

NaFe³⁺EDTA as a food fortificant: influence on zinc, calcium and copper metabolism in the rat

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The general acceptance of NaFe³⁺EDTA for food Fe fortification has been partly restricted by concern over the influence of EDTA on the metabolism of other nutritionally important trace elements and minerals. We have investigated the influence of NaFe³⁺EDTA, and of increasing dietary levels of Na₂EDTA, on Zn, Cu and Ca metabolism in rats fed on Zn-sufficient and Zn-deficient soya-bean-isolate-based diets. With the Zn-deficient diets, changing the dietary Fe compound from FeSO₄ to NaFe³⁺EDTA significantly ($P < 0.05$) increased mean apparent Zn absorption from 50.2 to 67.4%, urinary Zn excretion from 2.0 to 4.0% of intake, and Zn retention from 48.2 to 63.4%. Increasing the dietary EDTA level to 1000 mg/kg further increased Zn absorption to 78.1%, urinary Zn excretion to 15.6% of intake and Zn retention to 62.5%. Increased Zn retention was accompanied by a significant increase in weight gain indicating that the extra Zn was available for normal metabolic processes. With rats fed on the Zn-sufficient diet, NaFe³⁺EDTA and Na₂EDTA similarly increased the absorption, urinary excretion and retention of Zn but to a lesser extent. NaFe³⁺EDTA, however, had no influence on the absorption, urinary excretion and retention of Cu and Ca, and additional Na₂EDTA caused only minor increases in Cu absorption and retention and in the urinary excretion of Ca. We conclude that using NaFe³⁺EDTA as a food fortificant would have no detrimental effect on the metabolism of Zn, Cu and Ca and, in some situations, could improve Zn absorption and retention from low-bioavailability diets.

Iron: NaFe³⁺EDTA: Zinc: Calcium: Copper

Fe deficiency affects about one billion people and is a major nutritional problem in the world today (DeMaeyer & Adiels-Tegman, 1985). It is most prevalent amongst infants, children and women of child-bearing age, especially in developing countries where a major causative factor is the poor absorption of dietary Fe from cereal and legume-based diets (Charlton & Bothwell, 1983; Layrisse *et al.* 1990). Food fortification is generally considered to be the best long-term strategy to combat Fe deficiency (International Nutritional Anemia Consultative Group, 1977; Cook & Reusser, 1983); however, there are technical problems in the choice of the Fe compound as those compounds of high relative bioavailability, such as FeSO₄, often provoke unacceptable organoleptic changes, whereas the compounds which are organoleptically inert are often poorly absorbed (Hurrell, 1984, 1992). Even when the Fe compound is optimized by using ferrous fumarate or ferrous succinate (Hurrell *et al.* 1989, 1991), absorption from certain foods may still be low due to the presence of potent absorption inhibitors such as phytic acid (Hallberg *et al.* 1987) or polyphenolic compounds (Gillooly *et al.* 1983). One way of overcoming this inhibition is to add ascorbic acid as an absorption enhancer. Ascorbic acid increases the absorption of

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all non-haem-Fe compounds (Stekel *et al.* 1986; Forbes *et al.* 1989) and is commonly added with Fe when foods are fortified. It is, however, susceptible to losses during processing, storage and cooking (Hallberg *et al.* 1982).

An alternative strategy, which would appear to be particularly interesting in developing countries, would be to use $\text{NaFe}^{3+}\text{EDTA}$ as a food fortificant. This compound is stable during processing and storage and it is about two to four times better absorbed than FeSO_4 from foods such as cereals, legumes and milk which contain strong inhibitors of Fe absorption (Layrisse & Martinez-Torres, 1977; Martinez-Torres *et al.* 1979; McPhail *et al.* 1981). Pilot fortification trials using $\text{NaFe}^{3+}\text{EDTA}$ have resulted in an improved Fe status (Garby & Areekul, 1974; Ballot *et al.* 1989).

