

Competitive inhibition of iron absorption by manganese and zinc in humans¹⁻⁴

Lena Rossander-Hultén, Mats Brune, Brittmarie Sandström, Bo Lönnerdal, and Leif Hallberg

ABSTRACT Zinc and manganese may interfere with iron absorption because of similar physicochemical properties and shared absorptive pathways. The effects of zinc and manganese on iron absorption were studied in human subjects by using paired observations and a dual-radioisotope method (⁵⁵Fe and ⁵⁹Fe). Manganese inhibited iron absorption both in solutions and in a hamburger meal. Fractional iron absorption is strongly dose dependent. Adding 2.99 mg Mn to 0.01 mg Fe reduced iron absorption to the same extent as increasing the iron dose 300-fold to 3 mg, strongly indicating a direct competitive inhibition of manganese on iron absorption. In the same experiment with zinc, no inhibitory effect was observed, suggesting different pathways for the absorption of zinc and iron. An intraluminal interaction may occur, because a fivefold excess of zinc to iron (15 mg Zn/3 mg Fe) reduced iron absorption by 56% when given in a water solution but not when given with a hamburger meal. *Am J Clin Nutr* 1991;54:152-6.

KEY WORDS Iron absorption, human, manganese, zinc, trace element interactions

Introduction

Iron deficiency is very common in most developing countries and is also prevalent in many industrialized countries (1). Infants, children, teen-agers, and women of fertile age are the most vulnerable groups and various programs have been introduced to minimize this nutritional disorder (2, 3).

The main causes for nutritional iron deficiency are low dietary iron intake and low bioavailability of iron in the diet. The latter is due to an imbalance among dietary factors enhancing and inhibiting iron absorption (4). The main enhancing factors are ascorbic acid and meat and fish. We (5-7) previously identified and quantitated the inhibitory effects of phytate, iron-binding phenolic compounds, and calcium on iron absorption in humans. However, other dietary components that may interfere with iron absorption remain to be investigated.

Trace elements with similar physicochemical properties may interact with each other at the level of absorption. By forming ionic complexes in water that have similar configurations, a high concentration of one element may inhibit the mucosal uptake of another element provided that they share absorptive pathways (8, 9). Such an interaction was shown for zinc and copper (10), and this interaction has also been used in the treatment with zinc of patients with Wilson's disease, a genetic disorder of copper

overload (11). Similarly, a negative effect of high concentrations of iron on zinc absorption was demonstrated (12, 13).

In this study we investigated the effect of zinc and manganese on iron absorption. Zinc supplements have become more commonly used, and large pharmacological doses can be found in some mineral supplements. Manganese is less commonly found as a mineral supplement but is present in high concentrations in foods such as legumes, nuts, and tea (14, 15). We therefore found it important to evaluate and quantitate the individual effects of zinc and manganese on iron absorption in humans both when given as single supplements and when included as part of a composite meal. The access of two readily measurable radioactive iron tracers made it most feasible to study the interaction in absorption among zinc, manganese, and iron by primarily investigating the effect of zinc and manganese on iron absorption.

Methods

Subjects

Ninety-eight healthy volunteers participated in the study: 33 men and 65 women aged 19-50 y. Each series contained a number of regular blood donors to ensure variation in iron status among subjects. Participating subjects were given oral and written information about the aims and procedures of the study. The study was approved by the Ethical Committee of the University of Göteborg.

¹ From the Departments of Medicine and Clinical Nutrition, University of Göteborg, Sahlgren Hospital, Göteborg, Sweden; the Research Department of Human Nutrition, The Royal Veterinary and Agricultural University, Fredriksberg, Denmark; and the Department of Nutrition and Internal Medicine, University of California at Davis.

² Presented in part at the Sixth International Symposium on Trace Element Metabolism in Man and Animals (TEMA 6), Monterey, CA, 1987.

³ Supported by Swedish Medical Research Council project B90-19X-04721-15A, Swedish Council for Forestry and Agriculture research project 0572/89 L 136:1, and Swedish Agency for Research Co-operation with Developing Countries, project 9.49/SAREC 85/46:2.

⁴ Address reprint requests to L. Hallberg, Department of Medicine II, University of Göteborg, Sahlgren Hospital, S-413 45, Göteborg, Sweden. Received June 18, 1990.

