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Dry Cereals Fortified with Electrolytic Iron or Ferrous Fumarate Are Equally Effective in Breast-fed Infants[1–4]

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Abstract

Precooked, instant (dry) infant cereals in the US are fortified with electrolytic iron, a source of low reactivity and suspected low bioavailability. Iron from ferrous fumarate is presumed to be more available. In this study, we compared a dry infant rice cereal (Cereal L) fortified with electrolytic iron (54.5 mg iron/100 g cereal) to a similar cereal (Cereal M) fortified with ferrous fumarate (52.2 mg Fe/100 g) for efficacy in maintaining iron status and preventing iron deficiency (ID) in breast-fed infants. Ascorbic acid was included in both cereals. In this prospective, randomized double-blind trial, exclusively breast-fed infants were enrolled at 1 mo and iron status was determined periodically. At 4 mo, 3 infants had ID anemia and were excluded. Ninety-five infants were randomized at 4 mo, and 69 (36 Cereal L, 33 Cereal M) completed the intervention at 9 mo. From 4 to 9 mo, they consumed daily one of the study cereals. With each cereal, 2 infants had mild ID, a prevalence of 4.2%, but no infant developed ID anemia. There were no differences in iron status between study groups. Iron intake from the study cereals was (mean ± SD) 1.21 ± 0.31 mg·kg^{-1}·d^{-1} from Cereal L and 1.07 ± 0.40 mg·kg^{-1}·d^{-1} from Cereal M. Eleven infants had low birth iron endowment (plasma ferritin < 55 μg/L at 2 mo) and 54% of these infants had ID with or without anemia by 4 mo. We conclude that electrolytic iron and ferrous fumarate were equally efficacious as fortificants of this infant cereal. J. Nutr. doi: 10.3945/jn.110.127266.

Introduction

The iron in breast milk is highly bioavailable (1,2), but its concentration is low [0.2–0.4 mg/L (3.6–7.2 μmol/L)]. Therefore, once the birth iron endowment is exhausted at ~4–6 mo of age, the breast-fed infant largely depends on iron from sources other than breast milk to meet the high iron needs for growth. The occurrence of iron deficiency (ID)[8] in breast-fed infants, including its severe form, ID anemia (IDA), has been documented in a number of countries and localities (3–16). Severe ID at an early age can impair behavioral and neurocognitive development (17–20), so prevention of ID is important.

Most complementary infant foods contain little natural iron. Substantial amounts of iron are provided only by meats and iron-fortified foods, such as infant cereals. The latter are consumed by the great majority of infants in the US. In 2005–2007, 83.2% of infants aged 7.5–9 mo consumed infant cereals (21). Precooked, instant (dry) infant cereals in the US are fortified with electrolytic iron (A-131), a form of iron of small particle size but with low chemical reactivity that does not impart undesirable organoleptic characteristics to precooked instant cereal products (22). More than 90% by weight of the particles of A-131 are <30 μm in diameter and 40% by weight are <10 μm (22). The bioavailability of electrolytic iron has been questioned (23), largely on the basis of the findings of Elwood et al. (24). However, the particle size distribution of the electrolytic iron studied was markedly different from that of A-131 and the conclusion that A-131 is poorly available may not be justified. Although some studies in adults have shown poor availability of A-131 (25), others have shown availability as high as 86% that of ferrous sulfate (26). In the 1 study that examined the efficacy of A-131 as a fortificant of infant cereal (9), the fact that the cereal substantially reduced the prevalence of ID in both breast-fed and formula-fed infants is consistent with at least moderate efficacy of A-131.

Ferrous fumarate, which is more soluble than electrolytic iron, is suitable as a fortificant of infant cereals in that it does not

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[3] This trial was registered at ClinicalTrials.gov as NCT00841061.
[4] Supplemental Figure 1 is available with the online posting of this paper at jn.nutrition.org.
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[7] Deceased.
[8] Abbreviations used: CRP, C-reactive protein; ID, iron deficiency; IDA, iron deficiency anemia; Hb, blood hemoglobin; PF, plasma ferritin; sTfR, soluble transferrin receptor.

