Dietary Manganese Intake and Type of Lipid Do Not Affect Clinical or Neuropsychological Measures in Healthy Young Women\textsuperscript{1,2}

John W. Finley,\textsuperscript{3} James G. Penland, Ross E. Pettit* and Cindy D. Davis

U.S. Department of Agriculture, Agricultural Research Service, Grand Forks Human Nutrition Research Center, Grand Forks, ND and *Red River Neurology Clinic, Grand Forks, ND

ABSTRACT Because manganese (Mn) is potentially toxic, and because dietary fat type may affect Mn absorption, the objectives of the current study were to determine whether diets containing very low or very high amounts of Mn and enriched in either saturated or unsaturated fats affected measures of neuropsychological and basic metabolic function. Healthy young women were fed for 8 wk each, in a crossover design, diets that provided 0.8 or 20 mg of Mn/d. One half of the subjects received 15\% of energy as cocoa butter, and one half received 15\% of energy as corn oil. A meal containing \textsuperscript{54}Mn was fed after 4 wk, and subjects underwent whole-body counting for the next 21 d. Blood draws and neuropsychological tests were administered at regular intervals during the dietary periods. When subjects consumed the diets low in Mn, compared with the high Mn diets, they absorbed a significantly higher percentage of \textsuperscript{54}Mn, but had a significantly longer biological half-life of the absorbed \textsuperscript{54}Mn. Manganese intake did not affect any neurological measures and only minimally affected psychologic variables. These data show that efficient mechanisms operate to maintain Mn homeostasis over the range of intakes that may be encountered in a mixed Western diet. Thus, dietary intakes of Mn from 0.8 to 20 mg for 8 wk likely do not result in Mn deficiency or toxicity signs in healthy adults. J. Nutr. 133: 2849–2856, 2003.

KEY WORDS: • manganese • fat • humans • toxicity • neurological

Manganese (Mn), an essential nutrient, is toxic at high levels of exposure (1–4). The National Academy of Sciences has set an Adequate Intake of Mn at 1.8 (women) or 2.3 (men) mg/d, and an Upper Limit at 11 mg/d (5). Food is the main source of Mn exposure for most adults, and vegetarians may consume >10 mg Mn/d (5,7). The Environmental Protection Agency (EPA)\textsuperscript{4} and the FDA have not ruled out environmental Mn as a source of overexposure (6).

Although human deficiency is rare (8,9), Mn deficiency in animals may cause skeletal defects (10) and impaired lipid metabolism (11). Skin rash and abnormalities in lipid measures were reported in a subject fed a purified Mn-deficient diet (12). In contrast, toxicity is more common, and is characterized by a Parkinson’s-like neuromuscular condition with tremor and facial muscular disorders (3). Limited data suggest that trace element status, especially Mn status, is linked to aberrant behavior (13,14). Elevated hair Mn concentrations were reported to be linked with violence in a prison population (15); on the basis of this limited data a political action group is recommending nutritional modification of inmate diets, with an emphasis on Mn (16). Mn toxicity is reported primarily in individuals exposed to large amounts of Mn dust (e.g., miners), and there is little information to indicate whether Mn from high Mn foods may pose a problem (6). Gathering such information was a primary objective of this study.

We have demonstrated a strong link between Fe and Mn status. Women absorb a greater percentage of Mn than men, and absorption is correlated with Fe intake (17). Mn absorption is further enhanced in women with low Fe stores (18). Because saturated fatty acids promote Fe absorption in rats (19) and may alter Mn balance in humans (20), and because dietary fat affects heart Mn superoxide dismutase activity (21) and Mn status in women (22), a secondary objective of this study was to gather information regarding the interaction of dietary fat and Mn metabolism.

Daily Mn intake (mg/d, 10th–99th percentile) ranges from 0.8 to 5.9 (food) and 0.6–8.1 (supplements) (5). To study Mn homeostasis under extreme dietary conditions, women in the present study consumed 0.8 or 20 mg Mn/d. To study the effect of fat type on Mn, we enriched the diets with either corn oil (CO) or cocoa butter (CB) and included neurological and psychological tests to determine possible effects on psychomotor and behavioral variables.

\textsuperscript{1} 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.

\textsuperscript{2} Names are necessary to report factually on available data; however, the USDA neither guarantees nor warrants the standard of the product, and the use of the name by USDA implies no approval of the product to the exclusion of others that may also be suitable.

\textsuperscript{3} To whom correspondence should be addressed.

\textsuperscript{4} Abbreviations used: BDHI, Buss-Durkee Hostility Inventory; CB, cocoa butter; CO, corn oil; EPA, Environmental Protection Agency; IBS, Interpersonal Behavior Survey; STAXI, State-Trait Anger Expression Inventory.

0022-3166(03) $3.00 © 2003 American Society for Nutritional Sciences.
SUBJECTS AND METHODS

This study was approved by the Human Studies Internal Review Board of the University of North Dakota and all aspects of the study conformed to guidelines of the Department of Health and Human Services and the Helsinki Declaration. All subjects were informed in detail before the study began, and all subjects gave written consent.

