Proceedings of the Nutrition Society (1992) **51**, 211–218

Dose dependence of zinc and manganese absorption in man

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Intestinal absorption and excretion are the main regulatory mechanisms for maintenance of body homeostasis of most trace elements. In spite of considerable research on trace element metabolism in man and experimental animals, the knowledge about the mechanisms involved in these processes is still limited. The characteristics of trace element metabolism, such as low fractional absorption, the presence of the elements in intestinal juices and a rapid plasma turn-over, complicate specific studies of intestinal trace element absorption and excretion in the intact animal or man. Several in vivo and in vitro experimental models have, therefore, been developed, e.g. everted intestinal sacs, in situ loops, brush-border-membrane vesicle preparations and intestinal perfusion techniques, to study the kinetics of trace element absorption, usually administering aqueous solutions of the element at different concentrations or with the addition of ligands. However, the chemical environment at the site for intestinal absorption after ingestion of food is much more complex with the presence of partly digested food components, amino acids, organic acids, competing ions, etc. Therefore, the results from experimental models have to be judged with caution.

ZINC ABSORPTION

Site of Zn absorption. Zn seems to be absorbed throughout the entire small intestine. Intestinal perfusion studies of isolated intestinal segment in rats, using a perfusate containing 10 μg Zn/ml (0·15 mM), have suggested that the capacity of Zn absorption is highest in the ileum (Antonson *et al.* 1979). Davies (1980) used an intralumen injection of 10 μg Zn in 0·1 ml water labelled with ^{65}Zn to rats and showed that the duodenum contributed 60%, the ileum 30% and the jejunum 10% to the overall absorption of Zn. Negligible absorption occurred from the caecum and colon. Using a triple-lumen technique in humans and an electrolyte solution containing 0·1 mM-zinc acetate for the perfusion, Lee *et al.* (1989) observed the highest rate of absorption in the jejunum compared with the duodenum and ileum. As most of the hypotheses for Zn absorption mechanisms are based on results from studies of experimental animals, it would be important to find out whether the observed differences reflect true species differences or can be ascribed to differences in experimental design.

Zn absorption mechanisms. Experimental models indicate that Zn absorption is a carrier-mediated process and that uptake at the brush-border membrane is the rate-limiting step. Menard & Cousins (1983) used brush-border-membrane vesicles from rat intestine to study the kinetics of Zn absorption and concluded that Zn uptake was saturable and occurring by a carrier-mediated process, that did not seem to be energy dependent at 0·2 mM. At 1 mM it was non-saturable indicating a passive diffusion process. The results suggest a Michaelis constant (K_m) of 0·38 mM and a maximum uptake velocity (J_{max}) of 5·4 nmol/min per mg protein. Similar evidence of both

carrier-mediated, saturable and non-mediated, non-saturable components of Zn absorption was observed by Steel & Cousins (1985) using simultaneous lumen and vascular perfusion. The saturable component had a K_m of 55 μM and a J_{max} of 3·3 nmol/min, thus, saturation was reached at about 100 μM lumen Zn. Blakeborough & Salter (1987) used brush-border-membrane vesicles from the piglet and ^{65}Zn-labelled solutions of Zn. Their results suggest at least two separate mechanisms, a rapid saturable mechanism dominating at concentrations ≤10 μM and a component which was linear with time and only involved binding to the membrane. The K_m for the rapid Zn uptake was 67·0 μM and maximum uptake approximately 100 nmol/mg protein. The time-dependent uptake of Zn seemed to be shared with other divalent and trivalent ions and was assumed to be passive binding to anionic sites at, for example, phospholipids and sugar residues, and not a direct mechanism of absorption.

The conditions used in the previously quoted experiments and other similar in vitro experiments regarding pH, ionic strength, concentrations of studied elements and ligands are seldom comparable, and not always in the physiological range, which makes it difficult to evaluate and compare the results. However, results from studies using radioisotope techniques in intact animals are also consistent with a carrier-mediated saturable mechanism for Zn absorption. Jackson *et al.* (1981) administered oral doses of Zn labelled with ^{65}Zn in the range 0·25–5·0 μmol in 0·5 ml water and observed saturation at 1 μmol, which meant that under normal dietary Zn concentrations the quantity of Zn absorbed was proportional to the dietary Zn content.

