Zinc absorption in adult humans: the effect of iron fortification

BY LENA DAVIDSSON[1]*, ANNETTE ALMGREN[2], BRITTMARIE SANDSTRÖM[3] AND RICHARD F. HURRELL[1]*

[1] *Nestec Ltd., Nestlé Research Centre, PO Box 44, CH-1000 Lausanne 26, Switzerland*

[2] *Department of Clinical Nutrition, Gothenburg University, Annedalsklinikerna, S-41345 Göteborg, Sweden*

[3] *Research Department of Human Nutrition, The Royal Veterinary and Agricultural University, Rolighedsvej 25, DK-1958 Fredriksberg, Denmark*

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The effect of Fe fortification on the absorption of Zn was studied by radioisotopic labelling of single meals, followed by measurements of whole-body retention of ^{65}Zn at 14 d after intake. Healthy adult volunteers participated in the study. Weaning cereal, wheat bread and infant formula, foods that are all frequently Fe-fortified, were evaluated in the study. The amounts of Fe added as $FeSO_4$ were similar to the levels in commercial products in Europe and the USA, and were 200 or 500 mg Fe/kg (weaning cereal), 65 mg Fe/kg (white wheat flour) and 12 mg Fe/l (infant formula). For comparison, Zn absorption was measured in the same subjects, from identical test meals containing no added Fe. No statistically significant differences were found when Zn absorption from the Fe-fortified test meals was compared with that from non-Fe-fortified test meals. Fractional Zn-absorption values from Fe-fortified *v.* non-fortified meals were 31·1 (SD 11·9) *v.* 30·7 (SD 7·0)% (weaning cereal; 200 mg Fe/kg), 37·7 (SD 16·6) *v.* 30·2 (SD 9·9)% (weaning cereal; 500 mg Fe/kg), 36·5 (SD 14·4) *v.* 38·2 (SD 18·1)% (bread; 65 mg Fe/kg flour) and 41·6 (SD 8·1) *v.* 38·9 (SD 14·5)% (infant formula; 12 mg Fe/l). The addition of Fe to foods at the currently used fortification levels was thus not associated with impaired absorption of Zn and the consumption of these Fe-fortified foods would not be expected to have a negative effect on Zn nutrition.

Zinc: Iron: Humans: Radioisotope

Fe deficiency is one of the major nutritional problems in the world today, primarily affecting fertile women, infants and children (Bothwell *et al.* 1979; DeMaeyer & Adiels-Tegman, 1985). The major causative factors are low dietary intake as well as low bioavailability of Fe in the diet, in particular in the largely cereal-based diets in many developing countries (Charlton & Bothwell, 1983; Layrisse *et al.* 1990). One way of overcoming this problem is by increasing the intake of Fe, for example by fortification of foods regularly consumed by the groups at risk. This method is generally considered to be the best long-term strategy for reducing the prevalence of Fe deficiency (International Nutritional Anemia Consultative Group, 1977; Cook & Reusser, 1983). However, concern has sometimes been raised as to whether the increased intake of Fe, via food fortification programmes, could have a negative effect on the absorption of other minerals, in particular Zn (for a review see Solomons, 1986). A negative effect of increased Fe content on Zn absorption has been reported from studies in humans using the plasma response test after intake of water solutions, or Coca Cola, with high Zn concentrations (Solomons & Jacob, 1981; Solomons *et al.* 1983; Crofton *et al.* 1989). Later studies, using a radionuclide

* Present address: Laboratory for Human Nutrition, Swiss Federal Institute of Technology, PO Box 474, CH-8803 Rüschlikon, Switzerland.

technique based on whole-body retention measurements and with more 'physiological' levels of Zn present, confirmed the inhibitory effect of Fe on Zn absorption from water solutions (Sandström *et al.* 1985). However, when the Fe content of foods (as opposed to water solutions) was increased, no negative effect on Zn absorption was found (Solomons & Jacob, 1981; Valberg *et al.* 1984; Sandström *et al.* 1985). It can thus be argued that the increased Fe content of foods, from Fe-fortification, is of no concern for the absorption of Zn from the diet. However, the test meals used in the studies consisted of Atlantic oysters (Solomons & Jacob, 1981), turkey meat (Valberg *et al.* 1984) or rice and meat sauce (Sandström *et al.* 1985), none of which is representative of foods normally fortified with Fe. Furthermore, for groups of the population where Fe-fortified foods form a large fraction of the total intake of energy and nutrients, the issue about whether an increased intake of Fe could influence Zn nutrition negatively is of importance. Of special interest is the situation for infants who consume a limited number of foods of which a large proportion is usually Fe-fortified (infant formula and cereals) and for whom Zn is needed due to the rapid growth early in life (Walravens & Hambidge, 1975; Golden & Golden, 1981; Walravens *et al.* 1983, 1989).

