nutrition Research Center for the duration of the 120-d study. They were under supervision by nursing staff at all times during their stay. Physical fitness was maintained by two 5-km (3-mile) chaperoned outdoor walks daily. Equivalent treadmill walking was occasionally substituted for outdoor walks. The weights and vital signs of the volunteers were measured each morning after urination and before breakfast. Medications were not used during the study, with very few exceptions.

The experimental design is summarized in **Table 2.** The study was divided into two metabolic periods (MPs), a depletion period of 102 d and a repletion period of 18 d. The only variable between the two MPs was dietary molybdenum. ^{100}Mo was included in the diet on days 13, 49, and 91 in MP 1 and on day 109 in MP 2 to determine absorption and the excretion pattern of dietary molybdenum. ^{97}Mo was infused into an arm vein on days 1 and 85 in MP 1 to determine the routes of excretion of endogenous molybdenum. Complete urine and fecal collections were made throughout the experiment to determine molybdenum balance. Balance was calculated by subtracting urinary and fecal molybdenum from all dietary sources of molybdenum.

Health monitoring included urinalysis, a complete blood count, and blood chemistry indexes at the beginning of the study, the end of both MPs, and every 25–26 d during MP 1 (depletion). Blood and urinary uric acid, known to decline with molybdenum deficiency or molybdenum cofactor deficiency, were monitored at the same time points. Urinary sulfite excretion, which is high with molybdenum deficiency or molybdenum cofactor deficiency, was determined qualitatively daily by using sulfite screening strips. A physician made rounds weekly to record relevant clinical findings. Subjects were weighed and their temperature, heart rate, respiration, and blood pressure were measured daily.

Oral load tests were administered in an attempt to stress the molybdoenzymes xanthine oxidase, aldehyde oxidase, and sulfite oxidase. They were given at the beginning of the study, twice during depletion, and at the end of repletion to assess the effect of molybdenum depletion on the activity of the three molybdoenzymes. Loading doses of a purine (adenosine monophosphate; AMP), nicotinamide, and cysteine were given. Metabolites of these three compounds were analyzed and the results of these tests will be reported separately (19).

TABLE 2
Experimental design

	Depletion	Repletion
Length (d)	102	18
Dietary molybdenum (μg)	22	467
^{100}Mo included in diet		
Study day	13, 49, and 91	109
^{100}Mo (μg)	24	428
Food molybdenum (μg)	22	22
Total molybdenum (μg)	46	450
^{97}Mo infused		
Study day	1 and 85	—
^{97}Mo (μg)	33	—

Serum ferritin, plasma copper, ceruloplasmin, and superoxide dismutase (SOD) were determined to assess the interactions with copper and iron. Body composition, dark adaptation, immune function, and resting and exercise metabolic rates were assessed at intervals during the study. Results of these tests will also be reported separately.

Blood samples were taken on the day after the isotope feedings, before the isotope infusions, at six intervals after the infusions, and at the end of each metabolic period to determine isotopic enrichments and mineral concentrations. The molybdenum isotope and total molybdenum concentrations were too low to determine with thermal-ionization mass spectrometry (TIMS) or electrothermal atomic-absorption spectrometry (AAS) and these samples will be analyzed later by inductively coupled plasma mass spectrometry (ICP/MS).

Experimental diet

A 3-d rotating menu was used throughout the study. It comprised low-molybdenum foods. The low-molybdenum diet was low in other nutrients in addition to molybdenum and was supplemented with a liquid formula drink containing additional energy and essential minerals as well as a multiple vitamin tablet. The diet was planned to contain at least the recommended amounts of all known nutrients except molybdenum. The 3-d menu is shown in **Table 3.** The formula drink contained corn starch, dextrimaltose, cottonseed oil, sucrose, α-cellulose, $CaCO_3$, MgO, NaCl, KCl, KH_2PO_4, and trace elements. The NaCl and KCl used in the foods and formula were recrystallized to eliminate the molybdenum they contained. The diet, including supplements, contained calculated daily averages of 798 mg Ca, 355 mg Mg, 3015 mg K, 1971 mg Na, 9.8 mg Fe, 2 mg Cu, and 15 mg Zn. On the basis of the analysis of composites of the diets collected during the study, the diets contained 825 mg Ca, 348 mg Mg, 8.4 mg Fe, 1.6 mg Cu, and 13.4 mg Zn. In addition to the amounts of trace elements in the diet, 2.5 mg Mn, 0.15 mg I, 0.5 mg Cr, 1.5 mg F, and 0.05 mg Se were added to the formula. The daily energy intake of the diet ranged from 10 272 to 12 565 kJ (2455–3003 kcal) and averaged 11 360 kJ (2715 kcal). The diets provided 56 g protein/d. Carbohydrates comprised 61% of energy intake and fat 30%. The energy required for each volunteer to maintain initial body weight was calculated and increased as required with an extra-energy drink containing the same carbohydrate and fat sources as the formula given to all subjects. If an individual began to gain or lose weight, the energy content of the diet was adjusted by changing the amount of the extra-energy drink. The average body weight of the volunteers was 77 $\pm$ 10 kg at the beginning of the study and 76 $\pm$ 11 kg at the end. Weights at the end of the study were different from beginning weights by 0.1–1.9 kg.

