

Improving iron absorption from a Peruvian school breakfast meal by adding ascorbic acid or Na₂EDTA¹⁻³

Lena Davidsson, Thomas Walczyk, Nelly Zavaleta, and Richard F Hurrell

ABSTRACT

Background: Iron-fortified school breakfasts have been introduced in Peru to combat childhood iron deficiency.

Objective: We evaluated whether iron absorption from a school breakfast meal was improved by increasing the ascorbic acid content or by adding an alternative enhancer of iron absorption, Na₂EDTA.

Design: In a crossover design, iron absorption from test meals was evaluated by erythrocyte incorporation of ⁵⁸Fe and ⁵⁷Fe. The test meals (wheat bread and a drink containing cereal, milk, and soy) contained 14 mg added Fe (as ferrous sulfate) including 2.0–2.6 mg ⁵⁸Fe or 4.0–7.0 mg ⁵⁷Fe.

Results: Geometric mean iron absorption increased significantly from 5.1% to 8.2% after the molar ratio of ascorbic acid to fortification iron was increased from 0.6:1 to 1.6:1 ($P < 0.01$; $n = 9$). Geometric mean iron absorption increased significantly from 2.9% to 3.8%, from 2.2% to 3.5%, and from 2.4% to 3.7% after addition of Na₂EDTA at molar ratios relative to fortification iron of 0.3:1, 0.7:1, and 1:1, respectively, compared with test meals containing no added enhancers ($P < 0.01$; $n = 10$ for all). Iron absorption after addition of ascorbic acid (molar ratio 0.6:1) was not significantly different from that after addition of Na₂EDTA (molar ratio 0.7:1).

Conclusions: Ascorbic acid and Na₂EDTA did not differ significantly in their enhancing effects on iron absorption at molar ratios of 0.6:1 to 0.7:1 relative to fortification iron. Additional ascorbic acid (molar ratio 1.6:1) increased iron absorption significantly. Increasing the molar ratio of Na₂EDTA to fortification iron from 0.3:1 to 1:1 had no effect on iron absorption. *Am J Clin Nutr* 2001;73:283–7.

KEY WORDS Iron, iron absorption, stable isotope, food fortification, iron fortification, children, ascorbic acid, vitamin C, Na₂EDTA, Peru, childhood anemia, iron deficiency, pediatric nutrition

INTRODUCTION

Fortification of foods with iron is generally considered the most cost-effective approach for combating iron deficiency (1). Some food vehicles used in iron-fortification programs, such as wheat flour, salt, and sugar, are consumed by the general population, whereas other foods can be targeted to specific at-risk groups, such as infants and children. For example, iron fortification of infant formula has been a successful approach for reducing the prevalence of

iron deficiency in infants (2). Foods used in school meal programs are excellent potential vehicles for targeted iron-fortification programs. This is particularly true in countries, such as Peru, where the prevalence of childhood iron deficiency anemia is high.

The overall success of iron-fortification programs depends on several factors. One of the most important factors is the bioavailability of iron from the fortified foods. The iron-fortified school breakfast meals in Peru provide 14 mg Fe, as ferrous sulfate, a soluble iron compound with high relative bioavailability (1). However, the bioavailability of dietary iron, including iron-fortification compounds, depends on the overall composition of the meal, ie, the presence of enhancers and inhibitors of iron absorption. The school breakfast meals used in the Peruvian program are cereal-based with limited amounts of added ascorbic acid. Therefore, iron absorption would be relatively low, in part because of the presence of phytic acid, a potent inhibitor of iron absorption that is found in cereals (3).

The aim of the present study was to measure iron absorption from a meal used in the Peruvian school breakfast program and to evaluate the possibility of improving iron absorption by adding known enhancers of iron absorption. Two approaches for optimizing iron absorption from the meal were evaluated: increasing the ascorbic acid content (4) and adding Na₂EDTA (5).

SUBJECTS AND METHODS

Iron absorption was measured in Peruvian children by using the double stable-isotope technique developed by Kastenmayer et al (6). This technique enables the evaluation of iron absorption from 2 test meals administered on consecutive days. The technique analyzes the incorporation of iron stable-isotope labels into red blood cells 14 d after administration. The iron isotopic composition of blood samples was measured by using negative thermal ionization mass spectrometry (NTIMS) (7, 8).

