

## Bioavailability in infants of iron from infant cereals: effect of dephytinization<sup>1,2</sup>

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**ABSTRACT** Iron bioavailability from an infant cereal made of wheat flour with a low extraction rate (70%) and cow milk was measured in infants by using a stable-isotope technique. A dephytinized infant cereal was prepared by adding commercial phytase during manufacture, resulting in degradation of 88% of the native phytic acid. Paired comparisons were made to evaluate the effect of phytic acid on iron bioavailability. Both infant cereals contained identical amounts of ascorbic acid and had a molar ratio of ascorbic acid to iron of 2:1. Iron was added as ferrous sulfate. No difference in iron bioavailability was observed in this study; the geometric mean was 8.7% (range: 3.8–16.9%) and 8.5% (range: 3.4–21.4%) from the cereal with native phytic acid (0.08% phytic acid) and the dephytinized cereal (0.01% phytic acid), respectively. Dephytinization of infant cereals containing a relatively low native phytic acid content and high amounts of ascorbic acid is thus unnecessary to ensure adequate bioavailability of iron. *Am J Clin Nutr* 1997;65:916–20.

**KEY WORDS** Iron, bioavailability, infants, infant cereal, phytic acid, phytase, ascorbic acid

### INTRODUCTION

Iron deficiency anemia is still a major nutritional problem in the world, affecting primarily infants, children, and fertile women in the developing world but also in industrialized countries (1, 2). Iron deficiency anemia in infants is particularly important because it can lead to negative changes in psychomotor and mental development, some of which are irreversible (3). Breast-fed infants generally have an adequate iron status during the first 4–6 mo of life but after this time, when their iron stores have been depleted, additional dietary iron needs to be supplied to meet the infant's requirement (4). During this period of early life, weaning foods such as infant cereals can supply iron. Commercial infant cereals in industrialized countries are usually precooked, roller-dried cereal flours that are composed primarily of cereals with a low extraction rate, such as wheat, rice, and corn and that are fed with milk. Alternatively, complete infant cereals are produced with complementary protein added as cow milk or soy protein. Both types of infant cereals are normally fortified with iron.

Unfortunately, iron bioavailability from cereal products is usually low because of the presence of phytic acid (*myo*-inositol hexaphosphate), which is the major phosphorus storage compound in the grain. The strong inhibitory effect of phytic

acid on iron bioavailability, however, can be overcome by phytic acid degradation (5–7) or by the addition of sufficient amounts of ascorbic acid, a potent enhancer of iron absorption (7–10). Although the technology exists for phytic acid degradation in infant cereals, by adding exogenous phytase, this would be necessary only if satisfactory iron bioavailability could not be achieved more simply by the addition of ascorbic acid.

In this study we used a stable-isotope technique to measure iron bioavailability in infants of iron from an iron-fortified ( $\text{FeSO}_4$ ) infant cereal composed primarily of low-extraction wheat flour and cow milk. Iron bioavailability from a cereal containing its native phytic acid content was compared with the bioavailability from a similar cereal product in which phytic acid had been 88% degraded. Both infant cereals contained identical amounts of ascorbic acid and had a molar ratio of ascorbic acid to iron of 2:1.

### SUBJECTS AND METHODS

#### Subjects

Twelve full-term infants (eight boys and four girls) with a mean age of 32 wk (range: 21–39 wk) and a mean body weight of 8.4 kg (range: 7.1–9.1 kg) were recruited for the study at a health center in Paris. All infants participating in the study had been introduced to semisolid foods before enrollment in the study. The infants were fed an iron-fortified cereal product (Cérélac; Nestlé SA, Vevey, Switzerland) containing the native phytic acid content for  $\approx 2$  wk before the study. This prefeeding period was included in the protocol to ensure that the infants accepted the taste of the cereal and that the serving size could be consumed in one feed.

Information about the aims and the procedures of the study was given to the infants' parents and informed consent was obtained from at least one parent. The protocol was approved by the Ethical Committee of Paris-Cochin.