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cause discoloration and does not affect taste (27). The iron from ferrous fumarate added to a reconstituted dry cereal was only slightly less available to infants than the iron from ferrous sulfate in a wet-pack fruit-cereal baby food (28). Dry infant cereal made with ferrous fumarate was a more efficacious source of iron for infants than ferrous pyrophosphate when ascorbic acid was added after reconstitution (29). However, no comparison of the relative iron status of infants fed cereal fortified with ferrous fumarate compared with cereal fortified with A-131 electrolytic iron has been reported.

The present study was designed to compare in breast-fed infants ferrous fumarate with A-131 electrolytic iron with regard to efficacy in maintaining iron status and preventing ID when used as fortificants of dry infant cereal. Measures of efficacy were maintenance of iron status and protection from ID. We hypothesized that ferrous fumarate would be more efficacious than electrolytic iron. Because infant formula is a source of iron, infants in the present study were not permitted to receive formula. Because IDA may adversely affect mental development, infants were treated with therapeutic doses of iron when IDA was discovered at any time during the study, and their subsequent data were excluded.

Materials and Methods

Study design. This was a randomized, double-blind trial that involved infants who were exclusively breast-fed during the first 4 mo of life. They were enrolled at 1 mo and were followed through 12 mo of age. At 4 mo, infants were randomly assigned to 1 of 2 study cereals (Cereal L or M). Cereal L was fortified with electrolytic iron and Cereal M was fortified with ferrous fumarate. To be randomized at 4 mo, infants had to be exclusively breast-fed, meaning they received no infant formula and no complementary foods. The infants received the study cereals from 4 to 9 mo (study intervention). A follow-up assessment at 12 mo was included to determine whether there was a sustained effect of cereal on iron status.

Iron status was assessed at 2, 4, 5.5, 7.5, 9, and 12 mo. Infants who developed IDA [PF $<$ 10 μg/L and hemoglobin (Hb) $<$ 105 g/L] were treated with iron and, if IDA occurred by 4 mo, were not randomized. Infants with ID (PF $<$ 10 μg/L) without anemia were randomized. Randomization was performed with stratification by gender (males, females), birth weight ($<$3500 g, $\geq$3500 g), and PF at 2 mo ($<$150 μg/L, $\geq$150 μg/L). Randomization was by computer-generated sequence. Infants returned to the study center every 4 wk for anthropometry and to receive a new supply of study cereals. Capillary blood was obtained during visits at 2, 4, 5.5, 7.5, 9, and 12 mo.

Participants. We studied term infants of either gender with birth weight $>$ 2500 g who were considered normal by their physicians and the investigators. They were born between June 2003 and June 2005. Most were Caucasian with only 1 being mixed race African American/Caucasian. They were exclusively breast-fed at the time of enrollment and the mother intended to breast-feed to 9 mo and did not intend to use formula. The study team was not involved in providing health care to study infants. The study protocol was reviewed and approved by the University of Iowa Institutional Review Board and 1 parent provided written informed consent.

The flow of study infants is indicated in **Supplemental Figure 1**. A total of 111 infants were enrolled at 1 mo of age. Sixteen infants were excluded or were withdrawn by their parents due to scheduling problems. The remaining 95 infants were randomized at 4 mo. Fifty infants were assigned to Cereal L (22 females, 28 males) and 45 to Cereal M (18 females, 27 males). Eight infants did not finish the study because they refused to take the cereal (3 fed Cereal L, 5 fed Cereal M). Eleven infants did not finish the study because of their mothers' insufficient breast milk supply, which necessitated the feeding of formula, and 7 infants did not finish for reasons unrelated to the study, mostly scheduling difficulties. Thus, a total 69 infants (36 Cereal L, 33 Cereal M) completed the intervention at 9 mo and all of these infants completed the study to 12 mo.

