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EDTA and the absorption of iron from food[1–3]

A Patrick MacPhail, Razina C Patel, Thomas H Bothwell, and Rosario D Lamparelli

ABSTRACT Iron EDTA is an effective fortificant in meals of low iron bioavailability. Na_2EDTA, added to food to prevent oxidation, enhances iron bioavailability by chelating added iron. This study examines the optimal ratio of EDTA to iron causing enhanced iron absorption. Iron absorption from a rice-based meal of low iron bioavailability containing increasing molar ratios of EDTA to iron, was compared in 127 women volunteers by using standard double isotope techniques. Iron deficiency was present in 38% of the women. Mean standardized absorptions, at EDTA-iron ratios of 0.25, 0.5, and 1, were 11.3%, 13.5%, and 8.8%, respectively, compared with 3.8% when no Na_2EDTA was present. In meals of high iron bioavailability, Na_2EDTA (EDTA:Fe, 1.0) produced little enhancement (potato-based meal) nor inhibited iron absorption (apple-based meal). Na_2EDTA added to meals with molar ratios of EDTA to iron between 1.0 and 0.25 significantly increases iron absorption provided the meal is of low iron bioavailability. *Am J Clin Nutr* 1994;59:644–8.

KEY WORDS Iron EDTA, sodium EDTA, iron absorption, iron fortification

Introduction

Iron deficiency remains the most common cause of nutritional anemia. It is particularly prevalent in developing countries where cereals, rich in inhibitors of iron absorption such as phytate and polyphenols, are the major source of food (1). Iron in the form of NaFeEDTA has been shown to be well absorbed from a variety of cereals (2) and there is good evidence that iron in this chelated form is protected from inhibitors (particularly phytate) present in food (3). NaFeEDTA has been used successfully in three fortification trials in the developing world (4–6) but its general use has been hindered by the fact that it is not recognized as a food additive by the Joint Food and Agriculture Organization (FAO)/ World Health Organization (WHO) Expert Committee on Food Additives (JECFA) (7). However, sodium and calcium EDTA are recognized food additives and are used extensively to prevent oxidation and color changes in food. The acceptable daily intake (ADI) of EDTA set by the JECFA in 1974 is 2.5 mg EDTA$\cdot$kg body $wt^{-1}\cdot d^{-1}$ (7). However, the actual intake in the United States is probably much lower. Estimates vary widely but the most recent estimated daily intake (EDI), obtained by averaging the highest and lowest EDI values, is 10-fold lower than the ADI (8). Early work showed that high amounts of Na_2EDTA added to a standard American meal inhibited iron absorption (9) but a more recent study demonstrated that Na_2EDTA and $FeSO_4$ added to Baladi bread (a flat bread used extensively in the Middle East) in a molar ratio of EDTA to iron of 1.0 was associated with better iron absorption than when $FeSO_4$ was used alone (10). In the present study the effect of adding different molar ratios of EDTA to iron to a simple inhibitory meal was studied.

Subjects and methods

Subjects

A group of 127 parous Indian housewives living in Phoenix, near Durban, took part in the study. None was pregnant or lactating and all were unpaid volunteers. Although there is a high prevalence of iron deficiency among the women of this community (11), subjects were not selected on the basis of iron deficiency. However, 38% had depleted iron stores (serum ferritin $< 12\ \mu g/L$) whereas 15% had iron-deficiency anemia (no stores plus hemoglobin < 120 g/L).

Approval for the studies was obtained from the Committee for Research on Human Subjects of the University of the Witwatersrand, Johannesburg. Written informed consent was obtained from all subjects before each study. Each subject participated in one experiment only. The maximum potential whole-body irradiation dosage, presuming complete absorption of all the isotope, fell well within the permissible annual dose as reported previously (12).