We studied healthy, nonsmoking, premenopausal women (n = 17; 21–46 y old; mean, 35.7 ± 8.0 y; height, 164.6 ± 7.4 cm; weight, 72.9 ± 13.0 kg; BMI, 26.8 ± 4.2 kg/m²). The study was conducted in two similar parts (Fig. 1) with the overall experimental design identical for both parts. In Part I, subjects (n = 11) ate all meals and participated in testing procedures in a metabolic ward, but continued to live at their normal place of residence and conduct their daily affairs. In Part II, women (n = 6) lived in a metabolic ward for the duration of the study.

All diets used in the study (complete diets in Appendix, Table A1) were formulated on the basis of 8.4 MJ and were adjusted for individual energy intake; thus the actual nutritional composition of the diet depended on the energy consumed. However, supplemental nutrients, which included Mn, were set and did not vary with energy intake. In both parts of the study, subjects were divided into 2 groups on the basis of the predominate dietary fat; within the fat treatment, subjects consumed diets that supplied 0.8 or 20 mg of Mn/d in a crossover design. The basal diet had 20% energy from fat and an additional 15% of energy was added as CB (49.8% saturated, 41.7% monounsaturated, 8.4% PUFAs; total diet: 47% saturated, 42% monounsaturated, 11% PUFAs) or CO (26.5% saturated, 39.7% monounsaturated and 33.7% PUFAs; total diet 26% saturated, 38% monounsaturated, 36% PUFAs). Each woman consumed the same fat type throughout the study.

The basal diet was formulated with foods low in Mn and provided 0.8 mg Mn/d; the higher intake of Mn was provided as supplemental MnSO₄, hidden in juice. Before each dietary period, there was a 1-wk equilibration period (3.5 mg Mn/d), which was followed by an 8-wk dietary period (the purpose of the equilibration period was to ensure that gut contents and absorptive conditions were similar in the dietary groups before beginning consumption of treatment diets; it was not to equilibrate Mn status). This was followed by a second equilibration period and a second 8-wk dietary period with the other intake of Mn (126 d total).

Subjects and diets. All volunteers were given a general health screen before acceptance. They were allocated to treatments in a manner that equalized serum ferritin concentrations in the treatment groups before beginning consumption of the isotope (the counts were averaged to give the initial results and converted to percent of intake). Subjects smoking, premenopausal women (n = 17; 21–46 y old; mean, 35.7 ± 8.0 y; height, 164.6 ± 7.4 cm; weight, 72.9 ± 13.0 kg; BMI, 26.8 ± 4.2 kg/m²). The study was conducted in two similar parts (Fig. 1) with the overall experimental design identical for both parts. In Part I, subjects (n = 11) ate all meals and participated in testing procedures in a metabolic ward, but continued to live at their normal place of residence and conduct their daily affairs. In Part II, women (n = 6) lived in a metabolic ward for the duration of the study.

All diets used in the study (complete diets in Appendix, Table A1) were formulated on the basis of 8.4 MJ and were adjusted for individual energy intake; thus the actual nutritional composition of the diet depended on the energy consumed. However, supplemental nutrients, which included Mn, were set and did not vary with energy intake. In both parts of the study, subjects were divided into 2 groups on the basis of the predominate dietary fat; within the fat treatment, subjects consumed diets that supplied 0.8 or 20 mg of Mn/d in a crossover design. The basal diet had 20% energy from fat and an additional 15% of energy was added as CB (49.8% saturated, 41.7% monounsaturated, 8.4% PUFAs; total diet: 47% saturated, 42% monounsaturated, 11% PUFAs) or CO (26.5% saturated, 39.7% monounsaturated and 33.7% PUFAs; total diet 26% saturated, 38% monounsaturated, 36% PUFAs). Each woman consumed the same fat type throughout the study.

The basal diet was formulated with foods low in Mn and provided 0.8 mg Mn/d; the higher intake of Mn was provided as supplemental MnSO₄, hidden in juice. Before each dietary period, there was a 1-wk equilibration period (3.5 mg Mn/d), which was followed by an 8-wk dietary period (the purpose of the equilibration period was to ensure that gut contents and absorptive conditions were similar in the dietary groups before beginning consumption of treatment diets; it was not to equilibrate Mn status). This was followed by a second equilibration period and a second 8-wk dietary period with the other intake of Mn (126 d total).