The molybdenum content of the diet, based on analysis of four composites of each day of the 3-d rotating diet, was 20 μg on day 1 of the rotation, 22 μg on day 2, and 24 μg on day 3, averaging 22 μg/d during depletion. During the 18 d of MP 2, a solution of $(NH_4)_6Mo_7O_{14}\cdot 4H_2O$ dissolved in deionized water

TABLE 3
Low-molybdenum diet in a 3-d cycle[1]

	Day 1	Day 2	Day 3
Breakfast	Low-fat milk	Low-fat milk	Low-fat milk
	Melba toast	Melba toast	Melba toast
	Decaffeinated coffee	Apple sauce	Decaffeinated coffee
	Liquid formula	Decaffeinated coffee	Liquid formula
	Vitamin supplement	Liquid formula	Vitamin supplement
		Vitamin supplement	
Lunch	Casserole	Salad	Casserole
	Broiled steak	Tuna chunks	Chicken breast
	Canned corn	Lettuce	Canned tomatoes
	Pasta	Tomatoes	Pasta
	Butter	Oil	Butter
		Vinegar	
	Liquid formula		Liquid formula
		Orange juice	
		Liquid formula	
Dinner	Casserole	Tenderloin steak	Pasta
	Chicken breast		
	Pasta	Casserole	Pork loin
		Pasta	
	Salad	Carrots	Butter
	Lettuce	Onions	
	Celery	Butter	Salad
	Oil		Lettuce
	Vinegar	Liquid formula	Beets
			Oil
	Liquid formula		Vinegar
Snack	Mixed fruit	Canned peaches	Canned pears

[1] *See* text for composition of the liquid formula and mineral content of the diet.

was added to the formula portion of the meals, so the diet contained 467 μg Mo/d.

Isotope preparation and administration

The stable isotopes of molybdenum were purchased as metal powders (Oak Ridge National Laboratory, Oak Ridge, TN). Isotopes used were ^{94}Mo (93.77 atom %), ^{97}Mo (94.25 atom %), and ^{100}Mo (97.42 atom %). ^{100}Mo was added to the diet and ^{97}Mo was infused. ^{94}Mo was added to weighed samples before ashing to determine by isotope dilution the molybdenum content of the diets and to quantify the total molybdenum, ^{100}Mo, and ^{97}Mo in the urine and fecal collections. These procedures and calculations were described previously (20). The isotopes were dissolved in a 3:1 mixture of HCl and HNO_3 and diluted to the required concentrations with deionized water. The molybdenum concentrations of the isotope solutions were verified by isotope dilution by using a standard molybdenum solution (20).

The ^{97}Mo solution was prepared for intravenous administration by filtering through a 0.22-μm filter. The pH was adjusted to 2 and the solution was tested for sterility and endotoxins. The molybdenum concentration of the solution was 6.6 μg/g and 5 g was administered, for a total of 33 μg Mo/infusion. The solution was infused into an arm vein through a venous catheter on days 1 and 85. The syringe was flushed twice with saline solution to ensure complete transfer of the isotope.

The ^{100}Mo solution prepared for addition to the diet was stored frozen in 1-g amounts in microcentrifuge tubes. On the days the isotope was fed, tubes were thawed and the solution was added to the formula drink 1 h before each meal. The tubes were rinsed twice with deionized water to ensure complete transfer. On days 13, 49, and 91 (MP 1) 24 μg ^{100}Mo was added and on day 109 (MP 2) 428 μg was added in place of the molybdenum solution added on the other days of MP 2.