Preparation and administration of meals

In most of the studies a simple traditional Indian rice meal (200 g) consisting of boiled rice sweetened with cane sugar and prepared as described previously (12), was fed on consecutive days after an overnight fast. Only water was permitted during the meal and for 3 h afterward. A solution of $FeSO_4$ labeled with either ^{55}Fe (111 kBq, or 3 μCi) or ^{59}Fe (74 kBq, or 2 μCi) was added to each rice portion and mixed thoroughly shortly after cooking. The total iron in each portion was 3 mg, made up of 2.6 mg as $FeSO_4$ and 0.4 mg as the intrinsic iron in the rice. Thereafter, a solution of Na_2EDTA, of various concentrations depending on the design of the study, was added to each portion of iron-fortified rice and mixed in thoroughly. In the first group

[1] From the Department of Medicine, University of the Witwatersrand, Johannesburg, South Africa.

[2] Reprints not available. Address correspondence to AP MacPhail, Department of Medicine, Medical School, 7 York Road, Parktown 2193, South Africa.

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of studies 20 mg Na_2EDTA (molar ratio of EDTA to iron of 1.0) was added on the first day of each study, whereas in the second group no Na_2EDTA was added on the first day. On the second day various amounts of Na_2EDTA were added to give different molar ratios of EDTA to iron in each study (0.25, 0.5, 2.0, 3.0, and 4.0).

In a third group of studies two different meals were prepared. In the first study the meal consisted of mashed potato (200 g) containing a total of 3 mg Fe (1.4 mg as added $FeSO_4$, labeled with either ^{55}Fe or ^{59}Fe). On the first day no Na_2EDTA was added and on the second day 20 mg Na_2EDTA (molar ratio of EDTA to iron of 1.0) was added. The intrinsic ascorbic acid in the mashed potato was estimated from food tables (13) to be ≈ 40 mg/portion. In the second study an apple meal containing minimal quantities of inhibitors and enhancers of iron absorption was used. Fresh apples were peeled, chopped into small portions, and cooked until soft. The stewed apples were sweetened with cane sugar and made into a purée in a food blender. Iron (total 3 mg, 2.7 mg as $FeSO_4$, labeled with either ^{55}Fe or ^{59}Fe and 0.3 mg intrinsic Fe) and Na_2EDTA (none on day 1, 20 mg on day 2) were added to the purée (200 g) shortly before serving as described above. The ascorbic acid content of the apple purée was estimated from food tables to be ≈ 10 mg/portion (13).

Measurement of iron absorption

Two weeks after the administration of the radiolabeled meals, blood was obtained by venipuncture for the measurement of ^{55}Fe and ^{59}Fe, hemoglobin concentration, serum iron, total iron-binding capacity, and serum ferritin concentration. Samples were prepared for differential radioactive counting by the method of Eakins and Brown (14) and the absorption of each isotope was calculated as described previously (12).

In previous studies individual absorptions were standardized by multiplying the actual percent absorption by a factor of 40 divided by the percent iron absorption obtained from a reference dose of ferrous ascorbate (11, 12). This allowed for comparison of iron absorption between individuals of different iron status. The factor of 40 corresponds to a reference absorption of 40%, which represents approximately the amount of iron absorbed from ferrous ascorbate by subjects with borderline iron deficiency (15). In the present study, reference absorptions were derived from the regression of the percent absorption of a reference dose of ferrous ascorbate on the log of the serum ferritin concentration obtained in a previous study in a similar population of Indian women [reference absorption (%) = −37.3 × log serum ferritin + 93.9; r^2 = 0.8985] (16). Thus the calculation of the individual standardized absorptions was as follows:

$$\text{Standardized absorption (\%)} = \frac{\text{Actual absorption (\%)} \times 40}{(-37.3 \times \log \text{serum ferritin} + 93.9)}$$

The median calculated reference absorption for the whole group was 43%, indicating that the majority of the subjects had borderline or frank iron deficiency (15).

Chemical and statistical methods

Hemoglobin concentration, serum iron, total iron-binding capacity, and serum ferritin concentration were measured by standard techniques as described previously (12). The amount of intrinsic (not contaminant) iron in cooked rice was measured by using the method of Hallberg and Bjorn-Rasmussen (17) and was found to be close to published amounts (18). The concentration of ascorbate in potatoes and apples and phytate in rice, potatoes, and apples was obtained from published values (18).

