Iron bioavailability in infants from an infant cereal fortified with ferric pyrophosphate or ferrous fumarate[1,2]

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ABSTRACT

Background: Infant cereals are commonly fortified with insoluble iron compounds with low relative bioavailability, such as ferric pyrophosphate, because of organoleptic changes that occur after addition of water-soluble iron sources.

Objective: Our objective was to compare iron bioavailability from ferric pyrophosphate with an alternative iron source that is soluble in dilute acid, ferrous fumarate, and to evaluate the influence of ascorbic acid on iron bioavailability from ferrous fumarate in infants.

Design: Iron bioavailability was measured as the incorporation of stable iron isotopes into erythrocytes 14 d after administration of labeled test meals (25 g dry wheat and soy infant cereal, 100 g water, and 2.5 mg Fe as [^{57}Fe]ferric pyrophosphate or [^{57}Fe]ferrous fumarate). Ascorbic acid was added to all test meals (25 mg in study 1 or 25 or 50 mg in study 2). Infants were fed each test meal on 4 consecutive days under standardized conditions. The 2 different test meals within each study were administered 2 wk apart in a crossover design.

Results: Geometric mean iron bioavailability was significantly higher from [^{57}Fe]ferrous fumarate than from [^{57}Fe]ferric pyrophosphate [4.1% (range: 1.7–14.7%) compared with 1.3% (range: 0.7–2.7%); n = 8, P = 0.008]. In this study, doubling the ascorbic acid content did not further enhance iron bioavailability; the geometric means (range) were 3.4% (1.9–6.6%) and 4.2% (1.2–18.7%) for the test meals with 25 and 50 mg ascorbic acid added, respectively (n = 9).

Conclusion: Iron bioavailability from iron-fortified infant cereals can be improved by using an iron compound with high relative bioavailability and by ensuring adequate ascorbic acid content of the product. *Am J Clin Nutr* 2000;71:1597–1602.

KEY WORDS Iron, infants, ferrous fumarate, ferric pyrophosphate, bioavailability, stable isotopes, infant cereal

INTRODUCTION

Although the importance of iron-fortified infant formulas in the prevention of iron deficiency in infants is well recognized (1), the effect of iron-fortified infant cereals has been questioned because of concern over the bioavailability of the iron compounds used (2, 3). Industrially produced infant formulas are usually fortified with soluble iron compounds, such as ferrous sulfate, with high relative iron bioavailability. However, in contrast with infant formulas, infant cereals are difficult to fortify with soluble iron compounds because of unacceptable organoleptic changes such as rancidity and color and flavor changes in the products during storage. This has led to the use of less soluble, and hence less bioavailable, iron compounds, such as elemental iron and ferric pyrophosphate for iron fortification of infant cereals (4). An alternative iron compound, ferrous fumarate, has been proposed for iron fortification of infant cereals because ferrous fumarate was shown to be as well absorbed as ferrous sulfate in adults (5). Ferrous fumarate is less soluble than ferrous sulfate in water but is soluble in dilute acid such as gastric juice. Thus, this compound does not cause organoleptic changes during storage of fortified foods to the same extent as does ferrous sulfate but still has a high relative bioavailability. The previous evaluation of iron bioavailability from ferrous fumarate was done in adults by using a radiotracer technique (5). However, because of the potential influence of physiologic differences in gastric acid secretion between infants and adults, the data from the study by Hurrell et al (5) cannot be directly extrapolated to iron bioavailability from ferrous fumarate in infants. The aim of this study was to compare iron bioavailability from a compound commonly used to fortify infant cereals, ferric pyrophosphate, with that of ferrous fumarate in healthy 6–12-mo-old infants. Furthermore, the influence of increased ascorbic acid content on iron bioavailability from ferrous fumarate was evaluated in the second part of the study. Iron bioavailability was measured in healthy infants from a wheat and soy infant cereal by using a stable-isotope technique based on the incorporation of iron stable isotopes into erythrocytes 14 d after administration.