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### Test meals

Infant cereals composed primarily of white wheat flour (70% extraction) and skim milk powder were produced especially for the study at a Nestlé Research and Development Center (Orbe, Switzerland). The composition of the cereals was similar to that of a commercial product (Cérélac; Nestlé SA). The wheat flour used for cereal B was the same as that used for cereal A, but was dephytinized by the addition of phytase (EC 3.1.3.8; Alko Ltd, Helsinki) to the aqueous slurry. The slurry was then cooked by steam injection and roller-dried. The skim milk powder was dry-mixed with the roller-dried cereal base. The products were produced without added iron, but fortified with calcium ( $\text{CaCO}_3$ ) and a vitamin premix including ascorbic acid. Each test meal consisted of 25 g infant cereal mixed with 100 g hot deionized distilled water.

### Study design

Infants were randomly assigned to start with test meal A or B. Four test meals labeled with stable isotopes were given on 4 consecutive days in the order ABAB or BABA. All test meals were administered after an overnight fast, under close supervision by the investigators. Venous blood samples were drawn on days 1 (baseline) and 19 (14 d after intake of the last test meal) to measure iron-status indexes and stable-isotope composition of hemoglobin.

### Stable isotopes

Enriched stable isotopes of  $^{58}\text{Fe}$  and  $^{57}\text{Fe}$  were purchased from Medgenix (Ratingen, Germany) and Isotec (St Quentin, France) in the form of iron metal. Stable-isotope solutions were prepared as  $^{58}\text{FeSO}_4$  and  $^{57}\text{FeSO}_4$ , according to the procedure described earlier (11). The isotopic composition of the labels was measured by thermal-ionization mass spectrometry (TIMS) (model THQ; Finnigan MAT, Bremen, Germany). The  $^{58}\text{Fe}$  label contained 93.03%  $^{58}\text{Fe}$ , 6.901%  $^{57}\text{Fe}$ , and 0.0692%  $^{56}\text{Fe}$ . The  $^{57}\text{Fe}$ -enriched solution contained 95.18%  $^{57}\text{Fe}$ , 1.97%  $^{58}\text{Fe}$ , and 2.85%  $^{56}\text{Fe}$ . Each infant received a total dose of 22.2  $\mu\text{mol}$  (1.29 mg)  $^{58}\text{Fe}$  and 83.5  $\mu\text{mol}$  (4.76 mg)  $^{57}\text{Fe}$  during administration of the labeled test meals. Additional iron with the normal isotopic composition ( $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ , Merck, Darmstadt, Germany) was added to the  $^{58}\text{Fe}$  label to keep the total dose of iron constant between administrations. The total dose of iron was 44.8  $\mu\text{mol}$  (2.5 mg) per test meal.

### Analysis of infant cereals

Analysis of iron and calcium was made by atomic-absorption spectrometry (model 975; Varian Techtron, Mulgrave, Australia). Samples of infant cereals were ashed in quartz Erlenmeyer flasks in a muffle furnace at 520 °C for 48 h. Ash was dissolved in subboiled hydrochloric acid and diluted to 25 mL with ultrapure water. Iron was measured by using a standard addition technique. Lanthanum oxide (Fluka, Buchs, Switzerland) was added to the diluted digest to a final concentration of 1% lanthanum before measuring the calcium content. Reference materials—Wheat Flour 1567a (National Institute of Standards and Technology, Gaithersburg, MD) and Nonfat Milk Powder 1549 (National Institute of Standards and Technology)—were analyzed together with the diets. The analyzed values were found to be within the certified ranges for iron and calcium. Nitrogen was analyzed by the Kjeldahl technique and

protein was calculated as nitrogen  $\times$  6.25. Phytic acid was measured by cerium sulfate precipitation according to a modification of the method by Makover (12). Ascorbic acid was analyzed by electrometric titration with 2,6-dichlorophenol indophenol (13, 14).