A similar relationship between the amount of Zn absorbed from a test meal and the Zn content of the meal was observed by Coppen & Davies (1987). When dietary content increased from 5 to 40 mg Zn/kg, corresponding to a meal content of up to 3 μmol, the fractional Zn absorption appeared to decrease, while from 40 to 160 mg Zn/kg (approximately 3–10 μmol/meal) the amount of Zn absorbed increased linearly with increasing dose of Zn, indicating a diffusion process.

Intestinal perfusion studies in humans have also shown increasing Zn absorption at increasing Zn intakes. From Zn concentrations of 0·1 to 1·5 mM infused into the jejunum there were linear increases in the rate of Zn absorption, while the rate plateaued at higher concentrations (Lee *et al.* 1989). Intestinal absorption of Zn was significantly stimulated by the addition of 20 mM-glucose. Increasing Zn concentrations resulted in an increased glucose absorption and a decrease in sodium and water absorption. The authors speculate that Zn and glucose interact with a common carrier in the brush-border membranes.

The mucosal Zn-binding protein(s) have not been definitely defined. Kowarski *et al.* (1974) identified and partially purified a Zn-binding protein in rat jejunal mucosa with a molecular weight of approximately 100 000. Results from Steel & Cousins (1985) using homogenates from total small intestine from rat suggest Zn binding to both high-molecular-weight species and metallothionein. Two smaller Zn-binding proteins with molecular weights of 45 000 and 65 000 were observed in rat duodenum mucosa by Seal & Heaton (1987).

The role of low-molecular-weight ligands for Zn absorption has been subject to much debate. In experimental models of intestinal uptake both stimulating and depressing effects on Zn absorption rates of ligands such as picolinic acid and citrate, have been observed, depending on the experimental conditions used. The quantitative importance of small-molecular-weight ligands for Zn uptake from human diets is, however, not

clear. Addition of citrate to infant formulas has not shown any effect on Zn absorption (Sandström *et al.* 1983) and studies of Zn absorption from lactic acid-fermented vegetables (B. Sandström, unpublished results) does not imply an enhancing effect of organic acids. Addition of histidine, an amino acid with a reasonably high binding constant for Zn, to a Zn and iron solution improves Zn absorption (Sandström *et al.* 1985). However, the levels of free amino acids in the intestinal contents after intake of intact proteins are presumably too low to have much effect on Zn absorption.

DOSE DEPENDENCE OF Zn ABSORPTION IN HUMANS

Zn concentrations in human intestine. For evaluation of data from experimental models it might be useful to look at concentrations of Zn that can be found in the human intestine under normal dietary conditions. A typical content of Zn in a human diet is 10 mg (150 μmol)/d, corresponding to 20 mg/kg on a dry-weight basis. Most human foodstuffs have a high water content giving an actual Zn concentration of approximately 5 mg/kg wet weight, further diluted by an intake of 1–2 l liquid daily. In the stomach and small intestine the food is mixed with intestinal juices. Matseshe *et al.* (1980) observed, at the ligament of Treitz over 5 h after intake of a meal containing 400 ml liquid, a total flow between 1162 and 2626 ml with an overall mean of 1836 ml in four subjects. Thus, 3–5 l/d is probably secreted in connection with meal intake. A Zn concentration of 0·2 (SD 0·1) mg/l in pancreatic juice content has been reported after secretin and cholecystokinin stimulation (Casey *et al.* 1979), while the observations made by Matseshe *et al.* (1980) suggest a higher Zn concentration of intestinal juices, close to the plasma levels of 1 mg/l. However, even if these higher estimates of Zn content of intestinal juices are used, Zn concentration in the duodenum after a meal is probably always less than 2 mg/l (30 μM). In the presence of food components the 'free' Zn concentrations at the site for absorption is probably much lower. In vitro studies simulating intestinal digestion have shown that only a proportion of the Zn in a meal is dialysable at assumed small intestinal pH (Sandström *et al.* 1987*a*, 1989). Thus, human dietary and intestinal Zn concentrations are lower than those used in most experimental animal studies.