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SUBJECTS AND METHODS

Infants

Twenty apparently healthy, formula-fed infants (6–12 mo of age) were enrolled in the study. Infants were recruited from the outpatient clinic at the Department of Pediatrics, Warsaw University Medical School, and from the private practice of one of the investigators (HS). All infants had been introduced to weaning foods by the time of recruitment. To ensure acceptability of the cereal, the infants were fed, for ≥14 d before starting the study, a commercial infant cereal fortified with ferric pyrophosphate (Ceresoy; Nestlé, Vevey, Switzerland) equivalent to the wheat and soy product used in the study.

Ten infants were randomly allocated to each of 2 studies. On the basis of data from our previous study on the effect of ascorbic acid on iron bioavailability in infants (6), we estimated that 10 subjects per group would be a sufficient sample size to detect a nutritionally significant relative difference in iron bioavailability of 50% with 90% power and a type I error rate of 5%.

Parents were fully informed about the aims and procedures of the study and written consent was obtained from at least one parent. The study protocol was reviewed and approved by the Ethical Committee at the Warsaw University Medical School and the Ethical Committee at the Nestlé Research Centre Lausanne.

Infant cereal

An infant cereal based on white-wheat flour and soy flour was produced according to the specifications for a commercial product (Ceresoy) but without added iron and ascorbic acid at a Nestlé Product Development Center (Linor, Orbe, Switzerland). The nutritional composition was analyzed and the microbiologic safety was ensured before the product was released from the product development center.

Stable-isotope labels

[^{57}Fe]Ferric pyrophosphate and [^{57}Fe]ferrous fumarate were prepared in collaboration with one of the major commercial suppliers of iron fortification compounds, Dr Paul Lohmann GmbH KG (Emmerthal, Germany). The procedure used was similar to the industrial production of the equivalent fortification compounds but was done as a small-scale laboratory procedure. Elemental iron isotopically enriched with ^{57}Fe (96.00%) was purchased from Nippon Sanso Europe (Plaisir, France). For the preparation of [^{57}Fe]ferrous fumarate, 2.3 g ^{57}Fe was dissolved in sulfuric acid (20%) and combined with an aqueous solution of sodium fumarate. The iron fumarate precipitate was washed with ethanol until no residual sulfate could be detected, dried, and ground in an agate mortar to a fine powder. [^{57}Fe]Ferric pyrophosphate was prepared by dissolving 2.7 g ^{57}Fe in sulfuric acid (20%). After oxidation to Fe^{3+} by the addition of 4 g H_2O_2 (30%), 7.9 g $Na_2H_2P_2O_7$ was added and the resulting precipitate was washed with water and ethanol. The precipitate was then dried and ground as described above.

Total iron content was analyzed by atomic absorption spectrometry (AAS) (model 975; Varian Techtron, Mulgrave, Australia) after dissolution in 5 mol HCl/L. The isotopic composition of the metallic ^{57}Fe, [^{57}Fe]fumarate, and [^{57}Fe]pyrophosphate was measured by thermal ionization mass spectrometry (model THQ; Finnigan MAT, Bremen, Germany). Solubility of the ^{57}Fe-labeled compounds was compared with commercial equivalents by monitoring iron solubility in 0.06 mol HCl/L for 180 min. Duplicate aliquots equivalent to 20 mg Fe each were weighed into 500-mL flasks and 250 mL of 0.06 mol HCl/L (pH 1.2), preheated to 37°C, was added. The samples were placed in a shaking water bath at 37°C and 2-mL aliquots were taken after 10, 20, 30, 60, 90, 120, 150, and 180 min. Samples were centrifuged for 2 min at 1000 × *g* at 22°C, then the iron in the supernate was analyzed by AAS.

Labeled test meals

Study 1

Each test meal consisted of 25 g dry cereal and 2.5 mg Fe as [^{57}Fe]ferric pyrophosphate (A) or [^{57}Fe]ferrous fumarate (B) preweighed into plastic containers. Ascorbic acid (25 mg, food grade; Merck, Darmstadt, Germany) was preweighed into each portion of the cereal and iron mixture. At the time of feeding, 100 g hot deionized water was added and mixed well with the cereal.

Study 2

All test meals consisted of 25 g dry cereal and 2.5 mg Fe as [^{57}Fe]ferrous fumarate preweighed into plastic containers. Ascorbic acid (food grade, Merck) was preweighed into the cereal and iron mixture: 25 mg (A) or 50 mg (B) per test meal, respectively. At the time of feeding, 100 g hot deionized water was added and mixed well with the cereal.

