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Manganese absorption in humans: the effect of phytic acid and ascorbic acid in soy formula¹⁻²

Lena Davidsson, Annette Almgren, Marcel-A Juillerat, and Richard F Hurrell

ABSTRACT The absorption of manganese from soy formula was studied in adult volunteers by extrinsic labeling of test meals with ⁵⁴Mn, followed by whole-body retention measurements for ≈30 d after intake. Eight subjects participated twice in each of the two studies, acting as his or her own control. Soy formula containing the native content of phytic acid was compared with a similar dephytinized formula: geometric mean manganese absorption increased 2.3-fold from 0.7% (range: 0.2–1.1%) to 1.6% (range: 1.0–7.2%) (*P* < 0.01) with the dephytinized formula. In addition, the effect of the ascorbic acid content of the phytic acid-containing formula was investigated. Manganese absorption was not influenced by an increase in the ascorbic acid from 625 μmol/L (110 mg/L) to 1250 μmol/L (220 mg/L): the geometric mean manganese absorption was 0.6% (range: 0.3–1.0%) and 0.6% (range: 0.3–1.1%), respectively. In conclusion, fractional manganese absorption was approximately doubled by the dephytinization of soy formula but was not influenced by an increase in the ascorbic acid content of a soy formula containing the native amount of phytic acid. *Am J Clin Nutr* 1995;62:984–7.

KEY WORDS Manganese, soy protein, phytic acid, phytase, humans, radioisotope

INTRODUCTION

The absorption of the essential trace element manganese from foods and the factors influencing its absorption have not been studied extensively in humans. This lack of information is primarily related to the methodologic difficulties involved in studies of this trace element. Fractional manganese absorption is very low, typically ≈3–5% (1), which means that a very sensitive method with high precision is needed for studies of manganese bioavailability. In addition, excretion rather than absorption is believed to be the major regulator of homeostatic control (2). The reexcretion of absorbed manganese via bile into the feces makes it impossible to separate unabsorbed mineral from endogenous (re-excreted) manganese. The low absorption and the biliary excretion route for manganese make conventional techniques, such as chemical balance, of limited value for studies of manganese absorption. Furthermore, manganese only has one stable isotope; the use of stable-isotope techniques, based on isotope ratios, is therefore not an option.

Earlier we developed a technique for studies of manganese absorption based on the administration of extrinsically labeled

test meals to adult volunteers, using the radioisotope ⁵⁴Mn (3). Retention measurements are monitored for a period of ≈4 wk after intake of the labeled test meal in a sensitive whole-body counter. After excretion of nonabsorbed isotope, the whole-body retention measurements are used to calculate fractional absorption by extrapolation to the day of the intake of the labeled test meal (3).

A very low fractional manganese absorption ($\bar{x} \pm \text{SD}$: 0.7 ± 0.2%) from soy formula in adults was found in earlier studies (4). The significantly lower bioavailability of manganese from soy formula (compared with human milk and iron-fortified cow milk formula) was at least partly attributed to the relatively high content of phytic acid in soy isolate. Phytic acid has been demonstrated to have a negative effect on manganese absorption in an animal model (5). Phytic acid is a strong chelator of several nutritionally important minerals and trace elements such as iron and zinc. The removal of phytic acid from foods, in particular from infant foods containing this ligand, is currently considered a possible way to improve mineral bioavailability. In a recent study in infants we demonstrated a statistically significant twofold increase of mean iron absorption, from 3.9% to 8.7%, when dephytinized soy formula was compared with formula with the native phytic acid content (6). The influence of dephytinization on the bioavailability of other bivalent cations such as manganese has not yet been investigated in humans.

The aim of the present study was to evaluate the effect of dephytinization of soy protein isolate on manganese absorption in adults. In addition, we studied the effect of doubling the normal concentration of ascorbic acid on manganese absorption from soy formula containing the native amount of phytic acid. Manganese and iron share common absorption pathways (7) and after 100% dephytinization, we showed approximately the same increase in iron absorption as was demonstrated earlier by increasing the ascorbic acid content of the formula containing the native phytate content (6).