EDTA, however, is a strong metal chelator and there has been some concern over its effect on the metabolism of other nutritionally-important trace elements and minerals (McPhail *et al.* 1985). When $\text{NaFe}^{3+}\text{EDTA}$ is consumed, Fe is dissociated from the EDTA complex in the lumen of the gut and a small percentage of the EDTA (about 5%) is absorbed and excreted in the urine, while the majority passes through to the stools (Candela *et al.* 1984). The possibility that the released or the absorbed EDTA might bind other minerals and lead to increased losses of these minerals has delayed the general acceptance of $\text{NaFe}^{3+}\text{EDTA}$ as a food additive and prevented its more widespread use.

In the present paper we report on the influence of $\text{NaFe}^{3+}\text{EDTA}$ on the metabolism of Zn, Cu and Ca in the rat.

EXPERIMENTAL

Zn, Cu and Ca balances, together with measurements of bone Zn and Ca concentrations, were made in young rats consuming Zn-deficient or Zn-sufficient soya-bean-isolate-based diets fortified with FeSO_4 , $\text{NaFe}^{3+}\text{EDTA}$ or $\text{NaFe}^{3+}\text{EDTA}$ plus increasing levels of Na_2EDTA .

Test diets

Eight test diets (A–H) were prepared. All contained (g/kg): soya-protein isolate (Koreco, Konolfingen, Switzerland) 170, peanut oil 100, mineral mix 35.2, cellulose 30, vitamin mix 10 (AIN-76; ICN Nutritional Biochemicals, Cleveland, OH, USA), DL-methionine 5, choline bitartrate 2, glucose to 1000. The mineral mix was identical to AIN-76 (American Institute of Nutrition, 1977) except that it contained no Fe or Zn. Zn (23.9 mg/kg) was added as $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ to diets A–D to give a final Zn concentration of 30 mg/kg. Diets E–H were not Zn supplemented and contained 6.1 mg native Zn/kg.

The Fe concentration of all diets was adjusted to 50.1 mg/kg by addition of 31.6 mg Fe/kg, either as $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ in diets A and E, or as $\text{NaFe}^{3+}\text{EDTA}$ (W. R. Grace & Co., Nashua, NH, USA) to diets B, C, D, F, G and H. In addition, diets C and G were supplemented with $\text{Na}_2\text{EDTA} \cdot 2\text{H}_2\text{O}$ (E. Merck, Darmstadt, Germany) at 300 mg EDTA/kg, and diets D and H were supplemented with $\text{Na}_2\text{EDTA} \cdot 2\text{H}_2\text{O}$ at 800 mg EDTA/kg. All diets contained (g/kg): crude protein ($\text{N} \times 6.25$) 143, Ca 4.95 and Cu 0.0097.

Experimental design

Sixty-four male, weanling rats of the Sprague-Dawley strain (ICO, Lyon, France), weighing approximately 45 g, were housed individually in stainless-steel cages. They were randomized in a single block with eight rats receiving each dietary treatment. The test diets and deionized, distilled water were provided *ad lib*. Weight gain and food consumption were recorded each 3–4 d.

On day 18 the animals were transferred to plastic metabolism cages (Techniplast-gazzada, Bugugiatte, Italy) for a 4 d collection of urine and faeces. Before the balance

period the cages and the polyethylene containers used to collect urine and faeces were soaked in 1 M-HNO₃ and thoroughly rinsed in deionized, distilled water. Urine and faeces were collected every 24 h in the polyethylene bottles. The bottles for urine contained 1 ml 1 M-HNO₃. The collections were pooled and stored at 4°. On day 21, at the end of the balance period, the animals were killed by decapitation and the right femur was removed for Zn and Ca analysis.

Analytical methods

Cleaned femurs and faeces were dry-ashed in 50 ml quartz flasks in a muffle furnace for 48 h at 475°. The ash was dissolved in 2 ml concentrated HNO₃ plus 2 ml concentrated HCl, evaporated to about 1 ml on a hot plate, and when cool made up to 50 ml with 1 M-HCl. Portions of diet (about 500 mg) were wet-digested in quartz containers using 1 ml concentrated H₂SO₄ plus 5 ml of a mixture of concentrated HNO₃ and HClO₄ (19:1, v/v) in a continuous digestion system (VAO; Kürner Analysentechnik, Roseheim, Germany). Total urine was diluted without digestion to 100 ml in 1 M-HCl, a 10 ml portion was centrifuged for 10 min at 3000 rev./min and the supernatant fraction diluted to 100 ml in 1 M-HCl. All acids used for digestion and dilution were Suprapur from Merck.