Sample size. For regular feeding of an iron-fortified infant cereal to be considered a reliable means of preventing ID in breast-fed infants, we thought that no more than 3% of infants so fed should develop ID. We based our sample size calculation on the assumption that 20% of breast-fed infants who do not regularly receive medicinal iron or an iron-fortified food will become iron deficient at or before 9 mo of age. This value was taken from the literature (9,10). In a study we had completed earlier (16) only 17% of infants in the control group developed ID, but in that study infants were permitted to receive supplemental iron-fortified formula, which was prohibited in the present study. To demonstrate efficacy, i.e. ID in $\leq$3% of infants, for either of the study cereals, 37 infants per group were needed with α = 0.05 and power of 0.80 (30). We assumed that 70% of infants enrolled at 1 mo would complete the study as planned and we therefore planned to enroll 106 infants.

Feedings. Feeding decisions were entirely at the parents' discretion. From the time of enrollment, parents were supplied with vitamin drops that provided each day 0.45 mg (1.57 μmol) retinol acetate, 35 mg vitamin C, and 10 μg cholecalciferol. Other vitamin supplements were permitted. However, to remain in the study, infants could not receive medicinal iron, could not receive any nonstudy cereal, and could not receive any formula.

The study cereals were produced and provided free of charge by the Beech-Nut Nutrition Corporation. The cereals were identical in appearance, ingredients, and composition except for the nature of the fortification iron. The cereals were prepared by cooking an aqueous rice flour slurry containing the fortification iron and other nutrient additives by steam injection and drying with a drum dryer. Cereal L was a commercially available rice cereal fortified with electrolytic iron A-131 and contained 54.5 mg of iron/100 g of dry cereal. Cereal M was a specially prepared rice cereal fortified with ferrous fumarate that contained 52.2 mg of iron/100 g of dry cereal. Each serving (15 g) contained 200 mg calcium, 63 mg

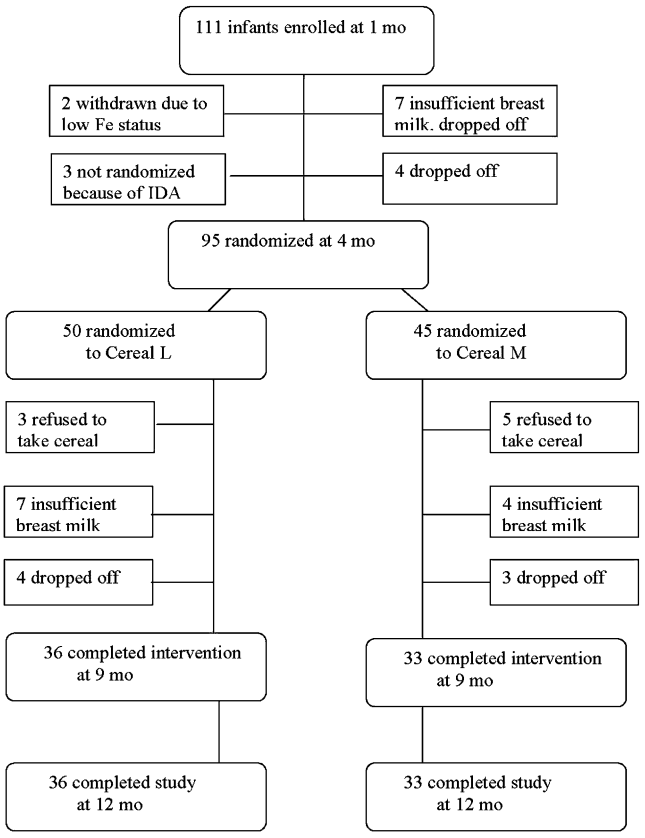

FIGURE 1

phosphorus, 2.0 mg zinc, and small amounts of other minerals, 8 water-soluble vitamins, and tocopherol. Ascorbic acid was included in both cereals at 250 mg/100 g of total dry ingredients prior to cooking and drying. The ascorbic acid level was <10% of the originally added amount 15 mo after production and <2% of the original amount 50 mo after production, which was 1 y after the final feeding.