¹From the Laboratory for Human Nutrition, Institute of Food Science, Swiss Federal Institute of Technology, Ruschlikon, Switzerland, and Instituto de Investigacion Nutricional, Lima, Peru.

²Supported by the International Atomic Energy Agency, Vienna (project PER/7/003).

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

Received February 29, 2000.

Accepted for publication July 10, 2000.

Subjects

Forty-seven apparently healthy boys and girls aged 6–7 y were recruited at a primary school participating in the school breakfast program in Lima, Peru. Children, parents, and teachers were fully informed about the aims and procedures of the study and written consent was obtained from at least one parent of each child. The protocol was reviewed and approved by the Ethical Committee at the Instituto de Investigacion Nutricional, Lima, Peru.

Test meals

A drink powder made from cereal, milk, and soy was prepared especially for the study from raw materials normally used for the preparation of school breakfast meals. The drink powder consisted of 16% extruded rice flour, 8% extruded wheat flour, 16% milk powder, and 16% extruded soy flour without added iron or ascorbic acid. Standardized test meals were prepared immediately before consumption by mixing 50 g drink powder with 200 g hot deionized water. White wheat bread (42 g) prepared from unfortified wheat flour was served together with the drink. All test meals were identical, except for the ascorbic acid and Na₂EDTA contents. Water solutions of ascorbic acid [L(+)-ascorbic acid (176.1 g/mol); Merck, Darmstadt, Germany] or Na₂EDTA [Na₂EDTA × 2H₂O (372.2 g/mol), Merck] were added to the test meal drinks immediately before consumption. Each test meal contained 14 mg added Fe as iron stable-isotope labels and iron with natural isotopic composition (food grade FeSO₄ × 7H₂O; Merck).

There were 5 studies that differed in terms of the composition of the breakfast drinks (test meals). Within each study, children were randomly assigned to receive either test meal A or test meal B on day 1. The other test meal (A or B) was consumed on the following day. Each child was his or her own control. The molar ratios of ascorbic acid to iron and of Na₂EDTA to iron in test meals A and B, respectively, in the 5 studies were calculated on the basis of the fortification iron content in the meal, ie, 14 mg Fe/test meal.

- 1) Study 1: ascorbic acid:iron molar ratio of 0.6:1 (A) versus ascorbic acid:iron molar ratio of 1.6:1 (B).
- 2) Study 2: ascorbic acid:iron molar ratio of 0.6:1 (A) versus Na₂EDTA:iron molar ratio of 0.7:1 (B).
- 3) Study 3: no added enhancers (A) versus Na₂EDTA:iron molar ratio of 0.3:1 (B).
- 4) Study 4: no added enhancers (A) versus Na₂EDTA:iron molar ratio of 0.7:1 (B).
- 5) Study 5: no added enhancers (A) versus Na₂EDTA:iron molar ratio of 1:1 (B).

The labeled test meals were administered under standardized conditions after the children had fasted overnight. All the children were supervised closely by the investigators during consumption of the test meals and for an additional 3 h, during which time no food or drink was allowed.

Stable-isotope labels

The stable-isotope solutions of ⁵⁷FeSO₄ and ⁵⁸FeSO₄ were gravimetrically prepared from isotopically enriched elemental iron (Chemgas, Paris) by dissolution in 0.1 mol H₂SO₄/L. The solutions were diluted by mass to an appropriate concentration for individual dose preparation. Solutions of ⁵⁷FeSO₄ and ⁵⁸FeSO₄ were stored in polytetrafluoroethylene containers that were flushed with argon to keep the iron in the +II oxidation

state. The isotopic composition of the iron in solution was determined by NTIMS with a magnetic sector field instrument (Finnigan MAT 262 thermal ionization mass spectrometer; Finnigan MAT, Bremen, Germany). Iron concentrations were determined by isotope dilution mass spectrometry against a diluted, commercially available iron standard (Titrisol, Merck). Stable-isotope doses were based on the precision attainable by positive thermal ionization mass spectrometry (6) and were therefore relatively high in Studies 1 and 2 (2.6 mg ⁵⁸Fe and 7.0 mg ⁵⁷Fe). Analyses of enriched blood samples obtained from children in Study 1 were used to evaluate certain aspects of our methods (8). The isotope doses used in Studies 3–5 were smaller (2.0 mg ⁵⁸Fe and 4.0 mg ⁵⁷Fe per subject) because of the improved precision of iron isotopic analysis by NTIMS (7).