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SUBJECTS AND METHODS

Subjects

Sixteen healthy adult volunteers (nine women and seven men aged 22–30 y) participated in the two separate manganese absorption studies, each study having eight subjects. Paired comparisons were made with each subject acting as his or her own control within each study. Subjects were randomly assigned to start with test meal a or b, followed by administration of the other test meal during the second part of the study. All volunteers were apparently healthy, nonpregnant, and had no known gastrointestinal disorders. The subjects were given oral and written information about the aims and procedures of the study. The study was approved by the Research Ethical Committee and the Isotope Committee at Sahlgren's Hospital, Göteborg, Sweden.

Test meals

Liquid infant formulas (ready-to-feed) based on soy isolate containing the native amount of phytic acid (formula 1) or virtually free from phytic acid prepared by the addition of phytase (formula 2) were produced for the studies at Alpura Koreco, Konolfingen, Switzerland. Soy isolate (Supro 1611; Protein Technologies International, St Louis) was used as the protein source. Soy formula 2 was dephytinized by the addition of phytase (EC.3.1.3.8). The enzyme (derived from *Aspergillus niger*) was purchased from Alko Ltd (Helsinki). Formulas were produced according to the specifications for a commercial soy formula (Nestlé SA, Vevey, Switzerland), but without added iron, zinc, and ascorbic acid. These nutrients ($\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$, ZnSO_4 , and L (+) ascorbic acid, Merck, Darmstadt, Germany) were added at the time of preparation of the labeled test meals at fortification concentrations equivalent to commercial European formulas. The following were added to each portion of soy formula (450 g): 48 μmol (2.7 mg) Fe, 34 μmol (2.25 mg) Zn, and either 284 μmol ascorbic acid (50 mg; studies 1a,b, and 2a) or 568 μmol ascorbic acid (100 mg; study 2b). In study 1, formula 1 was compared with formula 2. In Study 2, formula 1 was administered twice: the normal amount of ascorbic acid (625 $\mu\text{mol/L}$, or 110 mg/L) being compared with twice this amount.

Administration of labeled test meals and whole-body retention measurements

Each test meal was extrinsically labeled with 0.2 MBq ^{54}Mn (first test meal) or 0.4 MBq ^{54}Mn (second test meal) by adding almost carrier-free $^{54}\text{MnCl}_2$ solution ($>3.7 \text{ TBq/g Mn}$; Amersham International, Buckinghamshire, UK) during preparation of the test meals. Individual servings of infant formulas were labeled 16–18 h before consumption to allow equilibration. The activity of each individual test meal was measured in the whole-body counter before being served. All administrations of labeled test meals were made under close supervision by one of the investigators to ensure that the entire serving was consumed. No food or fluid was consumed during the following 3 h. After this time, the subjects were allowed to continue their normal diet. Leftovers were measured in the whole-body counter after intake to calculate the exact dose of radioactivity consumed by each subject. Whole-body retention was measured two to three times per week for ≈ 30 d after intake of the

labeled test meal. Manganese absorption was calculated by extrapolation to day 0 from retention measurements on days 10–30, according to the method described by Davidsson *et al* (3).

Food analysis

The amount of zinc, iron, and calcium in the soy formulas was analyzed by atomic absorption spectrometry (model 975; Varian Techtron, Mulgrave, Australia). Duplicate samples of freeze-dried formulas were ashed in quartz Ehrlenmeyer flasks in a muffle furnace at 520 °C for 48 h. Ash was dissolved in hydrochloric acid purified by sub-boiling distillation in a quartz still (Kümer Analysetechnik, Rosenheim, Germany) and diluted to 25 mL with ultrapure water. Iron was measured by the standard addition technique. Lanthanum oxide was added to the diluted digest to a final concentration of 10 g/L before the calcium content was analyzed. A reference material, Nonfat Milk Powder 1549 [National Institute of Standards and Technology (NIST) Gaithersburg, MD], was analyzed together with the formulas. The analyzed values were found to be within the certified ranges for zinc, iron, and calcium. Manganese was analyzed after mineralization of 1-g aliquots with 5 mL HNO_3 purified by sub-boiling distillation and 2 mL Suprapure H_2O_2 (300 mL/L, Merck) in a two-step heating procedure. Polytetrafluoroethylene digestion vessels and a microwave digestion system (MDS-81D; CEM Corporation, Matthews, NC) were used. The digest was transferred to polytetrafluoroethylene tubes and evaporated to 1 mL under filtered nitrogen gas. The manganese was analyzed by graphite-furnace atomic absorption spectrometry (Varian Techtron) after the samples were diluted to 25 mL with ultrapure water. Wheat Flour 1567a (NIST) was analyzed together with the formulas. The analyzed values were found to be within the certified range for manganese. Only ultrapure water (18 M Ω ; Milli-Q Water System, Millipore, Zürich, Switzerland) was used during mineral analysis. Nitrogen was measured by the Kjeldahl technique and protein was calculated by multiplying by the factor 6.25. Dry weight was determined after samples were dried at 105 °C for 24 h. Ascorbic acid was analyzed by electrometric titration with 2,6-dichlorophenol indophenol (8, 9). Phytic acid was measured by cerium (IV) sulfate precipitation according to a modification of the method by Makover (10).

