

Iron bioavailability from iron-fortified Guatemalan meals based on corn tortillas and black bean paste¹⁻³

Lena Davidsson, Triantafillia Dimitriou, Erick Boy, Thomas Walczyk, and Richard F Hurrell

ABSTRACT

Background: Corn masa flour is widely consumed in Central America and is therefore a potentially useful vehicle for iron fortification.

Objective: The goal was to evaluate the bioavailability of iron from meals based on corn tortillas and black bean paste that were fortified with ferrous fumarate, ferrous sulfate, or NaFeEDTA and to investigate the potential of Na₂EDTA to increase the bioavailability of iron from ferrous fumarate.

Design: With use of a crossover study design, iron bioavailability was measured in Guatemalan girls aged 12–13 y by a stable-isotope technique based on erythrocyte incorporation 14 d after intake.

Results: Geometric mean iron bioavailability from test meals fortified with ferrous fumarate was 5.5–6.2% and was not improved significantly by the addition of Na₂EDTA at molar ratios of 1:1 relative to fortification iron or to the total iron content of the fortified corn masa flour. Geometric mean iron bioavailability from test meals fortified with ferrous sulfate was 5.5% and was significantly higher in test meals fortified with NaFeEDTA (9.0%; $P = 0.009$, paired t test).

Conclusions: The bioavailability of iron from ferrous fumarate was not improved by the addition of Na₂EDTA, contrary to what was previously shown for ferrous sulfate in other cereal-based meals. However, the bioavailability of iron from the test meal was significantly enhanced when NaFeEDTA replaced ferrous sulfate. These results support the use of NaFeEDTA in the fortification of inhibitory staple foods such as corn masa flour. *Am J Clin Nutr* 2002;75:535–9.

KEY WORDS Iron bioavailability, stable isotopes, ferrous fumarate, corn tortillas, Na₂EDTA, disodium ethylenediaminetetraacetate, NaFeEDTA, sodium iron ethylenediaminetetraacetate, girls, Guatemala, corn masa flour

INTRODUCTION

Corn masa flour, often served as tortillas, is a major staple food in Central American countries, particularly in rural populations (1). It is thus a potentially useful vehicle for general iron fortification programs in countries such as Guatemala. Cereal flours, however, are difficult to fortify with soluble iron compounds because of the unacceptable organoleptic changes that result, such as rancidity and flavor changes during storage and

color changes during food preparation. This has led to the use in fortification programs of less soluble, and hence less bioavailable, iron compounds, such as elemental iron and phosphate compounds (2). Alternative iron compounds with high relative bioavailability that can be added to cereals without resulting unacceptable organoleptic changes are therefore needed. Evidence supports the use of NaFeEDTA to fortify cereal products (3). This compound was shown to increase iron absorption from inhibitory meals high in phytic acid 2- to 3-fold compared with ferrous sulfate (4) and was shown to protect against fat oxidation during storage of white-wheat flour (5). However, NaFeEDTA is a freely water-soluble iron compound and can result in unwanted color changes (6). Another potential iron fortificant for cereals is ferrous fumarate. This compound was proposed for iron fortification of infant cereals because it was shown to be as well absorbed as ferrous sulfate in adults and to cause less organoleptic problems (7).

The bioavailability of dietary iron (including fortification iron) depends on the overall composition of the meal, including the presence of enhancers and inhibitors of iron absorption. Corn masa flour contains relatively high amounts of phytic acid, a potent inhibitor of iron absorption (8). Thus, the bioavailability of fortification iron added to corn masa flour would be expected to be relatively low, unless an enhancer of iron absorption was added. Ascorbic acid is a potent enhancer of the absorption of fortification iron and its effect has been shown in adults (2) and in infants and schoolchildren (9–12). Ascorbic acid is widely used in industrial infant food products. However, because of the susceptibility of ascorbic acid to oxidation, especially when the food is also exposed to heat or humidity, its usefulness is limited when sophisticated packaging (4) or encapsulation is not affordable. Na₂EDTA could be a useful alternative

¹From the Laboratory for Human Nutrition, Swiss Federal Institute of Technology, Rueschlikon, Switzerland (LD, TD, TW, and RFH), and INCAP, Guatemala City (EB).

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³Reprints not be available. Address correspondence to L Davidsson, Laboratory for Human Nutrition, Institute of Food Science, Swiss Federal Institute of Technology, PO Box 474, CH-8803 Rueschlikon, Switzerland. E-mail: lena.davidsson@ilw.agrl.ethz.ch.