Subjects housed in metabolic ward

- **Corn oil diet group**
  - Week 1
  - Equilibration
  - 0.8 or 20 mg Mn/d (whole body counts weeks 6–9)
  - Weeks 2–9
  - Equilibration
  - 0.8 or 20 mg Mn/d (whole body counts weeks 6–9)
  - Week 10
  - Equilibration
  - 0.8 or 20 mg Mn/d (whole body counts weeks 6–9)
  - Week 11
  - Equilibration
  - 0.8 or 20 mg Mn/d (whole body counts weeks 6–9)
  - Week 12
  - Equilibration
  - 0.8 or 20 mg Mn/d (whole body counts weeks 6–9)

- **Cocoa butter diet group**
  - Week 1
  - Equilibration
  - 0.8 or 20 mg Mn/d (whole body counts weeks 6–9)
  - Weeks 2–9
  - Equilibration
  - 0.8 or 20 mg Mn/d (whole body counts weeks 6–9)
  - Week 10
  - Equilibration
  - 0.8 or 20 mg Mn/d (whole body counts weeks 6–9)
  - Week 11
  - Equilibration
  - 0.8 or 20 mg Mn/d (whole body counts weeks 6–9)
  - Week 12
  - Equilibration
  - 0.8 or 20 mg Mn/d (whole body counts weeks 6–9)

**Part II—Subjects housed in metabolic ward**

- **Corn oil diet group**
  - Week 1
  - Equilibration
  - 0.8 or 20 mg Mn/d (whole body counts weeks 6–9)
  - Week 2
  - Equilibration
  - 0.8 or 20 mg Mn/d (whole body counts weeks 6–9)
  - Week 3
  - Equilibration
  - 0.8 or 20 mg Mn/d (whole body counts weeks 6–9)
  - Week 4
  - Equilibration
  - 0.8 or 20 mg Mn/d (whole body counts weeks 6–9)
  - Week 5
  - Equilibration
  - 0.8 or 20 mg Mn/d (whole body counts weeks 6–9)

- **Cocoa butter diet group**
  - Week 1
  - Equilibration
  - 0.8 or 20 mg Mn/d (whole body counts weeks 6–9)
  - Week 2
  - Equilibration
  - 0.8 or 20 mg Mn/d (whole body counts weeks 6–9)
  - Week 3
  - Equilibration
  - 0.8 or 20 mg Mn/d (whole body counts weeks 6–9)
  - Week 4
  - Equilibration
  - 0.8 or 20 mg Mn/d (whole body counts weeks 6–9)
  - Week 5
  - Equilibration
  - 0.8 or 20 mg Mn/d (whole body counts weeks 6–9)

**FIGURE 1** Experimental design for healthy young women who consumed diets enriched with cocoa butter (n = 5, Part I; n = 2, Part II) or corn oil (n = 6, Part I; n = 4, Part II) and both 0.8 and 2.0 mg Mn/d.

Radioactive test meal and whole-body counting procedures. Manganese absorption and retention in the body were estimated from the retention of an orally administered (after an overnight fast) test dose of 0.037 mBq of 54Mn in the form of carrier-free MnCl₂ consumed in orange juice along with the regularly scheduled breakfast. (The total amount of juice varied depending on energy intake, whereas radioactive Mn intake was constant.) Identical breakfasts were consumed for each isotope administration. The breakfast and juice contained 1.3 MJ, 18 g protein, 3 g fat, 52 g carbohydrate, 1 g fiber, 238 mg Ca, 3 mg Fe, 48 mg Mg, 260 mg P, 759 mg K, 531 mg Na, 2 mg Zn, 0.3 mg Cu, 0.1 mg Mn, 74 mg vitamin C, 136 retinol equivalents vitamin A, 2 µg vitamin D, 0.4 mg α-tocopherol, 1 g saturated fatty acids, 2 g unsaturated fatty acids and 28 g cholesterol. To keep the specific activity relatively constant in the subjects, supplemental Mn was not added to the juice of subjects consuming the high-Mn diet.

After the test meal, total feces and urine were collected for the subsequent 28 d. Radioactive Mn in the feces was determined by using a custom-built, small animal whole-body counter equipped with an NDD2 multichannel analyzer (Nuclear Data Instrumentation, Schaumberg, IL) and calibrated with 137Cs. Whole-body 54Mn γ radiation was counted four times for 15 min each on the day of ingestion of the isotope (the counts were averaged to give the initial count, once each day for the subsequent 14 d and then twice weekly to the end of the dietary period. Counting was done using a method developed by Lykkken (23). Briefly, γ emissions were counted in a steel enclosed facility equipped with 32 NaI detectors, a 1024 mult-

Downloaded from https://academic.oup.com/jn/article-abstract/133/9/2849/4688166 by guest on 22 November 2018
tichannel analyzer (Nuclear Data, Schaumberg, IL) and an air-handling system that filtered and removed radon from the room air.

**Phlebotomy and clinical measurements.** Blood was taken at the end of each dietary period. Clinical measures were previously described (18,22,24).

**Chemical balance.** Chemical balance of Mn, Fe and Ca was determined over two 3-d periods during the last 6 d of each dietary period. Diet samples and feces collected during this period were digested and analyzed for Mn (18), and Fe and Ca (25).

**Neurological examination and motor steadiness.** During the last week of each dietary period, subjects were examined by a Board-Certified clinical neurologist (R.E.P.) for the presence and severity of >75 neurologic signs and symptoms (Appendix, Table A2). Examination included measures previously used to assess possible Mn toxicity (3,26,27), as well as those used to assess Parkinson's and related neurologic diseases, thus giving the most complete clinical examination possible.