Blood analysis

Iron-status indexes [hemoglobin, serum iron, and total iron-binding capacity (TIBC)] were measured at the clinical laboratory of Sahlgren's Hospital by using routine methods. Whole-blood manganese was analyzed by electrothermal atomic absorption (Perkin Elmer Zeeman/3030; Perkin Elmer, Überlingen, Germany) equipped with a graphite furnace (HGA 600; Perkin Elmer). Magnesium nitrate was used as a matrix modifier (11). All blood samples were drawn after an overnight fast.

Statistical methods

Because of the skewed distribution of the manganese absorption values, the results are given as geometric means ($\pm 1\text{SD}$) (12). Student's *t* test for paired observations was used for statistical comparisons. Values were converted to logarithms before statistical analysis and reconverted to antilogarithms to

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recover the original units according to the method used for studies of iron absorption (12).

RESULTS

The nutritional composition of the infant formulas is given in **Table 1**. All values for iron-status indexes and manganese in whole blood were found to be within the normal range of values found at Sahlgren's Hospital (hemoglobin: 116–149 and 132–166 g/L, serum iron: 10–30 and 15–35 $\mu\text{mol/L}$ for women and men, respectively, and TIBC: 43–70 $\mu\text{mol/L}$) and of reference values from our laboratory (0.12–0.30 $\mu\text{mol Mn/L}$). The fractional manganese absorption for each subject is given in **Table 2**. Geometric mean manganese absorption from the soy formula containing phytic acid was 0.7% (range: 0.2–1.6%; Study 1a). The mean absorption increased to 1.6% (range: 1.0–7.2%) after dephytinization (Study 1b). No effect was observed when the normal ascorbic acid concentration was doubled: the geometric mean absorption from formula with the native ascorbic acid concentration and that after doubling the concentration was 0.6% (range 0.3–1.0%) and 0.6% (range 0.3–1.1%), respectively.

DISCUSSION

The very low fractional absorption of manganese from infant formula based on soy isolate containing phytic acid found in the present study was in agreement with our earlier observations. The geometric mean manganese absorption of 0.7% and 0.6% from the formula containing the native amount of phytic acid as measured in Studies 1a and 2a was virtually identical to the earlier reported value of $0.7 \pm 0.2\%$ manganese absorbed from a similar soy formula (4). A statistically significant increase in manganese absorption was shown after dephytinization of the soy isolate: the geometric mean manganese absorption increased 2.3-fold from 0.7% to 1.6% ($P < 0.01$). A relatively large interindividual variation in manganese absorption was observed in the present studies. The absorption ranged from 0.2% to 1.6% (study 1a), from 1.0% to 7.2% (study 1b), and from 0.3% to 1.0% and 0.3% to 1.1% in studies 2a and 2b, respectively. These results thus confirm our earlier observations of the wide variation in manganese absorption between different subjects (3). However, repeated administrations of identical test meals to six volunteers demonstrated that the intraindividual variation was relatively small with CVs ($(\Sigma d^2/$

TABLE 1
Content of protein, manganese, calcium, zinc, iron, ascorbic acid, and phytic acid in infant formulas based on soy isolate¹

	Formula 1	Formula 2 ²
Protein (g)	17	17
Manganese (μmol)	5.0	5.5
Calcium (mmol)	12	10
Zinc (μmol)	16	17
Iron (μmol)	60	62
Ascorbic acid (μmol)	<6.0	<6.0
Phytic acid (% by wt)	0.035	0.001

¹ Values per kilogram ready-to-feed formula before the addition of iron, zinc, and ascorbic acid.

² Dephytinized.

