Chapter 12

Manganese and Lipid Metabolism as Affected by Dietary Manganese and Fat

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Two studies were conducted to investigate the effects of dietary manganese and fat on manganese and lipid metabolism. In Study I, 80 male, weanling rats were fed two levels of dietary fat (5% and 25%). Serum, liver and brain lipid concentrations, body weight change and fecal fat excretions were greater in rats fed the diet with 25% fat. Within each level of fat, total liver lipids decreased and liver cholesterol increased as level of dietary manganese increased. Serum cholesterol levels were highest when manganese was fed at 50 and 500 mg/kg diet. Manganese intake, fecal manganese excretion and whole blood manganese increased as level of dietary manganese increased. Dietary treatments had no effect on liver manganese concentrations. In Study II, young adult human subjects were fed two laboratory-controlled diets containing either 30% of total calories from fat (approximately 100 mg cholesterol; 10:10:10 PUFA to MUFA to SFA ratio) or 40% of total calories from fat (approximately 600 mg cholesterol; 4:14:14 fatty acid ratio). Two levels of manganese were fed (5 and 45 mg Mn/day) within each level of fat. The higher level of dietary fat generally increased fecal excretion of manganese and increased serum lipids. Dietary supplementation with 40 mg of manganese increased both fecal excretion and whole blood concentration of the mineral but had no effect on serum lipids or fecal fat.

At least in part because atherosclerosis and coronary heart disease continue to be the number one cause of death among North Americans, interest in interactions between dietary constituents

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0097-6156/87/0354-0123$06.00/0

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ACS Symposium Series; American Chemical Society: Washington, DC, 1987.

remains high. Several reports of an association of manganese with steroid biosynthesis and lipid metabolism have been published (1).

Curran and Clute (1) demonstrated an in vitro increase in cholesterol synthesis in rat liver cell clusters injected with manganese. In a similar experiment, Curran (2) injected several transition elements (V, Ti, Cr, Mn, Fe, Co, Ni, Cu and Zn) intraperitoneally into rats. After one hour the rats were sacrificed and the livers from each group were pooled and incubated with sodium acetate-1-C^{14}. Manganese and chromium were found to increase incorporation of acetate into cholesterol by 100 percent, whereas vanadium depressed cholesterol synthesis by 50 percent.

Manganese acts as a cofactor of mevalonate kinase and farnesyl pyrophosphate synthetase. Mevalonate kinase and possibly one other manganese-activated enzyme are necessary for the formation of mevalonate from acetate (3). Farnesyl pyrophosphate synthetase acts to add one 5-carbon unit to geranyl pyrophosphate to make farnesyl pyrophosphate (4) (Figure 1).

Little is known of the in vivo effect of manganese on cholesterol metabolism. Doisy (6) observed a decrease in serum cholesterol (from 206 to 80 mg/dl) in a single manganese deficient human subject. Reports of other human studies conducted to determine the influence of dietary manganese on cholesterol metabolism were not found.

Recently, Klimis-Tavantzis and coworkers (7,8) reported results of a series of studies designed to investigate the effects of dietary manganese deficiency on cholesterol and lipid metabolism in two experimental animal models. Day-old chicks were fed a manganese-deficient (4.8 μg/g) or a manganese-supplemented (104.8 μg/g) diet for 4 weeks after which an injection of estrogen was given. Manganese deficiency did not significantly alter plasma cholesterol or liver cholesterol. When older (36-week-old) laying hens were given similar diets (7), they demonstrated decreased hepatic manganese and cholesterol concentrations. These hens also tended to have increased total liver lipid concentrations.

Weanling, Wistar and RICO (genetically hypercholesterolemic) rats were placed on manganese-deficient (0.12 μg Mn/g) or manganese-sufficient (100.12 μg Mn/g) diets. Plasma total, VLDL- and HDL-cholesterol levels, and liver cholesterol and lipid concentrations were not affected by the treatment used. These results suggest that dietary manganese deficiency does not result in significant alterations in cholesterol and lipid metabolism in the rat (8).