Steadiness and the ability to control muscular tremor when making precise movements were assessed at the end of each diet period with a steadiness tester (Model TSD-801, BRS/LVE, Beltsville, MD). Steadiness was measured by having subjects hold a stylus free hand (both dominant and nondominant hands were tested) inside progressively smaller holes, or move a stylus along an elongated slot; errors were counted by electrical contact when the stylus touched the sides of the holes/slot.

**Psychological assessment.** Three standardized self-report measures were administered at the end of each dietary period to assess a broad spectrum of components related to hostility and anger. The Buss-Durkee Hostility Inventory (BDHI) (28) uses 75 true/false state-broad spectrum of components related to hostility and anger. The State-Trait Anger Expression Inventory (STAXI) (29) uses 44 statements rated on a 4-point scale to measure intensity of anger, the disposition to experience anger, propensity to express anger without provocation or when criticized or treated unfairly, the frequency of suppression of anger, the frequency of expression of anger and the frequency of an individual's attempt to control anger. The Interpersonal Behavior Inventory (IBS) (30) uses 272 true/false statements to measure the following: 1) general assertive behaviors, positive attitudes, self-assurance, and the willingness to ask for help and say "no" to unreasonable demands; and 2) general aggressive behaviors, feelings and attitudes, expression of anger in a direct, forceful manner and indirect expressions of anger such as stubbornness, negativism and complaining.

**Statistical analysis.** Because the experimental designs were identical, both parts of the study were combined for analysis. To account for statistical differences, Study Part was used as a blocking factor in the analysis. When the interaction between Study Part × Dietary Treatment was significant, it was used as the overall error term (this resulted in a reduction of the df). This statistical approach ensured that significant differences were not influenced by the part of the study in which a volunteer participated.

Absorption and biological half-life of 54Mn were estimated using data from the whole-body counter by plotting the natural logarithm of the percentage of 54Mn retained vs. time since administration of the dose; a regression line was fit to the linear portion of the curve (d 14–25; data not shown). The y-intercept was used as the estimate of absorption and biological half-life was computed as $-\ln(2)/$slope (17).

A two-component exponential model of the form $b_1e^{-kt_1} + b_2e^{-kt_2}$ was used to estimate the turnover rates of two compartments of Mn, where $b_1$ and $b_2$ represent the percentage of Mn turning over at rates $k_1$ and $k_2$, respectively. To allow the fit of the model, counts of each individual were examined for lag in tracer disappearance. Any counts after d 1 that were still 100% of initial counts (representing counts from unabsorbed tracer staying in the gut as a result of slow passage or the lack of a bowel movement, plus counts from absorbed tracer) were discarded and the model was fit to the reduced data.

Absorption estimates, clinical data, steadiness and psychological variables were analyzed statistically by using a 2 × 2 repeated-measures ANOVA with dietary Mn and dietary fat source as the independent factors. Because of the variability in the data, the Mn concentration in lymphocytes was reanalyzed by nonparametric rank-order analysis. To give the maximum time for variables to be affected by diet, only clinical data from the second blood draw (after 8 wk of treatment) were used. To reduce variation, some data were transformed to the natural log before statistical analysis.

Analyses of psychological variables were done on T-scores based on sex- and age-appropriate norms. IBS data were considered invalid and excluded from analysis when T-scores for validity scales (denial or impression management) exceeded 70. All variables were analyzed for inter-variables. If initial values were not different between treatments (e.g., steadiness), then statistical analysis was done on the actual value. If initial values did differ by treatment (e.g., psychological tests), statistical analyses were computed on change scores. Statistical analysis of dietary Mn and fat intakes on results of the neurologic exam were unnecessary because none of the subjects exhibited any aberrant neurologic signs or symptoms either before or after the dietary treatments. Differences were considered significant if $P < 0.05$.

**RESULTS**

**Absorption and biological half-life.** The percentage of Mn absorption calculated by linear regression was unaffected by dietary fat, but was almost 40% lower when subjects consumed the high Mn diet (Table 1). A double exponential model separated the tracer into quickly (unabsorbed material moving through the gut) and slowly excreted (absorbed) components; there was almost 40% less tracer in the slow component when subjects consumed high Mn, although the difference was not significant ($P = 0.14$) (Table 1). The total mass of Mn absorbed (calculated from dietary intake and absorption estimates) was $~0.03 \pm 0.003$ and $0.45 \pm 0.003$ mg from the low and high Mn diets, respectively, assuming that absorption of the tracer accurately estimated absorption of Mn from the whole diet.

The biological half-life (from the linear model) was almost twice as long when subjects consumed low compared with high Mn diets (Table 1). The exponential model predicted short (<2 d) and long (9–27 d) half-life components. Only the long half-life was affected by dietary Mn and it was shorter when subjects consumed the high (11.9d) vs. the low Mn diet (22.5d). The predicted percentage retention of tracer after 60 d was significantly greater when subjects consumed the low Mn diet. Assume that the percentage of tracer retention is an estimate of the percentage retention of daily dietary Mn. After subjects consumed the low Mn diet for 60 d, they still retained $6–8 \mu g$ of the dietary Mn consumed the day of the test meal, whereas after they consumed the high Mn diet, they retained $18–34 \mu g$ of dietary Mn consumed the day of the test meal. Retention of $54^m$Mn was significantly greater in subjects fed the diet enriched in CB.