Polyethylene glycol (PEG), a fecal marker not absorbed by the body, was fed at each meal containing the ^{100}Mo. Four capsules containing 0.5 mg PEG each were fed at each meal for a total of 6 g/d each day the ^{100}Mo was fed.

Sample collection

Precautions were taken to avoid trace element contamination during all phases of sample collection, preparation, and analysis. The precautions included handling of samples with powder-free plastic gloves; weighing, measuring, and processing samples under clear-plastic enclosures in a clean room or in a laminar flow bench; using ultrapure acids and acid-washed containers; and keeping samples covered.

Composites of the daily diets were weighed into large plastic containers on 4 different days for each of the three daily menus. Tumblers containing the formula drink were saved six times during MP 1 and four times during MP 2. Samples of the extra-energy formula were also saved. The diet samples were stored frozen.

Complete fecal collections were made throughout the study. Feces were collected in new plastic containers for 3-d periods. They were frozen as collected. The 3-d collections were later thawed and combined into 6-d pools.

Diet composites and fecal pools were homogenized by using a Polytron homogenizer (Brinkmann Instruments, Inc, Westbury, NY) with a stainless steel probe containing a polytetrafluoroethylene bearing. After homogenization, they were frozen again and lyophilized. When dry, they were weighed, crushed to a fine powder in large plastic bags, transferred to plastic jars, and stored in desiccators.

Urine was collected in 24-h periods, except on the days when isotopes were infused, when it was collected in 8-h periods. The 24-h collections were combined into 6-d pools, except after the isotope infusion, when the 8-h collections were stored separately. Urine samples were stored frozen until analyzed.

Samples were prepared for isotope ratio determinations as described previously (20). Five-gram subsamples of fecal collections, 200–250-g subsamples of urine collections, and 6-g subsamples of diet composites were weighed. A stable-isotope solution containing 3–7 μg ^{94}Mo was added to each sample and they were prepared for analysis. Briefly, samples were ashed in a muffle furnace and dissolved in 6 mol ultrapure HCl/L. Molybdenum was separated from the rest of the minerals in the samples and purified by ion-exchange chromatography (20). Recovery of molybdenum from the columns was checked by electrothermal AAS with an Instrumentation Laboratory 951 AA spectrophotometer (Lexington, MA) equipped with a model 655 Furnace Atomizer (Thermo Jarrel Ash Corp, Franklin, MA) or by isotope dilution.

Sweat and saliva samples were collected at several intervals, but the concentrations of molybdenum in these fluids were too low to obtain reliable measurements by AAS.

Laboratory analysis and calculations

Creatinine, uric acid, and nitrogen contents in urine collections were determined. Serum and urinary uric acid were determined with a centrifugal analyzer (Flexigem; Electro-Nucleonics, Inc, Fairfield, NJ). Urinary sulfite excretion was monitored with sulfite screening strips (Quantofix Sulphite, Gallard-Schlesinger Industries, Inc, Carle Place, NY). Urinary creatinine was determined with a Technicon Autoanalyzer IIC Plus System (Technicon Instruments Corp, Tarrytown, NY). Urinary nitrogen was measured with a Buchi Nitrogen Analysis System (Metrohm, Herisau, Switzerland). Fecal PEG was determined with a turbidimetric procedure with a Gilford Response UV-VIS spectrophotometer (21).

Molybdenum isotopic ratios were determined with a computer-controlled, 90° magnetic sector, thermal-ionization mass spectrometer (MAT model 261; Finnigan, Bremen, Germany) with modified software. A collector assembly with five Faraday collectors was used for simultaneous collection of molybdenum isotopes 94, 96, 97, 98, and 100. Purified samples of 1–2 μg Mo in an HCl solution were loaded inside a laminar-flow bench onto zone-refined rhenium double filaments with a silica-gel ionization-enhancement technique. The mass spectrometer, loading technique, and sample-heating and analysis procedures have been described in detail (20).