Because the percentages of iron absorptions and serum ferritin concentrations were positively skewed, results were expressed as geometric means and SD ranges. The significance of differences between the actual absorptions of the two isotopes in each study was calculated by using the signed-rank test for paired observations. Comparison of mean standardized absorptions between studies was performed by using analysis of variance on the logged data (19).

Results

The effect of increasing the molar ratio of EDTA to iron on iron absorption from a simple inhibitory rice meal is shown in a series of studies summarized in **Table 1.** Hemoglobin concentration and iron-related measurements show that the groups of women in each study had similar iron status. The iron absorption from the meals containing a molar ratio of EDTA to iron of 1.00 in each study showed little variation and, when standardized for iron status, were not significantly different (F = 1.12, $P > 0.3$). In contrast, the iron absorption from meals with molar ratios of EDTA to iron ranging from 0.25 to 4.0 varied considerably. In studies with ratios < 1.00 (meals A and B) absorption was significantly greater than when the ratio was unity, whereas in those studies with ratios > 1.00 (meals C, D, and E) absorptions were substantially less.

The standardized iron absorption from the rice meal containing $FeSO_4$ but no Na_2EDTA averaged 3.8% (**Table 2**). However, substantial improvement in iron absorption was obtained with the addition of Na_2EDTA (meals F, G, and H). This was greatest at molar ratio of EDTA to iron of 0.5 (meal G), for which a more than fivefold increase in iron absorption was observed ($P < 0.001$).

The change in iron absorption observed with different ratios of EDTA to iron is shown in **Figure 1.** Because these results were derived from studies on different women, the absorptions are standardized to values corresponding to borderline iron deficiency. The graph indicates that the maximum increase in iron absorption occurred at molar ratios of EDTA to iron between 0.25 and 1.00 (1:4 and 1:1). When the ratio of EDTA to iron was > 1.00 there was a marked reduction in iron absorption. Although the mean absorption obtained at a ratio of 4:1 was lower than when no Na_2EDTA was present, analysis of variance failed to show a significant difference.

Table 3 shows the results obtained when the rice meal was replaced by potato or apple. Although the addition of Na_2EDTA (EDTA:iron of 1.0) to the potato meal produced a moderate improvement in actual iron absorption ($P < 0.05$), it significantly reduced actual iron absorption from the apple meal ($P < 0.001$).

Discussion

The high prevalence of iron deficiency in the developing world can be attributed, in part, to the poor bioavailability of iron from staple cereal–based diets (1). This poor bioavailability is due to the presence in these diets of inhibitors of iron absorption, particularly phytate and polyphenols, as well as the relative absence

TABLE 1
The effect of varying the molar ratio of EDTA to iron (EDTA:Fe) on the absorption of iron from a test rice meal labeled with ^{59}Fe (day 2) compared with a standard meal, labeled with ^{55}Fe (day 1), containing an EDTA:Fe of 1.0*

Meal	Iron status			Na_2EDTA	EDTA:Fe	Iron absorption		
	Hemoglobin	Transferrin saturation	Serum ferritin			Actual	Standardized	Ratio†
	g/L	*%*	*μg/L*	*mg*		*%*	*%*	
A (*n* = 25)	134 ± 19‡	22 ± 13	12 (3–49)§					1.45
Day 1				20	1.00	9.3 (3.6–24.2)	7.7 (3.6–16.7)	
Day 2				5	0.25	13.7‖ (5.5–34.1)	11.3 (6.0–21.2)	
B (*n* = 18)	141 ± 13	28 ± 12	19 (5–74)					1.27
Day 1				20	1.00	10.5 (5.0–22.1)	10.6 (6.1–18.4)	
Day 2				10	0.50	13.4¶ (7.0–25.4)	13.5 (6.8–26.9)	
C (*n* = 14)	120 ± 12	32 ± 16	18 (5–66)					0.49
Day 1				20	1.00	8.4 (4.1–17.5)	8.0 (4.5–14.2)	
Day 2				40	2.00	4.1‖ (2.2–7.7)	3.9 (2.3–6.7)	
D (*n* = 7)	125 ± 8	32 ± 16	19 (6–59)					0.33
Day 1				20	1.00	12.8 (7.8–20.9)	11.8 (8.7–16.0)	
Day 2				60	3.00	4.2¶ (2.1–8.5)	3.9 (2.5–5.9)	
E (*n* = 7)	119 ± 13	25 ± 11	17 (5–61)					0.30
Day 1				20	1.00	9.0 (6.5–12.5)	8.2 (4.3–15.4)	
Day 2				80	4.00	2.7¶ (1.6–4.4)	2.4 (1.2–4.7)	