Zn absorption in man. Systematic studies of the kinetics of dietary Zn absorption in man are lacking. However, the results from studies of Zn absorption from aqueous solutions, single meals and total diets with different Zn contents give indirect information about the characteristics of Zn absorption in man. In Table 1 results from radioisotope studies of Zn absorption from aqueous solutions and single meals are listed. The meals selected for the compilation are those with negligible levels of known antagonists for Zn absorption and can be seen as examples of absorption at optimal dietary conditions. One important observation is the striking difference between absorption from aqueous solutions and from solid meals. Administration of an aqueous solution of Zn results in a percentage absorption above 50, even with doses corresponding to 50% of a total daily dietary intake of Zn. With increasing Zn levels of an aqueous solution the fractional absorption does not change much, resulting in a linear increase in the amount of Zn absorbed (Fig. 1). The absorption of Zn from the meals shows a different picture. Percentage absorption gradually decreases at higher intakes and the relationship between Zn content and absorbed Zn (Fig. 2) indicates a saturation of absorption at an intake of 70–80 μmol/meal resulting in 18–20 μmol Zn absorbed. This level of absorption is maintained after long-term supplementation with Zn (Sandström *et al.* 1990).

Table 1. *Zinc absorption from aqueous solutions and single meals measured by radioisotope techniques*

(Mean values and standard deviations)

Diet	Zn content (μmol)	*n*	Zn absorption % Mean	% SD	μmol Mean	μmol SD	Reference
Aqueous solutions	8	9	69	9	6	1	Valberg *et al.* (1984)
	40	6	74	5	30	2	Sandström *et al.* (1985)
	40	12	73	10	29	4	Sandström & Cederblad (1987)
	61	10	63	14	38	8	Valberg *et al.* (1984)
	92	14	61	15	56	13	Valberg *et al.* (1984)
	96	11	56	17	54	16	Valberg *et al.* (1984)
	200	6	46	13	92	26	Sandström & Cederblad (1987)
	230	12	38	7	87	16	Sandström *et al.* (1987*c*)
Single meals							
Liquid diets:							
Human milk	20	16	41	9	8	2	Sandström *et al.* (1983)
Cow's-milk formula	19	16	31	7	6	2	Sandström *et al.* (1983)
Cow's milk	24	6	28	15	8	2	Sandström *et al.* (1983)
Cow's-milk formula	41*	8	33	7	13	3	Lönnerdal *et al.* (1984)
Solid diets and foods:							
White bread	20*	11	25	8	5	2	Arvidsson *et al.* (1978)
Chicken meal	20	11	36	7	7	1	Sandström & Cederblad (1980)
White bread	25	24	37	11	9	2	Sandström *et al.* (1987*c*)
Beef meal	47	21	25	8	12	4	Sandström *et al.* (1987*c*)
Turkey	61*	11	28	8	17	5	Valberg *et al.* (1984)
Chicken meal	69*	8	24	5	17	4	Sandström & Cederblad (1980)
Beef meal	70	6	20	7	14	5	Sandström & Cederblad (1980)
Beef	70	12	24	9	18	6	Gallaher *et al.* (1988)
Beef + milk meal	75	5	21	3	16	3	Sandström *et al.* (1987*c*)
Beef meal	123*	8	15	3	19	4	Sandström & Cederblad (1980)
Bread meal	250*	12	10	2	23	5	Sandström *et al.* (1987*b*)

* Zn salts added.

The results from studies of Zn absorption from total diets, using stable-isotope techniques, show a similar relationship between Zn content and Zn absorbed to that with single meals (Table 2, Fig. 3). In several of these studies formula diets have been used and in most of the studies the isotopes have been administered at three or four meals. At the higher Zn intakes a substantial part of total Zn intake has been supplied from added Zn salts. Despite differences in the mode of administration and analytical techniques for the stable isotopes the observed Zn absorption values are reasonably consistent. The values give the impression of saturation at an intake of 150–200 μmol/d, with 80–100 μmol absorbed.