The present study was undertaken to evaluate further the effect of increased Fe intake from Fe-fortified foods on the absorption of Zn. Zn absorption from test meals fortified with Fe was measured in healthy adult volunteers by a radionuclide technique. Comparisons were made with identical test meals without extra Fe added. The test meals evaluated in this study consisted of weaning cereal, wheat flour (served as bread) and infant formula.

MATERIALS AND METHODS

Subjects

Forty healthy adult volunteers (twenty-eight women and twelve men; 19–38 years) participated in four separate Zn-absorption studies. Eight subjects participated in studies 1, 3 and 4 while in study 2 there were sixteen subjects. All were apparently healthy, non-pregnant, and without known gastrointestinal disorders. The subjects were given oral and written information about the aims and procedures of the study.

Zinc-absorption measurements

Zn absorption was measured according to the method of Arvidsson *et al.* (1978). Each subject's background radioactivity was measured before intake of the labelled test meal. The subjects were randomly allocated to the different test meals which were served after an overnight fast. No food or drink was allowed for 3 h after intake of the test meals. The whole-body retention was measured 10–14 d after intake of each test meal to allow for excretion of the non-absorbed fraction. Corrections were made for the excretion of initially absorbed isotope during the time between intake and retention measurements based on the mean rate of excretion of an intravenously administered dose of ^{65}Zn in a similar group of subjects (Arvidsson *et al.* 1978). After intake of the second test meal (approximately 14 d after intake of the first test meal), allowance was also made for the excretion of the residual radioactivity from the first test meal.

Test meals

The composition of the test meals is given in Table 1. The wheat flour and weaning cereal were prepared at a Nestlé Product Development Centre (Linor, Orbe, Switzerland).

Studies 1 and 2. A pre-cooked, roller-dried weaning cereal based on white wheat flour was prepared especially for the study without added Fe or vitamins. The cereal contained 858 g wheat flour/kg, 100 g sugar/kg and $CaCO_3$ was added at a level of 1·8 g/kg final

Table 1. *Composition of test meals, amount of iron added, level of iron fortification and iron:zinc ratio in the separate test meals*

Study	Ingredients	Test meal (a)	Fe:Zn ratio	Test meal (b)	Fe:Zn ratio
1	Weaning cereal (50 g) Milk (325 g) Water (150 g)	10 mg Fe (200 mg Fe/kg)	23·3:1	No Fe added	0·57:1
2	Weaning cereal (50 g) Milk (325 g) Water (150 g)	25 mg Fe (500 mg Fe/kg)	57·4:1	No Fe added	0·57:1
3	Bread rolls (60 g flour) Butter (10 g) Water (200 g)	3·9 mg Fe (65 mg Fe/kg)	8·8:1	No Fe added	1·2:1
4	Infant formula (450 g)	5·4 mg Fe (12 mg Fe/l)	10·2:1	No Fe added	0·17:1

product. Each serving consisted of 50 g cereal mixed with 325 g hot milk (30 g fat/l). The subjects were served 150 g ultra pure water (18 M omega; Milli-Q water system, Millipore AG, Zurich, Switzerland) together with the hot cereal meal. Fe was added to the cereal at the time of serving, corresponding to 200 mg (study 1) or 500 mg Fe/kg cereal (study 2). Wheat flour (5·3 g ash/kg) was used for production of the weaning cereal, as well as for preparing the bread rolls served in study 3.

Study 3. Dough was prepared from wheat flour, ultra pure water, salt and yeast. Rolls were made from weighed amounts of dough, each corresponding to 30 g wheat flour. Each test meal consisted of two bread rolls (60 g flour) served with 200 g ultra pure water and 10 g butter, Fe was added at a level of 65 mg Fe/kg flour during preparation of the dough.

Study 4. A commercial cow's milk-based, whey-adjusted infant formula without added Fe (Nestlé, Germany) was used in study 4. The test meal consisted of 450 g infant formula with or without Fe added at 12 mg/l.