Accepted for publication September 12, 1990.

Experimental design

The effect of manganese or zinc on iron absorption was studied by comparing in the same subject the absorption of iron, given as ferrous sulphate, with or without manganese or zinc and labeling the iron with two different radioiron isotopes, ^{55}Fe and ^{59}Fe . The effect of manganese and zinc was also studied when iron was supplied as nonheme iron in a composite hamburger meal.

In most experiments iron was given to fasting subjects as a solution containing 3 mg Fe, or 3 mg nonheme Fe in a meal, with or without 7.5–15 mg Mn or 15–45 mg Zn. To increase the sensitivity of the study, the absorption from iron doses 300-fold different in size, 3 and 0.01 mg Fe, were also compared and 2.99 mg Zn or Mn was added to the 0.01 mg Fe dose.

The iron solution or meal with (A) or without (B) added manganese or zinc was given on alternate mornings after an overnight fast on 4 consecutive days in the order ABBA or BAAB. Water (150 mL) was ingested with the meals. As mentioned above, the iron solutions or meals (A and B) were labeled with two different radioiron isotopes. A blood sample was drawn 2 wk after the last meal was served to determine the ratio of ^{55}Fe to ^{59}Fe in blood. Analysis of ^{55}Fe and ^{59}Fe was made by means of a modification of the method described by Eakins and Brown (16). Total retention of ^{59}Fe was measured by whole-body counting at the same time as the blood sample was drawn, whereas total retention of ^{55}Fe was calculated from the ratio of ^{55}Fe to ^{59}Fe in red cells. All procedures and methods of calculation were described previously (17).

Effects on iron absorption

The absorption of 3 mg Fe given as ferrous sulphate, together with 7.5 or 15 mg Mn (as manganese chloride) was studied (studies 1 and 2). In another experiment (study 4) iron absorption of 18 mg Fe was compared with absorption of 3 mg Fe given together with 15 mg Mn. Iron absorption was also studied from a hamburger meal containing 3 mg native nonheme Fe, given with or without 15 mg Mn (study 3). The nonheme iron was extrinsically labeled with a trace amount of high-specific-activity radioiron, ^{55}Fe or ^{59}Fe , when the meals were given with or without manganese. In one experiment (study 5) iron absorption from 3 and 0.01 mg Fe was compared, with 2.99 mg Mn given together with the 0.01-mg Fe dose.

The 3-mg Fe dose was given with two doses of zinc, 15 or 45 mg Zn as zinc sulfate (studies 6 and 8). In addition, iron absorption was compared from hamburger meals containing 3 mg native nonheme iron, extrinsically labeled with the two radioiron tracers, when the meals were given with or without 15 mg Zn (study 7). In one experiment the iron absorption from 3 and 0.01 mg Fe was compared when 2.99 mg Zn was given with the 0.01 mg Fe dose (study 9).

Iron absorption from 3 and 0.01 mg Fe was also compared to enable evaluation of the results above when the 0.01-mg Fe dose was given with 2.99 mg Mn or Zn (study 10).

The iron solutions contained 10 mL 0.01 mol HCl/L, iron as ferrous sulphate, zinc as zinc sulphate, and manganese as manganese chloride and were labeled with high-specific-activity ^{55}Fe or ^{59}Fe in the form of ferric chloride in 0.01 mol HCl/L. The 10-mL vials were rinsed twice with water, and this was also consumed. No food or drink was allowed for 3 h postdose. Each subject received a total of 37 kBq ^{59}Fe and 74 kBq ^{55}Fe .

The hamburger meal consisted of hamburger (110 g), green string beans (60 g), and mashed potatoes (150 g). The hamburger served was a commercial product containing 66 g minced meat. The meal contained 3 mg native nonheme Fe. Each meal contained 2.04 mg Zn and 0.36 mg Mn.

Results

Addition of 7.5 or 15 mg Mn reduced the absorption of 3 mg Fe by 21% and 34%, respectively (Table 1). A similar effect was found when 15 mg Mn was given in a meal containing 3 mg Fe; iron absorption decreased by 40%. Manganese had an effect on the fraction of iron absorbed similar to a corresponding increase in iron dose. The ratio between iron absorption from 18 and 3 mg Fe given with 15 mg Mn was 0.90 ± 0.07 , ie, no significant difference. The fraction of iron absorbed from a 3-mg Fe dose and a dose containing only 0.01 mg Fe and 2.99 mg Mn was almost identical.