Zn, Ca and Cu determinations were made on suitably-diluted solutions by flame atomic absorption spectrophotometry (model AA-975; Varian International AG, Zug, Switzerland). Ca concentrations were measured in the presence of 10 g La/l (as La₂O₃).

Statistical analysis

The results were submitted to a two-way analysis of variance. The interaction means were compared by Tukey's test using an estimated error for each comparison since there were some missing values. Differences were judged significant at the 5% level or as stated in the text.

RESULTS

Food intake, weight gain, femur zinc and femur calcium concentrations

The rats consuming for 21 d the Zn-sufficient diets A–D (30 mg Zn/kg) had a greater food consumption ($P < 0.01$) and body-weight gain ($P < 0.01$), a higher bone Zn concentration ($P < 0.01$), but a similar bone Ca concentration to the rats consuming the Zn-deficient diets E–H (6.1 mg Zn/kg) (Table 1). With the rats fed on the Zn-sufficient diet, changing the Fe fortification compound from FeSO₄ (diet A) to NaFe³⁺EDTA (diet B) had no significant influence on food intake, weight gain or femur Zn and Ca concentrations, although further increasing the EDTA concentration from 200 mg/kg (diet B) to 500 mg/kg (diet C) and 1000 mg/kg (diet D) significantly reduced the femur Zn concentration (Table 1). Increasing the dietary EDTA level had no influence on food intake, weight gain and femur Ca concentration.

On the Zn-deficient diets E–H, neither femur Zn nor femur Ca concentrations responded significantly to changing from FeSO₄ to NaFe³⁺EDTA or to increasing the dietary level of EDTA from 200 mg/kg to 1000 mg/kg. Changing from FeSO₄ (diet E) to NaFe³⁺EDTA (diet F), however, significantly increased both food intake and weight gain. Further increasing the dietary EDTA from 500 mg/kg (diet G) to 1000 mg/kg (diet H) decreased both food intake and weight gain. However, both food intake and weight gain of the rats fed on the highest-EDTA-containing diet (1000 mg/kg) were not significantly different from those receiving no dietary EDTA. Some rats did not eat normally when transferred to metabolism cages. These rats, which were evenly distributed between the control and test diets, have been omitted from the results presented in Tables 1–4, which include data from six to eight rats per dietary treatment.

Table 1. *Effect of different dietary EDTA levels on food intake, weight gain, femur zinc and femur calcium concentrations**

(Mean values with their standard errors)

Diet	Dietary EDTA† (mg/kg)	n	Food intake (g/21 d)		Wt gain (g/21 d)		Femur Zn (mg/g)		Femur Ca (mg/g)	
			Mean	SE	Mean	SE	Mean	SE	Mean	SE
Zn-sufficient (30 mg Zn/kg)										
A	0	6	273 ^a	7	129 ^a	3	180 ^{a, b}	6	188 ^a	7
B	200	7	272 ^a	6	129 ^a	2	184 ^a	5	181 ^a	8
C	500	7	273 ^a	5	122 ^a	7	166 ^b	3	175 ^a	4
D	1000	8	259 ^a	5	127 ^a	4	168 ^b	4	188 ^a	8
Zn-deficient (6.1 mg Zn/kg)										
E	0	8	222 ^a	6	89 ^a	4	72 ^a	6	182 ^a	4
F	200	6	248 ^b	7	106 ^{b, c}	5	81 ^a	5	172 ^a	7
G	500	7	258 ^b	8	113 ^b	5	75 ^a	2	177 ^a	7
H	1000	7	230 ^{a, b}	10	98 ^{a, c}	6	73 ^a	4	190 ^a	7

^{a, b, c} Within diets A–D and within diets E–H means with unlike superscript letters were significantly different ($P < 0.05$).