The study cereals were fed from 4 to 9 mo of age. They were packaged in identical code-labeled cartons. Mothers were instructed to reconstitute the cereal with expressed breast milk, water, or juice, but not with formula. When infants were between 112 and 140 d of age, parents could feed any amount of study cereal. This was done to accommodate the usual parental preference for initially feeding small amounts of complementary foods. When infants were 140 d of age and continuing through 280 d, the parents were requested to feed ~15 g (dry weight) of the study cereal daily, equivalent to 70 g of prepared cereal porridge (i.e. after mixing with liquid) and providing ~8 mg of iron. The amount of study cereal actually consumed was estimated as the difference in weight by weighing cereal containers before dispensing and weighing back returned empty and partially used containers at the next visit.

Procedures. Infants visited the Fomon Infant Nutrition Unit every 28 d up to 9 mo of age and again at 12 mo. Study visits occurred with very few exceptions within 4 d of the designated age. At each visit, weight and length were measured using established methods (31). Before each visit, parents completed a feeding questionnaire that asked whether the infant was fed formula and/or complementary foods and how much of these the infant typically consumed. The questionnaires were checked during visits for completeness by the nursing staff and any ambiguities were clarified. Blood was obtained at 2 mo (56 d), 4 mo (112 d), 5.5 mo (168 d), 7.5 mo (224 d), 9 mo (280 d), and 12 mo.

Blood collection and analysis. Capillary blood was collected by heel stick using a disposable spring-loaded device (Tenderfoot, International Technidyne). Approximately 1 mL of blood was collected into a heparin-treated tube. The Hb concentration and RBC indices (mean corpuscular volume, relative distribution width) were determined immediately on whole blood using a Coulter AcT diff Hematology Analyzer (Coulter). The blood was then centrifuged and plasma was used for the other measurements. Ferritin was measured (initial ~20% of determinations) by RIA using the Quantimune kit (Bio-Rad Laboratories). After the Quantimune kit was withdrawn from the market, ferritin was measured using an immunoradiometric procedure (Ramco catalog no. F-11). The interassay CV was 18.4%. Quantimune values were converted to Ramco values by multiplying by 0.739. Soluble transferrin receptor (sTfR) was measured by enzyme immunoassay using the Ramco kit (catalog no. TF-94). Iron was determined by a colorimetric method using ferrozine as the chromogenic substrate. C-reactive protein (CRP) was determined by a 2-sided ELISA developed in our laboratory (16).

Data analysis. ID was defined as PF < 10 μg/L and anemia was defined as Hb < 105 g/L before 9 mo and as Hb < 100 g/L at 9 mo and older (32). Although a ferritin cutoff of 12 μg/L is widely used, we prefer the more conservative cutoff of 10 μg/L (33), which indicates absence of iron stores. Data for the 5 infants (Table 2) who were not randomized because of poor iron status were not included in the data analysis. Data for infants who left the study were included up to the point of departure and data for infants who were excluded because of supplemental formula feeding (i.e. mothers' insufficient breast milk supply) were included up to the visit preceding the one where formula consumption was reported. A separate analysis was performed restricted to data for the 69 infants who completed the study to 12 mo (per protocol analysis).

Because ferritin is an acute-phase reactant, during inflammation or infection PF may be elevated and not reflective of iron stores. The presence of ID can thus be masked and the size of iron stores overestimated. Unfortunately, concurrent measurement of plasma CRP is not a reliable means of identifying PF values that are acute-phase elevated. From our longitudinal data, it is evident that PF may sometimes be normal in the presence of elevated CRP and, conversely, PF may be clearly elevated when CRP is normal. We presume that these circumstances reflect a lack of concurrence between the timing of increase and subsequent decrease of the 2 acute phase reactants (CRP and PF). We have therefore classified a PF value as elevated if it is 3-fold greater than the mean of the preceding and subsequent values. The classification of elevated PF values therefore rested entirely on 2 adjacent PF values and was not influenced by CRP. The classification was not applied to values at 56 d. Two PF values were identified in this fashion as elevated and were excluded from data analysis.

