© Copyright 2001 by Humana Press Inc.
All rights of any nature, whatsoever, reserved.
0163-4984/01/8201–3/0143 $13.75

Manganese Absorption and Retention in Rats Is Affected by the Type of Dietary Fat

John W. Finley* and Cindy D. Davis

United States Department of Agriculture, Agricultural Research Service, Grand Forks Human Nutrition Research Center, Grand Forks, ND 58203

Received November 18, 2000; Revised January 30, 2001; Accepted February 15, 2001

ABSTRACT

There is evidence that manganese (Mn) metabolism may be altered by the form and amount of dietary fat. Also, iron (Fe) absorption is greater with saturated fats, as compared to polyunsaturated fatty acids (PUFAs). The absorption of Fe and Mn are interrelated in many aspects; therefore, the form of dietary fat may indirectly alter Mn absorption. The reported studies were conducted to determine whether saturated fat, as compared to unsaturated fat, affected Mn absorption, retention, and metabolism. In experiment I, adult rats were fed diets containing either 0.7 or 100.4 μg/g Mn with the fat source as high-linoleic safflower oil or stearic acid. After 2 wk of equilibration, the animals were fed a test meal of ^{54}Mn followed by whole-body counting for 10 d. Manganese absorption was significantly ($p < 0.05$) lower in the stearic acid group (0.9–4.8%) than in the safflower oil group (20–33.8%); however, the biological half-life was shorter in the safflower oil group. Retention of ^{54}Mn and total Mn was always significantly ($p < 0.05$) greater in the safflower oil group when dietary Mn was low, but it was the same when dietary Mn was high. In experiment II, weanling rats were fed 1.3, 39.3, or 174.6 μg Mn/g and either stearate, high-oleic safflower oil or high-linoleic safflower oil for 8 wk.

*Author to whom all correspondence and reprint requests should be addressed.

The U.S. Department of Agriculture, Agricultural Research Service, Northern Plains Area, is an equal opportunity/affirmative action employer and all agency services are available without discrimination.

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

Long-term feeding of the stearate and low Mn-containing diet resulted in a significant ($p < 0.0001$) reduction in heart superoxide dismutase activity and kidney and liver Mn concentrations compared to the other diets. These data show that stearic acid inhibitits Mn absorption, but it may not inhibit Mn retention when dietary Mn is high.

Index Entries: Iron; manganese; fat; saturated fat; PUFA; safflower oil; stearic acid; rat.

INTRODUCTION

Manganese (Mn) is an essential element *(1)* and Mn deficiency causes severe problems in animals. Also, manganese is toxic when ingested in excessive amounts *(2);* therefore, it is essential that Mn retention be regulated. This is accomplished by variable absorption and excretion *(3).*

Manganese absorption may be influenced by several dietary factors *(3,4),* including calcium (Ca) *(5),* potassium (P) *(6),* nickel (Ni) *(7),* and the source of dietary carbohydrates *(8)* and protein *(9,10).* Dietary iron (Fe) has the greatest effect on Mn absorption *(3,4,11–13).* Iron status also affects Mn absorption; Mn absorption was negatively associated with serum ferritin concentrations of healthy young women fed 3–5 mg Mn/d *(14).* Subsequently, healthy women with low serum ferritin concentrations were found to absorb more Mn than women with high serum ferritin concentrations *(15),* and serum ferritin concentrations were associated more with the percentage of Mn absorption than with dietary Mn. This interaction may occur because Mn may be absorbed by the same mechanism as Fe; thus, factors that influence Fe absorption may indirectly affect Mn absorption. Iron absorption is affected by the type of dietary fat; saturated fats such as stearate (as compared to unsaturated fats such as safflower oil) enhance Fe absorption *(16,17),* which suggests that dietary fat also may affect Mn absorption.

Manganese is involved in cholesterol metabolism, and Mn deficiency may result in metabolic perturbations of cholesterol metabolism. Studies of Mn effects on cholesterol metabolism suggest that dietary fat may influence Mn absorption and/or retention also *(18,19).* Based on these reports and on studies showing an interaction between Fe and Mn absorption and between dietary fat and Fe absorption, the objective of the present investigation was to determine the effect of diets enriched in saturated fat (stearate) or polyunsaturated fat (PUFA; safflower oil) on Mn absorption, tissue distribution and retention, and biochemical measures.

MATERIALS AND METHODS

Experimental design

Experiment I was a 2 × 2 factorial design with diets that supplied 1.3 or 100.4 µg Mn/g diet and contained either stearate (as the free fatty acid)

or high-linoleic safflower oil (73% 18:2 [n-6] as the glycerol ester) as the fat source. Thirty-two rats were fed an equilibration diet for 2 wk and then allocated to four diets in a manner that equalized weights. On the same day that they were assigned to the diets, the rats consumed a test meal (dietary composition identical to their dietary treatment) that was labeled with carrier-free ^{54}Mn. Rats were placed in metabolic cages for complete urine and fecal collections and were counted daily for whole-body radiation for 10 d. Rats were killed and tissues collected on the d 10.

Experiment II was a 3 × 3 factorial design with diets that supplied low, adequate, or high concentrations of Mn (by analysis, 1.3-, 39.3-, or 174.6 µg Mn/g diet, respectively) and three types of dietary fat (stearate, high-oleic safflower oil [70% 18 : 1 {n-9} as the glycerol ester], and high-linoleic safflower oil). Six rats were fed each of these nine diets for 8 wk.