* For details of diets and procedures, see pp. 86–87.

† In diets B and F, total EDTA content was derived from NaFe³⁺EDTA which replaced the FeSO₄ in diets A and E; in diets C, D, G and H, EDTA was added as Na₂EDTA in addition to NaFe³⁺EDTA.

Zinc, copper and calcium balances

Apparent fractional Zn absorption (intake – faecal excretion) and Zn retention (intake – (faecal excretion + urinary excretion)) were 2–4-fold higher in rats fed on the Zn-deficient diets (E–H) than in those fed on the Zn-sufficient diets (A–D), and dietary EDTA concentrations had a profound effect on apparent Zn absorption, urinary Zn excretion and Zn retention in both the Zn-sufficient and Zn-deficient groups (Table 2). In the Zn-sufficient group, changing the Fe fortification compound from FeSO₄ (diet A) to NaFe³⁺EDTA (diet B) increased apparent Zn absorption from 16.0 to 24.2%, urinary Zn excretion from 0.7 to 1.7% and Zn retention from 15.3 to 22.5%. None of these changes was significant. In the Zn-deficient group, changing from FeSO₄ (diet E) to NaFe³⁺EDTA (diet F) significantly increased Zn absorption from 50.2 to 67.4%, urinary Zn excretion from 2.0 to 4.0%, and Zn retention from 48.2 to 63.4%.

As the EDTA concentration of the diet was increased from 0 to 1000 mg/kg, urinary Zn excretion increased progressively from 0.7 to 5.9% of intake on the Zn-sufficient diets and from 2.0 to 15.6% on the Zn-deficient diets. However, despite this large increase in urinary Zn excretion, urinary Zn had only a small influence on Zn retention which was significantly higher in all groups fed on the EDTA-containing Zn-deficient diets. In the Zn-sufficient group, only at a dietary concentration of 500 mg/kg did EDTA significantly increase Zn retention. There was no negative effect on Zn retention in rats receiving the highest level of EDTA (1000 mg/kg) compared with those receiving no EDTA.

Neither the dietary level of Zn nor the amount of EDTA in the diet markedly influenced the metabolism of Cu and Ca (Tables 3 and 4). Changing the Fe-fortification compound from FeSO₄ (diets A and E) to NaFe³⁺EDTA (diets B and F) did not change the apparent absorption, the urinary excretion or the retention of either Cu or Ca. Additional EDTA did not effect urinary Cu excretion and only at 500 mg/kg did it significantly increase Cu

Table 2. *Effect of different dietary EDTA levels on 4 d zinc balance in rats**
(Mean values with their standard errors)

Diet	Dietary EDTA† (mg/kg)	n	Zn intake (mg)		Apparent Zn absorption (% intake)		Urinary Zn (% intake)		Zn retention (% intake)	
			Mean	SE	Mean	SE	Mean	SE	Mean	SE
Zn-sufficient (30 mg Zn/kg)										
A	0	6	1.62 ^{a, b}	0.06	16.0 ^a	3.2	0.7 ^a	0.2	15.3 ^{a, c}	3.3
B	200	7	1.52 ^a	0.04	24.2 ^{a, c}	2.3	1.7 ^a	0.2	22.5 ^{a, b}	2.2
C	500	7	1.69 ^b	0.11	30.4 ^{b, c}	2.0	3.4 ^b	0.3	27.0 ^b	1.9
D	1000	8	1.50 ^a	0.04	20.4 ^a	2.9	5.9 ^c	0.4	14.5 ^c	3.0
Zn-deficient (6.1 mg Zn/kg)										
E	0	8	0.23 ^a	0.01	50.2 ^a	3.0	2.0 ^a	0.6	48.2 ^a	2.9
F	200	6	0.29 ^a	0.01	67.4 ^b	2.8	4.0 ^b	0.3	63.4 ^b	2.9
G	500	7	0.33 ^a	0.02	79.4 ^c	1.7	6.7 ^c	0.3	72.7 ^c	1.7
H	1000	7	0.25 ^a	0.02	78.1 ^c	1.9	15.6 ^d	0.7	62.5 ^b	2.1

a, b, c, d Within diets A–D and within diets E–H means with unlike superscript letters were significantly different ($P < 0.05$).