Indicators of iron status were compared between study groups by *t* tests and 2-factor (group, gender) ANOVA procedures. Time effects were tested by repeated-measures ANOVA. PF data were nonnormally distributed and statistical analysis was therefore also preformed after log transformation. Statistical results were similar and results obtained with log-transformed data are reported. However, only nontransformed values with arithmetic means and SD are shown. Because PF concentrations at 4 mo differed between study groups, the data also were analyzed by ANCOVA with PF at 4 mo as a covariate. Changes in PF and Hb concentrations were calculated over 2-mo intervals during the intervention. Pearson correlation coefficients were used to assess relationships between variables. Values in the text are means ± SD.

Results

Electrolytic iron vs. ferrous fumarate

Iron status. PF concentrations did not differ between infants who were fed Cereal L and Cereal M except at 7.5 mo when PF was lower in the Cereal L (electrolytic iron) than in the cereal M (fumarate) group ($P = 0.033$) (**Table 1**). However, after adjustment for the difference in PF at baseline (4 mo), the difference at 7.5 mo was no longer significant ($P = 0.06$). In both groups, concentrations showed the expected age-related changes: PF decreased with increasing age ($P < 0.001$). Hb and iron concentrations did not change and TfR initially increased ($P < 0.001$) and stabilized after 5.5 mo. There were no significant treatment-related differences in any of the other iron status indices. In the per protocol analysis, there were similarly no treatment-related differences. When changes in PF and Hb were calculated for 2-mo intervals during the intervention, there were no significant cereal-related differences, although there were the expected age-related changes.

ID. Three infants had ID at the time of randomization at 4 mo, all of whom were born with low iron endowment (**Table 2**). Two of them (1 in the Cereal L and 1 in the Cereal M group) were still iron deficient at 5.5 mo. Two additional infants were iron deficient at 5.5 mo [infant 10993 fed Cereal L and infant 10924 (not shown) fed Cereal M]. Thus, 4 infants (4.2% of those randomized) had ID, but none had IDA. At 12 mo (3 mo after cessation of the intervention), ID was present in 2 infants fed Cereal L and in 6 infants fed Cereal M.

Cereal consumption. The mean estimated consumption of study cereals exceeded the target amount of 15 g/d except during the period from 4 to 5.5 mo, when it was less (**Table 3**). However, the range of intakes was wide, with some infants consuming only about one-half the target amount and others consuming about twice the target amount. Corresponding intakes of iron from study cereals similarly showed a wide range. Overall, intake of cereal iron was 1.21 ± 0.31 mg·kg^{-1}·d^{-1} from Cereal L and 1.07 ± 0.40 mg·kg^{-1}·d^{-1} from Cereal M, which did not differ ($P = 0.10$). The intake of cereal iron during the 4- to 5.5-mo period was correlated with PF at 5.5 mo ($r = 0.32$; $P = 0.003$) and at 7.5 mo ($r = 0.29$; $P = 0.015$) but not at 9 mo ($r = 0.23$; $P = 0.065$). Intakes of cereal iron during the 5.5- to 7.5-mo and 7.5- to 9-mo periods were not correlated with PF at 7.5 or 9 mo, respectively (data not shown).

TABLE 1 Iron status of 95 infants who were randomized to receive either Cereal L (electrolytic iron) or Cereal M (ferrous fumarate) at 4 mo of age[1]