* The iron content of each meal was 3 mg (0.4 mg intrinsic iron and 2.6 mg as $FeSO_4$). Absorption values were standardized as described in the text.
† Ratio of the absorption on day 2 to that on day 1.
‡ $\bar{x} \pm$ SD.
§ $\bar{x}$; SD rnage in parentheses.
‖¶ Significantly different from day 1 (sign-rank test): ‖$P < 0.001$, ¶$P < 0.01$.

of promoters such as ascorbic acid and meat. It is recognized that the most cost-effective strategy for preventing iron deficiency is through the fortification of the diet with iron (1). This approach has, however, been hampered by the difficulty in finding suitable iron salts that are bioavailable yet do not alter the organoleptic properties of food. This problem has been solved to some extent by the use of the stable ferric chelate NaFeEDTA (2, 6), but it has not been possible to administer it on a large scale because it

TABLE 2
The effect of varying the molar ratio of EDTA to iron (EDTA:Fe) on the absorption of iron from a test rice meal labeled with ^{59}Fe (day 2) compared with a standard meal, labeled with ^{55}Fe (day 1), containing no EDTA*

Meal	Iron status			Na_2EDTA	EDTA:Fe	Iron absorption		
	Hemoglobin	Transferrin saturation	Serum ferritin			Actual	Standardized	Ratio†
	g/L	*%*	*μg/L*	*mg*		*%*	*%*	
F (*n* = 7)	132 ± 5‡	25 ± 8	10 (3–32)§					2.3
Day 1				0	—	6.8 (2.6–15.8)	5.1 (2.5–10.6)	
Day 2				5	0.25	15.1‖ (6.3–36.4)	11.5¶ (5.2–25.4)	
G (*n* = 12)	131 ± 16	22 ± 11	12 (3–43)					5.4
Day 1				0	—	3.8 (1.4–10.2)	3.1 (1.2–7.6)	
Day 2				10	0.50	20.5** (7.7–54.2)	16.6¶ (8.0–34.4)	
H (*n* = 10)	128 ± 25	27 ± 10	24 (7–81)					2.8
Day 1				0	—	3.3 (1.5–7.1)	3.6 (1.8–6.9)	
Day 2				20	1.00	9.6†† (4.3–21.6)	10.2¶ (5.1–20.1)	

* The iron content of each meal was 3 mg (0.4 mg intrinsic iron and 2.6 mg as $FeSO_4$). Absorption values were standardized as described in the text.
† Ratio of the absorption on day 2 to that on day 1.
‡ $\bar{x} \pm$ SD.
§ $\bar{x}$; SD range in parentheses.
‖**†† Significantly different from day 1 (sign-rank test): ‖$P < 0.05$, **$P < 0.001$, ††$P < 0.01$.
¶ Significantly different from day 1, $P < 0.05$ (Bonferroni *t* test).