* For details of diets and procedures, see pp. 86–87.

† In diets B and F, total EDTA content was derived from NaFe³⁺EDTA which replaced the FeSO₄ in diets A and E; in diets C, D, G and H, Na₂EDTA was added in addition to NaFe³⁺EDTA.

Table 3. *Effect of different dietary EDTA levels on 4 d copper balance in rats**
(Mean values with their standard errors)

Diet	Dietary EDTA† (mg/kg)	n	Cu intake (μg)		Apparent Cu absorption (% intake)		Urinary Cu (% intake)		Cu retention (% intake)	
			Mean	SE	Mean	SE	Mean	SE	Mean	SE
Zn-sufficient (30 mg Zn/kg)										
A	0	6	642 ^a	26	30.8 ^a	2.1	2.5 ^a	0.2	28.3 ^a	2.2
B	200	7	584 ^a	18	33.7 ^a	1.3	2.8 ^a	0.2	30.9 ^a	1.3
C	500	7	568 ^a	38	32.1 ^a	1.4	2.8 ^a	0.2	29.3 ^a	1.5
D	1000	8	603 ^a	16	33.1 ^a	1.8	2.8 ^a	0.2	30.2 ^a	2.0
Zn-deficient (6.1 mg Zn/kg)										
E	0	8	415 ^a	16	27.5 ^a	2.9	3.9 ^a	0.2	23.6 ^a	2.9
F	200	6	511 ^{b, c}	22	32.1 ^{a, b}	2.3	3.8 ^a	0.2	28.3 ^{a, b}	2.3
G	500	7	544 ^b	26	38.2 ^b	2.9	4.0 ^a	0.2	34.2 ^b	3.2
H	1000	7	460 ^{a, c}	32	31.1 ^{a, b}	2.7	4.1 ^a	0.2	27.0 ^{a, b}	2.8

a, b, c Within diets A–D and within diets E–H means with unlike superscript letters were significantly different ($P < 0.05$).

* For details of diets and procedures, see pp. 86–87.

† In diets B and F, total EDTA content was derived from NaFe³⁺EDTA which replaced the FeSO₄ in diets A and E. In diets C, D, G and H, Na₂EDTA was added in addition to NaFe³⁺EDTA.

Table 4. *Effect of different dietary EDTA levels on 4 d calcium balance in rats**
(Mean values with their standard errors)

Diet	Dietary EDTA† (mg/kg)	n	Ca intake (mg)		Apparent Ca absorption (% intake)		Urinary Ca (% intake)		Ca retention (% intake)	
			Mean	SE	Mean	SE	Mean	SE	Mean	SE
Zn-sufficient (30 mg Zn/kg)										
A	0	6	331 ^a	13	56.2 ^a	1.0	0.9 ^a	0.1	55.3 ^a	1.1
B	200	7	304 ^{a,b}	9	56.4 ^a	1.5	0.6 ^a	0.1	55.8 ^a	1.5
C	500	7	290 ^b	20	57.2 ^a	1.3	1.1 ^{a,b}	0.2	56.1 ^a	1.3
D	1000	8	312 ^{a,b}	8	58.8 ^a	1.5	1.7 ^b	0.2	57.1 ^a	1.6
Zn-deficient (6.1 mg Zn/kg)										
E	0	8	213 ^a	8	57.1 ^a	3.1	1.2 ^a	0.1	55.9 ^a	3.1
F	200	6	253 ^b	11	53.3 ^a	2.0	1.1 ^a	0.2	52.2 ^a	2.0
G	500	7	266 ^b	13	55.3 ^a	1.5	1.8 ^{a,b}	0.2	53.5 ^a	1.5
H	1000	7	233 ^{a,b}	16	57.7 ^a	2.1	2.3 ^b	0.5	55.4 ^a	1.9

^{a,b} Within diets A–D and within diets E–H means with unlike superscript letters were significantly different ($P < 0.05$).

* For details of diets and procedures, see pp. 86–87.

