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Manganese absorption and retention by young women is associated with serum ferritin concentration[1,2]

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ABSTRACT

Background: The interaction between iron and manganese in the gut is well characterized but iron status has not been shown to affect manganese absorption.

Objective: The objective of this study was to determine whether iron status as determined by serum ferritin concentrations affects manganese absorption, retention, balance, and status.

Design: The subjects were healthy young women; 11 had serum ferritin concentrations >50 μg/L and 15 had serum ferritin concentrations <15 μg/L. In a crossover design, subjects consumed diets that supplied either 0.7 or 9.5 mg Mn/d for 60 d. Manganese absorption and retention were assessed during the last 30 d of each dietary period by using an oral dose of ^{54}Mn; balance was assessed simultaneously.

Results: Dietary manganese did not affect manganese status, but high serum ferritin depressed arginase activity. The interaction of ferritin status and dietary manganese affected ^{54}Mn absorption and biological half-life. Absorption was greatest in subjects with low ferritin concentrations when they were consuming the low-manganese diet, and was least in subjects with high ferritin concentrations. Biological half-life was longest when subjects with high ferritin concentrations consumed the low-manganese diet, and was shortest in all subjects consuming the high-manganese diet. Manganese balance was only affected by the amount of manganese in the diet.

Conclusions: These results show that iron status, as measured by serum ferritin concentration, is strongly associated with the amount of manganese absorbed from a meal by young women. When greater amounts of manganese are absorbed, the body may compensate by excreting manganese more quickly. *Am J Clin Nutr* 1999;70:37–43.

KEY WORDS Manganese, ferritin, iron, women, humans, manganese absorption, manganese retention, manganese balance, manganese status

INTRODUCTION

Manganese is an essential trace element needed for catalytic activity or activation of several enzymes (1). The National Research Council has not determined a recommended dietary allowance (RDA) for manganese; however, an estimated safe and adequate daily dietary intake of 2–5 mg/d has been established (2). Despite the lack of documented cases of human manganese deficiency (3–5), there is concern about inadequate intake in humans because of the number and severity of deficiency problems in animals (3). At the other extreme manganese is a toxic element, although toxicity usually occurs in people who inhale large amounts of manganese-laden dust (6). Studies have shown that under some conditions, especially hepatic dysfunction, a person consuming normal amounts of manganese may accumulate manganese in the brain and such accumulation is associated with a neurologic condition that resembles acute manganese toxicity (7).

Regardless of whether the concern is about potential deficiency or toxicity of manganese, understanding the factors that affect manganese absorption and retention is essential. Relatively little is known about manganese absorption. The total percentage of dietary manganese absorbed from a meal is small; most studies suggest that humans absorb <5% (8–10). Dietary factors that affect manganese absorption have been studied in humans (10), laboratory animals (11), and cultured cells (12). These factors include the carbohydrate source in the diet (13), the presence of phytate (14), the presence of animal protein (15), the manganese content of the diet (11, 13), and the dietary content of other mineral elements (16, 17), especially iron (18–20).

High dietary iron has been shown to depress manganese absorption (11) and status (21) in rats. Increased non-heme iron intake depressed measures of manganese status in women (22), and iron added to an intestinal perfusate depressed manganese absorption (18). In a previous study, we showed that men and women absorbed significantly different amounts of manganese and this difference was associated with serum ferritin concentration (10). More specifically, absorption was significantly associated with serum ferritin in women, who had lower total ferritin concentrations than men, but was not associated with ferritin in men. Despite this association, no one has conclusively shown that iron stores, as assessed by serum ferritin concentrations, are related to manganese absorption or retention. We specifically studied manganese absorption in women with low or high con-

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TABLE 1
Four-day rotating menu of the mixed Western diet consumed by subjects