Blood samples

Venous blood samples (5 mL) were drawn into EDTA-treated tubes before the children consumed the first labeled test meal (baseline) and again 14 d after intake of the second labeled test meal. Baseline blood samples were analyzed for hemoglobin by the cyanomethemoglobin technique and for plasma ferritin by enzyme-linked immunosorbent assay. Blood samples drawn 14 d after intake of the second test meal were analyzed for the incorporation of stable-isotope labels (⁵⁷Fe and ⁵⁸Fe) into red blood cells. Body weight and height were measured at the time of blood sampling.

Analysis of isotopic composition of blood samples

During all of the experimental work, the guidelines for trace element analysis were followed strictly, including additional purification of the commercial chemicals and reagents and acid washing of all containers used during trace element analysis. All samples were handled under clean laboratory conditions to reduce the risk of sample contamination during analysis.

Each isotopically enriched blood sample was analyzed in duplicate to determine its iron isotopic composition under chemical blank monitoring. Whole blood samples were mineralized by using a mixture of nitric acid and hydrogen peroxide and microwave digestion. Sample iron was separated from the matrix by anion-exchange chromatography after a solvent-solvent extraction step into diethylether (6, 9). All isotopic analyses were performed by NTIMS with a magnetic sector field mass spectrometer (MAT 262 thermal ionization mass spectrometer; Finnigan MAT) equipped with a multicollector system for simultaneous ion beam detection (7). Iron separated from blood samples was loaded on barium fluoride-coated rhenium filaments of a double-filament ion source together with silver fluoride to promote the formation of FeF₄⁻ ions. Because of the high enrichment of the stable-isotope labels and the small amounts of stable-isotope labels incorporated into red blood cells, it was possible to normalize the acquired isotopic data internally for the natural ratio of ⁵⁴Fe to ⁵⁶Fe (10).

Calculation of iron absorption

The amount of circulating iron was calculated on the basis of blood volume and hemoglobin concentration. Blood volume was calculated by using empirically derived formulas that used height and weight (11). On the basis of the shift of iron isotope ratios in the blood samples and the calculated amount of iron circulating in the body, we calculated the amounts of ⁵⁷Fe and ⁵⁸Fe in the blood 14 d after test meal administration. The calculations followed the principles of isotope dilution and took into consideration that the

TABLE 1
Baseline iron status and iron absorption during the 5 studies in Peruvian schoolchildren¹

Test meals	Baseline		Iron absorption ²
	Hemoglobin <i>g/L</i>	Plasma ferritin <i>μg/L</i>	
Study 1 (<i>n</i> = 9)	124 (119, 129)	10 (6, 18)	
Meal A: AA:iron, 0.6:1 ³			5.1 ^a (2.7, 9.8)
Meal B: AA:iron, 1.6:1			8.2 ^b (4.1, 16.5)
Study 2 (<i>n</i> = 7)	119 (114, 125)	10 (7, 14)	
Meal A: AA:iron, 0.6:1			4.9 (2.5, 9.7)
Meal B: Na ₂ EDTA:iron, 0.7:1			4.1 (2.1, 8.0)
Study 3 (<i>n</i> = 10)	127 (119, 135)	18 (11, 27)	
Meal A: No enhancers			2.9 ^a (1.4, 5.7)
Meal B: Na ₂ EDTA:iron, 0.3:1			3.8 ^b (2.0, 7.0)
Study 4 (<i>n</i> = 10)	127 (121, 133)	22 (13, 37)	
Meal A: No enhancers			2.2 ^a (1.0, 5.2)
Meal B: Na ₂ EDTA:iron, 0.7:1			3.5 ^b (1.8, 6.5)
Study 5 (<i>n</i> = 10)	126 (122, 129)	18 (14, 24)	
Meal A: No enhancers			2.4 ^a (1.2, 4.7)
Meal B: Na ₂ EDTA:iron, 1:1			3.7 ^b (2.2, 6.4)

¹Geometric means (−1SD, +1SD). In each of the 5 studies, 2 test meals with different contents of iron-absorption enhancers [ascorbic acid (AA) and Na₂EDTA] were studied.

²Within each study, values with different superscript letters are significantly different, *P* < 0.01. Iron absorption was determined by measuring the incorporation of stable isotope labels (⁵⁷Fe and ⁵⁸Fe) into red blood cells.

