

Effects of age of introduction of complementary foods on iron status of breast-fed infants in Honduras¹⁻³

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See corresponding editorial on page 815.

ABSTRACT To evaluate the effect of introducing complementary foods before 6 mo of age, we randomly assigned 164 infants who had been exclusively breast-fed for 4 mo to continue being exclusively breast-fed until 6 mo (EBF group) or to receive iron-fortified foods in addition to breast milk from 4 to 6 mo (BF+SF group). At 6 mo, the BF+SF group had higher mean iron intake (4 mg/d compared with 0.2 mg/d in EBF infants) and higher hemoglobin, hematocrit, and ferritin values than the EBF group ($P < 0.05$). The percentage with low hemoglobin (< 103 g/L) did not differ significantly between groups, but fewer infants in the BF+SF group had a low hematocrit (< 0.33 ; 21.4% compared with 32.0%, respectively; $P < 0.05$). The percentage of infants with ferritin concentrations < 12 μ g/L at 6 mo was lower than the percentage with low hemoglobin or hematocrit, raising questions about the validity of the cutoffs at this age. Infants at greatest risk for anemia and low ferritin were those with birth weights < 2500 g; no infant with a birth weight > 3000 g had a low ferritin value at 6 mo. We conclude that the risk of iron deficiency is low among infants with birth weights > 3000 g who are exclusively breast-fed for 6 mo. Iron drops are recommended for low-birth-weight infants; for breast-fed infants with birth weights between 2500 and 3000 g, further research is needed to determine whether iron drops are more effective than complementary foods for preventing iron deficiency before 6 mo. *Am J Clin Nutr* 1998;67:878-84.

KEY WORDS Anemia, hemoglobin, iron status, breast-feeding, weaning, infants, complementary foods, hematocrit, plasma ferritin, C-reactive protein, birth weight, Honduras

INTRODUCTION

The optimal age of introduction of complementary foods to breast-fed infants is a topic of considerable debate. For several decades, the World Health Organization recommended that infants be given other foods beginning at 4-6 mo (1). However, recent resolutions by the World Health Assembly (2) used a different wording, stating that breast-fed infants should receive complementary foods from the age of "about six months." The risks and potential benefits of complementary feeding before 6 mo were reviewed recently (3). In developing countries this is an issue of particular importance because of the increased risk of diarrhea due to use of complementary foods in environments where sanitation is poor (4, 5). Even in industrialized countries, the evidence indicates that with regard to infant growth, there is no benefit to introducing foods other than

breast milk before 6 mo of age (3). However, there is less certainty about effects on micronutrient status, particularly iron status.

It is generally assumed that iron deficiency among full-term, breast-fed infants is rare during the first 6 mo of life. This is based on studies in affluent populations in which average birth weights, and thus iron reserves, are relatively high. Although the bioavailability of iron from human milk is high (6), its absolute concentration is low; thus, exclusively breast-fed infants must rely on iron reserves obtained in utero to supply most of the needs for red blood cell synthesis and growth. In low-birth-weight infants, who have lower iron reserves, the risk of iron deficiency is much greater (7). Thus, in developing countries in which there is a relatively high percentage of low-birth-weight infants, there is concern that exclusive breast-feeding for 6 mo may not be optimal with regard to infant iron status.

We recently reported the results of a randomized intervention trial in Honduras conducted to examine the effect of hygienically prepared, nutritionally enhanced, complementary foods on infant breast-milk intake, total energy intake, and growth between 4 and 6 mo of age (8). The results indicated that complementary foods largely displaced breast milk and did not improve growth. In this paper we present the results of iron status assessments of the same cohort of infants.

METHODS

Study design

Infants were recruited at birth from two public maternity hospitals in San Pedro Sula, Honduras. Subjects were eligible if the mother

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was primiparous, willing to breast-feed exclusively for 6 mo, not employed outside the home, living on a low income (<US\$150/mo), ≥ 16 y old, and healthy (taking no medication on a regular basis), and the infant was healthy, full-term (>37 wk), and weighed ≥ 2000 g at birth. Multiparous and working mothers were excluded because the study procedures required a 3-d stay in a central facility on three different occasions. The study protocol was approved by the Human Subjects Review Committee of the University of California, Davis, and by the Ministry of Health, Honduras.