Meal	Day 1	Day 2	Day 3	Day 4
Breakfast	Orange juice	Orange juice	Orange juice	Orange juice
	Corn cereal	Apple coffeecake	Pork sausage	Corn cereal
	Skim milk	Skim milk	Corn cereal	Skim milk
	Sugar		Skim milk	Sugar
	Mandarin oranges		Sugar	English muffin
				Margarine
Lunch	Baked hamburger	Root beer	Strawberry punch	Black cherry punch
	White bun	Pizza casserole	Beef taco salad	Chef's salad
	Ketchup	Cherry jello with pears	Taco sauce	Ranch dressing
	Coleslaw	Nondairy topping	Ranch dressing	Crackers
	Angel food cake		Chocolate cheesecake	Butter mints
	Vanilla frosting			
Dinner	Chicken rice casserole	Beef casserole	Chicken casserole	Hamburger pie
	Cheddar cheese	Cheddar cheese	Tossed salad	Fruit punch
	Lettuce salad	Lettuce salad	Italian dressing	Corn bread
	Ranch dressing	Ranch dressing	Cherries	Margarine
	Lemon pie	Raspberry sherbet	Angel food cake	Fruit salad
	Nondairy topping	Grape punch	Lemon frosting	Skim milk
	Skim milk	Crackers		Vanilla pudding
	Caramel sundae	Cream cheese		

centrations of serum ferritin who consumed different amounts of dietary manganese. Absorption and retention of manganese were measured with the aid of radioactive ^{54}Mn and whole-body counting.

SUBJECTS AND METHODS

Selection of subjects

Twenty-six healthy, nonpregnant, free-living women between the ages of 20 and 45 y were recruited for this study. All potential subjects were screened to ascertain that they were in good health and to determine their iron status. Iron status was determined by measuring serum ferritin concentration; C-reactive protein concentration was measured simultaneously to determine whether high ferritin concentration was a consequence of infection. If C-reactive protein concentration was high, both measures were repeated at a later date. Subjects were admitted into the study only if they were in the top or bottom 10% of the normal range of serum ferritin concentration (>50 or <5 μg/L). Fifteen subjects were enrolled in the low-ferritin group and 11 were enrolled in the high-ferritin group. Body mass index (in kg/m^2) did not differ significantly between the 2 groups (average of 27.9 ± 2.0 for the high-ferritin group and 26.1 ± 1.0 for the low-ferritin group).

The nature of the research was described in detail to all subjects, who provided their informed consent. The study was approved by the Institutional Review Board of the University of North Dakota and the US Department of Agriculture and followed the guidelines of the Department of Health and Human Services and the Helsinki Declaration regarding human subjects.

Diets

Subjects selected for the study lived at their usual place of residence and continued their normal activities. All foods consumed for the duration of the study were provided by the Grand Forks Human Nutrition Research Center. Within the 2 ferritin groups, subjects were provided with diets containing low or high amounts of manganese in a crossover design. Subjects consumed a mixed Western diet based on a 4-d rotating menu (**Table 1**) that was formulated to provide either 0.7 mg Mn/d (low-manganese diet) or 9.0 mg Mn/d (high-manganese diet) at an energy level of 8380 kJ (**Table 2**). The low-manganese diet was formulated by using foods low in manganese and the high-manganese diet was formulated by adding $MnSO_4$ monohydrate (Spectrum Chemical Manufacturing Co, Gardena, CA) to juice in amounts calculated to provide the desired manganese intake. The amounts of manganese consumed at the 8380-kJ/d level were checked by analyzing duplicate diets. On 27 occasions throughout the study, duplicate diets were prepared simultaneously with the subjects' diets. These diets were prepared for analysis and analyzed by inductively coupled argon plasma (ICAP) spectroscopy. Mean (±SD) dietary manganese intake from the low-manganese diet was 0.75 ± 0.09 mg/d (range: 0.60–0.91 mg/d). Manganese intakes from the high-manganese diet ranged from 8.9 to 9.2 mg/d. Mean iron intake was 14 mg/d, which was slightly below the 1989 RDA of 15 mg/d for women (2).