### Analysis of blood samples

The concentration of hemoglobin was analyzed with a Coulter Counter S 560 (Coultronics, Margency, France) and serum ferritin was measured by an enzyme-linked immunosorbent assay (15). Blood samples drawn on days 1 and 19 were analyzed in duplicate for their isotopic composition according to the method of Kastenmayer et al (11). Samples of whole blood ( $\approx$ 400 mg) were mineralized in a microwave digestion system (MDS-81D; CEM Corporation, Matthews, NC) with concentrated  $\text{HNO}_3$  and 30%  $\text{H}_2\text{O}_2$ . The clear digest was evaporated to dryness under filtered nitrogen and dissolved in concentrated hydrochloric acid twice before being taken up in 5 mol  $\text{HCl/L}$ . Iron was separated from matrix elements by anion-exchange chromatography by using a modified version of the method of Fasset et al (16). Total iron was determined by atomic-absorption spectrometry. Samples were evaporated to dryness in polytetrafluoroethylene tubes under filtered nitrogen and redissolved in 0.1 mol  $\text{HNO}_3/\text{L}$  to a final iron concentration of 0.05 mol/L. To check for contamination of samples during processing, chemistry blanks were prepared by using an  $^{57}\text{Fe}$ -enriched solution.

Isotope ratios were measured by using a computer-controlled TIMS (model THQ) equipped with a secondary electron multiplier (SEM), a 13-sample turret, and a reference pyrometer. Analysis was done with single rhenium filaments by using a silica gel ionization enhancement technique (17). Aluminum was added to further increase the ion yield (18). During analysis, the filaments were heated to 1320 °C within 30 min to obtain a beam of 3–5 V on the SEM operated at an amplification of 25 relative to the Farady detector. Three blocks with 12 scans each across the isotope pattern were collected in the peak jumping mode. Data were analyzed for outliers by a Dixon test (19). Measurements that had an internal SD greater than the target precision of 0.5% for  $^{57}\text{Fe}:^{56}\text{Fe}$  and 1% for  $^{58}\text{Fe}:^{56}\text{Fe}$  were discarded and the analysis was repeated. Accuracy of the mass spectrometric measurements had been verified earlier by analyzing standards containing known enrichments of  $^{57}\text{Fe}$  and  $^{58}\text{Fe}$  (11).

Only ultrapure water (18 M $\Omega$ ) from a Millipore system (Millipore AG, Zürich, Switzerland) and acids purified by subboiling distillation were used during the preparation of stable-isotope solutions as well as for all analytical work. Other chemicals were analytical grade purity. To minimize contamination through vessel materials, only acid-washed quartz, polytetrafluoroethylene, and polyethylene containers were used.

### Calculations of iron bioavailability

Iron bioavailability in infants was calculated based on the incorporation of  $^{58}\text{Fe}$  and  $^{57}\text{Fe}$  into red blood cells 14 d after administration (11). Of the initially absorbed iron, 90% was assumed to be incorporated into erythrocytes. Cross-contamination of stable isotopes was corrected for by assuming that the contribution of  $^{57}\text{Fe}$  from the  $^{58}\text{Fe}$  label was negligible (11); the

incorporation of  $^{57}\text{Fe}$  from the  $^{58}\text{Fe}$  label was calculated to represent  $2.0 \pm 0.4\%$  ( $\bar{x} \pm \text{SD}$ ) of the total  $^{57}\text{Fe}$  incorporated in the present study. The amount of  $^{58}\text{Fe}$  ingested with the  $^{57}\text{Fe}$  label, which was incorporated into red blood cells, was then calculated, assuming an identical absorption for both isotopes present in the  $^{57}\text{Fe}$  label (11).

### Statistics

Student's paired *t* test was used for comparisons of iron bioavailability. Values were converted to logarithms before statistical analysis and reconverted to antilogarithms to recover the original values (20). Values for fractional iron bioavailability are given as geometric means  $\pm 1$  SD.

### RESULTS

The amounts of protein, iron, calcium, phytic acid, and ascorbic acid in the test meals are given in Table 1. Eighty-eight percent of the phytic acid in the cereal was degraded in product B. The iron status of the infants (Table 2) showed a large interindividual variation, as was observed in our earlier studies (7, 11), and serum ferritin concentrations indicated that all infants had little or no iron stores. Infants who were iron deficient, or borderline iron deficient, were given iron supplements after the study.