Animals and Diets

Both studies used male, Sprague–Dawley rats (Sasco, Omaha, NE). Experiment I used 100-g rats and experiment II used weanling rats (58±4 g). In both experiments, rats were housed individually in stainless-steel wire-bottomed cages in a room with controlled temperature and light. They were provided free access to demineralized water and purified diet.

In experiment I, the equilibration diet was based on the AIN-76 diet and contained 35 µg Fe/g diet and 10 µg Mn/g diet (analyzed value, 11.4 µg Mn/g diet). Fat was 12% of the diet and was composed of 50% high-linoleic safflower oil and 50% stearate. After 2 wk on the equilibration diet, animals were randomly assigned to one of four dietary treatments, all based on the AIN-76 diet: (1) basal Mn (1.3 µg Mn/g diet by analysis) and 12% stearate; (2) 100 µg Mn/g diet (100.4 µg Mn/g diet by analysis) and 12% stearate; (3) basal Mn (1.6 µg Mn/g diet by analysis) and 12% safflower oil; (4) 100 µg Mn diet (97.4 µg Mn/g diet by analysis) and 12% safflower oil. All diets contained 35 µg Fe/g diet. Diets were prepared similar to Johnson et al. *(16)*, except 2% safflower oil was not included in the stearic acid diet. Stearic acid was added as the free fatty acid, which is a solid at room temperature (Pfalz and Bauer, Waterbury, CT). High-linoleic-acid safflower oil is a liquid composed of glycerol esters of the fatty acids (U.S. Biochemical, Cleveland, OH).

In experiment II, the experimental diets were all based on the AIN-76 diet with 12% of the diet from fat. The stearate-containing diets contained 10% stearate and 2% high-linoleic safflower oil to supply essential fatty acids. High-oleic safflower oil was purchased from California Fats and Oils (Richmond, CA).

Test Meal and Whole-Body Counting

The test meal was 3 g of the treatment diet labeled with 110 kBq ^{54}Mn. Whole-body counts (WBC) of retained ^{54}Mn were measured with a custom-built small-animal whole-body counter equipped with two NaI detec-

tors and a ND62 multichannel analyzer (Nuclear Data Instrumentation, Schaumberg, IL) set at 2048 channels and calibrated with ^{32}Na. Radioactivity of ^{54}Mn was measured between 700 and 890 keV. The measured radioactivity was corrected for background and decay. The absorption of ^{54}Mn was calculated by extrapolating the linear portion of a plot of ln(percentage of retention) versus time from d 4 to d 9 after isotope administration. Excretion rates were expressed as the biological half-life (BH), where BH = –(ln 2)/slope; the slope in this equation is the slope of the linear portion of the semilogarithmic plot of retention versus time.

Radioactivity in tissues, blood samples, and feces was measured in a gamma well counter equipped with a ND62 multichannel analyzer (Cobra-II Auto Gamma, Packard, Canberra). Tissue retention of ^{54}Mn was expressed as nanograms of Mn from a test meal, which was calculated from the ^{54}Mn in a tissue and the specific activity of Mn in the test meal.

Laboratory Analysis

Lyophilized tissues and diets were ashed with a nitric acid/hydrogen peroxide digestion. Mn was determined by atomic absorption spectrometry with bovine liver as the control (National Bureau of Standards). The certified value for Mn in bovine liver is 9.9 ± 0.8 μg/g; we found 10.3 ± 0.3 μg/g.

Plasma and high-density lipoprotein (HDL) cholesterol, triglyceride, and glucose concentrations were determined by using a Cobas Fara automated analyzer (Hoffman La Roche, Nutley, NJ). Heart and liver superoxide dismutase activities (experiment II) were determined by the inhibition of pyrogallol auto-oxidation; 0.1 mmol/L KCN was added to the reaction mixture to inhibit copper–zinc superoxide dismutase activity. One unit of superoxide dismutase (SOD) activity was defined as the amount of enzyme needed to obtain 50% inhibition of pyrogallol auto-oxidation.

Statistics

Data were analyzed as a 2 × 2 factorial that tested for fat source, dietary Mn, and the interaction of fat and Mn by using a SAS general linear model program *(20).* Absorption and biological half-life were transformed to the natural log before statistical analysis. Tukey's contrasts were used to differentiate among means for variables that had a significant ($p < 0.05$) effect of treatment *(21).*

RESULTS

Food Intake and Weight Gain

Dietary treatments did not affect the final weight of animals in either experiment. Food intake in experiment I was affected by the fat source

Table 1
The Effect of Dietary Fat Type and Mn Concentration on the Absorption of ^{54}Mn by Rats in Experiment I

Diet Mn	Diet Fat	ln (% absorption)[1,2]	Initial Disappearance (Slope days 1-3)	ln (Biological half-life)
Low	Stearate	1.32±0.24[b]	-0.91±0.06[b]	2.18±0.14[a]
		3.85 (2.94-4.76)[3]		9.0 (7.7 - 10.2)[4]
Low	Linoleic	3.24±0.24[c]	-0.56±0.06[a]	1.41±0.14[b]
		26.3 (20.1-32.5)		4.2 (3.6 - 4.8)
High	Stearate	0.11±0.24[a]	-1.28±0.06[c]	1.36±0.14[b]
		1.12 (0.88-1.41)		4.0 (3.4 - 4.5)
High	Linoleic	3.28±0.24[c]	-0.91±0.06[b]	0.30±0.14[c]
		27.3 (20.9-33.8)		1.4 (1.2 - 1.6)
Statistical effect determined by 2-way ANOVA:				
Mn		0.03	0.0001	0.0001
Fat		0.0001	0.0001	0.0001
Mn x Fat		0.02	NS	NS

[1] Values are means±SEM; n = 8. Dietary Mn notation is as follows: Low = 1.3 mg Mn/kg diet and High = 100.4 mg Mn/kg diet.