³Molar ratio of enhancer to fortification iron.

isotopically enriched ⁵⁷Fe and ⁵⁸Fe used as labels contained small amounts of other iron stable isotopes (8). We assumed 90% incorporation of the absorbed iron into red blood cells.

Food sample analysis

Food samples were mineralized by microwave digestion in a mixture of nitric acid and hydrogen peroxide (MLS 1200; MLS, Leutkirch, Germany) and the iron content was measured by electrothermal flame atomic absorption spectroscopy (SpectrAA 400; Varian, Mulgrave, Australia); standard addition technique was used to minimize matrix effects. The phytic acid content was determined by HPLC (12, 13).

Statistical analyses

Sample-size calculations were performed by using our previous data on the effect of ascorbic acid on iron absorption in infants (14). It was estimated that 8 children in each of the 5 studies would be a sufficient sample size to detect a nutritionally significant relative difference (50%) in iron absorption between the 2 test meals with 80% power and a type I error of 5%. Paired *t* tests were used to evaluate data from Studies 1–5. The values were logarithmically transformed before statistical analysis. The results are presented as geometric means +1SD and −1SD. Analysis of variance with Tukey's multiple comparison test was used to evaluate differences between studies on the basis of logarithmically transformed absorption ratios (Studies 3–5).

RESULTS

The test meals contained 2.3 mg native Fe and 122 mg phytic acid. Twenty-one girls and 25 boys with a mean body weight of 21.2 kg (range: 17.2–26.0 kg) participated. Each child participated in 1 of the 5 studies. One child in Study 2 did not complete the study. Ten children were anemic (hemoglobin < 120 g/L) and 13 children had no iron stores, as indicated by low ferritin con-

centrations (< 12 μg/L). Three children had iron deficiency anemia (hemoglobin < 120 g/L and ferritin < 12 μg/L). Anemic children with iron deficiency were treated with medicinal iron after the study. Geometric mean values for baseline hemoglobin and serum ferritin in the different study groups are shown in **Table 1**.

The enhancing effect of additional ascorbic acid was clearly shown in Study 1; iron absorption was significantly higher after meal B than after meal A (Table 1). In Study 2, mean iron absorption was similar after both test meals. In Studies 3–5, iron absorption was significantly higher after the addition of Na₂EDTA (ie, after meal B than after meal A). The ratios of geometric mean iron absorption from meals with added Na₂EDTA (meals B) to the meals without added Na₂EDTA (meals A) were 1.26, 1.54, and 1.59 in Studies 3, 4, and 5, respectively. These ratios were not significantly different from each other.

DISCUSSION

The school breakfast program was introduced in selected low-income areas of Peru in 1993 to improve the nutrition of children attending public primary schools. The program has expanded gradually and the distribution of fortified foods now reaches ≈800 000 to 1 million Peruvian children (ER Jacoby and G Lopez de Romaña, unpublished observations, 1998). The effects of the program have been measured as significantly increased intakes of energy and nutrients by participating children (G Lopez de Romaña, unpublished observations, 1997). In addition, the intervention has led to improvements in school attendance and school performance, as measured by a vocabulary test, in children living in a rural area of the Andes (15, 16).

The school breakfast meals evaluated in this study contained a relatively high amount of added iron, 14 mg/meal. Anemia is a major public health problem in Peruvian children and is thought to be largely the result of iron deficiency. The effect of the fortified foods has been evaluated by comparing the prevalence of anemia

before and after implementation of the program in selected areas. For example, in a central Andean town, the prevalence of anemia dropped from 66% to 14% in the 6 mo after the program was introduced (G Lopez de Romaña, unpublished observations, 1997). Although the decrease in anemia prevalence was impressive in this relatively small study, 14% of the children were still anemic after the dietary intervention. Low bioavailability of iron from the cereal-based meals could limit the effect of the program on iron deficiency anemia; therefore, a study of iron absorption from a typical meal used in the program was initiated. In addition, we evaluated the possibility of increasing iron absorption by adding additional ascorbic acid or Na₂EDTA to the school breakfast meals.