**Clinical measures.** Most indicators of Mn and Fe status were not affected by dietary Mn or fat (data not shown). When analyzed by parametric statistics, lymphocyte Mn concentration tended to be higher ($P < 0.06$) in subjects consuming the diet enriched with CO (10.2 ± 1.8 nmol Mn/g tissue) than in those consuming the diet enriched in CB (5.1 ± 1.4 nmol Mn/g). When data were analyzed by nonparametric rank-order, the effect of fat was significant ($P = 0.006$). The only lipid measure affected by diet was VLDL cholesterol, which was lower ($P < 0.02$) when subjects consumed low Mn diets (0.56 ± 0.01 and 0.61 ± 0.01 mmol/L for low and high Mn, respectively). Biliary function (assessed by alanine aminotransferase, aspartic aminotransferase and alkaline phosphatase activity, ammonia, bile acids and bilirubin concentrations
Manganese balance was not affected by fat type; however, ln(ferritin) tended (P = 0.07) to be lower in women who consumed the CO diet. When the diet was also low in Mn (P = 0.02) and greater anger expression in subjects consuming any other diet (P < 0.0006). On the STAXI, subjects consuming the diet high in CO reported more anger directed outward (P < 0.02) and greater anger expression in general (P < 0.02) than subjects consuming the diet high in CO.

**DISCUSSION**

Manganese toxicity is of increasing concern to regulatory groups such as the EPA (6). Although there is little scientific evidence to suggest that dietary Mn may pose a risk, some political action groups have campaigned for a reduction of Mn in the food supply (16). Dietary Mn may pose a risk to developing infants and children (31), but there is almost no evidence to suggest that it is a risk to physiological and/or neurological function of healthy adults. On the other hand, few data exist to rule out such effects in adults.

**Psychological assessment.** There were no effects of dietary Mn intake on any measure of hostility, anger or aggression assessed by the BDHI, STAXI or IBS (Table 3); however, there were significant effects of Mn or Mn × Fat on other psychological variables. Fat source did not affect any variables from the BDHI, but compared with low Mn intake, high Mn intake decreased self-confidence (P < 0.03), an assertiveness measure. Also, there was a significant Mn × Fat interaction for Requesting Help. Subjects consuming the diet high in CB reported more defensive assertiveness than subjects consuming the diet high in CO, when the diet was also low in Mn (P = 0.03). Also, subjects consuming the diet high in both Mn and CB reported less willingness to request help than subjects consuming any other diet (P < 0.0006). On the STAXI, subjects consuming the diet high in CB reported more anger directed outward (P < 0.02) and greater anger expression in general (P < 0.02) than subjects consuming the diet high in CO.

<table>
<thead>
<tr>
<th>Dietary Mn, mg/d</th>
<th>Corn oil (n = 10)</th>
<th>Cocoa butter (n = 6)</th>
<th>Probabilities</th>
</tr>
</thead>
<tbody>
<tr>
<td>Measures calculated by linear regression&lt;sup&gt;2&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Absorption&lt;sup&gt;3&lt;/sup&gt;, %</td>
<td>3.2 ± 0.4</td>
<td>3.4 ± 0.1</td>
<td>3.4 ± 0.1</td>
</tr>
<tr>
<td>Biological half-life&lt;sup&gt;3&lt;/sup&gt;, d</td>
<td>1.8 ± 0.4</td>
<td>2.7 ± 0.1</td>
<td>3.5 ± 0.1</td>
</tr>
<tr>
<td>Biological half-life mean, d</td>
<td>30</td>
<td>14</td>
<td>34</td>
</tr>
<tr>
<td>Biological half-life B1&lt;sup&gt;5&lt;/sup&gt; (range ± 1 SD), d</td>
<td>(28–33)</td>
<td>(13–16)</td>
<td>(31–38)</td>
</tr>
<tr>
<td>Tracer retention after 60 d, % total 54Mn</td>
<td>0.68 ± 0.06</td>
<td>0.09 ± 0.06</td>
<td>0.96 ± 0.06</td>
</tr>
<tr>
<td>Total Mn retained from day of test meal after 60 d,&lt;sup&gt;6&lt;/sup&gt; μg</td>
<td>6.1 ± 0.4</td>
<td>18 ± 12</td>
<td>8.6 ± 0.4</td>
</tr>
</tbody>
</table>

<sup>1</sup>Subjects ingested 0.037 mBq of 54Mn in the form of carrier-free MnCl<sub>2</sub> hidden in orange juice and consumed with the regularly scheduled breakfast. The corn oil or cocoa butter diets were consumed for 19 wk, and the two levels of Mn, supplied as MnSO<sub>4</sub> for 8 wk each. The test meal was administered 28 d after the beginning of each dietary period and subjects were counted at regular intervals for the subsequent 28 d.