		Age					
	Cereal	2 mo	4 mo	5.5 mo	7.5 mo	9 mo	12 mo
Infants,[2] *n*	L	50	50	43	37	36	36
	M	45	45	40	34	33	33
PF,[3] *μg/L*	L	165 ± 146	64 ± 50	39 ± 25	31 ± 16	31 ± 17	24 ± 16
	M	158 ± 96	79 ± 72	47 ± 49	41 ± 32*	37 ± 29	23 ± 17
Plasma sTfR, *mg/L*	L	4.26 ± 1.02	5.60 ± 1.09	5.19 ± 0.89	5.34 ± 0.80	5.48 ± 0.91	5.37 ± 0.88
	M	4.23 ± 1.04	5.63 ± 1.31	5.03 ± 1.23	5.68 ± 1.07	5.49 ± 0.84	5.59 ± 1.14
Ln[sTfR/ferritin]	L	1.49 ± 0.32	2.04 ± 0.36	2.20 ± 0.31	2.27 ± 0.22	2.28 ± 0.19	2.41 ± 0.22
	M	1.48 ± 0.30	1.98 ± 0.39	2.16 ± 0.38	2.22 ± 0.29	2.26 ± 0.29	2.47 ± 0.28
Plasma iron, *μmol/L*	L	13.0 ± 3.4	10.5 ± 4.0	10.1 ± 4.2	10.7 ± 3.0	10.8 ± 4.1	11.0 ± 4.0
	M	12.1 ± 3.5	10.0 ± 3.3	9.2 ± 2.8	9.4 ± 3.6	10.0 ± 3.8	10.5 ± 4.1
Blood Hb, *g/L*	L	114 ± 11	115 ± 7	118 ± 7	116 ± 9	116 ± 7	119 ± 7
	M	113 ± 11	117 ± 9	117 ± 8	118 ± 8	120 ± 7	120 ± 8
RDW,[4] *%*	L	14.2 ± 1.0	12.5 ± 0.9	13.0 ± 1.3	14.1 ± 1.2	14.3 ± 1.4	13.7 ± 1.0
	M	14.2 ± 1.1	12.4 ± 1.0	13.0 ± 1.3	13.9 ± 1.4	13.9 ± 1.2	13.6 ± 1.0
MCV, *fL*	L	92 ± 4	82 ± 4	78 ± 4	78 ± 5	79 ± 4	79 ± 4
	M	92 ± 4	82 ± 4	79 ± 4	79 ± 3	80 ± 3	80 ± 4

[1] Values are means ± SD. *Different from Cereal L, $P < 0.05$.
[2] Data for infants who left the study are included up to the point of departure.
[3] Decreased with age, $P = <0.001$.
[4] RDW, relative distribution width of erythrocytes; MCV, mean corpuscular volume of erythrocytes.

Growth. There were no differences between study groups in weight or length attained, nor in weight or length gained. Observed differences in gain were less than the detectable differences of 2.31 g/d and 0.046 mm/d with 69 infants. There were the expected gender-related differences, with boys being heavier and longer than girls ($P < 0.05$).

TABLE 2 PF and blood Hb concentrations of infants who were born with low iron endowment (PF ≤ 55 μg/L at age 2 mo) and consumed cereal L or M beginning at 4 mo of age

			Age					
Infant	Cereal		2 mo	4 mo	5.5 mo	7.5 mo	9 mo	12 mo
10913	L	PF, *μg/L*	49	8	8	28	22	21
		Hb, *g/L*	121	110	123	125	116	126
10933	L	PF, *μg/L*	32	9	13	19	38	15
		Hb, *g/L*	105	119	126	115	126	131
10937	M	PF, *μg/L*	34	8	6	12	34	23
		Hb, *g/L*	110	119	119	133	129	123
10991	M	PF, *μg/L*	36	13				
		Hb, *g/L*	107	138				
10993	L	PF, *μg/L*	44	13	9	12	11	14
		Hb, *g/L*	99	122	107	115	115	132
10998	M	PF, *μg/L*	50	15	17	16	17	22
		Hb, *g/L*	111	118	113			123
10935	NR[1]	PF, *μg/L*	29	5	10*	16*		24*
		Hb, *g/L*	105	101	100*	111*		112*
10939	NR	PF, *μg/L*	55	4	15*	18*	5*	17*
		Hb, *g/L*	100	105	114*	113*	109*	108*
10941	NR	PF, *μg/L*	39	2				
		Hb, *g/L*	102	100				
10946	NR	PF, *μg/L*	27	12	12*	14*	41*	
		Hb, *g/L*	142	100	111*	125*	121*	113*
10961	NR	PF, *μg/L*	40	21	18*	21*	20*	60*
		Hb, *g/L*	108	122	126*	125*	117*	132*

[1] NR, Not randomized. *Values obtained during and after Fe treatment.