Manganese has a further role as a lipotropic agent. Amdur and associates (9) found that hepatic lipid concentration was increased by manganese deficiency. Plumlee et al. (10) conducted four experiments to determine the effect of manganese deficiency in swine and found that total body fat and liver lipid concentrations were increased by manganese deficiency.

It has been characteristic of nutrition studies to use one nutrient alteration experimental design to investigate one possible effect. However, in order to elucidate dietary relationships with pathological conditions, it may be necessary to use interaction-type studies. Therefore, the overall objective of the research conducted in our laboratories was to investigate the effect of dietary fat-manganese interactions on cholesterol synthesis.

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Experimental Plan - Study I

Two studies were used to investigate the effects of dietary manganese and fat on manganese and lipid metabolism. The purpose of the first study was to determine the interactions among four levels of dietary manganese and two levels of dietary fat on manganese and lipid status of male weanling rats.

Male, weanling, Sprague-Dawley inbred rats (Harlan/Sprague/Dawley, Madison, WI) were used. After three days of adjustment, the 80 rats were randomly assigned to one of eight dietary treatment groups. Two levels of dietary fat (5 percent and 25 percent of the diets by weight) and four levels of dietary manganese (5, 50, 500 and 5000 mg/kg diet) were fed.

The composition of the basal 5 percent and 25 percent fat diets is shown in Table I. All ingredients were purchased from Teklad (Madison, WI) except for the hydrogenated vegetable oil and the corn starch which were purchased in single lots from a local supermarket. Corn starch was used to balance the fat content of the two basal diets. The animals were allowed feed and distilled water ad libitum for 56 days. Treatment variations are shown in Table II.

Table I. Composition of Experimental Rations

Ingredient	Amount/kg 5% Fat	25% Fat
Casein	20 g	20 g
DL-methionine	300 mg	300 mg
Crisco shortening	5 g	25 g
Corn starch	45 g	25 g
Sucrose	20 g	20 g
Cellulose	5 g	5 g
AIN mineral mix[1]	3.5 g	3.5 g
AIN vitamin mix	1 g	1 g
Choline bitartrate	200 mg	200 mg

[1]Mineral mix without manganese. Manganese to supply 5, 50, 500 and 5000 mg/kg diet as manganese carbonate (Teklad, Madison, WI) was added at the expense of sucrose to create rations varied in manganese content.

Feed intakes and body weights were recorded on a weekly basis. Feces were collected daily and composited into 7-day lots. At the end of the study, a 12-hour fasting blood sample was collected from each rat. The brain and liver of each animal was excised and frozen.

Liver, whole blood and feed manganese was measured using a Varian Techtron Atomic Absorption Spectrophotometer Model 1275. Total liver lipid was extracted from lyphilized tissue and determined by the method described by Folch et al. (11). Serum total cholesterol and HDL-cholesterol were also enzymatically assayed (12). Fecal fat analyses were performed using the Goldfisch method (13).

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Table II. Dietary Treatment Variations

Treatment Number	Fat (g/kg)	Manganese[1] (mg/kg)
1	5	5
2	25	5
3	5	50
4	25	50
5	5	500
6	25	500
7	5	5000
8	25	5000

[1]Manganese carbonate (Teklad, Madison, WI).

Results and Discussion - Study I

Mean serum total cholesterol levels are shown in Figure 2. For rats consuming the high fat diets, serum total cholesterol levels were highest when 50 mg Mn/kg diet was fed, although the value was not significantly higher than values attained on other high fat diets. For rats consuming the low fat diets, mean serum cholesterol levels were significantly higher for the 50 and 500 mg Mn/kg diet treatments ($P<0.05$). Perhaps the lower fat intake allowed the effect of manganese intake on lipid metabolism to become more pronounced.