After 10 measurements of the ion beam intensities of the five molybdenum isotopes, a data reduction module calculated the average of the 10 measurements for each ratio, correcting for amplifier baselines and relative gains. A second data reduction module then carried out isotope dilution and iterative normalization calculations by using ^{96}Mo:^{98}Mo to normalize ^{94}Mo:^{98}Mo, ^{97}Mo:^{98}Mo, and ^{100}Mo:^{98}Mo. The individual ratio measurements were subjected to the Dixon outlier test for extreme

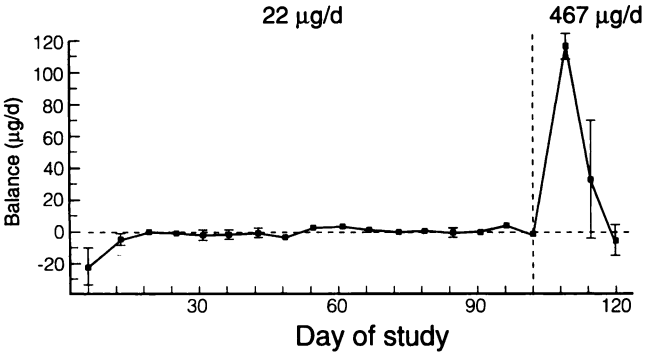

FIGURE 1. Molybdenum balance at 22 and 467 µg/d. $\bar{x} \pm$ SD; $n = 4$.

mean (22) and ratios that exceeded the 10% level of significance were eliminated from the averages.

Each set of 13 samples included an unenriched sample of the same matrix. These unenriched samples were used to monitor column recovery, detect sample contamination, and establish the natural isotopic ratios used in calculations (20). The total molybdenum contents and the isotope contents of fecal and urine samples were determined by triple isotope dilution by using normalized ^{94}Mo:^{98}Mo, ^{97}Mo:^{98}Mo, and ^{100}Mo:^{98}Mo from the enriched sample; unenriched samples of the same matrix; ^{100}Mo, ^{97}Mo, and ^{94}Mo solutions; the amount of isotopic diluent added; and the fraction of the total pool weighed for analysis (20). The molybdenum contents of the diet samples were calculated by using single-isotope dilution.

Statistics

Statistical analysis was performed with the personal computer version 6.04 of the Statistical Analysis System (23,24). PROC GLM was used to perform an analysis of variance (ANOVA) on the absorption, retention, and excretion of molybdenum. The three feeding periods within depletion were compared to examine the effects of adaptation to the low dietary molybdenum intake. In addition, the two infusion periods were compared within the depletion period. An ANOVA model was used to determine the effects of dietary molybdenum on molybdenum absorption, retention, and excretion. A significance level of 0.05 was used in all statistical tests.

Results

No clinical symptoms or adverse effects of molybdenum depletion were observed during the 102-d depletion period. Clinical laboratory values remained within normal ranges throughout the study. Urinary sulfite excretion was detectable occasionally in one subject and was observed more frequently late in the study than early in the study, but remained detectable after repletion. Serum and urinary uric acid did not change during the low-molybdenum diet. Serum uric acid averaged 333 ± 65 µmol/L at the beginning of the study, 357 ± 42 µmol/L at the end of depletion, and 357 ± 54 µg/L at the end of repletion. Urinary uric acid averaged 2.8 ± 0.5 mmol/d the first 6 d of the study, 2.7 ± 0.3 mmol/d at the end of depletion, and 2.9 ± 0.6 mmol/d at the end of repletion. Nitrogen balance was not affected by molybdenum depletion.

Molybdenum balance data, based on dietary, urinary, and fecal molybdenum throughout the study, are shown in **Figure 1.** Average balance during MP 1 ranged from −23.7 µg/d during the first 6 d of the study, or −18.2 µg/d after the ^{97}Mo infused was included, to +2.1 µg/d. Average balance was negative continuously through day 48 and after that was negative in four of the nine 6-d periods. After the infused ^{97}Mo (day 49) was included, the average 6-d balance was negative on three of the nine 6-d periods and was slightly positive, 0.3 µg/d, from days 49 to 102. Balance changed markedly during the course of MP 2, decreasing from an average of +114 µg/d in the first 6-d collections to +30.5 µg/d in the second, and −6.6 µg/d in the third. When the low-molybdenum diet was fed, ≈40% of the total molybdenum excreted was eliminated via the feces and 60% was excreted via the urine. After the dietary molybdenum was increased to 467 µg/d, < 10% of the total molybdenum excreted was eliminated in the feces and > 90% was excreted in the urine.