Study protocol

Labeled test meals were administered in the morning after an overnight fast or 3 h after the last formula feeding. Infants were randomly allocated to start with test meal A or B within the 2 studies. The 2 different test meals within each study were administered on 4 consecutive days (AAAA or BBBB) followed by the administration of the alternate test meal 2 wk later. All test meals were fed under close supervision by one of the investigators. Preweighed spoons, bibs, and wipes were used to estimate losses during feeding. All materials used during the administration of labeled test meals were weighed after the infants were fed to calculate the total intake of each labeled test meal. During study 1, any residual labeled test meal was collected after the 6 infants were fed and were analyzed for total iron by AAS and for ^{57}Fe by isotope-dilution mass spectrometry.

A baseline venous blood sample was drawn for determination of iron-status indexes and iron isotopic composition on the first day of the study. Body weight was measured before the serving of the first labeled test meal. No food or fluid was allowed during the next 3 h. On days 2–4 of the study, identical labeled test meals were administered under the same conditions. The second blood sample was drawn on day 18. The alternate labeled test meals were fed on days 18–21 by following the same protocol as used during the first part of the study. The final blood sample was drawn on day 35. Body weights were measured on days 18 and 35.

Blood analyses

Iron-status indexes

Hemoglobin was measured by the cyanomethemoglobin method (Micros OT 18 Automated System; Roche Diagnostic Systems, Vienna). A 3-concentration quality-control material (CBC 18 TM; R&D Systems Inc, Minneapolis) was analyzed together with the samples. Plasma ferritin was analyzed by nephelemetry (N-Latex Ferritin Kit; Behring, Marburg, Germany). Control materials at 2 ferritin concentrations supplied with the kit were analyzed in parallel for quality control.

Iron isotopic composition

Samples of whole blood were analyzed in duplicate for iron isotope composition according to the method of Kastenmayer et al (7) with the following modifications. Samples of whole blood (≈400 mg) were mineralized after the addition of concentrated nitric acid and 30% H_2O_2 by using a microwave digestion system with pressure control (MDS-2000; CEM Corp, Matthews, NC) and lined digestion vessel perfluoralcoxy copolymere bombs (max pressure 1.4×10^6 Pa, or 14 bars). The digest was evaporated to dryness under filtered nitrogen gas and dissolved twice in concentrated hydrochloric acid before being taken up in 5 mol HCl/L. Iron was separated from matrix elements by anion-exchange chromatography using disposable plastic columns for gravity flow (Poly-Prep column, bed volume 2 mL; Bio-Rad Laboratories, Glattbrugg, Switzerland) prefilled with anion exchange resin (AG1-X8, 200–400 mesh, Cl-form; Bio-Rad Laboratories). Columns were rinsed with 50 mL of 2 mol HNO_3/L and reconditioned with 80 mL of 1 mol HCl/L before use. The columns were washed with 25 mL of 5 mol HCl/L after sample loading. Iron was eluted with 15 mL of 0.5 mol HNO_3/L, evaporated to dryness, and dissolved in 1 mL concentrated HCl before being taken up in 5 mol HCl/L for a second passage through the ion-exchange column. Total iron was determined by AAS. Samples were evaporated to dryness and redissolved in 0.1 mol HNO_3/L to a final iron concentration of 0.05 mol/L. To check for sample contamination during processing, blanks were prepared by using ^{57}Fe-enriched samples.

Iron isotope ratios were measured by using a thermal ionization quadrupole mass spectrometer (model THQ; Finnigan MAT) as described previously (7). Accuracy of the isotope ratio measurements was verified by analysis of $Fe(NO_3)_3$ (Merck) as a standard for natural isotopic composition. Relative accuracy of the ^{54}Fe-^{56}Fe, ^{57}Fe-^{56}Fe, and ^{58}Fe-^{56}Fe ratios was within 1% (study 1) and 3% (study 2) of the accepted absolute iron isotopic composition determined by the Central Bureau for Nuclear Measurements, Geel, Belgium (8). Relative external precision was 0.3%, 0.2%, and 0.5% in study 1 (n = 11) and 0.5%, 0.2%, and 1.1% in study 2 (n = 12) for the ^{54}Fe-^{56}Fe, ^{57}Fe-^{56}Fe, and ^{58}Fe-^{56}Fe ratios. Isotope ratios of baseline blood samples were not significantly different from ratios of iron standards and relative external precision was comparable with that obtained from analysis of iron standards.