Infants born with low iron endowment

A low birth iron endowment, defined as a PF concentration < 55 μg/L (10th percentile value in the present dataset) at 2 mo of age, was present in 11 infants, for whom individual data are presented in Table 2. Two of these infants (10946, 10961) were withdrawn by their parents, received supplemental iron, and remained in good iron status. Three infants (10935, 10939, 10941) had IDA at 4 mo. They were not randomized and were instead referred for treatment. IDA responded slowly to treatment in infant 10935, but from 7.5 mo on the infant remained in good iron status. In infant 10939, IDA resolved promptly with treatment, but at 9 mo this infant had ID again; it is not clear how long iron therapy was continued. No follow-up information was obtained on infant 10941. The remaining 6 infants born with low iron endowment were randomized at 4 mo, at which time 3 (10913, 10933, 10937) had ID. A 4th infant (10993) developed ID at 5.5 mo.

Thus, among infants born with low iron endowment, 3 had IDA and 3 had ID without anemia by 4 mo of age, for a prevalence of any early ID (IDA plus ID) of 54%. All 3 cases of early IDA and 7 of 8 cases of early mild ID occurred in infants born with low iron endowment. By contrast, among infants with normal iron endowment, only 1 developed early ID without anemia before 6 mo.

Entire study population. Among all infants enrolled ($n = 111$), the prevalence of IDA, which occurred before 6 mo in each case, was 2.7%. Twelve infants had ID without anemia on 1 or 2 occasions, for a prevalence of infants with ID of 10.8%. ID occurred either early (5 infants) or late, i.e. at 12 mo (7 infants) or early and late (1 infant). The number of infants with any ID (ID and IDA) between 1 and 12 mo was 15 for a prevalence of 13.5%.

TABLE 3 Daily consumption of cereals L or M and of cereal iron during successive 2-mo periods by infants[1]

		Age interval		
	Cereal	4–5.5 mo	5.5–7.5 mo	7.5–9 mo
Infants, *n*	L	47	40	35
	M	40	34	33
Cereal,[2] $g \cdot d^{-1}$	L	11.3 ± 4.4	19.4 ± 7.4	23.6 ± 8.4
		(5.2–27.8)	(3.4–43.1)	(5.3–39.9)
	M	11.7 ± 5.2	19.1 ± 7.3	19.8 ± 9.3
		(2.9–25.1)	(9.0–33.3)	(2.4–39.0)
Cereal iron,[2] $mg \cdot d^{-1}$	L	6.1 ± 2.4	11.6 ± 7.0	13.9 ± 7.5
	M	6.1 ± 2.7	10.0 ± 7.3	10.3 ± 4.8
Cereal iron,[2] $mg \cdot kg^{-1} \cdot d^{-1}$	L	0.88 ± 0.34	1.39 ± 0.56	1.54 ± 0.54
	M	0.86 ± 0.39	1.28 ± 0.50	1.19 ± 0.54

[1] Values are mean ± SD (range).
[2] Increased with age, $P < 0.001$.

PF. At 2 mo of age, the PF concentration was 151 ± 96 μg/L (n = 106) with a range from 27 to 432 μg/L. The 5th, 10th, 50th, 90th, and 95th percentiles were 39, 55, 132, 259, and 318 μg/L, respectively. At 4 mo of age, PF was 68 ± 61 μg/L (n = 97) with a range from 2 to 379 μg/L. The 5th, 10th, 50th, 90th, and 95th percentiles were 8, 15, 54, 142, and 187 μg/L, respectively. PF was significantly higher in girls than in boys at 2, 4, and 5.5 mo but not at later ages (data not shown).

PF concentrations showed strong tracking, as indicated by significant correlation coefficients that decreased somewhat with increasing length of age intervals but remained significant for intervals as long as 8 mo (**Table 4**).

Discussion

This study involved healthy infants who were exclusively breast-fed at the time of enrollment at 2 mo and at the time of randomization at 4 mo. Thereafter, they began to consume various complementary foods but did not receive formula. The study cereals were provided from 4 to 9 mo and were consumed by all infants, with mean consumption approximating the intended amount (15 g/d). Iron provided by study cereals was, after the initial 2 mo, somewhat more than 1 $mg \cdot kg^{-1} \cdot d^{-1}$. Infants who developed IDA in the first month of life were excluded from the study but were followed.