The low-manganese diet was formulated to provide 16% of energy from protein, 49% from carbohydrate, and 35% from fat (linoleic to total saturated fatty acid ratio of 0.44; 231 mg cholesterol/d). The diet supplied all fat- and water-soluble vitamins in amounts near or above their respective RDAs. The diet supplied minerals in the following amounts daily (based on 8380 J/d): calcium, 941 mg/d; copper, 2.0 mg/d (1.4 mg from a supplement); iron, 10 mg/d; potassium, 1743 mg/d; magnesium, 294 mg/d (135 mg/d from a supplement); sodium, 3311 mg/d; phosphorus, 1219 mg/d; and zinc, 9 mg/d.

In addition to the foods listed on the menu (Table 1), subjects were allowed limited access to several optional foods (coffee, gum, diet soft drinks, and salt). A maximum of 0.014 mg/d of additional manganese could have been ingested if the optional foods were consumed in the maximal amounts allowed. All subjects' usual sources of drinking water were tested for manganese

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TABLE 2
Effect of serum ferritin concentration and dietary manganese intake on clinical measures of young women[1]

	Low-manganese diet		High-manganese diet	
	Low ferritin (n = 15)	High ferritin (n = 11)	Low ferritin (n = 15)	High ferritin (n = 11)
Ln(ferritin)	2.16 ± 0.06[2]	4.22 ± 0.08	2.19 ± 0.06	4.23 ± 0.08
Ferritin (μg/L)	8.67	68.03	8.93	68.72
Platelet manganese (pmol/10^9 cells)	17.7 ± 0.72	18.6 ± 0.72	17.3 ± 0.72	18.9 ± 0.72
Arginase (U/L packed cells)	8190 ± 400[3]	6390 ± 470	7550 ± 400	5780 ± 470
Mean platelet volume (fL)	9.44 ± 0.20	9.51 ± 0.23	9.63 ± 0.20	9.52 ± 0.23
GSH-Px RBC (U/mg hemoglobin)	3.25 ± 0.23	2.63 ± 0.27	3.41 ± 0.23	3.09 ± 0.27
Hematocrit	0.3928 ± 0.0039	0.4048 ± 0.0045	0.3896 ± 0.0039	0.4085 ± 0.0045
Hemoglobin (g/L)	129.9 ± 1.2	135.6 ± 1.4	129.8 ± 1.2	135.4 ± 1.4
Erythrocytes (× 10^{12} cells/L)	4.43 ± 0.04	4.47 ± 0.04	4.41 ± 0.04	4.49 ± 0.04
White cells (× 10^9 cells/L)	5.55 ± 0.22[3]	6.43 ± 0.26	5.35 ± 0.22	6.51 ± 0.26
Platelets (× 10^9 cells/L)	236.6 ± 4.9	248.3 ± 5.3	241.4 ± 4.9	258.7 ± 5.8

[1] $\bar{x}$ ± SEM; GSH-Px RBC, glutathione peroxidase activity in erythrocytes.
[2,3] Significant effect of ferritin status: [2] $P < 0.0001$, [3] $P < 0.05$.

and if the manganese concentration was too high, distilled water was provided. The maximum intake of manganese from water was <0.01 mg/d. Subjects consumed either the low-manganese or high-manganese diet for 60 d and were then given a break of ≈1 mo, during which they resumed their normal diet. Afterward, they returned to the study and were provided with the second diet for an additional 60 d.