Fe was added as food-grade $FeSO_4.7H_2O$ (Merck, Darmstadt, Germany) to all Fe-fortified test meals. Each test meal was extrinsically labelled with 0·05 MBq ^{65}Zn (first test meal) or 0·1 MBq ^{65}Zn (second test meal) by adding almost carrier-free $^{65}ZnCl_2$ solution (3·2–92·5 MBq/mg Zn; Amersham International, Bucks) during preparation of the test meals. The effective dose equivalent was calculated for each subject according to the data given by the International Commission on Radiological Protection (1987) and based on individual Zn-absorption values. Bread rolls were labelled by adding the isotope to the dough, while the hot cereal was labelled immediately before serving due to practical considerations. The infant formula was labelled about 16 h before consumption. The activity of each individual test meal was measured in the whole-body counter before being served. All administrations of labelled test meals were done under close supervision by one of the investigators to ensure that the entire serving was consumed. Leftovers were measured in the whole-body counter after intake in order to calculate the exact dose of radioactivity consumed by each subject.

Table 2. *Contents of nitrogen, calcium, zinc and iron in weaning cereal, wheat flour and infant formula, before iron fortification*

	Nitrogen (g)	Calcium (mg)	Zinc (mg)	Iron (mg)
Weaning cereal (50 g)	1·1	16·5	0·44	0·27
Wheat flour (60 g)	1·3	12·5	0·51	0·60
Infant formula (450 g)	1·2	227	0·54	0·09

Food analysis

Portions of the foods were analysed in duplicate for their content of Fe, Ca and Zn by atomic absorption spectrometry (AAS: model 975, Varian Techtron, Mulgrave, Australia). Samples of wheat flour and infant cereal were wet-ashed in silica flasks with a mixture of HNO_3 and H_2O_2 on a continuous wet-ashing system (VAO, Kürner Analysentechnik, Rosenheim, Germany). Samples of the infant formula were ashed in silica Ehrlenmeyer flasks in a muffle furnace at 510° for 48 h. Ash was dissolved in 4 ml concentrated HCl and diluted to 25 ml with ultra pure water. For Ca analysis, La_2O_3 was added to the mineralized samples equivalent to a final concentration of 10 g/l. Fe was analysed by graphite furnace AAS, using the model 975 AAS equipped with a GTA-95 graphite furnace and an auto sampler. Accuracy of the methods was tested by analysing the standard reference materials Wheat Flour (1567a) and Milk Powder (M1549; National Institute of Standards and Technology, Gaithersburg, MD, USA). Freeze-dried samples of milk were analysed for their content of Zn and Ca by AAS (Perkin Elmer Model 360, Perkin Elmer, Uberlingen, Germany) after dry- and wet-ashing respectively. Reference materials purchased from the National Bureau of Standards (Gaithersburg and Washington DC: Mixed Diet 8431, Bovine Liver 1577a and Orchard Leaves 157) were analysed together with the milk samples. N was analysed by the Kjeldahl technique.

Only ultra pure water was used during analysis.

Statistical analysis

Student's *t* test for paired observations was used for statistical comparisons.

Ethical considerations

The study was approved by the Research Ethical Committee and the Isotope Committee at Sahlgren's Hospital, Göteborg, Sweden.

RESULTS

The contents of N, Ca, Zn and Fe in the weaning cereal, wheat flour and infant formula, before the addition of Fe, are given in Table 2. Milk (30 g fat/l) added to the weaning cereal contained 1·6 g N, 380 mg Ca and 1·3 mg Zn/325 g. All values for the reference materials were found to be within the certified ranges.

Zn absorption from the different test meals showed no statistically significant difference related to the Fe content of the foods. Zn absorption from weaning cereal (200 mg Fe/kg) served with milk was 31·1 (SD 11·9) *v.* 30·7 (SD 7·0)% when no Fe was added. The same weaning cereal studied at a higher Fe-fortification level (500 mg Fe/kg) resulted in Zn absorption of 37·7 (SD 16·6) *v.* 30·2 (SD 9·9)% when no Fe was added. Wheat bread fortified at 65 mg Fe/kg flour resulted in Zn absorption of 36·5 (SD 14·4) *v.* 38·2 (SD 18·1)% for non-