In the present study, we chose to use ascorbic acid to improve iron absorption because it is one of the major enhancers of iron absorption in adults (4, 17–19) and in infants and children (14, 20, 21). Ascorbic acid is widely used in food products as an enhancer of iron absorption and as an antioxidant. However, because of its susceptibility to oxidation by exposure to air, especially when the food is also exposed to heat, humidity, or both, the usefulness of ascorbic acid as an enhancer of iron absorption is uncertain in some situations. Thus, a more stable enhancer of iron absorption is sometimes needed. This is especially important in developing countries, where the storage and packaging of food can pose serious problems (22). Na₂EDTA is a stable compound and a permitted food additive (23) that could be added to foods together with fortification iron in food fortification programs. Previous studies in adults showed significantly increased iron absorption after addition of Na₂EDTA together with ferrous sulfate to cereal-based meals (5, 24).

The geometric mean iron absorption was ≈5% from the test meals that were fortified with 14 mg Fe and contained ascorbic acid at a relatively low molar ratio relative to fortification iron (0.6:1; Studies 1 and 2). When we increased the ascorbic acid content to 70 mg (resulting in a molar ratio of 1.6:1), mean iron absorption increased significantly to 8.2% (Study 1). Our results are thus in agreement with earlier data from Derman et al (4) that showed a significant enhancing effect of ascorbic acid on iron absorption from an infant cereal at molar ratios in the range of 1.1 to 2.4 relative to added iron. Replacing ascorbic acid (molar ratio of 0.6:1) with Na₂EDTA at a molar ratio of 0.7:1 relative to fortification iron resulted in similar fractional iron absorption from the meals (4.9% compared with 4.1%, respectively; Study 2). The amount of Na₂EDTA added in Study 2 was determined on the basis of the acceptable daily intake for EDTA (2.5 mg/kg body wt) (23) and an estimated body weight of 20 kg in the study population.

Our observation that iron absorption was significantly enhanced after addition of Na₂EDTA at all molar ratios included in this study is supported by the results of other investigators. MacPhail et al (5) showed a positive effect of Na₂EDTA on iron absorption from a rice-based meal fortified with ferrous sulfate when it was added in molar ratios ranging from 0.25 to 1.0 relative to iron. Recent data (RF Hurrell, M Reddy, and JD Cook, unpublished observations, 1999) also showed an enhancing effect of Na₂EDTA on absorption of iron from ferrous sulfate when Na₂EDTA was added at molar ratios ≤0.7:1 in meals with low phytic acid contents and ≤1:1 in meals with high phytic acid contents.

The amount of iron absorbed from the school breakfast meals currently provided by the program is 0.7 mg/d because of the relatively high degree of iron fortification along with 27 mg added ascorbic acid (molar ratio of 0.6:1). An increase in the ascorbic

acid content to 70 mg/serving (molar ratio of 1.6:1) would increase the mean amount of iron absorbed to 1.15 mg/d. These quantities (0.7 and 1.15 mg/d) represent 70% and 115%, respectively, of the daily requirement for absorbed iron for young children (25). Thus, the expected effect on iron nutrition would be substantial if an adequate ascorbic acid content in the meals could be assured.

Addition of Na₂EDTA to school breakfast meals at a molar ratio relative to fortification iron of 0.7:1 would result in iron absorption similar to that from meals containing the lower ascorbic acid content (0.6:1) used in this study. Thus, Na₂EDTA could be a useful alternative to ascorbic acid as an enhancer of iron absorption in situations in which ascorbic acid stability is of concern. In addition, the presence of Na₂EDTA in cereal-based diets could have a positive effect on zinc nutrition because NaFeEDTA was shown to improve zinc absorption in women (26). 

We thank Graciela Respicio and Olga Villalobos for their excellent coordination of the fieldwork and we are grateful to all the children, parents, and teachers for their participation in the study.