Figure 3 shows the mean serum HDL-cholesterol levels of rats fed low and high fat dietary treatments as affected by level of dietary manganese. HDL-cholesterol levels were higher for rats fed the high fat diets than for rats fed the low fat diets at each level of dietary manganese except at the 500 mg Mn/kg diet level. When the data were grouped according to dietary manganese alone, serum HDL-cholesterol levels of rats fed the lowest level of manganese were significantly higher than were those of rats consuming the highest level of manganese.

As can be seen in Figure 4, liver lipid concentrations were higher for rats fed high fat diets (within each level of dietary manganese) than for rats fed low fat diets. These differences were significant for each level of dietary manganese fed ($P<0.05$). Within each level of fat fed, liver total lipid concentrations decreased as level of dietary manganese increased.

Figure 5 illustrates the increase in liver cholesterol concentration which occurs with an increase in dietary manganese. Within each level of manganese fed, liver cholesterol concentrations were higher for rats consuming the high fat diets, although these differences were only significant at the lowest and highest levels of dietary manganese ($P<0.05$).

Mean liver manganese concentrations are shown in Table III. Means did not differ significantly. Liver manganese concentrations did not seem to reflect the level of dietary manganese consumed.

Whole blood levels of manganese reflected differences in levels of dietary manganese. As shown in Figure 6, whole blood manganese concentrations of rats fed both low fat and high fat diets tended to increase as level of dietary manganese increased.

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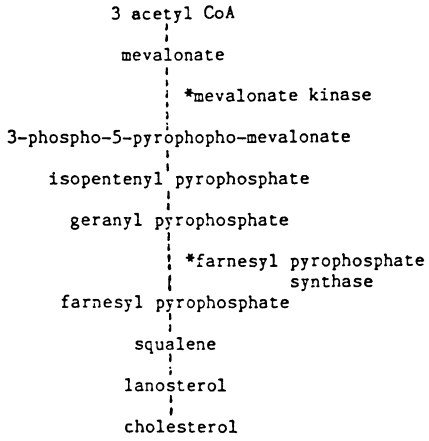

Figure 1. Pathway of cholesterol biosynthesis.

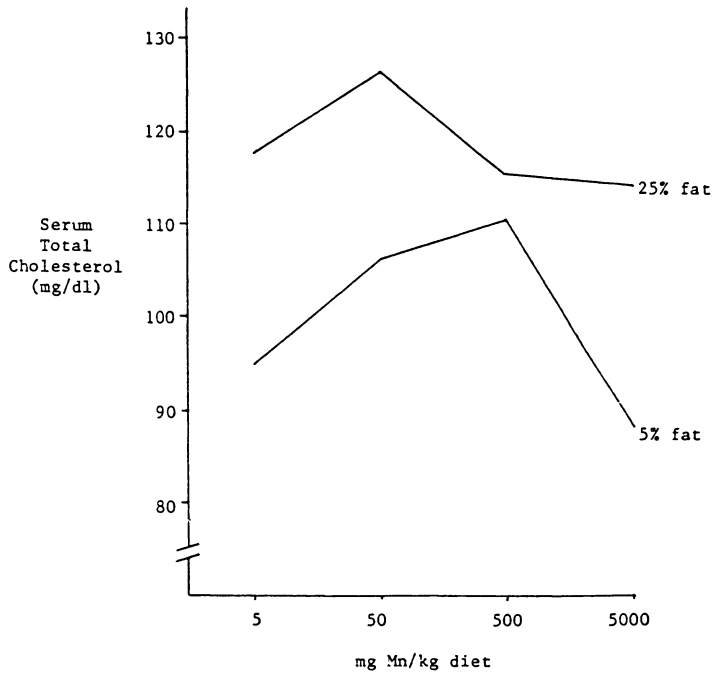

Figure 2. Serum total cholesterol levels (mg/dl) as affected by dietary manganese and fat.