<sup>2</sup>Measures calculated from a double exponential model<sup>7</sup>.

<sup>3</sup>Values are LS Means ± SE LSM unless otherwise specified; NS, nonsignificant.

<sup>4</sup>ln(x) = natural log of x.

<sup>5</sup>Values are the extremes of the range for biological half-life calculated from ln ± SD.

<sup>6</sup>Assuming that tracer retention is an accurate estimate of total Mn retention, total dietary Mn retained after 60 d was calculated by multiplying the percentage retention of the 54Mn tracer by the total amount of Mn consumed by the subject on the day of the test meal.

<sup>7</sup>Manganese toxicity is of increasing concern to regulatory groups such as the EPA (6). Although there is little scientific evidence to suggest that dietary Mn may pose a risk, some political action groups have campaigned for a reduction of Mn in the food supply (16). Dietary Mn may pose a risk to developing infants and children (31), but there is almost no evidence to suggest that it is a risk to physiological and/or neurological function of healthy adults. On the other hand, few data exist to rule out such effects in adults. Controlled absorption and retention of 54Mn calculated from whole-body counts of 54Mn radiation in women who consumed diets enriched in either corn oil or cocoa butter and both 0.8 and 20 mg Mn/d in a crossover design.
studies have provided as much as 15 mg Mn/d for up to 120 d (22,24). The current study provided an even greater amount, i.e., the maximum amount that might be encountered in mixed Western diets, representing the intake of Mn in the diet. Previous reports demonstrating that healthy adult women effectively regulate large variations in dietary Mn retention by a combination of variable absorption and elimination (17,18).

The lack of response of clinical and neuropsychological variables to changes in Mn intake are evidence of the strong homeostatic controls regulating Mn retention. The observed decrease in absorption with increased dietary Mn represents the first means of homeostatic regulation; a second homeostatic response was the observed increase in whole-body Mn turnover rate (estimated by biological half-life). Consequently, a 25-fold increase in Mn intake resulted in only a three- to fourfold increase in retention of whole-body Mn after 60 d (calculated as the product of biological half-life, the percentage absorption and total daily Mn intake). These data add to previous reports demonstrating that healthy adult women effectively regulate large variations in dietary Mn retention by a combination of variable absorption and elimination (17,18).

MRI is a sensitive method for visualizing Mn deposition in the brain (32), and MRI-visualized accumulation of Mn in the brain has been associated with neurological problems (33,34). Because Mn is excreted through the bile (35), Mn may accumulate in the brains of persons with hepatic dysfunction (36), leading to neuroses and tremor similar to those of Idiopathic Parkinson’s Disease (37). Aschner (37) suggested that environmental exposure to manganese (as distinguished from the well-documented route of occupational exposure in jobs such as mining) should receive renewed attention because of the addition of methylcyclopentadienyl manganese tricarbonyl as an anti-knock additive to gasoline. Gottschalk et al. (15) suggested that elevated Mn may be associated with behavioral changes because Mn concentrations were elevated in the hair of violent, but otherwise healthy prison inmates compared to nonviolent criminals. Although these data are preliminary at best, a political action organization has called for dietary intervention among prisoners as a way of decreasing criminal behavior (16).

Neuropsychological and behavioral measures have been

### TABLE 2

**Measures of steadiness by point and line steadiness testing in women who consumed diets enriched in either corn oil or cocoa butter and both 0.8 and 20.0 mg Mn/d in a crossover design**

<table>
<thead>
<tr>
<th>Dietary Mn, mg/d</th>
<th>Corn oil (n = 10)</th>
<th>Cocoa butter (n = 6)</th>
<th>Probabilities</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dominant hand</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Line steadiness (time), s</td>
<td>19.8 ± 1.8</td>
<td>19.4 ± 1.8</td>
<td>14.5 ± 1.5</td>
</tr>
<tr>
<td>Nondominant hand</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Point steadiness</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>32-mm hole, errors/60 s</td>
<td>49.9 ± 14.9</td>
<td>58.6 ± 14.9</td>
<td>126.6 ± 18.5</td>
</tr>
<tr>
<td>40-mm hole, errors/60 s</td>
<td>12.8 ± 8.9</td>
<td>15.0 ± 8.9</td>
<td>28.7 ± 11.1</td>
</tr>
<tr>
<td>48- to 128-mm holes, total errors/12 s</td>
<td>4.6 ± 1.7</td>
<td>14.8 ± 1.7</td>
<td>3.1 ± 2.2</td>
</tr>
</tbody>
</table>

1 Values are means ± SEM; NS, nonsignificant. Only measures showing effects with P < 0.10 are presented.
2 The corn oil or cocoa butter diets were consumed for 19 wk, and the two levels of Mn, supplied as MnSO₄, for 8 wk each.
3 An error was electronically registered each time the stylus touched the side of the hole.
used in previous nutritional studies and have been shown to respond to dietary intervention. Hand steadiness (and tremor) is a sensitive indicator of neurotoxicity (43), including manganese toxicity (44); it is responsive to nutritional supplementation (45) and treatment of nutritional deficiencies (46). Changes in mood states, particularly anger, hostility and aggression, are also sensitive indicators of neurotoxicity (43) and manganese toxicity (4,15). The BDHI and STAXI demonstrated sensitivity in association with cholesterol and lipid levels (47,48) and were clearly responsive to dietary fat in the current study. The IBS has not been used in prior nutrition studies but was included here in an attempt to distinguish aggression from assertiveness. Its responsiveness to dietary fat indicates some success in making that distinction, i.e., IBS measures of assertiveness but not aggressiveness varied with fatty acid content.