REFERENCES

- Hurrell RF. Preventing iron deficiency through food fortification. *Nutr Rev* 1997;55:210–22.
- Dallman PR. Progress in the prevention of iron deficiency in infants. *Acta Paediatr Scand* 1990;365:28–37.
- Cook JD, Reddy MB, Burri J, Juillerat MA, Hurrell RF. The influence of different cereal grains on iron absorption from infant cereal foods. *Am J Clin Nutr* 1997;65:964–9.
- Derman DP, Bothwell TH, MacPhail AP, et al. Importance of ascorbic acid in the absorption of iron from infant foods. *Scand J Haematol* 1980;25:193–201.
- MacPhail AP, Patel RC, Bothwell TH, Lamparelli RD. EDTA and the absorption of iron from food. *Am J Clin Nutr* 1994;59:644–8.
- Kastenmayer P, Davidsson L, Galan P, Cherouvrier F, Hercberg S, Hurrell RF. A double stable isotope technique for measuring iron absorption in infants. *Br J Nutr* 1994;71:411–24.
- Walczyk T. Iron isotope ratio measurements by negative thermal ionization mass spectrometry. *Int J Mass Spectrom Ion Proc* 1996;161:217–27.
- Walczyk T, Davidsson L, Zavaleta N, Hurrell RF. Stable isotope labels as a tool to determine iron absorption by Peruvian school children from a breakfast meal. *Fresenius J Anal Chem* 1997;359:445–9.
- Beer B, Heumann KG. Isotope dilution mass spectrometry of microelectronically relevant heavy metal traces in high purity cobalt. *Fresenius J Anal Chem* 1993;347:351–5.
- Taylor PDP, Maeck R, De Bièvre P. Determination of the absolute isotopic composition and atomic weight of a reference sample of natural iron. *Int J Mass Spectrom Ion Proc* 1992;121:111–25.
- Linderkamp O, Versmold HT, Riegel KP, Betke K. Estimation and prediction of blood volume in infants and children. *Eur J Pediatr* 1977;125:227–34.
- Sandberg A-S, Ahderinne R. HPLC method for determination of inositol tri-, tetra-, penta-, and hexaphosphates in foods and intestinal contents. *J Food Sci* 1986;51:547–50.
- Sandberg A-S, Carlsson N-G, Svanberg U. Effects of tri-, tetra-, penta-, and hexaphosphates on in vitro estimation of iron availability. *J Food Sci* 1989;54:159–61.
- Davidsson L, Galan P, Kastenmayer P, et al. Iron absorption in infants: the influence of phytic acid and ascorbic acid in formulas based on soy isolate. *Pediatr Res* 1994;36:816–22.
- Pollitt E, Jacoby E, Cueto S. Evaluation of a school breakfast program in Peru. In: *Nutrition, health and child development.*

- Washington, DC: Pan American Health Organization, 1998:119–27. (Scientific publication 566.)
16. Jacoby E, Cueto S, Pollitt E. Benefits of a school breakfast programme among Andean children in Huaraz, Peru. *Bull Nutr Food* 1996;17:54–64.
 17. Gillooly M, Torrance JD, Bothwell TH, et al. The relative effect of ascorbic acid on iron absorption from soy-based and milk-based infant formulas. *Am J Clin Nutr* 1984;40:522–7.
 18. Hallberg L, Brune M, Rossander L. Iron absorption in man: ascorbic acid and dose-dependent inhibition by phytate. *Am J Clin Nutr* 1989;49:140–4.
 19. Siegenberg D, Baynes RD, Bothwell TH, et al. Ascorbic acid prevents the dose-dependent inhibitory effects of polyphenols and phytates on nonheme-iron absorption. *Am J Clin Nutr* 1991;53:537–41.
 20. Stekel A, Olivares M, Pizarro F, Chadud P, Lopez I, Amar M. Absorption of fortification iron from milk formulas in infants. *Am J Clin Nutr* 1986;43:917–22.
 21. Davidsson L, Walczyk T, Morris A, Hurrell RF. Influence of ascorbic acid on iron absorption from an iron-fortified chocolate-flavored drink in Jamaican children. *Am J Clin Nutr* 1998;67:873–7.
 22. International Nutritional Anemia Consultative Group. Iron EDTA for food fortification. Washington, DC: The Nutrition Foundation/International Life Sciences Institute, 1993.
 23. Joint FAO/WHO Expert Committee on Food Additives. Toxicological evaluation of some food additives including anticaking agents, antimicrobials, antioxidants, emulsifiers and thickening agents. *World Health Organ Tech Rep Ser* 1974;539:1–40.
 24. El Guindi M, Lynch SR, Cook JD. Iron absorption from fortified flat breads. *Br J Nutr* 1988;59:205–13.
 25. National Research Council. Recommended dietary allowances. 10th ed. Washington, DC: National Academy Press, 1989.
 26. Davidsson L, Kastenmayer P, Hurrell RF. Sodium iron EDTA [NaFe(III)EDTA] as a food fortificant: the effect on the absorption and retention of zinc and calcium in women. *Am J Clin Nutr* 1994;60:231–7.