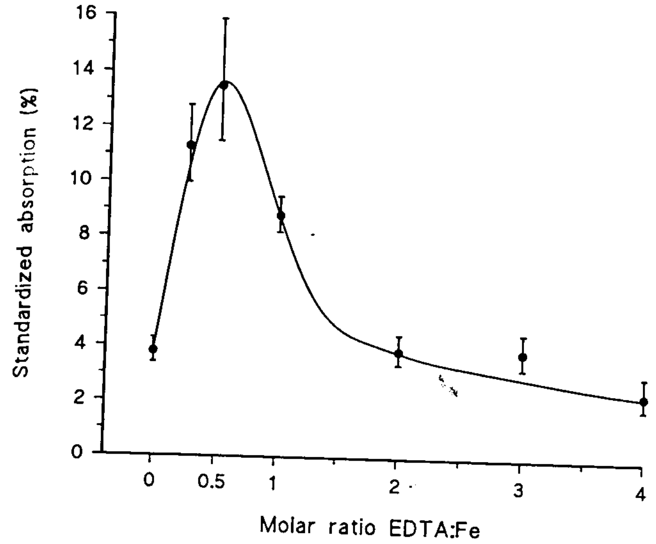

FIG 1. The effect of increasing amounts of Na_2EDTA on iron absorption (geometric $\bar{x} \pm$ SE) from a rice meal (0.4 mg Fe) fortified with $^{59}FeSO_4$ (2.6 mg Fe). Absorptions were standardized as described in the text.

is not yet approved as a food additive (7). The fact that Na_2EDTA and NaCaEDTA are approved food additives (7) has prompted investigations into the feasibility of using Na_2EDTA together with $FeSO_4$ as an alternative iron fortificant (10).

The present studies have confirmed that iron absorption from a rice meal containing a combination of Na_2EDTA and $FeSO_4$ in a molar ratio of between 0.25 and 1.00 is superior to $FeSO_4$ alone (Table 2). In a previous study (10) the improvement in iron absorption from fortified Baladi bread was between 2.6 and 3.4 times that found with $FeSO_4$ alone; the increase was similar to that found with a variety of cereal meals fortified with NaFe-EDTA (2). In the present study three different molar ratios of EDTA to Fe were compared with $FeSO_4$ when added to a rice meal (Table 2). The relative improvements in the presence of molar ratios of EDTA to iron of 1.0, 0.5, and 0.25 were 2.8, 5.6, and 2.3, respectively. The results of a direct comparison between meals containing a ratio of 1.00 and meals with a ratio of 0.25 and 0.5 indicated a moderate improvement in absorption with the lower ratios. (Table 1. meals A and B; Fig 1). However, when Na_2EDTA was present in excess (ratios of 2.0, 3.0, and 4.0) iron absorption was significantly less than with a ratio of 1.0 (Table 1, meals C, D, and E). Indeed, when Na_2EDTA was in a fourfold excess, iron absorption was less than when no Na_2EDTA was present (Fig 1).

The choice of rice as the standard meal was prompted by the need to have a simple inhibitory meal that was readily reproducible. The parboiled rice meal used in these studies contained no promoters of iron absorption but did have large amounts of phytate (0.2 mg/g) (18), which is a potent inhibitor of iron absorption. The poor bioavailability of iron from this meal was demonstrated by the low standardized absorptions ($\bar{x}$ 3.8%) observed when only $FeSO_4$ was added (Table 2, Fig 1). When a simple potato-based meal was used, estimated to contain ≈ 40 mg ascorbic acid and a third of the phytate (0.076 mg/g) (18) present in rice, the mean standardized absorption was 6.1% (Table 3) and the addition of Na_2EDTA in a molar ratio of EDTA to iron of 1.0 produced only a modest and barely significant rise in iron absorption. The last meal that was tested was an apple purée containing no phytate (18) and a low concentration of ascorbic acid. The mean iron absorption from $FeSO_4$ was high (20.8%) but was only 9.4% when Na_2EDTA was present (EDTA:iron of 1.0). A similar pattern was previously observed when the absorption of iron from $FeSO_4$ and NaFeEDTA, given in water, was compared (11). The reason for the high absorption from $FeSO_4$ and the modest absorption when Na_2EDTA was present, lies in the nature of the meal. In the absence of inhibitors, absorption from $FeSO_4$ is superior to that from NaFeEDTA (11). The presence of promoters, even the modest amounts of ascorbic acid present in the apple meal, has a marked promotive effect on absorption from $FeSO_4$ (20) but has little effect on iron absorption from NaFeEDTA (11). The nature of the meal may also explain the relatively poor absorption of iron found by Cook and Monsen (9) from a standard American dinner of high bioavailability to which Na_2EDTA had been added. However, in the presence of inhibitors, particularly