Absorption, excretion, and retention of the ^{100}Mo fed in the diet during depletion and repletion are summarized in **Table 4** both as a percentage of the dose fed and in micrograms. Absorption averaged 88 ± 0.5% ($\bar{x} \pm$ SE of pooled mean) in MP 1, significantly lower than the 93 ± 1% absorbed in MP 2. The 12-d retention of ^{100}Mo averaged 68 ± 2% during depletion and 21 ± 3% during repletion. The percent retained was significantly higher during depletion than repletion and the amount retained was greater during repletion than depletion. During depletion, only 20 ± 2% of the ^{100}Mo fed was excreted in the urine and 12 ± 0.5% was eliminated in the feces in the 12 d after the isotope was fed. During repletion, 71 ± 4% was excreted in the urine and 7.3 ± 0.9% in the feces over the same period of time.

The percent of ^{100}Mo absorbed and the percent retained over 12 d after each feeding are shown in **Figure 2A.** The amounts absorbed and retained are shown in Figure 2B. Neither absorption nor retention differed between the three administrations in MP 1. Fecal and urinary excretion after each feeding are shown

TABLE 4
Absorption, excretion, and retention of ^{100}Mo fed three times during depletion and once during repletion[1]

	Oral dose of molybdenum	
	24 µg	428 µg[2]
Absorption		
(µg)	21 ± 0.7	397 ± 1
(%)	88 ± 0.5	93 ± 0.9
Urine		
(µg)	4.8 ± 4.6	305 ± 8
(%)	20 ± 2	71 ± 4
Feces		
(µg)	2.8 ± 0.7	31 ± 1
(%)	12 ± 0.5	7.3 ± 0.9
Retention		
(µg)	16 ± 4	92 ± 8
(%)	68 ± 2	21 ± 3

[1] $\bar{x} \pm$ SEM; $n = 4$.
[2] All values significantly different from 24-µg values, $P < 0.05$.

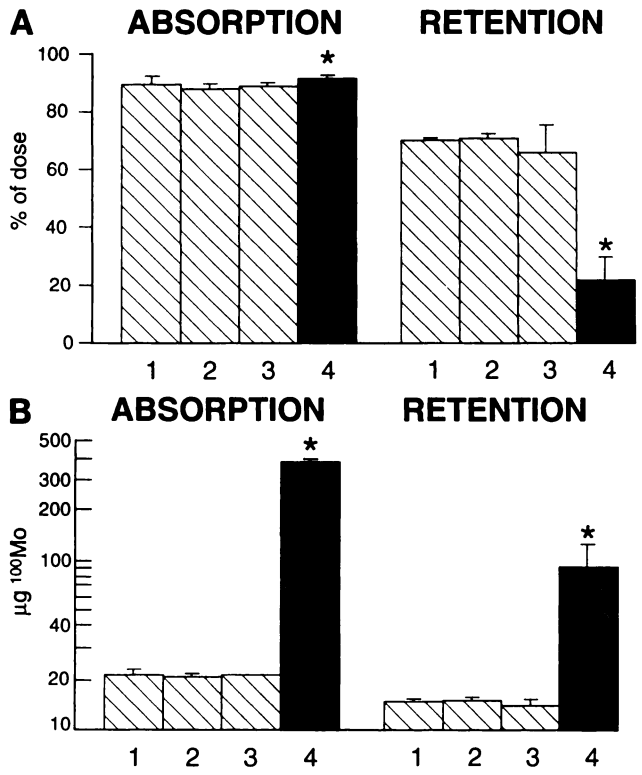

FIGURE 2. Absorption and 12-d retention of ^{100}Mo after each of four oral feedings at 22 and 467 µg Mo/d. $\bar{x}$ ± SD; n = 4. *P < 0.05.