Radioactive test meal and whole-body counting procedures

Manganese absorption and retention in the body were calculated by following the retention of an orally administered dose of ^{54}Mn. After consuming the respective diets for 30 d, subjects fasted overnight and then consumed a test dose of 0.037 mBq ^{54}Mn in the form of carrier-free $MnCl_2$. The radioactive manganese was consumed in ≈240 mL juice with the regularly scheduled breakfast (the total amount of juice varied depending on energy intake, whereas radioactive manganese intake was constant among subjects). The juice was consumed at will while eating the breakfast scheduled for day 3 in the menu cycle. The breakfast and juice contained 1278 kJ (305.5 kcal), 17.5 g protein, 2.7 g fat, 52.1 g carbohydrate, 0.8 g fiber, 238 mg Ca, 2.8 mg Fe, 48.4 mg Mg, 260 mg P, 759 mg K, 531 mg Na, 1.9 mg Zn, 0.3 mg Cu, 0.1 mg Mn, 74 mg vitamin C, 136 retinol equivalents vitamin A, 1.8 μg vitamin D, 0.4 mg α-tocopherol, 1 g saturated fatty acids, 1.7 g unsaturated fatty acids, and 28 mg cholesterol. Supplemental manganese was not added to the juice of subjects consuming the high-manganese diet.

Subjects collected total feces and urine for 21 d after consumption of the test meal. Radioactive manganese in the feces was determined by using a custom-built, small animal whole-body counter equipped with an ND62 multichannel analyzer (Nuclear Data Instrumentation, Schaumberg, IL) and calibrated with ^{137}Ce.

Whole-body ^{54}Mn gamma radiation was determined by using a method developed by Lykken (23). Gamma emissions were counted in a steel-enclosed facility equipped with 32 NaI detectors and a 1024 multichannel analyzer (Nuclear Data Instrumentation). An air-handling system filtered and removed radon from the room air. Subjects were counted 4 times for 15 min each on the day of ingestion of the isotope (these counts were averaged to calculate the initial count) and once daily for the next 14 d. After the first 2 wk of whole-body counting, subjects were whole-body counted twice weekly until the end of the dietary period.

Blood draws and clinical measurements

Blood samples were obtained on days 1, 30, and 60 of the study. Blood was analyzed to determine the complete blood count; hemoglobin; hematocrit; serum ferritin; platelet aggregation; C-reactive protein; manganese concentration in the plasma, white cells, erythrocytes, and platelets; whole blood arginase activity; and glutathione peroxidase activity in plasma and erythrocytes.

Chemical balance

Chemical balance of manganese and iron was calculated for all subjects by using feces and duplicate diets collected during the last 16 d of each dietary period. Total manganese and iron intakes were estimated from analysis of duplicate diets (corrected for differences in energy intake). Total fecal manganese and iron were estimated directly from fecal aliquots. Chemical balance was computed as total daily manganese or iron intake minus manganese or iron in the feces.

Chemical analyses

Manganese and iron in the diets and feces were determined by ICAP spectroscopy (Jarrell-Ash, Waltham, MA) (24). Samples were prepared for analysis by digesting freeze-dried material in nitric and perchloric acid (25). The detection limit of this method was 364 nmol/L for manganese and 895 nmol/L for iron. Subsample variation was always <10%. National Institute of Standards and Technology certified bovine liver (SRM #1577a) and total diet (SRM #1548) standards were used to ensure the accuracy of all analyses. Results were considered acceptable if concentrations of manganese and iron in standards fell within the certified range. Manganese concentrations in whole blood and plasma were analyzed by Zeeman graphite furnace atomic absorption spectroscopy (Zeeman model 3030; Perkin-Elmer, Norwalk, CT) (26) after digestion as described previously. The detection limit of this method was 9 nmol/L and sample-to-sample variation was between 5% and 12%. Standards (UTAK Trace Element Control Serum #66816; UTAK Laboratories, Inc, Valencia, CA) were analyzed with all samples to assess the accuracy of the method. Serum fer-

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TABLE 3
Absorption, biological half-life, and retention of ^{54}Mn after 60 d in young women who had high or low serum ferritin concentrations and consumed diets supplying 0.7 or 9.5 mg Mn/d[1]