TABLE 2
Fractional manganese absorption for studies 1 and 2¹

	Study 1		Study 2	
	Formula 1a	Formula 2a	Formula 1a	Formula 1b
Geometric mean	0.7	1.6	0.6	0.6
+1 SD	1.3	3.1	0.9	1.0
-1 SD	0.4	0.9	0.5	0.4

¹ Formula 2 was dephytinized; a has 625 mol ascorbic acid/L, b has 1250 mol ascorbic acid/L.

2n)^{0.5}) ranging from 0.7–2.1% between separate administrations (3). A study design including paired comparisons is therefore needed when evaluating the effect of dietary factors on manganese absorption from different diets (3). Furthermore, the present results also demonstrate a relatively large individual variation in the response to the removal of an inhibitory dietary factor, in this case phytic acid. A statistically significant increase in manganese absorption after removing the phytic acid from soy formula was clearly demonstrated in this study. In an earlier study, using a similar study design as in the present study, we were not able to show an inhibitory effect on manganese absorption by adding sodium phytate to cow milk formula in adults (13). Thus, the effect of adding sodium phytate to a test meal that does not normally contain any phytic acid (cow milk formula) could not predict the effect of the removal of native phytic acid from soy isolate.

Doubling the ascorbic acid concentration or removing the phytic acid from soy formula results in a similar increase in iron absorption in infants (6). However, in the present study, no effect could be found on manganese absorption after increasing the ascorbic acid concentration from 625 to 1250 $\mu\text{mol/L}$. The lower concentration of ascorbic acid (625 $\mu\text{mol/L}$) corresponds to the normal amount in the equivalent commercial product. The addition of a much higher excess ascorbic acid to cow milk formula (4545 $\mu\text{mol/L}$) in a previous study also showed no statistically significant effect on the absorption of manganese, although five of eight subjects absorbed less manganese when the ascorbic acid content was increased (13). These earlier data could indicate that when iron absorption is high, because of the excess ascorbic acid present in the test meal, manganese absorption might be reduced.

Manganese is an essential trace element that is of special interest in infant nutrition (14, 15). The requirement for manganese and, consequently, the amount of manganese that needs to be supplied by the infant's diet is not well understood. Because of the limited information on manganese requirements, no exact recommendations for this trace element have been issued. The Food and Nutrition Board (16) established estimated safe and adequate daily dietary intakes for manganese corresponding to 5.5–11.0 μmol (0.3–0.6 mg) for infants 0–6 mo and 11–18 μmol (0.6–1.0 mg) per day for infants 6–12 mo old. No cases of documented deficiencies have been reported in infants, although the risk for developing manganese deficiency has been discussed, especially for premature and low-birth-weight infants (17, 18). The lack of information on manganese deficiency and impaired manganese status is, at least partly, due to the absence of diagnostic tests to measure manganese status. The potential for manganese toxicity during infancy might be of more concern than the risk for manganese

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deficiency because manganese homeostasis is regulated mainly via bile excretion and biliary excretion is not well established early in life (19).

Studies in adult humans have demonstrated a very low fractional manganese absorption from infant formulas but it is not known whether the manganese absorption values found in adults can be extrapolated to other age groups, such as infants, because the influence of age on manganese absorption has not been evaluated in humans. A pronounced effect of age on manganese absorption was shown in rats, with higher absorption in younger animals (14). The very limited information available from studies in human infants indicates that apparent manganese retention is relatively high. When the chemical balance technique was used to study manganese retention in infants fed human milk and infant formula, the mean apparent manganese retention was 43% and 20%, respectively (20). Because of endogenous manganese losses in feces, the chemical balance technique would tend to underestimate the true manganese absorption. Mean manganese absorption measured in adults by radioisotopic technique from human milk- and cow milk-based infant formulas ranged from 1.7% to 8.2% (4), much lower than the values found in infants. If the infants respond to the degradation of phytic acid as the adults did in the present study, then the manganese absorption by this age group could be increased considerably by the dephytinization of soy formula. Infant formulas contain higher concentrations of manganese than does human milk and the intake of manganese is thus higher in formula-fed infants than in breast-fed infants (21, 22). More information about the absorption and retention of manganese during early life is clearly needed before the requirement for manganese and the optimal manganese content in infant foods can be established.

In conclusion, the results from the present study show that the fractional manganese absorption was approximately doubled (mean absorption increased from 0.7% to 1.6%) by the dephytinization of soy formula, whereas increasing the ascorbic acid content of a phytic acid-containing soy formula had no effect on manganese absorption in adults. ■

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