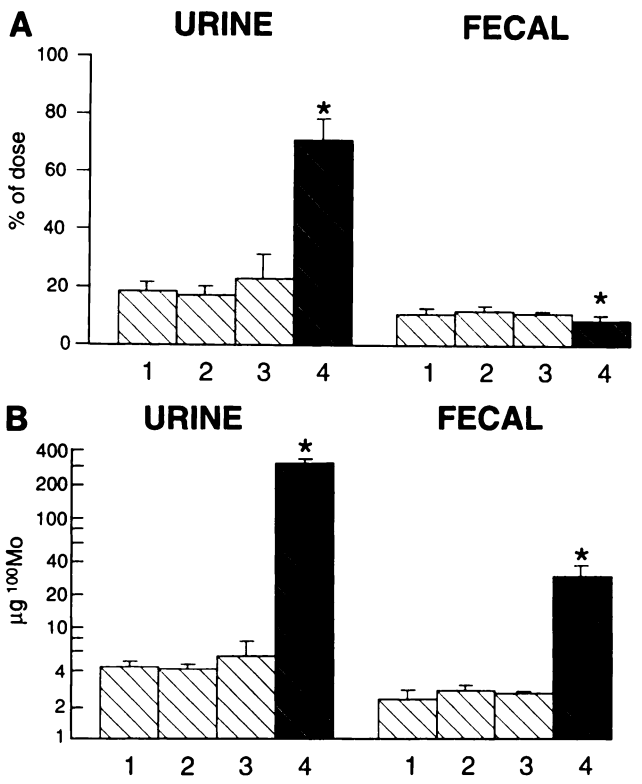

FIGURE 3. Fecal and urinary excretion of ^{100}Mo in the 12-d period after each of four oral feedings at 22 and 467 µg/d. $\bar{x}$ ± SD; n = 4. *P < 0.05.

in **Figure 3** A and B. They did not differ between the three administrations in MP 1.

The pattern of excretion of ^{97}Mo in the urine and feces after the two infusions during depletion are shown in **Figure 4**: 2 ± 0.2% of each dose was excreted into the gastrointestinal tract and eliminated in the first 6 d after each infusion. Fecal excretion remained low, gradually declining after that; 36 ± 2% was excreted in the urine in the first 6 d after each infusion, followed by a rapid decline in urinary excretion in the next 6 d.

No ^{97}Mo was infused during repletion, but there was a significant increase in urinary excretion of ^{97}Mo when dietary molybdenum increased (Figure 4). In the 6-d period 19–24 d after the first infusion, only 3 ± 0.4% of the infused dose was excreted in the urine, but in the 6-d period 19–24 d after the second infusion, when dietary molybdenum was increased, 23 ± 0.4% of the infused dose was excreted in the urine. A similar pattern was observed with ^{100}Mo. In the 6-d period from 13 to 18 d after the first ^{100}Mo feeding, only 1% of the fed dose was eliminated in the urine, whereas 10% was eliminated 13–18 d after the third feeding, at the time dietary molybdenum was increased. Fecal excretion of the tracers was not influenced by the increase in dietary molybdenum.

Discussion

No clinical symptoms of molybdenum deficiency were observed during the course of the 102-d depletion period, which suggests that 102 d of a low-molybdenum diet does not result in a risk of molybdenum deficiency. Clinical signs of molybdenum deficiency have never been observed in normal healthy individuals, suggesting that most diets are adequate in molybdenum. Diarrhea, weakness, and weight loss were observed in an individual with Crohn's disease and small-bowel resections, because of his medical condition (25). In addition, high plasma methionine concentrations and low serum uric acid concentrations and low urinary uric acid excretion were observed. Urinary sulfite excretion was high and sulfate low. After the addition of 300 µg Mo/d to the TPN infusion, these biochemical abnormalities were reversed (25). None of these abnormalities were observed in our subjects during depletion. The lack of biochemical abnormalities suggests that individuals can function without known risk for ≥ 102 d on a very low (22 µg/d) intake of molybdenum.

The balance data from this study could suggest that a molybdenum intake of 22 µg/d is less than adequate to maintain molybdenum status. However, balance was negative less often after day 49 of the 102-d depletion period, and overall balance during that period was slightly positive. This suggests adaptation to the low-molybdenum diet, after which balance can be maintained. We expect that a dietary intake of 25 µg/d would be sufficient to achieve balance in all subjects after the first 6 d of the low-molybdenum diet. Because we observed none of the symptoms or biochemical changes that accompany molybdenum deficiency or molybdenum cofactor deficiency, it is possible that the minimum dietary requirement, the amount to prevent frank deficiency, is less (26). We suggest 25 µg/d as a tentative minimum dietary requirement for healthy adult men, but until a lower amount is tested, we cannot be sure that the minimum requirement is not less than this. There appears to be