The incorporation of iron isotopes in whole blood drawn from infant number 2 was found to be below the detection limit. The results for iron bioavailability are therefore based on data from 11 infants (Table 2). The mean geometrical bioavailability was 8.7% (range: 3.8–16.9%) and 8.5% (range: 3.4–21.4%) from test meals A and B, respectively. The values were not significantly different.

### DISCUSSION

During the weaning period there is a gradual increase in energy and nutrients provided by semisolid and solid foods as they replace human milk or infant formula. With the introduction of a more varied diet, dietary components such as phytic acid will be introduced because vegetables and infant cereals are often among the first semisolid foods to be presented to the infant. Traditionally, commercial infant cereals are produced from low-extraction cereal flours with a relatively low content of this strong inhibitor of iron bioavailability.

Few data have been reported on the bioavailability to infants of iron from infant foods, although it was shown recently that high amounts of phytic acid in infant cereals based on wheat and soy are inhibitory (21). In the present study, we investigated an infant cereal composed primarily of low-extraction wheat flour and cow milk before and after dephytinization.

TABLE 1  
Contents of protein, iron, calcium, phytic acid, and ascorbic acid in the infant cereals

	Protein	Iron <sup>2</sup>	Calcium	Phytic acid	Ascorbic acid
	mg	$\mu\text{mol}$	$\mu\text{mol}$	%	$\mu\text{mol}$
Infant cereal A	157	1.91	152	0.08	4.02
Infant cereal B	156	1.92	154	0.01	4.01

<sup>1</sup> Values are given per 1 g dry product.

<sup>2</sup> Values include iron added as stable isotopes and with normal isotopic composition.

TABLE 2  
Geometric mean values for iron-status indexes and iron bioavailability in the infants

	Hemoglobin	Serum ferritin	Iron bioavailability	
			Cereal A	Cereal B
	g/L	$\mu\text{g/L}$	%	
Mean	112	4.6	8.7	8.5
+ 1 SD	124	12.9	14.5	15.6
- 1 SD	101	1.7	5.2	4.6

<sup>1</sup> *n* = 11.

This infant cereal had a much lower content of phytic acid than did the infant cereals used earlier. We found that, in the presence of the relatively high concentration of ascorbic acid in the test meal (equivalent to commercial weaning cereals), there was no significant difference in iron bioavailability between the two test meals.

The lack of effect of the enzymatic degradation of phytic acid in the cereal composed primarily of low-extraction wheat flour used in our study can most likely be attributed to the relatively high content of ascorbic acid, which counteracted the inhibitory effect of phytic acid on iron bioavailability. Ascorbic acid was added at a molar ratio of 2:1 relative to iron, resulting in a relatively high iron bioavailability,  $\approx 8.5\%$ , from both the phytic acid-containing infant cereal and the dephytinized product. Ascorbic acid has on several occasions been shown to overcome the inhibitory effect of phytic acid on iron bioavailability (7–10). The ability of ascorbic acid to overcome the inhibitory effect of phytic acid clearly depends on both the native content of phytic acid in the food and the amount of ascorbic acid added. The amount of ascorbic acid added to the infant cereal containing the native content of phytic acid (1.21  $\mu\text{mol/g}$ ) in the present study resulted in a molar ratio of ascorbic acid to phytic acid of 3.3:1, which presumably overcame the inhibitory effect of phytic acid on iron absorption. In an earlier study with infant cereals containing high-extraction wheat and soy flours (21), the initial amount of phytic acid (11.67  $\mu\text{mol/g}$ ) was much higher and, even though the amounts of ascorbic acid added were similar to those in the present study, the molar ratio of ascorbic acid to phytic acid was only 0.4:1. In this study, iron bioavailability in infants measured with a stable-isotope technique was low (range: 1.0–5.4%) (21).