[2] Means in a column with different superscripts are significantly different (p > 0.05) as determined by Tukey's contrasts. NS = not significant.

[3] Absorption (%), In(mean)±SEM; mean and range of –1 to +1 SEM back-transformed from In values are indicated in parentheses.

[4] Biological half-life (d), In(mean)±SEM; Mean and range of –1 to +1 SEM back-transformed from In values are indicated in parentheses.

(p < 0.0006), and animals fed stearate consumed more diet (19.7 g/d) than animals fed high-linoleic safflower oil (16.8 g/d).

Absorption and Biological Half-life

There was a significant Mn and fat interaction (p < 0.02) for percent absorption in experiment I (Table 1). This occurred because, overall, more Mn was absorbed when high-linoleic safflower oil was the fat source (0.88–4.76% for stearate and 20.1–33.8% for high-linoleic safflower oil;

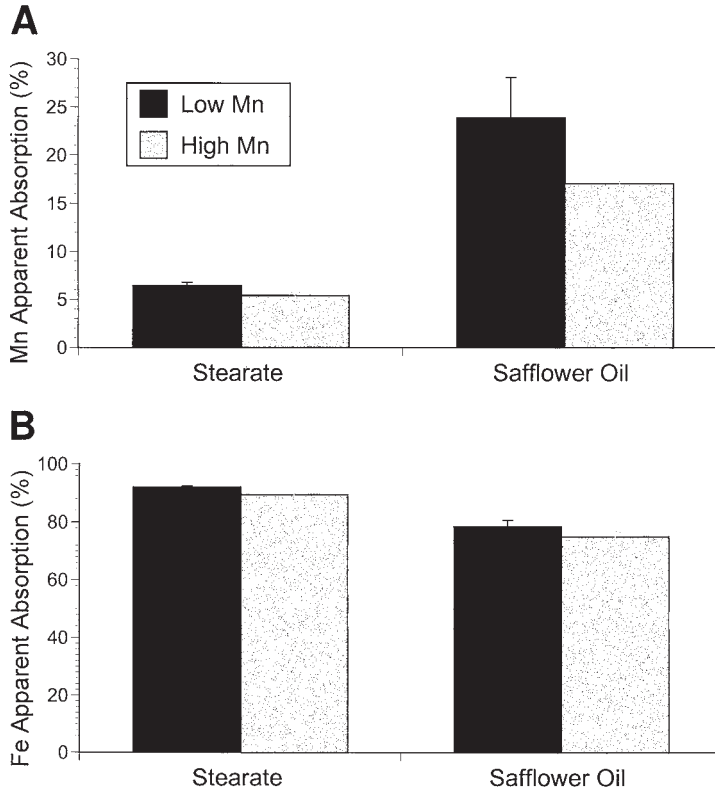

Fig. 1. Apparent absorption of manganese **(A)** and iron **(B)** determined by chemical balance in rats fed diets containing low or high manganese and stearate or high-linoleic safflower oil. Values are means ±SEM; n = 8.

range of mean ± 1 SE), but high dietary Mn only decreased Mn absorption when stearate was the fat source.

The slope of the WBC curve for the first 4 d is reflective of initial gut turnover. It was more negative (Table 1) when stearate was the fat source (–0.74 and –1.09 for high-linoleic safflower oil and stearate, respectively) and when high dietary Mn was fed (–0.74 and –1.10 for low and high Mn, respectively). The biological half-life (reflective of long-term Mn turnover) was significantly greater when stearate was the fat source (mean of 5.9 d and 2.4 d for stearate and high-linoleic safflower oil, respectively) and when rats were fed low Mn (mean of 6.0 d and 2.3 d for animals fed low and high Mn, respectively).

Chemical Determination of Fe and Mn Apparent Absorption

Manganese absorption, as determined by chemical balance (Fig. 1), was similar to absorption determined by whole-body scintillation counting. More Mn was absorbed when high-linoleic safflower oil was the

dietary fat source ($p < 0.0001$) and less Mn tended ($p < 0.08$) to be absorbed when dietary Mn concentrations were high (as compared to low dietary Mn). Iron absorption was 3–5% greater ($p < 0.04$) when dietary Mn was low than when it was high.

Mn Distribution

Experiment I. There was a significant dietary Mn and fat interaction that affected the distribution of ^{54}Mn in every tissue (Table 2). On average, more Mn from the test meal was retained in the tissues and organs of rats fed high-linoleic safflower oil than those fed stearate. Also, more Mn was retained when high dietary Mn was fed (as compared to low dietary Mn). However, when dietary Mn was high, Mn retention was not significantly different between animals fed stearate and animals fed high-linoleic safflower oil.

High dietary Mn increased total Mn in the heart ($p = 0.0006$) and spleen ($p = 0.0001$) (Table 3). Total Mn concentration in erythrocytes was significantly increased in rats fed high-linoleic safflower oil ($p = 0.002$). When dietary Mn was low, Mn concentrations in the kidney and liver were depressed by stearate (depression of 16% and 33% in kidney and liver, respectively). When the diet was high in Mn, the fat type did not affect kidney Mn concentrations ($p > 0.05$), and liver Mn concentrations were elevated in the stearate-fed animals (significant interaction between dietary Mn and fat; $p = 0.005$, kidney; $p = 0.0001$, liver).