This observed dose dependence of Zn absorption has several implications. The distribution of Zn intake over the day's meals can influence utilization. In studies of the effect of other dietary components and comparison between different types of diet differences in Zn content of the meals or diets have to be taken into account. The linear

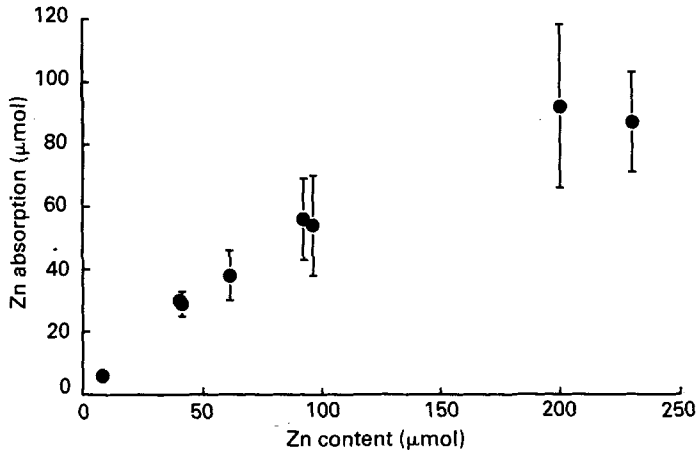

Fig. 1. Zinc absorption in relation to Zn content of aqueous solutions administered, measured by radioisotope techniques. Values are means and standard deviations, represented by vertical bars. Values from Valberg *et al.* (1984), Sandström *et al.* (1985, 1987*c*) and Sandström & Cederblad (1987).

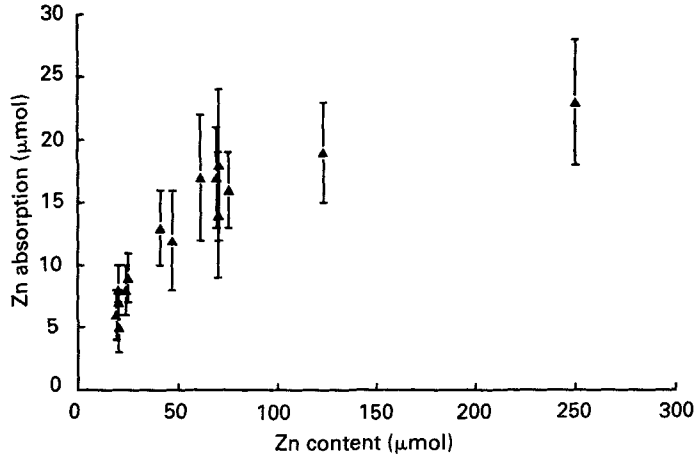

Fig. 2. Zinc absorption in relation to Zn content of single meals measured by radioisotope techniques. Values are means and standard deviations, represented by vertical bars. Values from Arvidsson *et al.* (1978), Sandström & Cederblad (1980), Sandström *et al.* (1983, 1987*b*,*c*), Lönnerdal *et al.* (1984), Valberg *et al.* (1984) and Gallaher *et al.* (1988).

increase in uptake from an aqueous solution has implications for supplementation programmes as the mode of administration will strongly affect the amount of Zn absorbed.

MANGANESE ABSORPTION

The chemical similarity and other observations have suggested that Mn shares or competes for absorptive mechanisms with Fe. Addition of Mn to an Fe solution or a meal depresses Fe absorption in a way that gives the impression that the body cannot distinguish between Fe and Mn (Rossander-Hultén *et al.* 1991). However, no significant

Table 2. *Zinc absorption observed in total diet studies using stable-isotope techniques*

(Mean values and standard deviations)