Lactation guidance was provided to all mothers throughout the study. At 16 wk postpartum, subjects who were still eligible were randomly assigned (by week of birth) to one of three groups: 1) infants exclusively breast-fed to 26 wk (EBF group), with no other liquids (water, milk, or formula) or solids ($n = 63$); 2) infants introduced to solid foods at 16 wk, with ad libitum breast-feeding ($n = 51$); or 3) infants introduced to solid foods at 16 wk, with maintenance of preintervention breast-feeding frequency ($n = 50$).

Because the intake of solid foods in the second and third groups was similar, for this analysis subjects in those two groups were pooled into one group, herein referred to as the BF+SF group. Subjects were not informed of their assignment until they had completed the first 16 wk of the study. At 16 wk, subjects stayed at a central facility for baseline measurements of infant breast-milk intake (48 h), 24-h breast-milk sampling, and maternal and infant anthropometry. Women assigned to the BF+SF group gave their infants their first solid food just before leaving the facility, and received instructions on feeding at home. Between 16 and 26 wk mothers in both groups were visited weekly so that infant growth and morbidity could be assessed and baby foods and feeding instructions could be delivered to the BF+SF group. All mothers returned to the facility at 21 and 26 wk for the above measurements and quantification of infants' solid food intake in the BF+SF group. In addition, home visits were conducted at 19 and 24 wk to complete 12-h observations of nursing frequency (both groups) and to weigh all foods consumed by the infants (BF+SF group).

At the end of the intervention (26 wk), a venous blood sample was collected from all infants. Blood samples were not collected at the beginning of the intervention period for fear of increasing attrition. Infants with a hemoglobin concentration < 110 g/L at 6 mo were provided with iron supplements ($5 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$) for 3 mo and retested at 9 mo of age. Parents were asked monthly about infant intake of the supplements provided, and nearly all reported regular usage. After the intervention phase, all subjects were visited in the home each month from 6 to 12 mo so that infant growth and feeding practices could be assessed. At 12 mo, blood samples were collected again from all infants whose parents consented.

Complementary foods

Commercial baby foods in jars (supplied by Gerber Products Company, Fremont, MI) were provided to all subjects in the BF+SF group during the intervention period from 16 to 26 wk. The foods included iron-fortified rice cereal, iron-fortified rice cereal with egg yolk (formulated specifically for this study), non-iron-fortified fruit (banana, papaya, and pineapple, each containing $123 \mu\text{g}$ vitamin C/g), and vegetables (carrots, squash, and mixed vegetables, containing 3, 63, and $2 \mu\text{g}$ vitamin C/g, respectively). The ingredients in these foods are locally available and commonly used as complementary foods. The foods were chosen so that their combined nutrient density (per MJ) would meet or exceed the nutrient density needed to satisfy the recom-

mended dietary allowances for infants (9). In the first 2 wk the infants were given the standard iron-fortified rice cereal product, which contained $53 \mu\text{g}$ Fe/g ($50 \mu\text{g}$ from ferrous sulfate) and $123 \mu\text{g}$ vitamin C/g. Thereafter, they used the rice cereal-egg yolk combination, which contained $75 \mu\text{g}$ Fe/g ($50 \mu\text{g}$ from ferrous sulfate) and $90 \mu\text{g}$ vitamin C/g.

Fruit and vegetables were added one at a time after the first week. Foods were given in two meals by spoon ≈ 1 h after breast-feeding. The morning meal included cereal consumed with a fruit, and the afternoon meal included cereal eaten with a vegetable. Women were advised to feed the infant until the child rejected any further food, to use each jar only once, to give away or discard any excess, and not to give the infant any other foods or liquids. At 26 wk all mothers were given a baby food grinder and taught how to prepare their own baby foods.

Anthropometry

Birth weight was recorded from the birth certificate or from parental recall (and was highly correlated with infant weight measured at 1 wk: $r = 0.81$). Infant weight was measured weekly [to the nearest 100 g with a suspended spring scale (Perspective Enterprises, Kalamazoo, MI)] during the intervention phase and monthly thereafter. Weights were adjusted (by linear interpolation) when they were measured more than 1 wk from the target date. Infant length was measured to the nearest 0.1 cm with a recumbent length board at weeks 16, 21, and 26 and monthly from 7 to 12 mo. Maternal height (to the nearest 0.1 cm) and weight (to the nearest 0.2 kg) were measured at 16 wk postpartum.