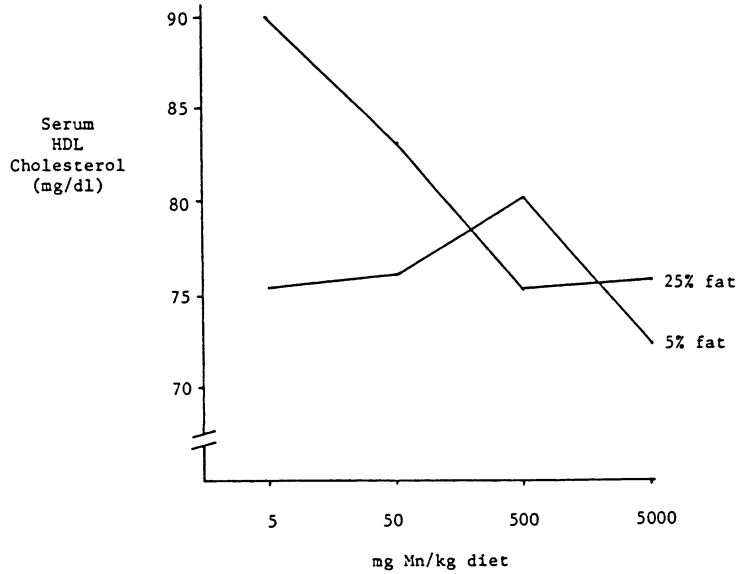

Figure 3. Serum HDL-cholesterol levels (mg/dl) as affected by dietary manganese and fat.

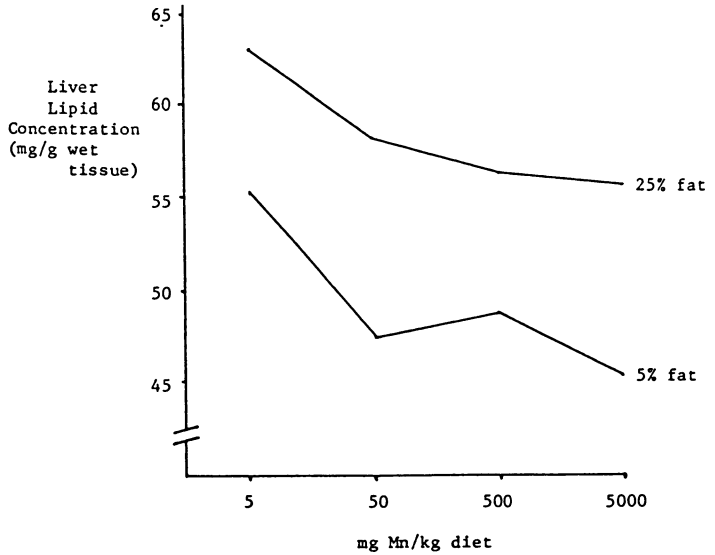

Figure 4. Liver lipid concentrations (mg/g wet tissue) as affected by dietary manganese and fat.

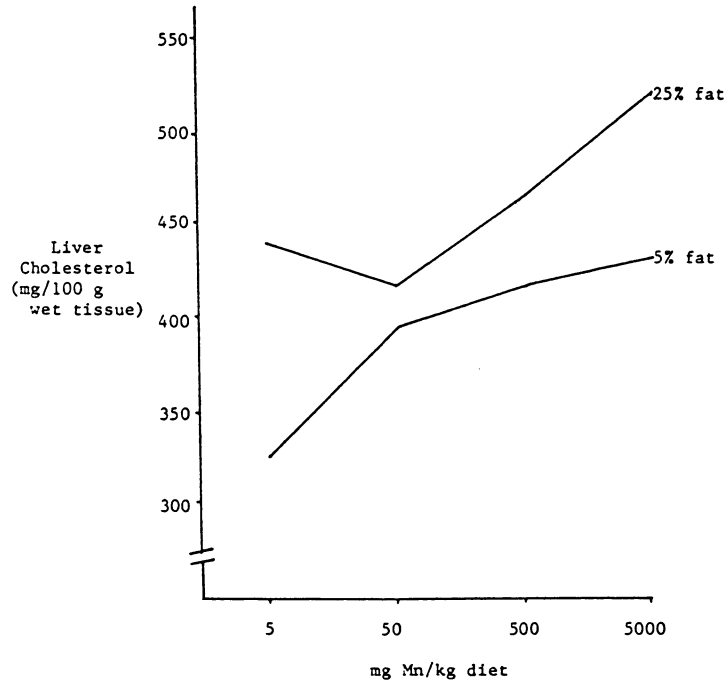

Figure 5. Liver cholesterol concentrations (mg/100 g wet tissue) as affected by dietary manganese and fat.