In the present study, dietary variables, including Mn, did affect several neuropsychological and behavioral variables, suggesting that the test measures did respond to dietary intervention. However, self-reported psychological measures did not suggest that high dietary Mn intakes increase hostility, anger or aggressiveness, nor did neurological exams find any evidence that high Mn intake caused neuropsychological or neuromotor impairment. These conclusions are based on the limits of this study and should not be extrapolated to infants, young children with incompletely developed hepatic and biliary excretion systems, or adults with hepatobiliary dysfunction (38). However, ethical considerations, as well as the practical aspects of controlled dietary studies, dictate that all human studies of a potentially toxic substance be limited in scope initially and more extensive studies be conducted only when there is absolutely no evidence of impairment at the present level of Mn intake.

Homoostatic controls on Mn retention intake were apparent from measures of whole-body $^{54}$Mn and not from measurement of Mn chemical balance. Mn retention was zero or slightly negative when subjects consumed the low-Mn diet, but 13–16 mg/d were retained when subjects consumed the high-Mn diet, suggesting that 0.8 mg of Mn/d is insufficient to maintain positive balance and 20 mg of Mn intake/d may result in Mn accumulation. Balance measures have inherent variability and unreliability, and our balance periods were relatively short, thus complicating these problems. Tracer Mn was administered as a single test meal, and it is possible that tracer data are not indicative of what happens from the whole diet. However, the lack of changes in clinical, psychological and neurological measures is more in line with tracer, rather than balance data. It also is possible that a much longer time is required to completely adapt all pools of Mn within the body to the new intake of Mn. Total balance represents excretion and retention in pools that are not completely adapted and is still responding to a previous intake. Because of the reliance of dietary reference intake data on balance measures, the inconsistency between balance and tracer methodology warrants further examination.

In the present study, saturated fat tended ($P = 0.06$) to increase Mn chemical balance and did significantly increase calculated retention of $^{54}$Mn after 60 d. These findings are consistent with the hypothesis that factors that increase Fe absorption also increase Mn absorption (17,19,39). Mn absorption is primarily responsive to controls on Fe absorption (17,18), and Fe absorption has been reported to be enhanced by saturated fat (19,39). However, these results are inconsistent with a report that Mn absorption in rats was increased by unsaturated fats (40).

The diets enriched in saturated fatty acids (CB diets) also increased motor tremor (decreased point steadiness) and increased anger expression and anger directed outward. These findings are consistent with reports of a connection between dietary fat and aberrant behavior, linking decreased cholesterol concentrations and/or a decreased ratio of polyunsaturates:saturated fat intake to an increased propensity for aggressive behavior and violent death (41,42). On the basis of these results, we speculate that previously reported associations between Mn status and aberrant behavior (15) may have been because Mn status was slightly affected by a variable that also altered lipid status. Consequently, there may not have been a cause and effect relationship between Mn status and behavior; rather, what was detected is a variable that affected Mn status and lipid composition simultaneously, and changes in behavior were the result of changes in lipid metabolism.

In conclusion, the present study demonstrated that retention of dietary Mn is regulated over a wide range of intakes in healthy adult women. Large variations in Mn intake had little effect on various clinical markers and there was no evidence that extremes in dietary Mn intake increased aggressive behavior. Conclusions regarding the possible health problems of low and high Mn intake in these groups depends on properly designed and controlled studies that examine specifically the interaction of Mn nutrition with these conditions.

LITERATURE CITED

18. Finley, J. W. (1999) Manganese absorption and retention by young...


APPENDIX

TABLE A1

Four-day rotating menu for healthy young women who consumed diets enriched with cocoa butter (n = 7) or corn oil (n = 10) and both 0.8 and 2.0 mg Mn/d