Experiment II. Maganese SOD activity in the hearts and livers of animals in experiment II changed in response to dietary Mn concentration and fat source ($p < 0.0001$). Animals fed low dietary Mn had a 25% and 33% reduction in heart and liver MnSOD activities, respectively, compared to animals fed either medium or high dietary Mn. Both activities were significantly different depending on the fat source, with animals fed high-linoleic safflower oil showing the highest heart and liver MnSOD activities, followed by animals fed high-oleic safflower oil; animals fed stearate diets had the lowest activities. Similar to experiment I, there was a Mn and fat interaction that affected kidney Mn ($p = 0.005$) and liver Mn ($p = 0.0001$). Manganese concentrations were depressed when animals were fed low dietary Mn in conjunction with stearate, as compared to the other fat sources. Femur Mn was also lower ($p < 0.0001$) when animals were fed low dietary Mn rather than medium or high dietary Mn, and when they were fed stearate rather than high-oleic or high-linoleic safflower oil.

Fe Status

There were no significant effects ($p > 0.05$) of fat type or dietary Mn on any measures of Fe status (hematocrit, hemoglobin, spleen or liver iron concentrations) in experiment I, although liver Fe concentrations

Table 2
The Effect of Dietary Fat Type and Mn Concentration on the Retention of Mn from a ^{54}Mn-Labeled Test Meal

Diet Mn	Diet Fat	Brain[2,3]	Heart	Spleen	Kidney	Liver	Gut	Carcass
Low	Stearate	0.71±0.08[a]	1.05±0.07[a]	0.92±0.09[a]	3.49±0.07[a]	5.89±0.10[a]	4.09±0.13[a]	5.70±0.08[a]
		2.1 (1.9-2.2)	3.00 (2.7-3.1)	2.5 (2.3-2.7)	33 (31-35)	362 (327-399)	60 (52-68)	299(276-324)
Low	Linoleic	2.00±0.08[b]	2.24±0.07[b]	1.94±0.09[b]	4.56±0.07[b]	7.00±0.10[c]	5.71±0.13[b]	6.91±0.08[b]
		7.4 (6.8-8.0)	9.4 (8.8-10.1)	7.0 (6.4-7.6)	96 (89-102)	1097(992-1212)	302(265-344)	1002(925-1086)
High	Stearate	4.14±0.08[c]	3.88±0.07[c]	3.11±0.09[c]	5.62±0.07[c]	6.72±0.10[b,c]	6.33±0.13[c]	8.44±0.08[c]
		63 (58-68)	48(45-52)	22(20-24)	276(257-296)	829(750-916)	561(493-639)	4628(4273-5014)
High	Linoleic	4.11±0.08[c]	3.77±0.07[c]	3.09±0.09[c]	5.44±0.07[c]	6.39±0.11[b]	7.21±0.14[d]	8.35±0.08[c]
		61 (56-66)	43 (40-46)	22 (20-24)	231 (215-247)	596(534-665)	1352(1176-1556)	4230(3405-4583)
Statistical effect determined by 2-way ANOVA:								
Mn		0.0001	0.0001	0.0001	0.0001	NS	0.0001	0.0001
Fat		0.0001	0.0001	0.0001	0.0001	0.0006	0.0001	0.0001
Mn x Fat		0.0001	0.0001	0.0001	0.0001	0.0001	0.01	0.0001

Note: Animals were fed the test meal and whole-body radiation was counted for 10 d before animals were killed and dissected. Individual organs and tissues were counted in a gamma well counter and the specific activity of Mn/^{54}Mn in the test meal was used to calculate the total amount of test meal Mn retained in the whole organ or tissue.

[1] Values are means±SEM for ln(ng Mn from test meal); $n = 8$ in all Mn × fat cells except for 100 Mn, linoleic, liver ($n = 6$) and 100 Mn, linoleic, gut and carcass ($n = 7$). Dietary Mn notation is as follows: Low = 1.3 mg Mn/kg diet and High = 100.4 mg Mn/kg diet.

[2] Second row of values = ng Mn (range of Mn retention within tissue); ng Mn calculated from specific activity of test meal and back-transformed from ln (amount of ^{54}Mn retained in tissue).

[3] Means in a column with different superscripts are significantly different ($p > 0.05$) as determined by Tukey's contrasts.

Table 3
Effect of Dietary Fat Type and Mn Concentration on the Concentration of Mn in Rat Tissues in Experiment I

		Mn concentration (µg/g)					
Diet Mn	Diet Fat	Brain[1,2]	Heart	Spleen	Kidney	RBC	Liver
Low	Stearate	0.37±0.05	0.32±0.012	0.13±0.013	0.65±0.010[a]	0.015±0.003	0.95±0.02[a]
Low	Linoleic	0.38±0.005	0.30±0.032	0.16±0.013	0.77±0.05[b]	0.023±0.003	1.40±0.04[b]
High	Stearate	0.39±0.03	0.36±0.03	0.20±0.01	0.97±0.015[c]	0.016±0.003	2.39±0.02[d]
High	Linoleic	0.40±0.10	0.34±0.011	0.22±0.02	0.89±0.04 [b,c]	0.034±0.004	2.18±0.05[c]
Statistical effect determined by 2-way ANOVA:							
Mn		NS	0.0006	0.0001	0.0001	NS	0.0001
Fat		NS	NS	NS	NS	0.002	0.003
Mn x Fat		NS	NS	NS	0.005	NS	0.0001

[1] Values are means±SEM; $n = 8$ for all Mn × fat cells except for high Mn, linoleic, liver ($n = 6$); low Mn stearate, spleen ($n = 7$); high Mn, linoleic, spleen ($n = 4$). Dietary Mn notation is as follows: Low = 1.3 mg Mn/kg diet and High = 100.4 mg Mn/kg diet.