Dietary intake

Breast-milk intake was measured by test weighing for 48 h at 16, 21, and 26 wk, and corrected for insensible water loss as described elsewhere (8). After the 48-h test-weighing period, breast-milk samples were collected over a 24-h period using the alternate breast expression method (10). Milk samples were analyzed for energy content as described previously (8) and for iron concentration by flame atomic-absorption spectrophotometry (11). In the BF+SF group, intake of solid foods was measured by weighing the baby food jars before and after each feeding during the 48-h test-weighing periods at 21 and 26 wk and during the 12-h home observations at 19 and 24 wk. Energy, iron, and vitamin C intakes from solid foods were calculated based on manufacturer's data.

Blood sampling and analysis

Blood samples were collected by venipuncture with a butterfly needle into heparin-containing tubes at 26 wk and 12 mo. Hemoglobin and hematocrit values were measured at a local laboratory using an automated system (Cell-Dyne 610; Abbott Laboratories, Abbott Park, IL). Plasma was stored at $\approx -20^\circ\text{C}$ until transported for analysis of ferritin and C-reactive protein at the University of California, Davis. Ferritin in 26-wk samples was analyzed by radioimmunoassay (Diagnostic Products Company, Los Angeles); ferritin in 12-mo samples was not analyzed because of a freezer malfunction. C-reactive protein was analyzed by radial immunodiffusion with a kit from The Binding Site (Birmingham, United Kingdom); concentrations > 10 mg/L were considered elevated.

Data analysis

Data were analyzed with PC-SAS (SAS Institute Inc, Cary, NC). Characteristics and iron status of the two groups (EBF and BF+SF)

TABLE 1
Characteristics of the study groups

	Exclusively breast-fed (<i>n</i> = 50)	Breast-fed + solid foods (<i>n</i> = 89)
Maternal age (y)	19.8 ± 3.0 ¹	20.4 ± 3.7
Maternal education (y)	6.1 ± 3.1	7.1 ± 2.8
Family income (\$/mo)	125 ± 57	120 ± 62
Maternal height (cm)	154 ± 6	153 ± 6
Maternal BMI at 16 wk (kg/m ²)	22.5 ± 3.3	22.7 ± 3.4
Maternal prenatal iron supplementation (% yes)	58	62
(no./mo)	2.6 ± 2.7	2.9 ± 3.0
Infant sex (% male)	52	41
Infant birth weight (g)	2991 ± 505	2832 ± 463

¹ $\bar{x} \pm SD$. There were no significant differences between groups for any variable.

were compared by using Student's *t* test and chi-square statistics. Analysis of variance was used to compare iron status by birth-weight category (<2500, 2500–3000, or >3000 g). Multiple-logistic-regression analysis was used to evaluate the factors associated with low hemoglobin, hematocrit, or ferritin at 6 mo. Factors associated with ferritin concentration at 6 mo were determined by using multiple linear regression, after log transformation of ferritin values to normalize the distribution. Variables included in the regression models (both logistic and linear) were group (EBF or BF+SF), birth weight, weight gain from 0 to 6 mo, and months of reported maternal prenatal iron supplementation. Changes in hemoglobin and hematocrit from 6 to 12 mo were evaluated by using repeated-measures analysis of variance, including time, intervention group, and iron supplementation as the main effects.

For data analysis, two different cutoff values were used to define low hemoglobin: 1) the standard value of 110 g/L used for children (12) and 2) a lower cutoff of 103 g/L based on recent data for infants. Several studies have shown that at 6 mo, the cutoff of 110 g/L results in an overestimation of anemia (13, 14). On the basis of data for well-nourished infants in Denmark, Michaelsen et al (13) concluded that a value of 105 g/L at 6 mo was more appropriate. Emond et al (14) suggested a cutoff of 97 g/L (for capillary blood) by using data for 1175 infants aged 8 mo in England. The cutoff used herein (103 g/L) is intermediate between these two estimates. Two different cutoff values were also used to define low ferritin (<12 or <15 µg/L), because of uncertainty about the appropriate value to use during infancy.

RESULTS

Of the 164 subjects who entered the intervention phase at 16 wk, 23 dropped out of the study before 26 wk (13 in the EBF group and 10 in the BF+SF group), primarily because they moved out of the area (*n* = 4), went back to work (*n* = 5), their husbands refused to allow participation (*n* = 10), or (in the EBF group) they discontinued exclusive breast-feeding (*n* = 4). The dropouts did not differ significantly from the subjects who completed the intervention in any of the maternal or infant characteristics assessed. Blood samples could not be obtained from two subjects at 6 mo.