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because it is stable under normal food processing and storage conditions and is a permitted food additive in specified foods (13). The enhancing effect of Na₂EDTA on iron absorption from ferrous sulfate in humans was shown previously in studies of low-bioavailability meals (12, 14–16). No information is available on the effect of Na₂EDTA on the bioavailability of iron from other iron compounds such as ferrous fumarate.

The aim of this study was to evaluate the bioavailability of iron from test meals based on corn tortillas and black bean paste fortified with ferrous fumarate, ferrous sulfate, and NaFeEDTA and to investigate the possibility of increasing the bioavailability of iron from ferrous fumarate by adding Na₂EDTA. Iron absorption was measured by a stable-isotope technique based on the incorporation of stable isotopes into erythrocytes 14 d after intake (17).

SUBJECTS AND METHODS

Study subjects

Thirty-three apparently healthy adolescent girls (aged 12–13 y; maximum body weight: 45 kg; 11 girls per study) were recruited at public high schools in Guatemala City. Exclusion criteria included consumption of iron supplements or medication.

The subjects and their parents were fully informed about the aims and procedures of the study and written consent was obtained from at least one parent. The Ethical Committees at INCAP, Guatemala City, and the Swiss Federal Institute of Technology, Zürich, reviewed and approved the protocol.

Study design

Girls were randomly assigned to studies 1, 2, or 3. Further randomization was done within each study: either 5 or 6 girls started with test meal A and 5 or 6 girls with test meal B. Each test meal was administered as breakfast after the girls had fasted overnight and again as lunch on 2 consecutive days. The alternate test meals were administered 14 d later according to the same study protocol. No food or drinks were allowed between the intake of the labeled breakfast and lunch meals and for 3 h after the intake of the labeled lunch meals.

Venous blood samples (5 mL) were drawn into EDTA-treated tubes before the intake of the first labeled test meal (day 1) and again on days 16 and 31. Samples were analyzed for iron-status indexes (hemoglobin and plasma ferritin) and for the incorporation of ⁵⁷Fe into red blood cells. Body weight and height were measured at each blood sampling.

Test meals

Test meals were representative of typical Central American meals and consisted of corn tortillas (made from 50 g industrially lime-treated corn masa flour) served with refried black bean paste (50 g) and water (250 g deionized water). Test meals were prepared from locally available foods purchased in bulk. Each test meal contained 2 mg added ⁵⁷Fe (as isotopically labeled ferrous fumarate, ferrous sulfate, or NaFeEDTA). Isotopically labeled iron compounds and Na₂EDTA were added to the bean paste. Individual servings of tortillas were prepared for each meal. Refried black bean paste was spread on the tortillas (2 per serving) and the tortillas were folded and heated in a microwave oven before being served.

In study 1, test meal A was fortified with ferrous fumarate. Test meal B was fortified with ferrous fumarate and contained added

Na₂EDTA at a 1:1 molar ratio relative to total iron (fortification iron plus native iron in the corn masa flour). In study 2, test meal A was fortified with ferrous fumarate. Test meal B was fortified with ferrous fumarate and contained added Na₂EDTA at a 1:1 molar ratio relative to fortification iron. In study 3, test meal A was fortified with ferrous sulfate. Test meal B was fortified with NaFe(III)EDTA (ferric chloride mixed with Na₂EDTA); the Na₂EDTA was added at a molar ratio of 1:1 relative to fortification iron.

Stable-isotope labels

[⁵⁷Fe]Ferrous fumarate was prepared in collaboration with one of the major commercial suppliers of iron fortification compounds, Dr P Lohmann (Emmerthal, Germany). The method used for the industrial production of ferrous fumarate was modified to a small-scale laboratory procedure. The physicochemical properties of the ferrous fumarate were tested and found to be similar to those of the commercial compound. This material has been used previously in human studies (18).

Solutions of ⁵⁷FeSO₄ and ⁵⁷FeCl₃ were prepared from isotopically enriched elemental iron by dissolution in sulfuric acid or hydrochloric acid and dilution to appropriate concentrations. The solution of ⁵⁷FeSO₄ was stored in polytetrafluoroethylene containers flushed with argon to keep the iron in the +II oxidation state. The isotopic composition of the stable-isotope labels was determined by negative thermal ionization mass spectrometry with a magnetic sector field instrument (Finnigan MAT 262 thermal ionization mass spectrometer; Finnigan MAT, Bremen, Germany). The iron concentration of the labels was determined by isotope dilution mass spectrometry against a standard prepared gravimetrically from an iron isotope reference material (IRM-014; Institute of Reference Materials and Measurements, Geel, Belgium).