All acids used during analysis of iron and calcium as well as for preparation of iron samples for mass spectrometric analysis were purified by sub-boiling in a quartz still (Kürner Analysentechnik, Rosenheim, Germany). Other chemicals were analytic grade purity. Only ultrapure water (18 MΩ) was used. To minimize contamination with vessel materials, only acid-washed quartz, polytetrafluoroethylene, and polyethylene containers were used.

Calculation of iron bioavailability

The amount of ^{57}Fe incorporated into erythrocytes 14 d after administration of the labeled test meals was calculated on the basis of the shift in iron isotope ratios in blood samples and the total amount of circulating iron in the body as described in detail previously (7). Circulating iron was estimated from blood volume and hemoglobin concentration. Blood volume calculations were based on body weight and the assumption of 65 mL blood/kg body wt (9). For calculations of iron bioavailability, 90% incorporation of newly absorbed iron into erythrocytes was assumed.

Food analysis

Aliquots of the infant cereals used for acceptability testing and for preparation of labeled test meals were analyzed in duplicate for their iron and calcium contents by AAS. Samples were reduced to ash in quartz Erlenmeyer flasks in a muffle furnace at 520 °C for 48 h. The ash was redissolved in 4 mL sub-boiled HCl and diluted to 25 mL with ultrapure water before analysis of calcium and iron. For calcium analysis, lanthanum was added to a final concentration of 1%. Iron was analyzed by a standard addition technique. Accuracy of the methods was tested by analyzing 2 standard reference materials: Whole Meal Flour (BCR no. 189; Community Bureau of References, Brussels) and Total Diet (no. 1548; National Institute of Standards and Technology, Gaithersburg, MD). Ascorbic acid was measured by electrometric titration with 2,6-dichlorophenol indophenol (10, 11) and phytic acid content was determined by the HPLC technique of Sandberg and Ahderinne (12). Energy content was measured by bomb calorimetry.

Statistics

The statistical analysis of iron bioavailability in the 2 studies was done by crossover analysis with analysis of variance according to Senn (13). Values for iron bioavailability were logarithmically transformed before statistical analysis. Results are presented as geometric means and ranges.

RESULTS

Stable-isotope labels

The iron contents of [^{57}Fe]ferrous fumarate and [^{57}Fe]ferric pyrophosphate were 34.5% and 20.2%, respectively. Equivalent commercial iron compounds analyzed in parallel contained 33.6% Fe and 22.2% Fe. The isotopic composition of the labeled compounds was identical to the isotopic composition of the metallic ^{57}Fe.

Solubility of the labeled compounds was similar to that found for commercial iron fortificants. Iron fumarate dissolved completely in 0.06 mol HCl/L after 20 min whereas the solubility of iron pyrophosphate was only 7–8% after 180 min.

Food analysis

The wheat and soy infant cereal used for the preparation of the labeled test meals contained 0.50 (study 1) or 0.36 (study 2) mg native Fe, 67.5 (study 1) or 61.5 (study 2) mg Ca, 46 (study 1) or 50 (study 2) mg phytic acid, and 465 kJ/serving (25 g dry material). No ascorbic acid was detected in the cereal products. The cereal products used for the acceptability testing contained 2.9–3.4 mg Fe, 62.5–70.5 mg Ca, and 23.1–24.1 mg ascorbic acid/25 g dry material.

Infant characteristics

Fourteen boys and 6 girls aged 6–12 mo and weighing 6.8–11.4 kg participated in the 2 studies. At the start of the study, individual hemoglobin concentrations ranged from 97 to 138 g/L. Six infants were anemic (hemoglobin < 110 g/L). Plasma ferritin was in the range of 6–95 μg/L; 2 infants had plasma ferritin concentrations below the detection limit of 5 μg/L (**Table 1** and **Table 2**). The infants consumed ($\bar{x}$ ± SD) 97.6 ± 0.8% of the labeled test meals during study 1 and 97.9 ± 0.7% during study 2.