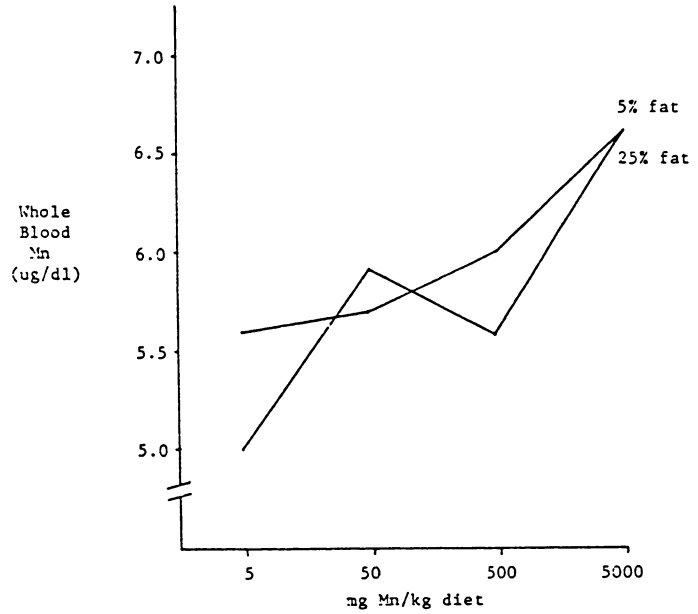

Figure 6. Whole blood manganese levels (μg/dl) as affected by dietary manganese and fat.

Table III. Mean Liver Manganese Concentrations (μg/g wet tissue) in Rats Fed Varying Levels of Manganese and Fat

Dietary Manganese mg/kg	Dietary Fat (%) 5	25
5	4.0 ± 1.0	5.0 ± 0.9
50	3.7 ± 1.7	3.5 ± 1.4
500	4.7 ± 2.4	4.0 ± 1.7
5000	4.5 ± 2.7	3.8 ± 2.1

As shown in Figure 7, rats excreted approximately five to six times more fat when receiving the high fat diets compared to the low fat diets. Since the high fat diets contained five times as much fat as did the low fat diets it appeared that fecal fat excretion of the rats was proportional to the fat content of the diets. This is perhaps due to the relatively poor ability of the rat to utilize fat. For the high fat treatments, fecal fat excretions of rats fed the lowest and highest levels of dietary manganese were significantly different from each other and from excretions of rats fed the other two levels of dietary manganese ($P<0.05$). With the lwo fat ration, however, there were no significant differences in fecal fat excretion attributable to level of dietary manganese.

Body weight changes (g/8 week period) as affected by dietary manganese and fat are shown in Figure 8. Rats consuming 50 mg Mn/kg diet gained the most weight on each level of dietary fat, while those consuming the diets containing the lowest and highest levels of manganese gained the least. No significant main effects of dietary manganese occurred. Thus, significant differences in body weight change may be attributed to differences in dietary fat alone.

Experimental Plan - Study II

The purpose of the second study was to determine the effect of changes in kind and amount of dietary fat, with or without manganese supplementation, on blood serum cholesterol and triglyceride levels and on manganese status of human adults. The project was comprised of a 5-day pre-period and four, 14-day experimental periods.

During the experimental periods, two constant, laboratory-controlled diets were fed. The "usual" U.S. diet (U.F.) was formulated to contain 40 percent of total calories from fat, 600 mg cholesterol, and polyunsaturated to monounsaturated to saturated fatty acids in a ratio of 4:14:14. The modified fat diet (M.F.) contained 30 percent of total calories from fat, approximately 100 mg cholesterol, and a polyunsaturated to monounsaturated to saturated fatty acid ratio of approximately 10:10:10.