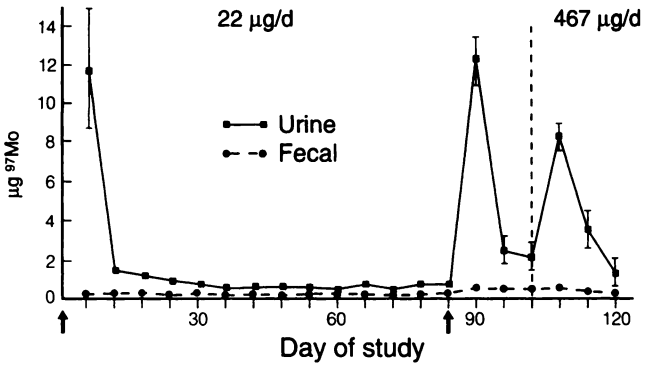

FIGURE 4. Urinary and fecal excretion of ^{97}Mo after two infusions. Arrows indicate day of infusion of 33 µg ^{97}Mo. Numerals at the top of the figure are the amounts of daily dietary molybdenum. $\bar{x} \pm$ SD; $n = 4$.

little if any risk of dietary molybdenum deficiency in healthy individuals, because this is well below the usual dietary intake. To achieve this low amount in our diets, it was necessary to purify the salts to reduce the molybdenum content of the diet. The lower end of the ESADDI, 75 µg/d, is three times our estimate and appears higher than necessary. Lowering the present recommended value to perhaps 30–50 µg/d would provide a substantial margin of safety and appear to present no known risk to normal, healthy young men.

The ^{100}Mo stable-isotope data demonstrate that molybdenum is very efficiently absorbed at both dietary intakes. Therefore, molybdenum retention is not regulated at the level of intestinal absorption. The increased efficiency of absorption at the higher dietary intake was not expected. Other elements we have studied, such as zinc and copper, are absorbed most efficiently at lower intakes and the efficiency of absorption declines as intake increases (27, 28). Those minerals are absorbed much less efficiently than molybdenum at all dietary amounts. The mechanism for this response is not clear, but data suggest that molybdenum absorption is exclusively passive and not saturable, that some dietary components bind a small fraction of the molybdenum, and that the molybdenum binding capacity of the diet is limited.

The ^{97}Mo data demonstrate that very little (< 1%/d) endogenous molybdenum is excreted into the gastrointestinal tract, so there is no regulation of molybdenum retention due to fecal excretion, and that dietary molybdenum does not influence excretion into the gastrointestinal tract.

Urinary excretion of molybdenum appears to be the only point of regulation of molybdenum retention. This was demonstrated in this study by data on total molybdenum excretion and the excretion of both oral and intravenous tracers. When dietary intake was very low, ≈60% of total molybdenum excreted was via the urine. When dietary intake increased to 467 µg/d, > 90% of the total molybdenum excreted was via the urine. The data also suggest that mechanisms to conserve molybdenum retention act to reduce urinary excretion when intake is low. This effect was illustrated most clearly by the urinary excretion of the intravenous dose of ^{97}Mo 19 d after the first compared with the second infusion of ^{97}Mo. At that time interval after the first infusion, when dietary intake was low, 3% of the infused dose was excreted in the urine over a 6-d period, whereas 19 d after the second infusion, when dietary molybdenum was increased, 23% of the dose was excreted in 6 d, more than a sevenfold increase. This demonstrates that an increase in dietary molybdenum increases urinary excretion of endogenous molybdenum and results in more rapid turnover of the body's molybdenum stores.

The fraction of molybdenum excreted in the urine is very different from that of most minerals. Extremely small amounts of copper are eliminated in the urine and under usual conditions the amount does not vary with dietary intake (29). More zinc is excreted in the urine, but this is also a small fraction of dietary intake (30). These minerals exist primarily as cations, whereas molybdenum exists mainly as anions in living organisms. Several minerals, including the electrolytes potassium and sodium, are excreted primarily in the urine, as is selenium (17,31).

In summary, the results of this study suggest that the minimum dietary molybdenum requirement is ≈25 µg/d or possibly less, that deficiency is unlikely in normal healthy men, that molybdenum retention and turnover are regulated by urinary excretion, and that excess molybdenum is rapidly excreted in the urine.

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