	Low-manganese diet		High-manganese diet	
	Low ferritin	High ferritin	Low ferritin	High ferritin
Absorption (%)[2]	4.86 ± 0.58[a] [11]	0.97 ± 0.68[b] [15]	2.3 ± 0.58[b] [11]	1.03 ± 0.63[b] [15]
Ln(biological half-life)[3]	2.82 ± 0.18[a,b] [7]	3.53 ± 0.21[a] [5]	2.56 ± 0.18[b] [7]	2.46 ± 0.19[b] [6]
Half-life (d)[4]	17.0; 20.1–14.0	36.6; 42.1–27.7	13.0; 15.5–10.8	11.8; 14.2–2.27
Retention after 60 d (%)[5]	0.50 ± 0.09[a] [7]	0.26 ± 0.03[a] [5]	0.07 ± 0.09[b] [7]	0.03 ± 0.10[b] [6]

[1] Values determined by linear regression of whole-body counts for days 15–30; values are $\bar{x}$ ± SE unless otherwise indicated; values in same row with different superscript letters are significantly different with P as indicated; n in brackets.
[2] Significant effect of ferritin status × dietary manganese, $P < 0.001$.
[3] Significant effect of dietary sequence, only data for first test meal used in analyses; significant effect of ferritin status × dietary manganese, $P < 0.05$.
[4] Values are half-lives, transformed from ln values [mean; (mean + 1 SEM)–(mean − 1 SEM)].
[5] Significant effect of dietary manganese, $P < 0.01$.

ritin was measured by using a diagnostic kit (IMX Ferritin; Abbott Laboratories, Abbott Park, IL). Complete blood counts, hemoglobin, and hematocrit were determined with a cell counter (CellDyne; Abbott Laboratories). Arginase activity was determined by using the method of Colombo and Konarska (27), glutathione peroxidase activity was determined by using the method of Paglia and Valentine (28), and plasma selenium was determined by using Zeeman graphite furnace atomic absorption spectroscopy.

Data analysis

Whole-body count data were analyzed by using the regression procedure in SAS/STAT, version 5 (SAS Institute, Cary, NC) (29). For each subject, the natural logarithm of the percentage of ^{54}Mn retained was plotted against time elapsed after the dose was administered and a regression line was fit to the linear portion of the curve from day 15 to day 30. Biological half-life was computed as $-\ln(2)$/slope and the y intercept was used as the estimate of percentage absorption (10). A two-factor repeated-measures analysis of variance (ANOVA) was performed to determine the effects of ferritin status and dietary manganese (30). Dietary sequence (ie, low-manganese diet first followed by high-manganese diet or vice versa) was included in an initial ANOVA model. If the effect was not significant it was removed from the model. If the effect was significant then only data for the initial diet consumed by a subject were included and the analysis was repeated. Pearson's product-moment correlation coefficients, followed by Tukey's contrasts where appropriate, were computed among ln(serum ferritin), manganese absorption, ln(biological half-life of manganese), arginase activity, plasma manganese, and manganese balance.

RESULTS

Clinical measures

The effects of dietary manganese intake and ferritin status on clinical measures are shown in Table 2. Dietary manganese intake was not significantly associated with any clinical measure. The only measure of manganese status that was affected by ferritin status was arginase activity, which was significantly depressed in subjects with high ferritin (mean of 6090 ± 330 compared with 7870 ± 280 U/L packed cells for high and low ferritin, respectively). Subjects with high ferritin status also tended to have depressed glutathione peroxidase activity in erythrocytes.

Manganese absorption, excretion, and retention

The kinetics of the disappearance of the orally administered dose of ^{54}Mn (data not shown) were similar to those described in previous studies (8–10) that used similar methods. After a brief delay (1–3 d for most subjects), ^{54}Mn disappeared from the body quickly. Between 6 and 10 d postdosing, ^{54}Mn disappeared at an intermediate rate and from 11 d until the end of the study, ^{54}Mn disappeared from the body at a slow but linear rate. The disappearance of ^{54}Mn from the body was mirrored by the appearance of ^{54}Mn in the feces (data not shown).