The four experimental periods were divided into parts A and B, with each part composed of two experimental periods. Within each part the following variations were used: basal diet alone (either U.F. or M.F.) or the basal diet plus a 40 mg manganese supplement (as manganese gluconate amino acid chelate) (Table IV).

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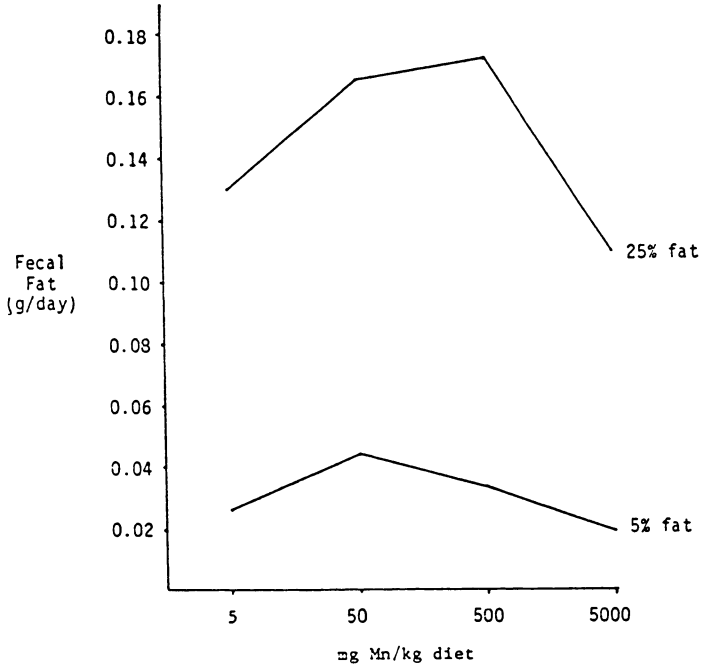

Figure 7. Mean fecal fat excretion (g/day) as affected by dietary manganese and fat.

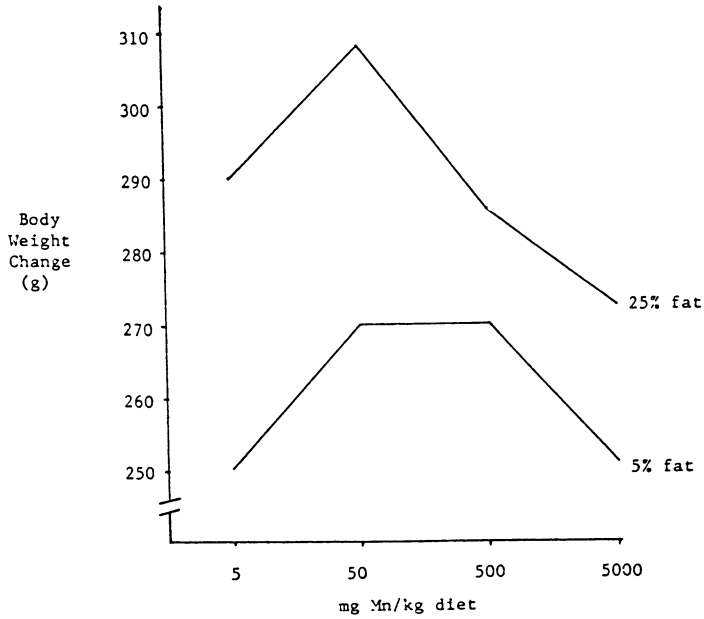

Figure 8. Body weight change (g/8 week period) as affected by dietary manganese and fat.