Na₂EDTA doses

Water solutions of Na₂EDTA were prepared from Na₂EDTA·2H₂O (Merck, Darmstadt, Germany) and were added to the test meals at the time of serving. Labeled NaFeEDTA was prepared immediately before administration by mixing solutions of [⁵⁷Fe]ferric chloride and Na₂EDTA (molar ratio of iron:Na₂EDTA, 1:1).

Analysis of iron-status indexes

Blood samples were portioned for the analysis of hemoglobin, and plasma was separated (2000 × g, 10 min, room temperature), portioned, and frozen for later analysis of ferritin. Hemoglobin was measured with a kit by the cyanmethemoglobin method (Sigma, St Louis) and plasma ferritin by use of enzyme-linked immunosorbent assay kits (Ramco Laboratories, Houston). Commercial quality-control materials (DiaMed, Cressier sur Morat, Switzerland, and Ramco Laboratories) were analyzed together with all samples analyzed for hemoglobin and plasma ferritin.

Analysis of the isotopic composition of the blood samples

Each isotopically enriched blood sample was analyzed in duplicate under chemical blank monitoring. Whole blood samples were digested by microwave digestion with a nitric acid–hydrogen peroxide mixture, followed by separation of the sample iron from the matrix by anion-exchange chromatography and a subsequent solvent-solvent extraction step into diethylether (17, 19, 20). All isotopic analyses were performed by negative thermal ionization mass spectrometry with a magnetic sector field mass

TABLE 1

Hemoglobin (Hb), ferritin, and iron absorption from corn tortillas and black bean paste fortified with ferrous fumarate without added Na₂EDTA (test meal A) and with added Na₂EDTA at a molar ratio of 1:1 relative to total iron (test meal B): study 1

Subject no.	Test meal A			Test meal B		
	Hb	Ferritin	Iron absorption	Hb	Ferritin	Iron absorption
	g/L	μg/L	%	g/L	μg/L	%
8	133	28	2.4	129	20	8.2
10	147	28	10.9	143	22	2.3
18	139	9	17.4	133	8	12.5
19	139	22	2.5	136	18	2.4
38	143	14	8.2	138	12	12.2
39	138	40	7.3	137	42	3.5
11	143	16	12.1	150	18	22.0
13	124	20	0.6	135	19	3.5
14	146	24	4.5	149	24	8.4
15	134	24	5.4	130	32	5.9
16	129	10	12.3	133	10	12.8
Mean ¹	138	20	5.5	137	18	6.7
+1 SD	145	31	14.8	144	30	14.2
-1 SD	131	12	2.0	131	11	3.1

¹Geometric \bar{x} .

spectrometer (Finnigan MAT 262) equipped with a multicollector system for simultaneous ion beam detection (21). Iron separated from the sample was loaded on barium fluoride-coated rhenium filaments of a double-filament ion source together with silver fluoride to promote the formation of negatively charged FeF₄⁻ ions. Because of the high enrichment of the isotopically enriched labels and the low amounts of isotopic label incorporated into the red blood cells, data were normalized for the natural ratio of ⁵⁴Fe to ⁵⁶Fe (22).

Calculation of iron absorption

On the basis of the shift in the iron isotope ratios in the blood samples and the amount of iron circulating in the body, the amounts of ⁵⁷Fe in the blood 14 d after the administration of the test meals were calculated. The principles of isotope dilution were taken into account, considering that the iron isotopic labels were not monoisotopic (20). Circulating iron was calculated based on blood volume and hemoglobin concentration (17). Blood volume calculations were based on height and weight according to Brown et al (23). For calculations of fractional absorption, 80% incorporation of the absorbed iron into red blood cells was assumed. Corrections for enriched baseline values were made when calculating iron absorption from the second test meal.

Food analysis

Freeze-dried samples of tortillas and black bean paste were mineralized by microwave digestion in a nitric acid-hydrogen peroxide mixture and analyzed for iron and calcium by electrothermal flame atomic absorption spectroscopy (SpectraAA 400; Varian, Mulgrave, Australia) according to a standard addition technique to minimize matrix effects. Phytic acid content (pentainositol phosphate and hexainositol phosphate) was determined by an HPLC technique (24, 25).