There was a significant interaction between ferritin status and dietary manganese for manganese absorption and biological half-life (**Table 3**). The lowest percentage absorption of manganese was in subjects with high ferritin status who consumed diets low in manganese; subjects with low ferritin status who consumed diets low in manganese absorbed almost 5-fold more manganese. The interaction between diet and ferritin status occurred because manganese absorption was similar for all subjects with high ferritin status; however, in subjects with low ferritin status, low dietary manganese intake resulted in an almost 2-fold enhancement of manganese absorption.

There was a significant effect of the sequence of dietary treatments (ie, whether subjects were on the low- or high-manganese diet first) on biological half-life of ^{54}Mn and retention of ^{54}Mn after 60 d. Consequently, the data were reanalyzed by using only the first dietary period of each subject. Although this change resulted in reduced significance levels, the trends and significant effects were similar to when all data were used. There was a significant interaction between ferritin status and dietary manganese intake for biological half-life (Table 3) because subjects consuming the low-manganese diet with high ferritin status had a biological half-life more than twice as long as subjects with low ferritin status consuming the low-manganese diet. However, there was little effect of ferritin status when manganese intake was high. Biological half-life was similar for all subjects consuming the high-manganese diet and subjects with low ferritin consuming the low-manganese diet.

Retention of the dose of ^{54}Mn after 60 d was calculated from the percentage absorption and the biological half-life. There was a significant effect of dietary manganese intake; subjects consuming the low-manganese diet retained a greater percentage of the test dose of ^{54}Mn than subjects consuming the high-manganese diet (0.38 ± 0.07% compared with 0.049 ± 0.07% for the low- and high-manganese diets, respectively). When retention of

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TABLE 4
Manganese chemical balance in women with high or low serum ferritin concentrations who consumed diets supplying 0.7 or 9.5 mg Mn/d[1]

	Low-manganese diet		High-manganese diet	
	Low ferritin (n = 11)	High ferritin (n = 15)	Low ferritin (n = 11)	High ferritin (n = 15)
	mg/d			
Manganese intake	0.73 ± 0.03	0.75 ± 0.04	8.99 ± 0.03	9.09 ± 0.04
Manganese in feces[2]	0.75 ± 0.36	0.86 ± 0.49	7.46 ± 0.36	8.50 ± 0.49
Manganese balance[3]	−0.01 ± 0.37	−0.12 ± 0.49	1.53 ± 0.37	0.59 ± 0.49

[1] $\bar{x}$ ± SEM.
[2,3] Significant effect of dietary manganese: [2] $P < 0.0001$, [3] $P < 0.05$.

dietary manganese after 60 d was estimated from the ^{54}Mn retention data, subjects consuming the high-manganese diet retained 4.41 μg and subjects consuming the low-manganese diet retained 2.66 μg Mn from a day's total manganese intake.

Manganese balance

The effects of ferritin status and dietary manganese intake on manganese chemical balance measures, as calculated from the last 16 d of the fecal collection periods, are shown in **Table 4**. Fecal manganese and manganese balance were significantly affected by dietary manganese intake; the low-manganese diet resulted in a slightly negative balance (−0.06 mg Mn/d) compared with the high-manganese diet (1.06 mg Mn/d). Iron balance was positive in all subjects and was not affected by diet or manganese status (data not shown).

Correlations between measures of manganese metabolism and ferritin status

When all data were used (**Table 5**), there was a significant negative association between ln(serum ferritin) and manganese absorption and a significant negative association between manganese absorption and ln(manganese half-life). There was a strong positive association between ln(serum ferritin) and ln(manganese half-life) and a marginal positive association between ln(manganese half-life) and plasma manganese.