Table IV. Experimental Plan for Study II

Period[1]	No. of Days	Diet Type and Modification
Pre-period	5	Self-selected, self-recorded
Part A		
1	14	Controlled, usual U.S. diet
2	14	Controlled, usual U.S. diet + 40 mg Mn/day
Part B		
1	14	Controlled, fat modified diet
2	14	Controlled, fat modified diet + 40 mg Mn/day

[1]Fourteen subjects; order of periods arranged according to a double, cross-over design.

Subjects were 14 healthy men and women who lived in the human metabolic unit of the Department of Human Nutrition and Food Service Management at the University of Nebraska-Lincoln. All meals were prepared and consumed in the metabolic diet kitchen. Subjects made complete urine and fecal collections throughout the study and donated blood samples biweekly.

The manganese content of the basal diets and of urine, feces and whole blood was measured using a Varian Techtron Atomic Absorption Spectrophotometer Model 1275. Urine samples were read directly using a graphite furnace attachment (Model GTA-95). Fecal composites were analyzed for fat content (13). Serum total cholesterol and HDL-cholesterol were enzymatically assayed (12,14). Serum triglycerides were assayed spectrophotometrically based on the method of Fletcher (15).

Results and Discussion - Study II

The mean manganese intakes are shown in Table V. Mean manganese intakes differed significantly among dietary treatments. Whole wheat bread, a good source of manganese, supplied extra calories to those subjects who began losing weight during the experimental portion of the study. Since weight loss was more prevalent during the M.F. periods than when the U.F. diet was fed, the mean manganese intake was higher when the M.F. diet was fed. Manganese intakes during the supplemented periods were at least six times greater than during the non-supplemented periods. Therefore, effects of manganese intakes on manganese and lipid metabolism may be attributed to supplementation versus non-supplementation.

Fecal manganese losses (Table V) were significantly higher during periods of manganese supplementation than during non-supplemented periods. Significantly more manganese was excreted during the U.F. + Mn period than during the M.F. + Mn period suggesting that manganese utilization from this supplement was affected by differences in fat content of the two diets (either total amount or type of dietary fat).

Table V. Mean Manganese Intakes (mg/day) and Fecal Manganese Excretions (mg/day) of Humans Fed Varying Levels of Manganese and Fat

	Diet[1]				
	Pre-	MF	MF+Mn	UF	UF+Mn
Manganese intake (mg/day)	5.68[d] ±2.70	7.18[c] ±0.85	47.20[a] ±0.80	5.85[d] ±0.88	45.61[b] ±0.54
Fecal manganese (mg/day)	3.68[c] ±1.55	5.55[c] ±2.43	32.29[b] ±12.14	5.73[c] ±2.84	46.89[a] ±11.69

[1]Diet Code: Pre- = Self-selected diet
UF = Usual U.S. diet
MF = Modified fat diet
Mn = Manganese supplement (40 mg/day)

As shown in Table VI, urinary excretion of manganese did not differ significantly among the four dietary treatment periods. No explanation can be offered for the high urinary manganese losses that occurred during the pre-period.

Mean manganese balances are also shown in Table VI. Manganese balances were significantly higher during the MF+Mn period than during any other period. This agrees with the data on fecal manganese excretion and indicates that supplemental manganese may be absorbed and retained by the body more efficiently during consumption of a low fat diet than during consumption of a high fat diet.

Table VI. Mean Urinary Manganese Excretion (μg/day), Manganese Balance (mg/day) and Whole Blood Manganese (μg/dl) in Humans Fed Varying Levels of Manganese and Fat

	Diet[1]				
	Pre-	MF	MF+Mn	UF	UF+Mn
Urinary manganese (μg/day)	9.86[a] ±4.11	6.99[b] ±3.04	7.80[ab] ±3.07	7.93[ab] ±3.19	7.70[ab] ±2.40
Manganese balance (mg/day)	1.70[b] ±3.21	1.63[b] ±2.45	14.91[a] ±12.47	0.11[b] ±2.77	-1.37[b] ±11.72
Whole blood manganese (μg/dl)	2.43[c] ±0.36	2.70[b] ±0.28	2.90[a] ±0.15	2.55[bc] ±0.29	2.91[a] ±0.17

[1]Diet Code: Pre- = Self-selected diet
UF = Usual U.S. diet
MF = Modified fat diet
Mn = Manganese supplement (40 mg/day)

Mean whole blood manganese levels (Table VI) were significantly higher during periods of manganese supplementation. This would not have been expected during the UF+Mn period since manganese balance for this period was -1.37 mg/day. This would suggest that the high fecal manganese excretion during this period was actually due to an

increase in endogenous secretion of manganese into the gastrointestinal tract rather than to a decreased absorption of the mineral.