Whether sufficient ascorbic acid could be added to infant cereals containing higher amounts of phytic acid to give an adequate iron bioavailability is not known and phytic acid degradation achieved by adding phytase appears to be an interesting approach with these products. No data are available

on the influence of dephytinization of infant cereals on mineral bioavailability in infants. However, in earlier studies of the effect of the phytic acid degradation via activation of the native phytase in wheat bran, wheat flour, and rye (served as bread), a significantly increased iron absorption was observed in adult subjects (5, 22).

Although we did not show that the wheat-flour, cow milk-based infant cereal was inhibitory to iron bioavailability in the absence of ascorbic acid, there was theoretically enough phytic acid in infant cereal A to bind all iron. The initial amount of phytic acid in a serving of infant cereal A was relatively low (30  $\mu\text{mol}/25$  g cereal) because it was manufactured from low-extraction wheat flour and milk powder. The test meal contained 48  $\mu\text{mol}$  Fe, mainly as added  $\text{FeSO}_4$ , resulting in a molar ratio of iron to phytic acid of 1.6:1. However, there was theoretically enough phytic acid to bind all the iron because 1 mol phytic acid can bind up to 6 mol ferrous iron, assuming that other metal ions or amino acid side chains had not occupied the phosphate binding sites. In an earlier study measuring iron absorption in adults fed a liquid test meal based on soy isolate (6), phytic acid in the absence of ascorbic acid was shown to be a potent inhibitor of iron absorption until the phytic acid was reduced to  $<15$   $\mu\text{mol}$  in a 280-g meal, resulting in a molar ratio of iron to phytic acid  $>7:1$ . The small amount of phytic acid in the dephytinized infant cereal fed in the present study (molar ratio of iron to phytic acid of 12.5:1) would therefore not be expected to inhibit iron bioavailability.

Both test meals fed in this study contained added ascorbic acid because commercial infant cereals in Europe normally contain ascorbic acid to improve iron bioavailability. However, from an experimental point of view, this study design complicates the interpretation of the results because we cannot explain why there was no increase in iron bioavailability from the dephytinized cereal product. In theory, three explanations are possible. First, as we proposed, the high amount of ascorbic acid in both test meals completely overcame the inhibitory effect of the relatively low amounts of phytic acid in both cereal products and enhanced iron bioavailability similarly from both test meals. Alternatively, it could be argued that neither of the cereal products was inhibitory to iron absorption. This is unlikely because we showed a fourfold increase in iron absorption in adult humans after dephytinization of a similar wheat flour served as a porridge (23). The third possibility is that both cereal products were inhibitory and that the addition of ascorbic acid did not overcome this negative effect. This is also unlikely because ascorbic acid is a well-known iron absorption enhancer and as yet unpublished data from our group have shown that iron absorption from the same 25 g low-extraction wheat flour containing 2.5 mg Fe as ferrous sulfate served as a porridge with water was increased from 2.5% to 8.5% in adults after the addition of 50 mg ascorbic acid (JD Cook, MB Reddy, J Burri, M-A Juillerat, Hurrell RF, unpublished observations, 1995).

Most commercial infant foods are iron fortified and the importance of this food fortification in infant formulas on the decline of the frequency of anemia has been recognized (24). However, the effect of iron fortification of other infant foods, eg, weaning foods such as infant cereals, on iron nutrition is not well established (25). Infant formulas are usually fortified with soluble, and highly bioavailable, iron compounds such as ferrous sulfate. Cereal products on the other hand are much more

difficult to fortify with iron because of the problems related to rancidity, color changes, and off flavors during storage after addition of soluble iron compounds (26). Thus, iron is often added as less-soluble (and less bioavailable) compounds to cereals. Infant cereals are commonly fortified with ferric pyrophosphate or ferrous fumarate in Europe and with electrolytic elemental iron in the United States. The relative bioavailability of these compounds compared with ferrous sulfate has not been investigated in infants. However, in adults fed a wheat- and milk-based infant cereal similar to that used in the present study, the relative absorption of iron was 1.0 (ferrous fumarate) and 0.4 (ferric pyrophosphate) (27). The bioavailability of electrolytic iron in adults or infants has not been evaluated because of difficulties in producing a radiolabeled or stable-isotope-labeled compound with particle size and solubility characteristics similar to those of the commercial compound. One study showed a relative absorption of 0.75, relative to ferrous sulfate, when measured in adults fed a farina meal (28). The data from this study are difficult to interpret, however, because of differences in the characteristics of the labeled compound compared with commercial electrolytic iron; thus, the results could be an overestimate of iron bioavailability from the labeled compound (26).