Associations calculated within each ferritin concentration and dietary manganese group are not shown in table format. Of the 60 associations measured (15 per treatment group) only 8 were significant, and 5 of the 8 were in the low-manganese, low-ferritin group. The significant associations ($P < 0.05$) in the low-manganese, low-ferritin group were a negative association between ln(serum ferritin) and manganese absorption and positive associations between ln(serum ferritin) and manganese balance and manganese absorption and ln(manganese half-life) (P = 0.058). Positive associations were noted between manganese absorption and manganese balance and ln(manganese half-life) and plasma manganese. There was only a single significant association within each of the other treatment groups, and all these associations were negative, as follows: between manganese absorption and ln(manganese half-life) in the high-manganese, high-ferritin group; between arginase activity and manganese balance in the low-manganese, high-ferritin group; and between manganese absorption and ln(manganese half-life) in the high-manganese, low-ferritin group.

DISCUSSION

A previous study by this laboratory found a strong association between ferritin status and the absorption and retention of manganese (10). Other investigators have shown an interaction between iron and manganese that occurred primarily in the gut and was strongest with nonheme iron (11, 18–20, 22). Anemia induced by a deficiency of dietary iron increased manganese absorption, but anemia induced by bleeding had little effect on manganese absorption (31). A possible explanation is that iron and manganese share a common cellular transport mechanism (18, 32, 33) and thus excesses of one element inhibit transport of the other. If this is the mechanism of manganese absorption, then depressed iron stores should turn on iron transport and, as a consequence, also concurrently increase manganese transport. However, there is no direct evidence suggesting that manganese absorption and retention are affected by the iron stores of a healthy individual. In fact, previous studies have found no association between the iron status of normal, nonanemic individuals and manganese absorption (8, 34, 35).

This study provided evidence of a strong association between iron stores, as assessed by serum ferritin concentrations, and manganese absorption, as determined by regression of whole-body

TABLE 5
Associations among various measures of iron status, manganese status, and manganese metabolism in all study subjects, who were young women differing in ferritin status and consuming diets either low or high in manganese[1]

	Manganese absorption	Ln(manganese half-life)	Arginase activity	Plasma manganese	Manganese balance
Ln(serum ferritin)	−0.664	0.411	NS	NS	NS
Manganese absorption		−0.344	NS	NS	NS
Ln(manganese half-life)			NS	0.287	NS
Arginase activity				NS	NS
Plasma manganese					NS

[1] Values are Pearson's product-moment correlation coefficients (r) that are significant at $P < 0.05$, except for ln(manganese half-life) and plasma manganese, for which P = 0.053; NS, not significant ($P > 0.053$); n = 26 (all study subjects) for all analyses.

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radioactivity counts, in normal individuals. The regression method of determining absorption has inherent problems (36) that may be related to more than one rate of excretion of ingested tracer, possibly as a result of fast biliary excretion of absorbed ^{54}Mn (17, 37–39). We showed previously that the number of days used for regression analysis affects the absorption estimate (10). For this study, absorption was defined as the y intercept of a regression line through data from day 15 to day 30, thus we estimated absorption from the retained manganese and did not consider ^{54}Mn that may have been absorbed and rapidly reexcreted.

With this model of absorption, the serum ferritin concentration of an individual had more influence on manganese absorption than did the dietary manganese intake. Within a diet group, individuals with low ferritin absorbed 3–5-fold more ^{54}Mn than individuals with high ferritin. Further evidence of the influence of iron status on manganese absorption is the overall strong negative association between ln(serum ferritin) and manganese absorption. The relation is not simple however, because when associations were measured within treatment groups the only significant association was within the low-manganese, low-ferritin group.