Statistics

The effect of added Na₂EDTA on the bioavailability of iron from the test meals (studies 1 and 2) and the bioavailability of iron from ferrous sulfate compared with NaFeEDTA (study 3) were evaluated by paired *t* tests with each girl acting as her own

control. Values were logarithmically transformed before statistical analysis. Results are presented as geometric means (+1 SD, -1 SD). The analyses were performed in EXCEL 97 (Microsoft, Redmond, WA).

RESULTS

Test meals contained 2.2 mg native Fe, 58.3 mg Ca, and 427 mg phytic acid per serving. Individual data on hemoglobin, ferritin, and iron absorption are given in **Tables 1-3**. Hemoglobin concentrations ranged from 124 to 155 g/L. None of the girls had a hemoglobin concentration < 120 g/L. The geometric mean plasma ferritin concentration was 22 μg/L (range: 5-48 μg/L) at baseline and 19 μg/L (range: 5-42 μg/L) at day 16.

The bioavailability of iron from ferrous fumarate was not significantly influenced by the addition of Na₂EDTA. Geometric mean iron bioavailability was 5.5% (no added Na₂EDTA) and 6.7% (added Na₂EDTA) in study 1 (Table 1). In study 2, geometric mean iron bioavailability was 6.2% (no added Na₂EDTA) and 5.8% (added Na₂EDTA) (Table 2).

Geometric mean iron bioavailability from test meals fortified with ferrous sulfate (test meal A, study 3) was 5.5%. After the addition of labeled NaFeEDTA, iron bioavailability increased significantly to 9.0% (Table 3).

DISCUSSION

The traditional Central American meal based on corn and black beans used in this study contained a relatively high amount of phytic acid (427 mg) and no enhancers of iron absorption because neither animal tissue nor ascorbic acid-containing foods or drinks were included. Although no direct comparisons between ferrous fumarate and ferrous sulfate were made in this study, the data indicate that fractional iron bioavailability from the test meal fortified with ferrous fumarate was similar to that from the meal fortified with ferrous sulfate, the iron compound generally used as the reference in iron bioavailability studies. This result agrees with the results of an earlier study in adults by Hurrell et al (7). Relatively high fractional iron bioavailability

TABLE 2

Hemoglobin (Hb), ferritin, and iron absorption from corn tortillas and black bean paste fortified with ferrous fumarate without added Na₂EDTA (test meal A) and with added Na₂EDTA at a molar ratio of 1:1 relative to fortification iron (test meal B): study 2

Subject no.	Test meal A			Test meal B		
	Hb	Ferritin	Iron absorption	Hb	Ferritin	Iron absorption
	<i>g/L</i>	$\mu\text{g/L}$	%	<i>g/L</i>	$\mu\text{g/L}$	%
25	148	40	8.6	134	42	3.7
26	142	23	7.1	143	20	5.0
27	148	21	15.0	141	18	23.3
28	138	48	2.2	144	40	1.9
29	138	28	5.2	133	20	9.1
40	155	30	3.6	143	22	3.6
31	144	36	4.4	144	34	3.5
32	146	24	7.3	146	32	7.9
33	146	10	4.2	146	19	3.9
35	142	10	12.1	142	20	9.8
36	143	28	8.6	143	24	8.2
Mean ¹	144	24	6.2	142	25	5.8
+1 SD	149	41	10.8	146	35	11.5
-1 SD	140	15	3.6	137	18	2.9

¹Geometric \bar{x} .

from ferrous sulfate and ferrous fumarate was found in the present study (geometric mean values of 5.5–6.7%), presumably because the young girls participating in the study had limited storage iron and high requirements for absorbed iron. In previous studies with adult subjects, the absorption of iron from test meals based on corn flour was reported to be lower (1, 8).

The results from study 3 indicated that the bioavailability of iron from ferrous sulfate was inhibited by the composition of the test meal because iron bioavailability was significantly higher when NaFeEDTA was added. NaFeEDTA was suggested for use in the fortification of cereal products (3) because of its high iron absorption from meals with a high phytic acid content (4). The results from the present study confirm the usefulness of NaFeEDTA as an iron fortificant for staple foods with high contents of phytic acid (such as corn masa flour), provided that this compound can be added without causing adverse sensory changes

during storage or food preparation. Although NaFeEDTA is not currently used in large-scale food fortification programs, the recent evaluation by the Joint FAO/WHO Expert Committee on Food Additives concluded that NaFeEDTA can be considered safe when used in supervised food fortification programs providing $\approx 0.2 \text{ mg Fe} \cdot \text{kg body wt}^{-1} \cdot \text{d}^{-1}$ (26). The use of this iron compound will presumably increase in the future.