Diet	Zn content (μmol)	*n*	Zn absorption % Mean	% SD	μmol Mean	μmol SD	Reference
Total diet studies (stable isotopes)							
Formula	12	5	93	1	11	1	Taylor *et al.* (1991)
Low-Zn foods	84	6	49	7	41	5	Wada *et al.* (1985)
Formula	85	5	38	3	32	3	Taylor *et al.* (1991)
Low-Zn foods	90	9	64	15	58	14	August *et al.* (1989)
Chicken-based	102	10	72	13	73	13	Janghorbani *et al.* (1982)
Chicken-based	110	1		47		50	Jackson *et al.* (1984)
Chicken-based	230*	1		32		73	Jackson *et al.* (1984)
Formula	230*	4	34	12	78	23	Turnlund *et al.* (1984)
Low-Zn foods	243*	9	39	9	95	22	August *et al.* (1989)
Low-Zn foods	250*	6	25	6	67	14	Wada *et al.* (1985)
Chicken-based	470*	1		21		98	Jackson *et al.* (1984)

* Zn salts added.

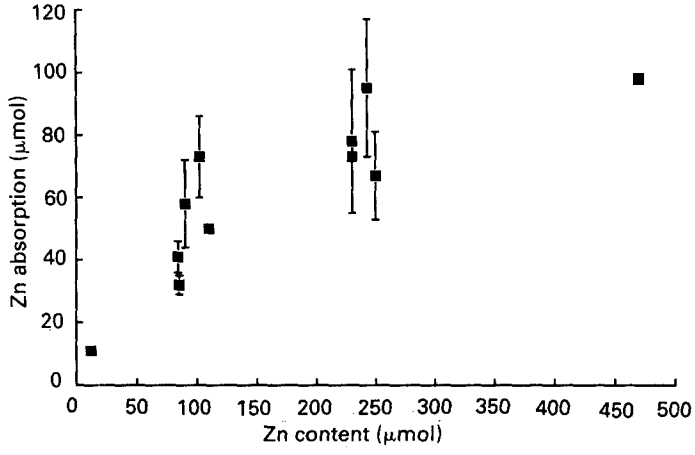

Fig. 3. Zinc absorption in relation to Zn content of total diets studied using stable-isotope techniques. Values are means and standard deviations, represented by vertical bars. Values from Janghorbani *et al.* (1982), Jackson *et al.* (1984), Turnlund *et al.* (1984), Wada *et al.* (1985), August *et al.* (1989) and Taylor *et al.* (1991).

effects of increasing Fe content or Mn absorption from an infant formula (Davidsson *et al.* 1989) or a bread (Davidsson *et al.* 1991*b*) have been observed.

The percentage absorption of Mn is low, most often below 10 (Table 3). Neither the mode of administration nor the level of Mn seem to have any significant impact on the percentage absorption. Also there is no indication of a saturation of Mn absorption up to levels corresponding to total daily intakes. This is contrary to what is known for Fe, where the percentage absorption is high at low levels of intake and decreases with increasing dose. Thus, the few findings available for Mn absorption in man do not

Table 3. *Manganese absorption measured by radioisotope techniques*

(Mean values and standard deviations)

Diet	Mn content (μmol)	*n*	Mn absorption % Mean	% SD	μmol Mean	μmol SD	Reference
Human milk	0·13	8	8	3	0·01	0·004	Davidsson *et al.* (1989)
Cow's-milk formula	0·42	13	6	5	0·02	0·002	Davidsson *et al.* (1989)
Human milk + Mn	4·9	8	3	2	0·16	0·07	Davidsson *et al.* (1991*b*)
White bread	10	8	3	2	0·25	0·2	Davidsson *et al.* (1991*b*)
Mangold	13	6	6	3	0·78	0·4	Davidsson *et al.* (1991*a*)
Mineral supplement	46	12	9	3	4·1	1·4	Sandström *et al.* (1987*b*)
Aqueous solution	55	9	8	3	4·6	1·6	B. Sandström and Å. Cederblad (unpublished results)

indicate any plausible mechanism for Mn absorption. The increased amount absorbed with increasing doses means that body homeostasis of Mn has to rely on an efficient excretion in order to avoid an accumulation of Mn at high intakes.

CONCLUSIONS

The size of the administered dose determines the amount absorbed of both Zn and Mn. Absorption of Mn and of Zn administered as an aqueous solution shows a linear increase in uptake with increasing dose suggesting a diffusion process. Dietary Zn uptake is saturated at or below levels that can be found in normal meals and diets.

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Printed in Great Britain