Addition of 15 or 45 mg Zn interfered significantly with absorption of 3 mg Fe given as a water solution (Table 2). The absorption of iron was reduced to less than half. When 15 mg Zn was given with 3 mg Fe in a meal, however, there was no effect on iron absorption. The fraction of iron absorbed from a 3-mg Fe dose and a dose containing 0.01 mg Fe and 2.99 mg Zn differed markedly. The fractional iron absorption from the lower iron dose was 2.5 times higher.

Fractional iron absorption from 0.01 mg Fe as compared with 3 mg Fe was considerably higher (Table 3). This ratio was not statistically different from the ratio found when 2.99 mg Zn was added to 0.01 mg Fe but was markedly different from the absorption ratio when 2.99 mg Mn was added to the 0.01-mg Fe dose.

Discussion

The individual variability in iron absorption in large, even in normal healthy subjects (18). This implies that in any group of

TABLE 1
Effect of manganese on iron absorption*

Study†	Iron absorption		Absorption ratio (with Mn:without Mn)
	Without Mn	With Mn	
	%		
1: 3 mg Fe \pm 7.5 mg Mn; 10 F [3]	20.9 \pm 3.43	16.3 \pm 3.02	0.79 \pm 0.07‡
2: 3 mg Fe \pm 15 mg Mn; 5 M, 4 F [2]	30.5 \pm 7.40	18.1 \pm 4.09	0.66 \pm 0.08‡
3: 3 mg Fe \pm 15 mg Mn; 9 M [3], 2 F	14.0 \pm 2.87	8.2 \pm 1.80	0.60 \pm 0.04‡
4: 18 mg Fe/3 mg Fe + 15 mg Mn; 1 M [1], 9 F [2]	18.0 \pm 3.12	15.3 \pm 2.50	0.90 \pm 0.07
5: 3 mg Fe/0.01 mg Fe + 2.99 mg Mn; 7 M, 2 F	27.5 \pm 3.98	26.0 \pm 2.69	1.02 \pm 0.09

* $\bar{x} \pm \text{SE}$.

† F, female; M, male. Number of blood donors given in brackets.

‡ $P < 0.001$.

§ 3 mg Fe as nonheme iron in a hamburger meal.

TABLE 2
Effect of zinc on iron absorption*

Study†	Iron absorption		Absorption ratio (with Zn:without Zn)
	Without Zn	With Zn	
	%		
6: 3 mg Fe ± 15 mg Zn; 9 F [2]	22.9 ± 3.54	12.5 ± 4.01	0.44 ± 0.07‡
7: 3 mg Fe§ ± 15 mg Zn; 1 M [1], 9 F [2]	11.1 ± 2.51	11.5 ± 2.27	1.11 ± 0.08
8: 3 mg Fe ± 45 mg Zn; 3 M, 7 F [2]	24.9 ± 4.08	11.1 ± 2.52	0.45 ± 0.07‡
9: 3 mg Fe/0.01 mg Fe + 2.99 mg Zn; 3 M, 7 F	18.7 ± 4.24	40.5 ± 6.08	2.50 ± 0.22

* $\bar{x} \pm SE$.

† F, female; M, male. Number of blood donors given in brackets.

‡ $P < 0.001$.

§ 3 mg Fe as nonheme iron in a hamburger meal.

subjects, the mean value for iron absorption is likely to be different from another group of subjects of the present size. Therefore, we chose to evaluate the inhibitory effect of manganese and zinc on iron absorption in each experiment by using paired observations in the same subject and taking the ratio between iron absorption with and without the factor to be studied. Thus, several sets of experiments using different groups of subjects can be compared.

There are two kinds of iron in the diet with respect to mechanisms of absorption. Heme iron forms a minor part of dietary iron and is present as hemoglobin and myoglobin. Nonheme iron usually constitutes $\geq 90\%$ of the dietary iron and can be uniformly labeled with an extrinsic inorganic radioiron tracer. The added inorganic iron salt can thus be considered to form a common pool with nonheme-iron compounds in the diet (19). The access of two suitable radioiron tracers has made it possible to comprehensively validate this pool concept for iron (20). The same pool concept has been applied for several other inorganic nutrients such as manganese and zinc. Recently, by using two manganese tracers, ^{52}Mn and ^{54}Mn , it was shown that there was a complete isotope exchange between manganese chloride and manganese in chicken liver (21).