[2] Means in a column with different superscripts are significantly different ($p < 0.05$) as determined by Tukey's contrasts.

tended to be higher in the stearic acid group. In contrast, long-term feeding of diets (Table 5) high in dietary Mn (compared to low dietary Mn) depressed liver and kidney Fe ($p < 0.04$). Animals consuming stearate had higher hemoglobin, liver iron, and kidney iron concentrations ($p < 0.04$) than animals fed diets rich in oleic or linoleic acids. An interaction between dietary Mn and fat affected femur Fe ($p = 0.02$) because Fe concentrations changed in response to dietary fat only when dietary Mn was low.

Cholesterol, Triglyceride, and Glucose Concentrations.

There were no interactions between Mn and fat that affected blood indices of lipid or glucose metabolism. Animals fed low dietary Mn had lower HDL cholesterol ($p < 0.0001$) than animals fed medium or high dietary Mn and lower total plasma cholesterol ($p < 0.007$) than animals fed high dietary Mn. Animals fed high dietary Mn tended to have lower glucose concentrations ($p < 0.07$) than animals fed low or medium dietary Mn. Animals fed the diets containing stearate had higher triglycerides ($p < 0.0001$) than animals fed the oleic-acid or linoleic-acid-containing diets and higher plasma cholesterol ($p < 0.02$) than animals fed the linoleic-acid-containing diets.

Table 4
Effect of Dietary Fat Type and Mn Concentration on Superoxide Dismutase Activity and on the Concentration of Mn in Rat Tissues in Experiment II

Diet Mn	Diet Fat	Heart MnSOD (U/mg protein)[1,2]	Liver MnSOD (U/mg protein)	Kidney Mn (μg/g)	Liver Mn (μg/g)	Femur Mn (μg/g)
Low	Stearate	3.04 ± 0.13^{E}	1.41 ± 0.55^{D}	1.94 ± 0.08^{F}	2.26 ± 0.14^{C}	0.113 ± 0.008^{D}
Low	Oleic	4.06 ± 0.10^{D}	1.73 ± 0.10^{CD}	3.28 ± 0.14^{DE}	6.00 ± 0.22^{B}	0.176 ± 0.009^{D}
Low	Linoleic	4.69 ± 0.15^{C}	2.07 ± 0.09^{CD}	3.17 ± 0.12^{E}	6.13 ± 0.24^{B}	0.239 ± 0.007^{D}
Medium	Stearate	4.96 ± 0.20^{BC}	2.07 ± 0.09^{BC}	3.74 ± 0.14^{CD}	7.91 ± 0.43^{A}	0.366 ± 0.021^{CD}
Medium	Oleic	5.20 ± 0.13^{AB}	2.58 ± 0.11^{AB}	4.17 ± 0.09^{ABC}	9.25 ± 0.16^{A}	0.414 ± 0.031^{BC}
Medium	Linoleic	5.46 ± 0.17^{AB}	2.79 ± 0.12^{A}	4.29 ± 0.14^{AB}	9.03 ± 0.39^{A}	0.492 ± 0.054^{ABC}
High	Stearate	4.75 ± 0.09^{C}	2.18 ± 0.69^{B}	3.98 ± 0.09^{BC}	8.71 ± 0.27^{A}	0.440 ± 0.018^{ABC}
High	Oleic	5.19 ± 0.11^{AB}	2.61 ± 0.15^{AB}	4.52 ± 0.12^{A}	9.08 ± 041^{A}	0.543 ± 0.020^{AB}
High	Linoleic	5.78 ± 0.13^{A}	2.98 ± 0.15^{A}	4.42 ± 0.12^{AB}	9.39 ± 0.49^{A}	0.551 ± 0.043^{A}
Statistical effect determined by 2-way ANOVA:						
Mn		0.0001	0.0001	0.0001	0.0001	0.0001
Fat		0.0001	0.0001	0.0001	0.0001	0.0001
Mn x Fat		NS	NS	0.005	0.0001	NS

[1] Values are mean±SEM; $n = 6$. Dietary Mn notation is as follows: Low = 1.3 mg Mn/kg diet and High = 100.4 mg Mn/kg diet.

[2] Means in a column with different superscripts are significantly different ($p < 0.05$) as determined by Tukey's contrasts. NS=not significant.

Table 5
Effect of Dietary Fat Type and Mn Concentration on Iron Status Indicators in Experiment II

Diet Mn	Diet Fat	Hematocrit[1,2] (%)	Hemoglobin (g/L)	Liver Fe (μg/g)	Kidney Fe (μg/g)	Femur Fe (μg/g)	Plasma Fe (μg/L)
Low	Stearate	43.7±0.7	14.5±0.2	491±22[B]	290±8[A]	108±4[A]	1.97±0.15
Low	Oleic	43.1±0.4	14.5±0.1	435±33[AB]	260±4[AB]	84±5[BC]	1.81±0.13
Low	Linoleic	41.6±1.5	14.1±0.2	426±41[AB]	264±10[AB]	66±6[D]	1.54±0.22
Medium	Stearate	44.5±0.7	14.9±0.2	454±17[AB]	268±6[AB]	89±1[B]	1.86±0.14
Medium	Oleic	43.9±1.0	14.6±0.4	406±14[AB]	250±1[BC]	75±3[BCD]	1.67±0.22
Medium	Linoleic	42.8±1.7	14.3±0.6	393±27[AB]	241±8[BC]	69±3[CD]	1.48±0.15
High	Stearate	44.8±1.2	15.2±0.2	456±22[AB]	257±9[A]	85±4[BC]	1.85±0.14
High	Oleic	41.9±0.6	14.0±0.2	358±32[B]	202±10[D]	74±3[CD]	1.62±0.25
High	Linoleic	43.2±0.4	14.5±0.1	363±23[B]	219±7[CD]	69±4[CD]	1.76±0.19
Statistical effect determined by 2-way ANOVA:							
Mn		NS	NS	0.04	0.0001	0.005	NS
Fat		NS	0.04	0.003	0.0001	0.0001	NS
Mn x Fat		NS	NS	NS	NS	0.02	NS