Contrary to our hypothesis, the study produced no evidence that ferrous fumarate is a more efficacious fortificant of infant cereal than electrolytic iron in maintaining iron status and preventing ID in breast-fed infants. With either source of iron, the prevalence of ID was 4.2% and the prevalence of IDA was 0%. It must be kept in mind that in this study, parents were asked to feed the cereals every day and in relatively large amounts. The suggested daily amounts of cereal were intended to ensure intakes of iron that the investigators deemed necessary to maintain good iron status. The amounts consumed were similar to those reported by Walter et al. (9). There were no difficulties reported in infants consuming the suggested amounts and actual intakes varied over a wide range. But it appears likely that free-living infants received lesser amounts of cereals and may not have received cereal every day. Although the prevalence of ID (4.2%) was slightly higher than assumed in our power calculation, ID was in all cases mild and transient.

TABLE 4 Pearson correlations between PF concentrations of infants who consumed cereal L or M at different ages

Age	4 mo	5.5 mo	7.5 mo	9 mo	12 mo
			r[1]		
2 mo	0.91	0.82	0.72	0.67	0.54
4 mo		0.87	0.84	0.82	0.67
5.5 mo			0.82	0.75	0.61
7.5 mo				0.82	0.64
9 mo					0.74

[1] All $P < 0.05$.

In contrast, in our earlier study (16) in which the parents of control infants freely chose complementary foods, 14.3% of infants developed ID between 4 and 9 mo of age, including 2 infants with IDA. Not all infants in that study consumed cereal, but quite a few consumed some formula. In comparison, infants in the present study consumed no formula but still had much less ID, suggesting that the regular consumption of these iron-fortified cereals offered relative protection from ID.

Because infants with the worst iron status, i.e. 3 infants with early IDA, were excluded from the study proper, it remains unknown whether the cereals would have been able to normalize iron status in these infants.

That electrolytic iron appeared to be equally effective as ferrous fumarate came somewhat as a surprise, because the literature led us to expect that the fumerate form would outperform electrolytic iron. Several explanations appear possible. One is that the cereals provided a surfeit of iron, so that even in the presence of a difference in bioavailability each provided sufficient absorbed iron. Another explanation is that enough ascorbic acid, a known enhancer of iron availability (34), may have survived the cooking and drying process and may have enhanced iron availability to the point where any difference between the 2 fortificants was eliminated. Because the currently marketed dry infant cereals in the US no longer include ascorbic acid as an ingredient, the present results should not be extrapolated to current U.S. infant feeding practice.

The study produced further evidence that infants who are born with a low iron endowment are at increased risk of ID, in particular during the first 4 mo of life. A full 54% of infants born with low storage iron developed ID in the first 4 mo of life, including all 3 who developed IDA. We (15,16) and others (35–37) have previously reported that infants with a low birth iron endowment are at increased risk of ID and IDA early in life. This increased risk during the first 6 mo of life occurs at a time during which ID has traditionally not been expected.

The study had some limitations. Because the feeding of supplemental formula was not permitted, infants depended exclusively on complementary foods as a source of iron. Among free-living breast-fed infants, supplemental formula is often fed and provides a readily available source of iron (ferrous sulfate). Infants with manifest IDA were excluded from the study. Therefore, nothing can be said about the efficacy of iron-fortified infant cereals in preventing or correcting IDA. However, because IDA had developed by 4 mo, the age at which complementary foods are beginning to be introduced, iron-fortified cereals cannot be expected to prevent early IDA. Because the study had no placebo group, inferences regarding the overall effectiveness of the cereals in preventing ID could only be drawn from comparisons with historical data.

In conclusion, the results suggest that the regular consumption of iron-fortified infant cereals can afford breast-fed infants at least

partial protection against ID. The 2 dry infant cereals fortified with electrolytic iron (A-131) and ferrous fumarate and containing ascorbic acid appeared to be equally efficacious in maintaining iron status and protecting breast-fed infants against ID.

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