TABLE 1
Individual data on iron status and iron bioavailability in the infants in study 1

Subject	Hemoglobin	Ferritin	Iron bioavailability	
			Ferric pyrophosphate	Ferrous fumarate
	g/L	*μg/L*	*%*	
1	113	12	2.6	12.0
2	109	14	1.0	3.8
3	124	95	<0.66	1.7
4	138	29	1.4	1.8
5	115	37	<0.66	10.9
6	118	6	1.2	14.7
7	116	13	2.7	6.9
8	101	28	1.5	1.7
9	123	37	0.7	2.6
10	123	59	0.7	2.0
Geometric mean	—	—	1.3	4.1[1]
+1 SD	—	—	2.2	9.9
−1 SD	—	—	0.8	1.7

[1] Significantly different from ferric pyrophosphate, $P < 0.008$.

Iron bioavailability

Individual data for iron bioavailability are given in Tables 1 and 2. In study 1, iron bioavailability was significantly higher from [^{57}Fe]ferrous fumarate (geometric mean: 4.1%; range: 1.7–14.7%) than from [^{57}Fe]ferric pyrophosphate (geometric mean: 1.3%; range: 0.7–2.7%; $n = 8$, $P = 0.008$). The mean difference in iron bioavailability was 212% (ferrous fumarate compared with ferric pyrophosphate; 95% CI: 52%, 539%). Because of the more pronounced variation in the results in study 2 compared with our earlier data (6), we were not able to detect any significant change in iron bioavailability after doubling the ascorbic acid content of the test meal. Geometric mean iron bioavailability was 3.4% (range: 1.9–6.6%) and 4.2% (range: 1.2–18.7%; $n = 9$, $P = 0.31$) from the test meals with 25 and 50 mg ascorbic acid added, respectively. Erythrocyte incorporation was below the detection limit for iron bioavailability calculations (<0.66%) in 2 infants after intake of [^{57}Fe]ferric pyrophosphate (study 1) and in 1 infant after intake of the test meals with 25 mg ascorbic acid/serving (study 2). Data from these infants were excluded from the statistical analysis. A sensitivity analysis replacing values below the detection limit with the value 0.33% did not change the results.

DISCUSSION

Infants are nutritionally vulnerable during the weaning process, when human milk or infant formula is gradually replaced by semisolid foods. Iron nutrition during the weaning period is of particular concern because the timing of the introduction of weaning foods usually coincides with increased iron requirements due to rapid growth at ≈4–6 mo of age (14). Thus, it is very important to ensure adequate quantities of bioavailable iron in weanlings' diets (15). Cereal is often one of the first foods given to infants in both industrialized and developing countries. Industrially produced infant cereals provide an excellent vehicle for targeted food fortification of infants and young children in societies in which the cost of these foods does not limit their use. The effect of iron-fortified infant cereals on iron nutrition depends on the composition of the product, the quantity consumed, the fortification level, and the bioavailability of the iron compound. One of the main positive determinants of iron stores in 8-mo-old British infants was shown to be consumption of commercial baby foods (16). Although no detailed information was given about the composition of the products consumed by the infants in that study, it can be assumed that they contained added iron and ascorbic acid. A study in Chile showed that iron-fortified infant rice cereal (550 mg elemental Fe/kg dry product) contributed substantially to preventing iron deficiency anemia when fed together with infant formula containing ascorbic acid (17). However, these results are difficult to extrapolate to the normal feeding pattern in most countries because cereal consumption in the cited studies was considerably higher than usually observed. A recent study in Honduras indicated that the introduction of iron-fortified complementary foods containing ferrous sulfate (50 μg Fe/g wet product) is beneficial for iron nutrition in breast-fed infants (18). However, although infants fed iron-fortified foods had significantly higher iron intakes, hematocrit, and hemoglobin and serum ferritin concentrations than did the exclusively breast-fed infants at 6 mo of age, the introduction of iron-fortified foods was not sufficient to prevent anemia in all of the study infants.