The present studies on the interaction among iron, zinc, and manganese are only valid for nonheme iron, which exchanges

with inorganic iron. It is not known to what extent there are manganese and zinc compounds in the diet that do not exchange with extrinsic inorganic tracers of these elements. On the basis of the wide exchangeability of various iron compounds with inorganic iron in the gastrointestinal tract, it may be assumed that this is probably also valid for most manganese and zinc compounds in the diet and their corresponding inorganic tracers. The present results may therefore be considered to be representative for interactions between nonheme iron and the majority of the zinc and manganese in the diet.

Manganese was found to have a strong inhibitory effect on the fractional absorption of iron. At a manganese-iron ratio of 2.5:1, iron absorption decreased by 22% as compared with when no manganese was given and at a ratio of 5:1, absorption decreased by 34%. In the presence of a meal, a 5:1 ratio of manganese to iron reduced iron absorption to the same extent, 40%. Thus, the presence of dietary ligands that could bind iron and/or manganese did not affect the manganese-iron interaction. This suggests that the mechanism for absorption of nonheme iron is strongly influenced by manganese and that dietary binding ligands have a similar effect on both elements. That the same absorption ratio was obtained when 18 mg Fe was given alone and 3 mg Fe was given with 15 mg Mn also suggests that the intestinal mucosa cannot distinguish between iron and manganese at the level of absorption. If manganese did not affect iron absorption, a higher fractional absorption should have been obtained from 3 mg than from 18 mg Fe.

A strong support for the interpretation that iron and manganese use the same critical step in the mucosal absorption is the present observation that the fractional absorption was the same from 3 and 0.01 mg Fe, despite the 300-fold difference in dose size, when the 0.01 mg Fe dose was given with 2.99 mg Mn (Fig 1). Note that the observed absorption ratio close to 1 (1.02) was obtained even though there was a wide variation in iron absorption among subjects (range 15.1–60.8%).

An interaction between iron and manganese was previously shown in experimental animals (22–26). Iron supplementation of infant formula resulted in lower liver manganese concentrations in weanling mice (25). That the interaction occurred at the level of absorption was supported by the strong correlation between the ratio of iron to manganese in the small intestinal mucosa and in the liver. Large amounts of dietary manganese were shown to cause anemia in rats (22), indicating a negative effect of manganese on iron absorption and subsequently on

TABLE 3
Iron absorption from 3 and 0.01 mg Fe

Study*	Iron absorption		Absorption ratio (0.01 mg Fe:3 mg Fe)	Absorption†	
	3 mg Fe	0.01 mg Fe		3 mg Fe	0.01 mg Fe
	%			%	
10: 3 mg Fe/0.01 mg Fe; 4 M [2], 6 F [2]	22.3 ± 4.22‡	51.1 ± 6.80	2.30 ± 0.17	40	91.7
9: 3 mg Fe/0.01 mg Fe + 2.99 mg Zn§; 3 M, 7 F	18.7 ± 4.24	40.5 ± 6.08	2.50 ± 0.22	40	86.4

* M, male; F, female. Number of blood donors given in brackets.

† Values adjusted to correspond to an absorption of 40% from the 3-mg Fe dose.

‡ $\bar{x} \pm SE$.

§ From Table 2, included as a comparison.