TABLE 3
The effect of varying the molar ratio of EDTA to iron (EDTA:Fe) on the absorption of iron from potato and apple test meals labeled with ^{59}Fe (day 2) compared with a standard potato or apple meal, labeled with ^{55}Fe (day 1), containing no EDTA*

Meal	Iron status: Hemoglobin	Iron status: Transferrin saturation	Iron status: Serum ferritin	Na_2EDTA	EDTA:Fe	Iron absorption: Actual	Iron absorption: Standardized	Ratio†
	g/L	*%*	*μg/L*	*mg*		*%*	*%*	
Potato (*n* = 8)	128 ± 9‡	32 ± 8	55 (21–144)§					
Day 1				0	—	3.7 (1.3–10.1)	6.1 (2.8–13.2)	0.59
Day 2				20	0.25	6.2‖ (2.1–17.9)	10.3 (4.3–24.5)	
Apple (*n* = 19)	132 ± 14	22 ± 10	30 (9–97)					
Day 1				0	—	18.1 (7.8–41.9)	20.8 (9.9–44.1)	2.2
Day 2				20	0.25	8.1¶ (3.2–21.0)	9.4 (4.7–18.7)	

* The iron content of each meal was 3 mg. Absorption values were standardized as described in the text.
† Ratio of the absorption on day 2 to that on day 1.
‡ $\bar{x} \pm$ SD.
§ $\bar{x}$; SD range in parentheses.
‖¶ Significantly different from day 1 (sign-rank test): ‖$P < 0.05$, ¶$P < 0.001$.

phytate, absorption from NaFeEDTA or a combination of $FeSO_4$ and Na_2EDTA would be expected to be better than from $FeSO_4$ alone because of the protective nature of the chelate (2, 3).

In the setting of an inhibitory meal the ratio of Na_2EDTA to iron appears to be of critical significance (Table 1, Fig 1). When EDTA is present in excess, absorption is no better than from $FeSO_4$ alone, and at high doses iron absorption is inhibited (9). When, however, the available iron is present in equivalent quantities or in excess, Na_2EDTA promotes iron absorption. Although this phenomenon clearly relates to the relative affinity for iron and the concentration of EDTA and various other ligands in the meal, the exact mechanisms involved remain to be elucidated. In this regard, it is of interest that the absorption of the intrinsic iron in the diet is enhanced by the presence of NaFeEDTA (11), which suggests that the chelate exerts its influence at molar ratios of EDTA to iron of < 1.00.

The present findings suggest that Na_2EDTA, an approved food additive, can be used to enhance the absorption of intrinsic and added iron when the molar ratio of EDTA to iron is ≤ 1.00 and the meal contains significant amounts of phytate. Such diets are typical of those consumed in many developing countries, where iron fortification is needed to combat iron deficiency. Although long-term data in humans are scanty (5, 6), the fear that prolonged use of Na_2EDTA may lead to trace element depletion, particularly of zinc, is not borne out by animal experiments and consideration of the relative affinities of EDTA for other metals (8). Fortification trials using NaFeEDTA in humans extending over 2 y have failed to produce changes in serum zinc concentrations in the participants (5, 6). However, exchange of iron for zinc does occur in the lumen of the small intestine and, as with iron, the effect of EDTA on zinc balance depends on the molar ratio of EDTA to zinc in the diet (3). Animal studies have shown that the use of both Na_2EDTA and NaFeEDTA in the fortification range has no detrimental effect on zinc balance. Indeed zinc balance may actually improve if the diet has a low zinc content or is of low zinc bioavailability (3, 21).

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