Very limited information on the bioavailability of different iron compounds in infants has been reported and no data on the bioavailability of iron from ferric pyrophosphate or ferrous fumarate have been available until now. In the present study, we measured iron bioavailability in healthy infants from these 2 iron fortificants added to an infant cereal. Special care was taken to ensure that the characteristics of the labeled iron compounds were comparable with commercial equivalents added to infant cereals to be able to extrapolate our results to iron bioavailability from commercial iron-fortified infant cereals. The fortification level of the cereals used in this study was similar to that of industrially manufactured products, ie, 10 mg Fe/100 g dry product. The results from the present study clearly show that iron bioavailability from the alternative iron compound, ferrous fumarate, which is soluble in dilute acid, is significantly higher than that from the commonly used compound, ferric pyrophosphate. Bioavailability from a soluble iron compound, ferrous

TABLE 2
Individual data on iron status and iron bioavailability in the infants in study 2[1]

Subject	Hemoglobin	Ferritin	Iron bioavailability	
			25 mg ascorbic acid	50 mg ascorbic acid
	g/L	*μg/L*	*%*	
11	119	8	2.1	1.9
12	125	58	1.9	2.9
13	104	<5	4.0	18.7
14	97	23	4.0	1.2
15	131	20	<0.66	4.2
16	112	23	3.4	2.6
17	137	<5	3.8	6.4
18	97	10	5.6	10.6
19	106	47	2.0	4.5
20	115	9	6.6	3.8
Geometric mean	—	—	3.4	4.2
+1 SD	—	—	5.3	9.3
−1 SD	—	—	2.2	1.9

[1] In this study, all infants were fed cereal fortified with ferrous fumarate. There was no significant difference in mean iron bioavailability between groups.

sulfate, is usually used as a reference in studies in which bioavailability from different iron compounds is evaluated. Although no direct comparison with ferrous sulfate was included in the present study, the results of our previous study in healthy infants (19) indicated that iron bioavailability from ferrous sulfate added to a similar wheat and soy cereal was in the same range (<0.9–9.1%; $n = 6$) as that from the test meals labeled with ferrous fumarate used in the present study.

Iron bioavailability from iron fortification compounds depends not only on the characteristics of the compounds per se but also on the overall composition of the diet. The infant cereal used in this study was based on white-wheat flour and soy and contained moderate amounts of phytic acid, a strong inhibitor of iron absorption in infants (6). In this study, ample amounts of ascorbic acid, a potent enhancer of iron absorption in infants (6, 20), were added to all test meals, resulting in molar ratios of 3.2:1 to 6.3:1 relative to added iron. Earlier studies of iron absorption from infant cereals fortified with ferrous sulfate in adult women showed significant increases in iron absorption after addition of ascorbic acid. Derman et al (21) reported that mean iron absorption was increased 3.7- and 6.2-fold by the addition of ascorbic acid at molar ratios of 1.1:1 and 2.4:1 relative to added iron, respectively. In a study of iron bioavailability in infants, we reported relatively high mean iron bioavailability (≈8.5 %) from ferrous sulfate added to infant cereals with low contents of phytic acid (22). The addition of ascorbic acid in that study was equivalent to a molar ratio of 2:1 relative to iron (22). However, the addition of approximately the same amount of ascorbic acid to infant cereals made from high-extraction rate wheat flour and soy flour (less refined), containing relatively high contents of phytic acid, resulted in lower iron bioavailability (19). Thus, the ability of ascorbic acid to overcome the inhibitory effect of phytic acid clearly depends both on the amount of phytic acid in the food and on the amount of ascorbic acid present (23). On the basis of these data from study 2, iron bioavailability from the infant cereal fortified with ferrous fumarate in the present study would not be expected to be greatly inhibited by the content of phytic acid in the test meals because of the relatively high molar ratios of ascorbic acid to phytic acid, 2.1:1 and 4.1:1, respectively, for 25 and 50 mg added ascorbic acid.

The results of the present study show that intake of one serving of an infant cereal fortified with ferric pyrophosphate or ferrous fumarate would provide a mean of 32.5 compared with 102.5 μg bioavailable Fe, equivalent to 4.3% and 14%, respectively, of the estimated daily requirement of absorbed iron in infants aged 4–12 mo (0.75 mg/d) (24). The energy content per serving (465 kJ) would contribute ≈13% of the average energy allowance for infants aged 6–12 mo (25). Thus, the amount of bioavailable iron supplied by the infant cereal fortified with ferric pyrophosphate is limited and products fortified with this iron compound may not be expected to have any major effect on iron nutrition. When ferrous fumarate was used as the iron fortificant, the cereal provided a balanced amount of bioavailable iron relative to the energy content. These results show that infant cereals with relatively low phytic acid content, in combination with sufficient amounts of added ascorbic acid, can provide adequate quantities of iron to rapidly growing infants, provided that an iron fortificant with high relative bioavailability is added.