In our study we measured iron bioavailability from infant cereals after the addition of iron in the form of ferrous sulfate. This study design was chosen because of the considerable technical problems (and cost) involved in the preparation of stable-isotope-labeled iron compounds of the type normally added to cereal products. However, the infant cereal used in the present study would normally be fortified with ferrous fumarate and the results can thus be regarded as representative of the bioavailable iron from this particular type of product. Iron bioavailability from infant cereals fortified with elemental iron or ferric pyrophosphate would be expected to be lower. The amount of iron in the infant cereal used in the present study was  $\approx 1.9$   $\mu\text{mol}/\text{g}$  (0.1 mg/g), slightly higher than the 1.34  $\mu\text{mol}/\text{g}$  (0.075 mg/g) normally used to fortify similar European infant cereals, which are reconstituted with water. A 25-g serving of the infant cereals used in this study would provide  $\approx 473$  kJ (113 kcal) and  $\approx 10\%$  of the infant's daily energy requirement (29). At a fractional iron bioavailability of 8.5%, the serving would provide 3.8  $\mu\text{mol}$  (212  $\mu\text{g}$ ) Fe, equivalent to  $\approx 20\%$  of the daily requirement of absorbed iron for boys aged 6–12 mo (30). The infant cereal used in this study can thus be regarded as a useful source of bioavailable iron for rapidly growing infants.

There are few other studies of the bioavailability in infants of iron from weaning cereals. In a study by Fomon et al (31), iron bioavailability from rice cereals with added fruits or fortified with protein were evaluated in infants by incorporation of  $^{58}\text{Fe}$  into red blood cells. The results showed a mean incorporation of 5.4% (range: 2.7–15.4%) from the rice cereal containing fruits and  $\text{FeSO}_4$ , 4.4% (range: 0.6–19.0%) from the protein-fortified cereal containing  $\text{FeSO}_4$ , and 4.0% (range: 2.1–7.8%) from a protein-fortified cereal with ferrous fumarate. Because of differences in the total iron content of the test meals (50.1, 19.7, and 82.4  $\mu\text{mol}$ ; 2.8, 1.1, and 4.6 mg), and in the study design, no conclusions can be drawn regarding the iron bioavailability from the two different iron compounds used. A study of iron absorption in infants from weaning foods containing added  $\text{FeSO}_4$  (32) reported mean iron bioavailability of

3.0% (range: 1.1–21.2%) from a test meal of whole-wheat breakfast cereal containing 39.4  $\mu\text{mol}$  (2.2 mg) Fe and 3.1% (range: 1.2–15.4%) from whole-meal bread with 37.6  $\mu\text{mol}$  (2.1 mg) Fe. The bioavailability of iron increased twofold from both meals when 284  $\mu\text{mol}$  (50 mg) ascorbic acid was added (32).

The data from the present study clearly indicate that infant cereals can supply iron with a relatively high bioavailability, similar to that from infant formula, provided that the iron compound added to cereals has the same relative bioavailability as ferrous sulfate. The fractional iron bioavailability from the infant cereals used in the present study,  $\approx 8.5\%$ , was roughly equivalent to the iron bioavailability observed earlier in infants consuming cow milk-based infant formula (11) or dephytinized soy formula (7), as measured with the same stable-isotope technique and containing similar amounts of iron. It seems probable therefore that the addition of ascorbic acid can overcome the inhibitory effect of phytic acid in low-extraction infant cereals ( $< 0.1\%$  phytic acid). If this is confirmed, it would not be necessary to dephytinize this type of product to obtain adequate iron bioavailability in infants. ■

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