Table 3. *Study 1. Individual zinc-absorption values for weaning cereal*

Subject	Sex	Zn absorption (%) Weaning cereal (a) (200 mg Fe/kg)	Weaning cereal (b) (no added Fe)
1	F	30·7	24·5
2	M	44·8	33·5
3	F	23·0	33·4
4	M	33·4	29·6
5	F	26·8	25·3
6	M	20·2	27·1
7	F	51·7	45·8
8	F	18·3	26·5
Mean		31·1	30·7
SD		11·9	7·0

Table 4. *Study 2. Individual zinc-absorption values for weaning cereal*

Subject	Sex	Zn absorption (%) Weaning cereal (a) (500 mg Fe/kg)	Weaning cereal (b) (no added Fe)
1	F	40·3	15·2
2	M	27·3	24·8
3	F	14·9	54·8
4	M	30·5	24·9
5	F	28·7	31·5
6	F	18·6	30·8
7	F	65·5	19·8
8	M	49·7	17·9
9	F	55·5	28·9
10	F	32·0	33·7
11	F	65·8	41·0
12	M	45·8	37·1
13	F	21·0	21·2
14	F	28·2	36·9
15	F	24·1	30·6
16	F	55·9	34·3
Mean		37·7	30·2
SD		16·6	9·9

Fe-fortified bread. The Zn absorption from infant formula was also unaffected by the level of Fe-fortification; 41·6 (SD 8·1) *v.* 38·9 (SD 14·5) % was absorbed from formula containing 12 mg Fe/l compared with when no Fe was added. The fractional Zn-absorption values for each subject are given in Tables 3–6.

The mean effective dose equivalent was calculated to be 0·57 mSv (range 0·25–0·97 mSv) per subject.

DISCUSSION

Mild Zn deficiency has been related to poor growth early in life since a positive effect of Zn supplementation has been demonstrated in infants and preschool children (Walravens *et al.* 1983, 1989). It is thus very important to ensure that Zn absorption from foods normally

Table 5. *Study 3. Individual zinc-absorption values for bread rolls*

Subject	Sex	Zn absorption (%) Bread rolls (a) (65 mg Fe/kg flour)	Bread rolls (b) (no added Fe)
1	F	51·2	73·9
2	F	50·3	42·4
3	M	32·7	41·4
4	F	15·8	14·6
5	F	21·2	26·0
6	M	27·5	29·7
7	F	40·2	48·8
8	M	53·2	28·5
Mean		36·5	38·2
SD		14·4	18·1

Table 6. *Study 4. Individual zinc-absorption values for infant formula*

Subject	Sex	Zn absorption (%) Infant formula (a) (12 mg Fe/l)	Infant formula (b) (no added Fe)
1	F	29·3	45·0
2	F	48·5	54·2
3	F	35·1	26·1
4	M	48·6	55·1
5	F	45·5	48·6
6	F	40·1	16·3
7	M	34·0	25·6
8	F	51·5	40·0
Mean		41·6	38·9
SD		8·1	14·5

consumed by infants and young children, e.g. infant formulas and weaning cereals, is optimal. Since these foods are commonly fortified with Fe it was considered important to evaluate the effect of Fe fortification on Zn absorption.

The results from the present study confirm the earlier observations that, although Fe inhibits Zn absorption from water solutions, an increased Fe content in foods does not impair Zn absorption (Valberg *et al.* 1984; Sandström *et al.* 1985). The difference between water solutions and more complex matrices containing dietary ligands, such as foods, is also demonstrated by the results from the study by Sandström *et al.* (1985) where the addition of a Zn-ligand, histidine, to the water solution decreased the inhibitory effect of Fe. In a similar study by Rossander-Hultén *et al.* (1991), looking at the effect of high Zn levels on Fe absorption, a strong negative effect on Fe absorption was found when increased amounts of Zn were added to water solutions, while no effect was observed when the test meal consisted of a composite meal. These results taken together thus suggest that when dietary ligands are present, either in foods or by addition to water solutions, there is little or no interaction between the absorption of Fe and Zn. A negative effect on Zn absorption due to increased Fe intake from foods, via Fe-fortification compounds, would

therefore not be expected. The effect of considerably higher Fe intakes on Zn nutrition, for example in the form of dietary supplements, has been investigated in some recent studies. Decreased concentrations of Zn in serum were found in Fe-supplemented pregnant women (Bloxam *et al.* 1989; Dawson *et al.* 1989) as well as in non-pregnant women (Newhouse *et al.* 1993).