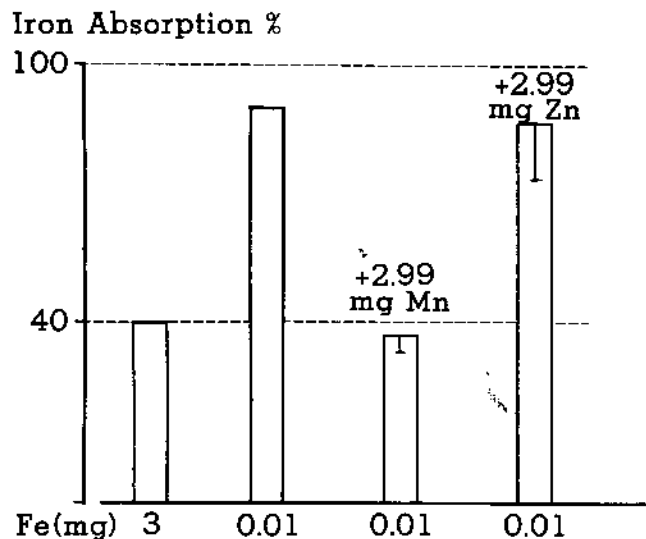


FIG 1. Effect of manganese and zinc on iron absorption. Absorption of iron from 3- and 0.01-mg Fe doses; the latter are given with or without 2.99 mg Mn or Zn. To facilitate comparisons the results are normalized to correspond to an absorption of 40% from the 3-mg Fe dose.

hematopoiesis. We therefore conclude that manganese interferes with iron absorption and that this occurs over a range of iron intakes and also in the presence of a composite meal. It is well known that there is a marked individual variation in iron absorption mainly related to mucosal regulation of the iron absorption according to physiological demands for iron. The facts that the fractional absorption of iron was the same from 3 and 0.01 mg Fe when the latter dose was given with 2.99 mg Mn and that the interindividual variation in iron absorption was very marked in this study (fourfold) strongly suggest that the common steps in the transfer of iron and manganese through the intestinal mucosa include unknown steps regulating iron absorption.

The interference of manganese with the absorption of iron should be taken into account when the inhibitory effects of various dietary components on iron absorption in humans are evaluated. The competitive interaction between manganese and iron implies that the effect expected depends on the relative amounts of these elements in a meal. For example, the inhibitory effect of tea on iron absorption is mainly due to its content of iron-binding phenolic compounds (6, 27). If much tea is consumed with a meal low in iron, part of the observed inhibition may also be related to the high manganese content of tea (~1–2 mg Mn per cup of tea).

In contrast to manganese, zinc had no detectable effect on iron absorption in the very sensitive experiment in which the absorption from 3 and 0.01 mg Fe were compared and 2.99 mg Zn was added to the low-iron dose (Fig 1). Thus, this finding indicates that iron and zinc do not share a transfer system through the intestinal mucosa. Therefore, the observed inhibition of iron absorption when 15 or 45 mg Zn was given with 3 mg Fe suggests that this interaction occurs in the intestinal lumen and with hypothetical ligands that normally favor iron absorption. This interpretation is also compatible with the present observation that the inhibitory effect of zinc on iron absorption was abolished in the presence of a meal. These findings suggest that when di-

etary ligands are present, physiologically there is no interaction between the absorption of iron and zinc.

In a previous study (13) it was shown that iron can inhibit zinc absorption but that this effect is not observed when these elements are given with a composite meal. That zinc is absorbed via another pathway in the presence of dietary ligands was supported by the finding that addition of histidine, a chelator of zinc, also abolished the interaction between iron and zinc.

The findings in the present study indicate that fortification with zinc or consumption of diets high in zinc do not interfere with iron absorption. However, if multimineral supplements are taken apart from a meal, a negative effect of zinc on iron absorption may be exerted. This is supported by a recent study by Crofton et al (28), who gave zinc supplements to human subjects and studied iron absorption. It should be noted, though, that this effect only occurs at a high zinc-iron ratio and that most supplements would be higher in iron than in zinc. □