Serum lipid values are shown in Table VII. There were no significant effects of either dietary fat or manganese on serum cholesterol levels during the experimental periods. However, total cholesterol levels tended to be lower when the MF diet was fed than when the UF diet was used ($P<0.10$).

Serum HDL-cholesterol values were significantly higher for the UF diet treatments than for the MF diet treatments ($P<0.05$). There were no differences, however, in manganese-supplemented versus non-supplemented periods within the same level of dietary fat.

While mean triglyceride levels were higher for the UF and UF+Mn periods than for the lower fat periods, the difference was only significant for the manganese-supplemented (MF+Mn versus UF+Mn, $P<0.005$) period. No effect of dietary manganese on serum triglyceride levels was seen.

Table VII. Mean Serum Cholesterol, HDL-Cholesterol and Triglyceride Levels (mg/dl) in Humans Fed Varying Levels of Manganese and Fat

	Diet[1]				
	Pre-	MF	MF+Mn	UF	UF+Mn
Serum total cholesterol (mg/dl)	207.4^{a} ±34.2	169.1^{b} ±30.8	177.0^{b} ±30.8	192.8^{ab} ±33.7	191.1^{ab} ±48.7
Serum HDL-cholesterol (mg/dl)	46.7^{b} ±9.0	47.6^{b} ±5.74	48.0^{b} ±6.46	63.6^{a} ±6.58	62.6^{a} ±9.65
Serum triglycerides (mg/dl)	55.6^{c} ±14.6	80.2^{ab} ±11.5	73.3^{b} ±8.1	88.1^{a} ±13.9	88.3^{a} ±11.5

[1]Diet Code: Pre- = Self-selected diet
UF = Usual U.S. diet
MF = Modified fat diet
Mn = Manganese supplement (40 mg/day)

No significant differences were found in mean fecal fat excretions (Table VIII). Failure to detect differences in fecal fat may indicate that the increase in total fat content of the usual versus the modified fat diet is compensated for by an increase in absorption of fat. Increased absorption of fat, then, could account for the higher serum HDL-cholesterol and triglycerides levels caused by the two higher fat treatments.

In conclusion, serum, liver and brain lipid concentrations, body weight change and fecal fat excretions were greater in rats fed a diet with 25% fat than in rats fed a lower fat diet. Within each level of fat, total liver lipids decreased and liver cholesterol concentration increased as level of dietary manganese increased. However, in 14 adult, human subjects fed two levels of dietary fat, dietary manganese had no effect on serum lipid parameters or fecal fat excretion.

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Table VIII. Mean Fecal Fat Excretions (g/day) of Humans Fed Varying Levels of Manganese and Fat

	Diet[1]				
	Pre-	MF	MF+Mn	UF	UF+Mn
Fecal fat (g/day)	2.39[a] ±1.40	2.21[a] ±1.10	2.36[a] ±1.33	1.57[a] ±0.68	2.21[a] ±1.09

[1]Diet Code: Pre- = Self-selected diet
UF = Usual U.S. diet
MF = Modified fat diet
Mn = Manganese supplement (40 mg/day)

Acknowledgments

Published as Paper Number 8243, Journal Series, Agricultural Research Division, University of Nebraska, Lincoln, Nebraska 68583. Supported by U.S.D.A., C.S.R.S. Regional Research Projects NC-167 and W-143 and University of Nebraska Agricultural Research Division Project 91-031.

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RECEIVED July 17, 1987