[1] Values are mean±SEM; $n = 6$. Dietary Mn notation is as follows: Low = 1.3 mg Mn/kg diet and High = 100.4 mg Mn/kg diet.
[2] Means in a column with different superscripts are significantly different ($p < 0.05$) as determined by Tukey's contrasts. NS=not significant.

Table 6
Effect of Dietary Fat Type and Mn Concentration on Cholesterol, Triglyceride, and Glucose Concentrations in Experiment II

Diet Mn	Diet Fat	Plasma Cholesterol (mg/dL)	HDL Cholesterol (mg/dL)	Triglycerides (mg/dL)	Glucose (mg/dL)
Low	Stearate	77 ± 4^{B}	45 ± 4^{C}	84 ± 8^{A}	239 ± 23
Low	Oleic	81 ± 5^{AB}	49 ± 2^{ABC}	44 ± 3^{BC}	227 ± 12
Low	Linoleic	75 ± 6^{B}	44 ± 3^{C}	46 ± 9^{BC}	225 ± 25
Medium	Stearate	95 ± 3^{AB}	60 ± 2^{AB}	77 ± 8^{AB}	195 ± 15
Medium	Oleic	88 ± 3^{AB}	48 ± 3^{BC}	35 ± 4^{C}	222 ± 13
Medium	Linoleic	85 ± 4^{AB}	56 ± 3^{ABC}	44 ± 9^{BC}	223 ± 16
High	Stearate	107 ± 12^{A}	63 ± 3^{A}	79 ± 14^{AB}	172 ± 16
High	Oleic	96 ± 3^{AB}	58 ± 4^{ABC}	48 ± 6^{ABC}	223 ± 18
High	Linoleic	76 ± 6^{B}	54 ± 3^{ABC}	24 ± 2^{C}	190 ± 21
Statical effect as determined by 2-way ANOVA:					
Mn		0.007	0.0001	NS	NS
Fat		0.02	NS	0.0001	NS
Mn x fat		NS	NS	NS	NS

[1] Values are mean±SEM; $n = 6$. Dietary Mn notation is as follows: Low = 1.3 mg Mn/kg diet and High = 100.4 mg Mn/kg diet.

[2] Means in a column with different superscripts are significantly different ($p < 0.05$) as determined by Tukey's contrasts. NS=not significant.

DISCUSSION

Previous studies have hinted at an inverse relationship between the amount of unsaturated fat in the diet and the status of Mn and other nutritionally important metals. Lukaski and Johnson *(22)* reviewed the effects of dietary fat on mineral metabolism and concluded that the increased PUFA content of a diet may depress zinc (Zn) and copper (Cu) status. Johnson et al. *(19)* demonstrated that Mn excretion in human feces increased when the total dietary fat was increased and the ratio PUFA : mono-saturated and saturated fats was decreased. Malecki et al. *(23)* fed rats different amounts of corn oil and found no effect on biliary excretion

of Mn. However, rats fed 20%, as compared to 5%, corn oil had slightly increased Mn concentrations in the liver and pancreas *(24).* Davis and Greger *(25)* showed that women in the lowest quintile of fat intake had significantly less lymphocyte MnSOD activity and lower serum Mn concentrations than women in the other four quintiles. As previously reviewed, Fe has a large influence on Mn absorption, and Fe absorption is improved by saturated fats *(3,4,16,17),* which also suggests that the fat type may affect Mn absorption.

The design of the present studies allowed us to assess the effect of the fat source on Mn absorption in animals that had not adapted to their diet, as well as in animals that had equilibrated with their diet. Experiment I fed the radioactively labeled test meal simultaneous with beginning dietary treatments. The guts of these animals were not adapted to their diets; therefore, the observed differences were a result of dietary conditions alone. Rats used in Experiment II were fed diets for 8 wk before they were killed and measurements were made; thus, these animals had sufficient time to adapt to their diet and any differences were a result of dietary conditions as well as differences in metabolism.

Both experiments clearly demonstrate that the form of dietary fat diet affects Mn absorption, retention and distribution. However, the effect of dietary fat type on Mn absorption and retention measures were opposite to those previously reported for Fe. Diets enriched in saturated fats have been reported to enhance Fe absorption *(16,17),* but the present results show Mn absorption to be decreased by saturated fats.

Similar to other reports *(14,15),* changes in Mn absorption were offset by reciprocal changes in the biological half-life of Mn in most tissues and organs. Consequently, for rats fed high dietary Mn diets enriched in linoleic acid resulted in a 24-fold increase in absorption of Mn from the test meal, relative to animals fed diets enriched in stearic acid. However, in the same rats, the retention of Mn from the test meal after 10 d was not significantly affected by the form of dietary fat. Animals fed diets for 8 wk (Experiment II) did show a small, but significant, increase in MnSOD activity in the hearts and livers of animals fed high dietary Mn and high-linoleic safflower oil (as compared to high-Mn stearate-fed animals), whereas concentrations of Mn in the femur, kidney, and liver were not significantly different.