<table>
<thead>
<tr>
<th>Day 1</th>
<th>Day 2</th>
<th>Day 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Breakfast</td>
<td>Breakfast</td>
<td>Breakfast</td>
</tr>
<tr>
<td>Orange juice</td>
<td>Orange juice</td>
<td>Orange juice</td>
</tr>
<tr>
<td>Cinnamon bread</td>
<td>Pork sausage</td>
<td>Corn chex</td>
</tr>
<tr>
<td>Skim milk</td>
<td>Blueberry muffin</td>
<td>White sugar</td>
</tr>
<tr>
<td></td>
<td>Skim milk</td>
<td>Banana bread</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Margarine</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Skim milk</td>
</tr>
<tr>
<td>Lunch</td>
<td>Lunch</td>
<td>Lunch</td>
</tr>
<tr>
<td>Mountain Dew</td>
<td>Beef taco salad</td>
<td>Ham sandwich</td>
</tr>
<tr>
<td>Canadian bacon</td>
<td>Taco sauce</td>
<td>Fat-free</td>
</tr>
<tr>
<td>pizza1</td>
<td>Ranch dressing1</td>
<td>mayonnaise</td>
</tr>
<tr>
<td>Lettuce salad</td>
<td>Tortilla chips</td>
<td>Peach crisp2</td>
</tr>
<tr>
<td>Ranch dressing1</td>
<td>Lemon Cake</td>
<td></td>
</tr>
<tr>
<td>Cherry crisp2</td>
<td>Lemon pudding</td>
<td></td>
</tr>
<tr>
<td>Supper</td>
<td>Supper</td>
<td></td>
</tr>
<tr>
<td>Beef patty</td>
<td>Chicken rice casserole1</td>
<td>Baked chicken1</td>
</tr>
<tr>
<td>Mashed potatoes</td>
<td>Lettuce Salad</td>
<td>Lettuce salad</td>
</tr>
<tr>
<td>with pork gravy1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Carrot/celery sticks</td>
<td>Fat-free thousand island</td>
<td>Ranch dressing1</td>
</tr>
<tr>
<td>Pears</td>
<td>Cheese biscuit1</td>
<td>Ritz crackers</td>
</tr>
<tr>
<td>Orange sherbert</td>
<td>Red grapes</td>
<td>Mandarin orange cake</td>
</tr>
<tr>
<td>Skim milk</td>
<td></td>
<td>Light vanilla ice cream</td>
</tr>
<tr>
<td>Optional foods</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Salt</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
| A maximum of 6 g/3-d menu cycle; salt was weighed once every 3 d after being set on d 4 of the study. 355 mL (12-oz) cans as desired  
| Black cherry sparkling water  |                       |                       |
| Diet Coke with        |                       |                       |
| caffeine              |                       |                       |
| Diet Mountain Dew     |                       |                       |
| with caffeine         |                       |                       |
| Diet Sprite caffeine-free|                   |                       |

1 Corn oil only.
2 Cocoa butter only.
TABLE A2

Neurologic signs and symptoms experienced by healthy young women who consumed diets enriched with cocoa butter \( (n = 7) \) or corn oil \( (n = 10) \) and both 0.8 and 2.0 mg Mn/d\(^1\)

<table>
<thead>
<tr>
<th>History of the following (self-reported)</th>
<th>Currently experiencing (by examination)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Head or spinal injury</td>
<td>Abnormal EEG,(^2) CAT or MRI</td>
</tr>
<tr>
<td>Loss of consciousness or blackouts</td>
<td>Psychiatric disturbance</td>
</tr>
<tr>
<td>Seizures or convulsions</td>
<td>Neuroleptic drug use</td>
</tr>
<tr>
<td>Current experience (self-reported)</td>
<td>Confusion</td>
</tr>
<tr>
<td>Depression</td>
<td>Fainting or dizziness</td>
</tr>
<tr>
<td>Excessive sleepiness</td>
<td>Loss of balance</td>
</tr>
<tr>
<td>Diminished libido or impotence</td>
<td>Tremors or shakes</td>
</tr>
<tr>
<td>Insomnia</td>
<td>Numbness</td>
</tr>
<tr>
<td>Nervousness or irritability</td>
<td>Hot or cold sensation abnormalities</td>
</tr>
<tr>
<td>Sudden laughter or crying</td>
<td>Urinary urgency or incontinence</td>
</tr>
<tr>
<td>Hallucinations</td>
<td>Cramps</td>
</tr>
<tr>
<td>Memory problems</td>
<td>Constipation</td>
</tr>
<tr>
<td>Mental slowing</td>
<td>Aches and pains</td>
</tr>
<tr>
<td>Current experiencing (by examination)</td>
<td>Speech disorders</td>
</tr>
<tr>
<td>Masked facies</td>
<td>Diminished volume</td>
</tr>
<tr>
<td>Myerson’s sign</td>
<td>Halted speech</td>
</tr>
<tr>
<td>Vision disorders</td>
<td>Slurred speech</td>
</tr>
<tr>
<td>Gaze palsy</td>
<td>Monotonous, hoarse speech</td>
</tr>
<tr>
<td>Absence of blinking</td>
<td>Swallowing difficulty</td>
</tr>
<tr>
<td>Eye tracking abnormalities</td>
<td>Sialorrhoea</td>
</tr>
<tr>
<td>Nystagmus</td>
<td></td>
</tr>
<tr>
<td>Pupillary abnormalities</td>
<td></td>
</tr>
</tbody>
</table>

1 Items were compiled from tests used in previous studies of Mn toxicity (3,26,27).
2 EEG, electroencephalogram; CAT, computer axial tomography.