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REFERENCES

1. Dallman PR. Progress in the prevention of iron deficiency in infants. Acta Paediatr Scand 1990;365:28–37.
2. Fomon SJ. Bioavailability of supplemental iron in commercially prepared dry infant cereals. J Pediatr 1987;110:660–1.
3. Fuchs GJ, Farris RP, DeWier M, et al. Iron status and intake of older infants fed formula versus cow milk with cereal. Am J Clin Nutr 1993;58:343–8.
4. Hurrell RF. Preventing iron deficiency through food fortification. Nutr Rev 1997;55:210–22.
5. Hurrell RF, Furniss DE, Burri J, Whittaker P, Lynch SR, Cook JD. Iron fortification of infant cereals: a proposal for the use of ferrous fumarate or ferrous succinate. Am J Clin Nutr 1989;49:1274–82.
6. Davidsson L, Galan P, Kastenmayer P, et al. Iron bioavailability studied in infants: the influence of phytic acid and ascorbic acid in infant formulas based on soy isolate. Pediatr Res 1994;36:816–22.

7. 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.
8. 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.
9. Fomon SJ, Janghorbani M, Ting BTG, et al. Erythrocyte incorporation of ingested 58-iron by infants. Pediatr Res 1988;24:20–4.
10. AOAC method 967:21. In: Hilrich K, ed. Official methods of analysis of the AOAC. Arlington, VA: AOAC International, 1990:1058–9.
11. Harris LJ, Mapson LW, Wang YL. Vitamin methods. A simple potentiometric method for determining ascorbic acid suitable for use with coloured extracts. Biochem J 1992;36:183–95.
12. 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.
13. Senn S. The AB/BA design with normal data. In: Barnett V, ed. Cross-over trials in clinical research. Chichester, United Kingdom: John Wiley & Sons Ltd, 1993:31–71.
14. Dallman PR. Changing iron needs from birth through adolescence. In: Fomon SJ, Zlotkin S, eds. Nutritional anemias. Nestlé nutrition workshop series. New York: Raven Press, 1992:29–39.
15. Dallman PR. Iron deficiency in the weanling: a nutritional problem on the way to resolution. Acta Paediatr Scand Suppl 1986;323: 59–67.
16. Wharf SG, Fairweather-Tait SJ, Cook JD. Factors affecting iron stores in infants 4–18 mo of age. Eur J Clin Nutr 1997;51:504–9.
17. Walter T, Dallman PR, Pizarro F, et al. Effectiveness of iron-fortified infant cereal in prevention of iron deficiency anemia. Pediatrics 1993;91:976–82.
18. Dewey KG, Cohen RJ, Rivera LL, Brown KH. Effects of age of introduction of complementary foods on iron status of breast-fed infants in Honduras. Am J Clin Nutr 1998;67:878–84.
19. Davidsson L, Mackenzie J, Kastenmayer P, et al. Dietary fiber in weaning cereals: a study of the effect on stool characteristics and absorption of energy, nitrogen, and minerals in healthy infants. J Pediatr Gastroenterol Nutr 1996;22:167–79.
20. Fairweather-Tait S, Fox T, Wharf SG, Eagles J. The bioavailability of iron in different weaning foods and the enhancing effect of a fruit drink containing ascorbic acid. Pediatr Res 1996;37:389–94.
21. 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.
22. Davidsson L, Galan P, Cherouvrier F, et al. Bioavailability in infants of iron from infant cereals: effect of dephytinization. Am J Clin Nutr 1997;65:916–20.
23. 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.
24. Fomon SJ. Iron. In: Fomon SJ, ed. Nutrition of normal infants. St Louis: Mosby Year Book Inc, 1993:239–60.
25. National Research Council. Recommended dietary allowances. 10th ed. Washington, DC: National Academy Press, 1989.