When dietary Mn was low, a different pattern of Mn retention was observed. Absorption of Mn was only increased sevenfold by high-linoleic safflower oil (as compared to stearate), but there was significantly greater tissue/organ retention of Mn in safflower-fed animals. Likewise, long-term-fed animals (Experiment II) that consumed diets enriched in high-linoleic safflower oil also had significantly increased Mn concentrations in several organs and significantly increased MnSOD activity in the heart. The discrepancy in results between animals fed low Mn and animals fed high Mn could be evidence for distinct metabolic pathways dependent on the amount of Mn available. Chua et al. *(26)* reported high-affinity and

low-affinity uptake of Mn by erythrocytes, and the high-affinity process only occurred in a low-ionic-strength medium. Kies *(27)* reviewed factors affecting Mn absorption and concluded that active transport is the primary method when dietary Mn is low and passive diffusion is more important at higher levels of dietary Mn.

The effect of dietary fat type on Mn absorption is more difficult to explain. It is possible that stearic acid inhibited Mn absorption by rendering it insoluble or by chelating it. However, the initial steeply declining portion of the WBC curve (d 1–3) represents elimination of unabsorbed Mn from the gut tract. If Mn were rendered insoluble, then the gut rate of the passage of ^{54}Mn should increase and, consequently, this portion of the curve should change. Although this portion of the curve was significantly steeper when the mean values of stearic-acid-fed animals were compared to mean values of safflower-oil-fed animals, the slopes were identical in animals fed stearic acid/low Mn and in animals fed safflower oil/high Mn, whereas percent absorption was greatly different (3.8% vs 27.3% for stearate and safflower oil, respectively).

Another possibility is that Mn absorption is determined largely by Fe absorption, and stearate had a direct effect on Fe absorption. Johnson et al. *(28)* studied Fe absorption in moderately anemic rats fed diets enriched in safflower or coconut oil. Iron absorption was improved from 79% in safflower oil diets to 87% in the coconut oil diet. Johnson et al. *(16)* reported increases of 168% in hemoglobin concentration, 159% in plasma Fe, and 129% in liver Fe concentration in animals fed 20% stearic acid and 2% safflower oil as compared to 22% safflower oil. Droke and Lukaski *(17)* also found rats fed diets enriched in cocoa butter or beef tallow showed a 13% enhancement of Fe absorption over that of rats fed safflower oil. (The present data support these previous observations, as apparent absorption of Fe was enhanced in animals fed stearate and long-term feeding of stearate-rich diets significantly increased hemoglobin and Fe concentrations in liver and kidney.)

The enhanced absorption of Fe by stearate then resulted in an inhibition of Mn absorption. The absorptive interaction between Mn and Fe may have a biochemical basis with the recent characterization of a putative Fe transport protein, DCT1 (Nramp2), that may be responsible for the absorption of Fe and other trace elements, including Mn *(29)*. However, the opposite effects of fat type on Mn and Fe absorption are difficult to explain and require further experimentation.

Weigand et al. *(30)* reported that the apparent absorption of Mn by weanling rats was not affected by dietary Mn concentration, but true absorption, calculated after taking endogenous excretion into account, varied significantly from 5.4% (animals fed 100 µg Mn/g diet) to 25.7% (animals fed 1.5 µg Mn/g diet). In the present study, although rats fed the high-linoleic safflower oil diet consistently absorbed more Mn than rats fed stearate and although rats fed the low-Mn diet consistently had depressed organ/tissue Mn concentrations when stearate was the fat

source, paradoxically tissue and organ concentrations of Mn were unaffected by dietary fat type when dietary Mn was high. This seeming paradox may be explained by changes in endogenous excretion. When dietary Mn was high, animals fed stearate may have compensated for the increased absorption by increasing biological half-life (i.e., increasing the rate of endogenous excretion), but when dietary Mn was low an increase in biological half-life was insufficient to overcome the inhibition of absorption by stearate which resulted in lower tissue concentrations of Mn.

In summary, we have demonstrated that a greater percentage of dietary Mn is absorbed when high-linoleic safflower oil, as compared to stearate, is used as a dietary fat source. Differences in absorption do not always result in differences in tissue retention of Mn, because biological half-life changes increase or decrease the endogenous excretion of absorbed Mn. Although there are several possible biochemical bases for this interaction, none can be proven or disproven at the present time. However, these data do add to the body of evidence that factors that affect Fe absorption also affect Mn absorption.

ACKNOWLEDGMENTS

We wish to thank B. Gregoire and D. Schafer for assistance with animal care and experimental procedures. We also wish to thank M. Rydell for assistance with manuscript preparation and Fariba Roughead and Eugene Korynta for help with the whole-body counting procedures.