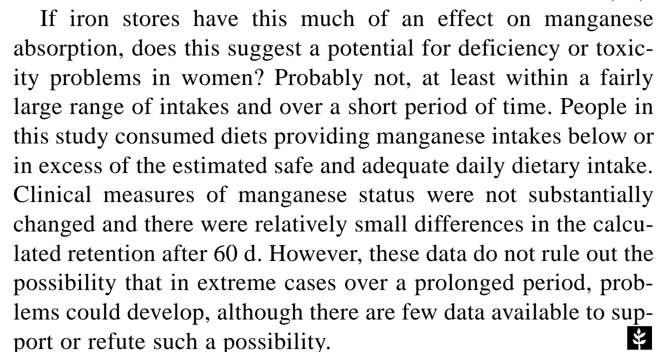
Subjects with low serum ferritin concentrations absorbed more ^{54}Mn while consuming the low-manganese diet (compared with the high-manganese diet), although the amount of dietary manganese had no effect on absorption by subjects with high serum ferritin concentrations. This may show that manganese absorption is regulated in part by the amount of manganese in the diet. An alternative explanation, however, is that true absorption was unaffected and the difference in apparent absorption was a consequence of determining absorption by regression of whole-body counts, which is actually a measure of absorption minus quick excretion. Subjects consuming the high-manganese diet may have absorbed the same amount of ^{54}Mn as subjects consuming the low-manganese diet while increasing the amount of ^{54}Mn rapidly reexcreted into the gut by way of the bile.

Rapid biliary excretion of absorbed manganese might partially explain the strong negative association between manganese absorption and serum ferritin concentration when all data were used, as well as the lack of such an association in all groups except the low-ferritin, low-manganese group when associations were measured within individual diet × ferritin groups. True absorption may have been significantly (negatively) associated with manganese absorption as measured in the low-ferritin, high-manganese group, but true absorption may have been underestimated because subjects may have absorbed much manganese that was quickly eliminated through the bile.

Despite the large differences in intake, the ultimate retention of manganese by the body was effectively controlled. Calculated percentage retention of ^{54}Mn after 60 d was 5–10-fold greater for the low-manganese diet than for the high-manganese diet. However, when the retention of dietary manganese after 60 d was estimated from ^{54}Mn absorption and turnover, subjects consuming the diet high in manganese retained less than twice as much manganese (6.3 and 2.7 μg Mn retained for low and high serum ferritin, respectively) as subjects consuming the diet low in manganese (3.5 and 1.8 μg Mn retained for low and high serum ferritin, respectively).

Evidence that much of this efficient homeostatic control is achieved by variable excretion is provided by the association between biological half-life and absorption. Absorption was significantly and negatively associated with biological half-life in subjects with high or low serum ferritin consuming high-manganese diets. Absorption was marginally associated with biological half-life in subjects with low serum ferritin consuming low-manganese diets, but was not associated in subjects with high serum ferritin who consumed low-manganese diets. Subjects with high serum ferritin and low dietary manganese absorbed minimal manganese and consequently elimination pathways were relatively unutilized. However, when ferritin stores were low, the body was bringing in much iron and manganese and consequently, even though dietary manganese was low, relatively large amounts of manganese may have been absorbed and needed to be eliminated.

Previous studies showed that excretion is an important means of controlling manganese homeostasis. Mena et al (40) showed that increases in potentially toxic amounts of manganese absorbed as a consequence of anemia were partially ameliorated by increased manganese excretion. Mahoney and Small (41) found that an anemic subject retained a dose of ^{54}Mn longer after beginning iron therapy than before iron therapy started. Although the primary route of elimination of endogenous manganese is presumed to be the bile, a recent study by Finley et al (42) showed that substantial amounts of manganese were present inside the epithelial cells of pigs up to 11 d after a dose of ^{54}Mn was given. Because it is assumed that a large portion of gut cells would have sloughed into the lumen in that period, this may show that manganese is transported back into the gut epithelia from the blood. Evidence supporting this idea also came from a study of cultured intestinal epithelial cells (CACO-2) that were shown to actively transport manganese in a serosal to luminal direction much faster than in a luminal to serosal direction (12).



If iron stores have this much of an effect on manganese absorption, does this suggest a potential for deficiency or toxicity problems in women? Probably not, at least within a fairly large range of intakes and over a short period of time. People in this study consumed diets providing manganese intakes below or in excess of the estimated safe and adequate daily dietary intake. Clinical measures of manganese status were not substantially changed and there were relatively small differences in the calculated retention after 60 d. However, these data do not rule out the possibility that in extreme cases over a prolonged period, problems could develop, although there are few data available to support or refute such a possibility.

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