References

1. DeMaeyer E, Adiels-Tegman M. The prevalence of anaemia in the world. *World Health Stat Q* 1985;38:302–16.
2. Baker SJ, DeMaeyer EM. Nutritional anemia: its understanding and control with special reference to the work of the World Health Organization. *Am J Clin Nutr* 1979;32:368–417.
3. Dallman PR, Siimes MA, Stekel A. Iron deficiency in infancy and childhood. *Am J Clin Nutr* 1980;33:86–118.
4. Hallberg L. Bioavailability of dietary iron in man. *Annu Rev Nutr* 1981;1:123–47.
5. Hallberg L, Brune M, Rossander L. Iron absorption in man: ascorbic acid and dose-dependent inhibition by phytate. *Am J Clin Nutr* 1989;49:140–4.
6. Brune M, Rossander L, Hallberg L. Iron absorption and phenolic compounds. Importance of different phenolic structures. *Eur J Clin Nutr* 1989;43:547–58.
7. Hallberg L, Brune M, Rossander L. Calcium: effect of different amounts on nonheme- and heme-iron absorption in humans. *Am J Clin Nutr* 1991;53:112–9.
8. Hill CH, Matrone G. Chemical parameters in the study of in vivo and in vitro interactions of transition elements. *Fed Proc* 1970;29:1474–81.
9. Hurley LS, Keen CL, Lönnerdal B. Aspects of trace element interactions during development. *Fed Proc* 1983;42:1735–9.
10. Fischer PWP, Giroux A, L'Abbe MR. Effect of dietary zinc on intestinal copper absorption. *Am J Clin Nutr* 1981;34:1670–5.
11. Brewer GJ, Hill GM, Prasad AS, Cossack ZT, Rabbani P. Oral zinc therapy for Wilson's disease. *Ann Intern Med* 1983;99:314–20.
12. Solomons AB, Jacob RA. Studies on the bioavailability of zinc in humans: effect of heme and nonheme iron on the absorption of zinc. *Am J Clin Nutr* 1981;34:475–82.
13. Sandström B, Davidsson L, Cederblad Å, Lönnerdal B. Oral iron, dietary ligands and zinc absorption. *J Nutr* 1985;115:411–4.
14. Gibson RS, Schytes CA. Trace element intakes in women. *Br J Nutr* 1982;48:241–8.
15. Mena I. Manganese. In: Bronner F, Coburn JW, eds. *Disorders of mineral metabolism*. Vol I. New York: Academic Press Inc, 1981: 233–69.
16. Eakins JD, Brown DA. An improved method for the simultaneous determination of iron-55 and iron-59 in blood by liquid scintillation counting. *Int J Appl Radiat Isot* 1966;17:391–7.
17. Björn-Rasmussen E, Hallberg L, Magnusson B, Rossander L, Svanberg B, Arvidsson B. Measurement of iron absorption from composite meals. *Am J Clin Nutr* 1976;29:772–8.
18. Magnusson B, Björn-Rasmussen E, Hallberg L, Rossander L. Iron absorption in relation to iron status. Model proposed to express

- results of food iron absorption measurements. *Scand J Haematol* 1981;27:201-8.
19. Hallberg L. The pool concept in food iron absorption and some of its implications. *Proc Nutr Soc* 1974;33:285-91.
 20. Hallberg L. Bioavailability of dietary iron in man. *Annu Rev Nutr* 1981;1:123-47.
 21. Davidsson L, Cederblad Å, Hagebö E, Lönnerdal B, Sandström B. Intrinsic and extrinsic labelling for studies of manganese absorption in humans. *J Nutr* 1988;118:1517-21.
 22. Hartman RH, Matrone G, Wise GH. Effect of high dietary manganese on hemoglobin formation. *J Nutr* 1955;57:429-39.
 23. Diez-Ewald M, Weintraub LR, Crosby WH. Interrelationship of iron and manganese metabolism. *Proc Soc Exp Biol Med* 1968;129:448-57.
 24. Thomson ABR, Olatunbosun D, Valberg LS. Interrelation of intestinal transport system for manganese and iron. *J Lab Clin Med* 1971;78:642-55.
 25. Keen CL, Fransson G-B, Lönnerdal B. Supplementation of milk with iron bound to lactoferrin using weanling mice. II. Effect on tissue manganese, zinc and copper. *J Pediatr Gastroenterol Nutr* 1984;3:642-55.
 26. Gruden N. The effect of iron dose on manganese absorption in neonatal and weanling rats. *Nutr Rep Int* 1986;34:21-7.
 27. Disler PB, Lynch SR, Charlton RW, et al. The effect of tea on iron absorption. *Gut* 1975;16:193-200.
 28. Crofton RW, Gvozdanovic D, Gvozdanovic S, et al. Inorganic zinc and the intestinal absorption of iron. *Am J Clin Nutr* 1989;50:141-4.

Ca
theMa
FraAB
com

Dur

curr

a po

not t

for :

qu

P, tr

with

a do

cons

4.57

for f

and

fract

157-

KEY

inci

Intr

D

incr

of ca

ciati

of ca

sona

port

caffè

ever

(9)

won

unit

CI 1

A

but

olise

stud

asso

14).

fract

but

Am