REFERENCES

1. L. Hurley and C. Keen, Manganese, in *Trace Elements in Human and Animal Nutrition,* W. Mertz, ed., Academic Press, San Diego, pp. 185–224 (1987).
2. C. Keen, S. Zidenberg-Cherr, and B. Lonnerdal, Nutritional and toxicological aspects of manganese intake: an overview, in *Risk Assessment of Essential Elements,* W. Mertz, C. O. Abernathy, and S. S. Olin, eds., ILSI, Washington, DC, pp. 221–236 (1990).
3. C. Davis, T. Wolf, and J. Greger, Varying levels of manganese and iron affect absorption and gut endogenous losses of manganese by rats, *J. Nutr.* **122,** 1300–1308 (1992).
4. A. B. R. Thomson, D. Olatunbosun, L. S. Valberg, and J. Ludwig, Interrelation of intestinal transport system for manganese and iron, *J. Lab. Clin. Med.* **78,** 642–655 (1971).
5. L. Davidsson, A. Cederblad, B. Lonnerdal, and B. Sandstrom, The effect of individual dietary components on manganese absorption in humans, *Am. J. Clin. Nutr.* **54,** 1065–1070 (1991).
6. K. Wedekind, E. Titgemeyer, A. Twardock, and D. Baker, Phosphorus, but not calcium, affects manganese absorption and turnover in chicks, *J. Nutr.* **121,** 1776–1786 (1991).
7. J. Finley and P. Monroe, Mn absorption: the use of CACO-2 cells as a model of the intestinal epithelium, *Nutr. Biochem.* **8,** 92–101 (1997).
8. D. Lee and P. Johnson, Factors affecting absorption and excretion of ^{54}Mn in rats, *J. Nutr.* **118,** 1509–1516 (1988).

9. C. Keen, J. Bell, and B. Lonnerdal, The effect of age on manganese uptake and retention from milk and infant formulas in rats, *J. Nutr.* **116,** 395–402 (1986).
10. R. Wapnir, Nutritional status and the effect of protein and other foodstuffs on the absorption of manganese, molybdenum and vanadium, in *Protein Nutrition and Mineral Absorption,* Boca Raton, FL, pp. 243–257 (1990).
11. N. Gruden, The effect of age and sex upon iron–manganese interaction in different segements of the rat's intestine, in *Trace Elements in Man and Animals,* L. S. Hurley, C. L. Keen, B. Lönnerdal, and R. Rucker, eds., Plenum, New York, pp. 557–558 (1989).
12. P. Flanagan, J. Haist and L. Valberg, Comparative effects of iron deficiency induced by bleeding and a low-iron diet on the intestinal absorptive interactions of iron, cobalt, manganese, zinc, lead and calcium, *J. Nutr.* **110,** 1754–1763 (1980).
13. L. Rossander-Hulten, M. Brune, B. Sandström, B. Lönnerdal, and L. Hallberg, Competitive inhibition of iron absorption by manganese and zinc in humans, *Am. J. Clin. Nutr.* **54,** 152–156 (1991).
14. J. Finley and P. Johnson, Sex affects manganese absorption and retention by humans from a diet adequate in manganese, *Am. J. Clin. Nutr.* **60,** 949–955 (1994).
15. J. W. Finley, Manganese absorption and retention by young women is associated with serum ferritin concentration, *Am. J. Clin. Nutr.* **70,** 37–43 (1999).
16. P. Johnson, H. Lukaski, and E. Korynta, Effects of stearic acid and beef tallow on iron utilization by the rat, *Proc. Soc. Exp. Biol. Med.* **200,** 480–486 (1992).
17. E. Droke and H. Lukaski, Dietary iron and fat affect nonheme iron absorption, iron status, and enterocyte aconitase activity and iron concentration in rats, *Nutr. Res.* **16,** 977–989 (1996).
18. C. Davis, D. M. Ney, and J. L. Greger, Manganese, iron and lipid interactions in rats, *J. Nutr.* **120,** 507–513 (1990).
19. J. Johnson, C. Kies, and H. Fox, Dietary fat and manganese interactions in human adults, *Nutr. Rep. Int.* **38,** 1269–1281 (1988).
20. SAS Institute, *SAS Users Guide: Statistics,* version 5, SAS Institute, Cary, NC (1985).
21. R. Steel and J. Torrie, *Principles and Procedures of Statistics,* McGraw-Hill, New York (1980).
22. H. Lukaski and P. E. Johnson, Dietary fats and minerals, in *Fatty Acids in Foods and Their Health Implications,* C. K. Chow, ed., Marcel Dekker, New York, pp. 501–516 (1992).
23. E. A. Malecki, G. M. Radzanowski, T. J. Radzanowski, D. D. Gallaher, and J. L. Greger, Biliary manganese excretion in conscious rats is affected by acute and chronic manganese intake but not by dietary fat, *J. Nutr.* **126,** 489–498 (1996).
24. E. Malecki, D. Huttner, and J. Greger, Manganese status, gut endogenous losses of manganese, and antioxidant enzyme activity in rats fed varying levels of manganese and fat, *Biol. Trace Element Res.* **42,** 17–29 (1994).
25. C. D. Davis and J. Greger, Longitudinal changes of manganese-dependent superoxide dismutase and other indexes of manganese and iron status in women, *Am. J. Clin. Nutr.* **55,** 747–752 (1992).
26. A. C. G. Chua, L. M. Stonell, D. L. Savigni, and D. H. Morgan, Mechanisms of manganese transport in rabbit erythroid cells, *J. Physiol.* **493,** 99–112 (1996).
27. C. Kies, Bioavailability of manganese, in *Manganese in Health and Disease,* D. Klimis-Tavantzis, ed., CRC, Boca Raton, FL, pp. 39–58 (1994).
28. P. E. Johnson, H. Lukaski, and T. Bowman, Effects of level and saturation of fat and iron level and type in the diet on iron absorption and utilization by the rat, *J. Nutr.* **117,** 501–507 (1987).
29. J. Fleet, Identification of Nramp2 as an iron transport protein: another piece of the intestinal iron absorption puzzle, *Nutr. Rev.* **56,** 88–89 (1998).
30. E. Weigand, M. Kirchgessner, and U. Helbig, True absorption and endogenous fecal excretion of manganese in relation to its dietary supply in growing rats, *Biol. Trace. Element Res.* ***10,*** 265–279 (1986).