† In diets B and F, total EDTA content was derived from $\text{NaFe}^{3+}\text{EDTA}$ which replaced the FeSO_4 in diets A and E; in diets C, D, G and H, Na_2EDTA was added in addition to $\text{NaFe}^{3+}\text{EDTA}$.

absorption and retention on the Zn-deficient diet. Additional dietary EDTA had no influence on Ca absorption and retention but increased urinary Ca excretion slightly but significantly at both the 500 mg/kg (diet G) and 1000 mg/kg (diet H) levels (Table 4).

DISCUSSION

EDTA forms stable, water-soluble, metal chelates stoichiometrically with virtually every metal in the periodic table. The stability constants of EDTA with different metals vary considerably and are also highly dependent on pH (West & Sykes, 1960). Fe^{3+} has the highest stability constant followed by Cu^{2+} , Zn, Fe^{2+} , Ca and Mg. The optimum pH for complex formation is pH 1 for Fe^{3+} , pH 3 for Cu^{2+} , pH 4 for Zn, pH 7.5 for Ca and pH 10 for Mg. It would seem likely, therefore, that Fe added to foods as $\text{NaFe}^{3+}\text{EDTA}$ would be strongly chelated to EDTA while in the gastric juice, but that as the partially-digested food enters the duodenum and passes down the intestine Fe will exchange with other minerals depending on their stability constant, the pH of the environment, as well as on the metal concentration and the presence of other metal-binding ligands in the food. There is a justifiable concern, therefore, that small amounts of $\text{NaFe}^{3+}\text{EDTA}$ fed over a long period of time might negatively affect the nutritional status of metals other than Fe due to the formation of non-metabolizable EDTA complexes which are either excreted directly in the stools or absorbed from the gut and excreted unchanged in the urine. The present study, however, does not support this hypothesis and, on the contrary, indicates that $\text{NaFe}^{3+}\text{EDTA}$ added to foods could have a beneficial effect on Zn and possibly Cu nutrition, while having no effect on Ca nutrition.

Our results (Table 2) show an improved Zn absorption and retention from the Zn-deficient diets when $\text{NaFe}^{3+}\text{EDTA}$ replaced FeSO_4 as the dietary Fe compound. The increased Zn bioavailability was manifested as increased weight gain (Table 1). Earlier

animal studies with 150–300 mg Na₂EDTA/kg soya-bean-based diet have also demonstrated increased Zn bioavailability and weight gain in turkey poults (Kratzer *et al.* 1959), chicks (Scott & Ziegler, 1963) and rats (Forbes, 1961). Oberleas *et al.* (1966) measured Zn availability from casein-based diets with or without phytate and EDTA. They demonstrated that EDTA increased Zn absorption when the diet contained phytate but not in its absence. They concluded that EDTA increased Zn bioavailability by forming soluble complexes with Zn and, thus, preventing it reacting with phytate. Soya-bean-protein as used both in our study and in previous studies is rich in phytate which is a major factor contributing to its negative influence on Zn and Fe absorption (Lönnerdal *et al.* 1988; Hurrell *et al.* 1992).

There is an indication from the present study that EDTA added to foods might also improve Cu absorption (Table 3), at least from low-bioavailability Zn-deficient diets, but that it would have no influence on Ca absorption or excretion (Table 4). Forbes (1961) similarly fed rats on a soya-bean-protein-based diet with 230 mg Na₂EDTA/kg and 8.5 mg Zn/kg and reported increased body-weight gain and Zn absorption but no effect on Ca or Mg balances. The non-effect with Ca is perhaps not surprising since there was a 200-fold lower molar concentration of EDTA than Ca in our rat diets. This is in contrast with an 8-fold molar excess of EDTA over Zn in the Zn-deficient diets, a 1.5 molar excess of EDTA over Zn in the Zn-sufficient diets, and a 4.5-fold molar excess of EDTA over Cu. A human daily diet containing the recommended daily allowance (mg) for different minerals (i.e. Ca 800, Mg 350, Zn 10, Cu 2) and fortified with 10 mg Fe as NaFe³⁺EDTA would contain about a 1.5-fold molar excess of EDTA over Zn, an 8-fold excess of EDTA over Cu, but some 80-fold less EDTA than Ca and 50-fold less EDTA than Mg on a molar basis. Ca and Mg nutrition would seem, therefore, not to be of concern in diets fortified with NaFe³⁺-EDTA.