The Fe compound used to fortify foods in the present study was $FeSO_4$. This compound is commonly used to fortify infant formulas and occasionally used to fortify bread and wheat flour which is stored for short periods of time (Hurrell, 1992). When added to weaning cereals, $FeSO_4$ causes fat oxidation during storage and also causes unwanted colour reactions. $FeSO_4$ added to wheat flour or other cereals promotes fat oxidation during long-term storage, which leads to rancid products. Wheat flour and weaning cereals are consequently often fortified with elemental Fe, ferric orthophosphate or ferric pyrophosphate (Hurrell, 1992). Other Fe-fortified foods commonly consumed by children, such as breakfast cereals and chocolate drink powder, are also normally fortified with the same compounds. These Fe compounds are less soluble in the gastric juice and less bioavailable than $FeSO_4$ and would be expected to have even less effect on Zn absorption. Furthermore, the level of Fe-fortification varies between different products and also between different countries (Lynch & Hurrell, 1990). In the present study we used the higher levels of commonly practised Fe-fortification, i.e. 65 mg Fe/kg wheat flour as used in Sweden and 12 mg Fe/l infant formula which is the content of Fe in infant formulas in the USA. The cereal was evaluated at the current level of Fe-fortification in both European and US commercial weaning cereal; 200 and 500 mg Fe/kg cereal. The test meals in the present study therefore represent a more stressed situation than normally present in Fe-fortified foods, i.e. a highly bioavailable Fe compound was added at relatively high levels. Therefore, it is not very likely that Zn absorption would be influenced by the commonly used Fe-fortification practices.

The present study, as well as earlier studies, have used adult human subjects because radioisotopes were administered. Both men and women were included in the studies since no sex differences in Zn absorption have been reported to our knowledge. The radionuclide technique used in the present study has been described in detail (Arvidsson *et al.* 1978). The 100% dose of radioactivity ingested by each subject was calculated as the difference between the measured activity in the test meal, minus activity in the leftovers measured after administration. Alternatively, subjects could be measured in the whole-body counter immediately after intake of the labelled test meals. We did not choose the latter approach due to the uneven distribution of the ingested isotope shortly after intake. Immediately after ingestion of the labelled test meal the dose of radioactivity must still be regarded as a point source, surrounded by the body. The systematic error introduced by measuring the point source before administration compared with measurements after ingestion has been estimated to be a maximum of 10% (A. Cederblad, personal communication, 1994). The error is relatively small due to the characteristics of the whole-body counter used in our studies. Large detectors and the choice of using the 'valley' spectral window are important features of this whole-body counter (Arvidsson *et al.* 1978).

Since infants and children are among the most vulnerable groups to be affected by impaired absorption of dietary Zn, it still remains to confirm that Fe-fortification has no effect on Zn absorption in this age group. Stable-isotope techniques are now available making it possible to measure Zn absorption in infants, but due to the much more elaborate study protocol needed, i.e. complete faecal collections over several days, this has not yet been done. The results from a metabolic balance study in infants (Haschke *et al.* 1986) did not demonstrate any effect of Fe-fortification in infant formulas (2·5 *v.* 10·2 mg Fe/l) on Zn absorption.

Long-term effects on Zn status of the consumption of Fe-fortified foods have not been addressed in the present study. In several other studies this issue has been investigated without finding any influence. For example, no significant effect on the level of Zn in serum in infants could be related to the Fe-content of the infant formulas fed in the study by Bradley *et al.* (1993), and in a study of low-birthweight infants by Salvioli *et al.* (1986) the addition Fe given as medicinal Fe, together with Fe-fortified formula, did not influence plasma Zn levels. Furthermore, no effect on serum Zn levels was observed after 3 months of Fe-supplementation in the form of 30 mg Fe/day, administered in close proximity to breakfast to 1-year-old children (Yip *et al.* 1985). However, Craig *et al.* (1984) reported significantly lower plasma Zn levels in a small group of infants given Fe-fortified formula, compared with low-Fe formula. Thus, while there are no conclusive data on the long-term effects of an increased Fe intake on Zn nutrition in infants, the results from most studies have not demonstrated any negative effect.

In conclusion, Zn absorption was not affected by the increased content of Fe in foods, when added at commonly used levels for Fe-fortification in the present study. Thus, the consumption of Fe-fortified foods does not appear to influence Zn nutrition negatively.

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