Previous studies clearly showed that the bioavailability of iron from ferrous sulfate can be significantly enhanced by adding Na₂EDTA to cereal-based meals. Iron absorption from Egyptian bread fortified with ferrous sulfate was significantly increased after the addition of Na₂EDTA at a molar ratio of 1 relative to iron (14), and Na₂EDTA added at molar ratios of 0.25–1.0 relative to iron increased iron absorption significantly from a rice-based test meal (15). In addition, in 6–7-y-old children (12) and adults (16), we recently showed a significant

TABLE 3

Hemoglobin (Hb), ferritin, and iron absorption from corn tortillas and black bean paste fortified with ferrous sulfate (test meal A) and with NaFeEDTA (test meal B): study 3

Subject no.	Test meal A			Test meal B		
	Hb	Ferritin	Iron absorption	Hb	Ferritin	Iron absorption
	<i>g/L</i>	$\mu\text{g/L}$	%	<i>g/L</i>	$\mu\text{g/L}$	%
21	144	21	17.3	145	16	18.2
22	150	14	3.7	149	16	12.4
23	139	24	17.4	139	14	25.6
24	139	23	2.8	139	22	2.2
37	145	30	1.8	145	24	3.4
01	140	5	13.8	152	5	24.2
02	148	18	8.4	142	18	18.8
03	125	48	0.6	140	28	1.6
04	136	26	7.1	152	24	6.0
12	150	10	9.4	137	10	18.2
30	150	34	5.0	140	32	5.6
Mean ¹	142	20	5.5	144	17	9.0 ²
+1 SD	150	37	16.5	149	29	25.5
-1 SD	135	11	1.8	138	10	3.2


¹Geometric \bar{x} .

²Significantly different from test meal A, $P = 0.009$ (paired t test).

enhancing effect on iron absorption of Na₂EDTA added at molar ratios of 0.33, 0.69, and 1 relative to iron in cereal-based meals fortified with ferrous sulfate.

An enhancing effect of Na₂EDTA on iron absorption was not shown, however, in the present study with ferrous fumarate. Iron bioavailability was not significantly influenced by adding Na₂EDTA to ferrous fumarate at a molar ratio of 1:1 relative to either fortification iron or the total iron content of the corn tortillas. The difference in the influence of Na₂EDTA on iron bioavailability from a water-soluble iron compound (ferrous sulfate) and a compound soluble in dilute acid (ferrous fumarate) illustrates the importance of carefully evaluating the effect of potential enhancers of iron absorption on iron compounds with different physicochemical properties. The lack of effect of added Na₂EDTA on the bioavailability of iron from ferrous fumarate could be assumed to be related to the solubility properties of this iron compound in the gastric juice and the complex formation between the EDTA moiety, iron, and other minerals and trace elements present in the meal. In addition, the relatively high pH of the lime-treated corn masa flour used in this study could have negatively influenced the formation of EDTA-iron complexes because iron has the highest binding constant at low pH (4).

It was recently shown that Na₂EDTA does not enhance the absorption of iron from ferric pyrophosphate added to infant cereal (16) and there is therefore some doubt that it will enhance the absorption of iron from elemental iron compounds, although this remains to be confirmed. At the present time, the usefulness of Na₂EDTA as an enhancer of iron bioavailability seems to be relevant only to water-soluble iron fortification compounds and native iron in foods.

In conclusion, iron bioavailability was ≈6% in 12–13-y-old girls fed a typical Guatemalan meal based on corn tortillas and black bean paste fortified with ferrous sulfate or ferrous fumarate. No enhancing effect of Na₂EDTA on iron bioavailability was found with the test meals fortified with ferrous fumarate, indicating that Na₂EDTA is not an alternative to ascorbic acid as an enhancer of iron absorption for iron compounds that are less soluble than ferrous sulfate. NaFeEDTA was significantly more bioavailable than was ferrous sulfate from this inhibitory meal, providing further support for the use of NaFeEDTA in cereal flour fortification when the sensory quality of the food is not affected. 

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