From the previous discussions on the chemical properties of EDTA it is evident that the form in which EDTA is added to the diet is of little importance in determining its influence on trace element metabolism. EDTA added to foods as Na₂EDTA, CaNa₂EDTA or NaFe³⁺EDTA will form more or less the same metal-EDTA chelates in the digestive tract. What is more important is the molar ratio EDTA:metal concerned. There appears to be an optimum EDTA:metal molar ratio below which absorption is not increased and beyond which absorption is no longer improved. Higher levels of EDTA might even decrease absorption as has been reported for Fe in humans (Davis & Deller, 1967; Cook & Mosen, 1976) and Zn in rats (Suso & Edwards, 1968). Another major effect of high dietary EDTA levels might be an increased urinary Zn excretion. In our studies (Table 3), increasing dietary EDTA from 0 to 1000 mg/kg increased urinary Zn excretion in rats fed on a Zn-deficient diet from 2.0 to 15.6% of Zn intake with a corresponding fall in Zn retention. Rats consuming the same diets increased their urinary Ca excretion from 1.2 to 2.3% of intake (Table 4), although this had no measurable effect on Ca retention.

The enhancing effect of EDTA on Zn and Fe absorption can be explained by EDTA forming soluble chelates from which the metal is potentially absorbable while, at the same time, preventing the metal reacting with other ligands such as phytate which inhibit its absorption. EDTA, thus, exerts its maximum effect in diets containing absorption inhibitors. Fe, for instance, was less well absorbed from NaFe³⁺EDTA than from FeSO₄ when consumed with sugar-cane syrup containing no absorption inhibitors but two to four times better absorbed when consumed with wheat and maize (Martinez-Torres *et al.* 1979). Most of the Fe would appear to be separated from the chelate before absorption (Candela *et al.* 1984; McPhail *et al.* 1985), although in man it has been reported that about 15% of the Fe absorbed from NaFe³⁺EDTA is absorbed as the intact chelate and rapidly excreted in the urine (Martinez-Torres *et al.* 1979; McPhail *et al.* 1981). It would seem likely that

most of the Zn is also separated from the EDTA chelate before absorption, although there is evidence in chicks that the intact Zn chelate can be absorbed (Koike *et al.* 1964), and the increase in urinary Zn excretion in our studies (Table 2) would be best explained by the Zn-EDTA chelate being absorbed and excreted unchanged in the urine. The small increment in urinary Ca excretion (Table 4) could likewise be due to the Ca-EDTA chelate being absorbed and excreted unchanged in the urine, although it is possible that EDTA was absorbed combined with another metal which was then replaced by Ca at physiological pH, and that the Ca-EDTA chelate was excreted.

We would now conclude that using NaFe³⁺EDTA as a food additive would have no detrimental effect on the metabolism of other nutritionally-important minerals and trace elements. In some situations it might improve Zn absorption from low-Zn-bioavailability diets, although this needs further evaluation in man. There are two previous reports concerning the influence of foods fortified with NaFe³⁺EDTA on Zn nutrition in man and neither is conclusive. In the first, a recent Fe-fortification trial with NaFe³⁺EDTA-fortified curry powder, no change in serum Zn was reported after 2 years of feeding, although Fe status improved significantly (Ballot *et al.* 1989). Serum Zn, however, is not a sensitive indicator of Zn status (El-Khoury, 1991). In the second study, Solomons *et al.* (1979) reported that NaFe³⁺EDTA added to a low-bioavailability Guatemalan meal did not influence Zn absorption as measured by plasma Zn after ingesting 25 mg Zn as ZnSO₄ with the meal. In that study using a Zn-tolerance test, however, there was a 10-fold molar excess of Zn over EDTA and any improvement of Zn absorption would be unlikely.

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