Characteristics of the EBF and BF+SF groups are shown in **Table 1**. There were no significant differences between groups in

maternal age, education, family income, height, body mass index, reported prenatal iron supplementation, or infant sex or birth weight. Energy intake from solid foods averaged 451 ± 224 kJ/d (108 ± 54 kcal/d) at 21 wk and 501 ± 229 kJ/d (120 ± 55 kcal/d) at 26 wk. Intake of solid foods in the home at 19 and 24 wk was ≈13% lower than intake in the central unit at 21 and 26 wk, after differences in infant age were adjusted for (8). Total energy intake (from breast milk plus solid foods) did not differ significantly between groups during the intervention period because breast-milk intake declined in the BF+SF group (8).

Mean iron intake at 21 and 26 wk of age from breast milk and solid foods is shown in **Table 2**. Mean total iron intake was 4.32–4.76 mg/d in the BF+SF group compared with 0.16–0.17 mg/d in the EBF group (*P* < 0.001). Vitamin C intake from solid foods in the BF+SF group averaged 11.7 mg at 21 wk and 12.5 mg at 26 wk.

The hematologic results at 6 mo of age are shown in **Table 3**. Mean hemoglobin and hematocrit values were significantly higher in the BF+SF group than in the EBF group. The percentage of infants with hemoglobin <110 or 103 g/L, however, did not differ significantly between groups when either chi-square tests or logistic regression analysis controlling for potential confounders (birth weight, weight gain from 0 to 6 mo, and maternal prenatal iron supplementation) were used. When the cutoff of 110 g/L was used, the prevalence of low hemoglobin was much higher than the prevalence of hematocrit <0.33, whereas the hemoglobin cutoff of 103 g/L yielded prevalence estimates that were similar to those for a low hematocrit. With chi-square analysis, the percentage of infants with a low hematocrit did not differ significantly between groups (*P* = 0.17), but in logistic regression analysis with adjustment for birth weight, the difference became significant (*P* < 0.05).

Plasma ferritin data are also shown in **Table 3**. The mean ferritin concentration was significantly higher in the BF+SF group than in the EBF group (*P* < 0.05) when birth weight was controlled for; this was also true when log ferritin concentration was compared (*P* < 0.01). We measured plasma C-reactive protein concentration as a marker of infection or inflammation, which is associated with increased ferritin concentrations (15). Only

TABLE 2
Iron intake at 21 and 26 wk¹

	Exclusively breast-fed (<i>n</i> = 50)	Breast-fed + solid foods (<i>n</i> = 89)
	<i>mg/d</i>	
21 wk		
Breast milk	0.17 ± 0.09	0.18 ± 0.12
Solid foods		
Endogenous iron	0	1.40 ± 0.84
Fortificant iron	0	2.74 ± 1.57
Total	0	4.14 ± 2.32
Total	0.17 ± 0.09	4.32 ± 2.32 ²
26 wk		
Breast milk	0.16 ± 0.08	0.18 ± 0.12
Solid foods		
Endogenous iron	0	1.57 ± 0.86
Fortificant iron	0	3.01 ± 1.55
Total	0	4.58 ± 2.35
Total	0.16 ± 0.08	4.76 ± 2.32 ²

¹ $\bar{x} \pm SD$.

² Significantly different from exclusively breast-fed, *P* < 0.001.

TABLE 3
Hematologic results at 6 mo

	Exclusively breast-fed (n = 50)	Breast-fed + solid foods (n = 89)
Hemoglobin (g/L)	104 ± 10 ¹	109 ± 10 ²
< 110 g/L (%)	66.0 (33/50)	55.1 (49/89)
< 103 g/L (%)	32.0 (16/50)	24.7 (22/89)
Hematocrit (l)	0.335 ± 0.028	0.347 ± 0.026 ²
< 0.33 (%)	32.0 (16/50)	21.3 (19/89) ³
Ferritin (µg/L)		
Total sample	48.4 ± 44.2	67.3 ± 64.5 ²
Excluding high CRP ⁴	47.1 ± 45.2 [46] ⁵	67.1 ± 65.8 ² [82]
Median (25th, 75th percentile)	39.7 (16.8, 65.9)	47.8 (34.8, 73.1)
< 12 µg/L (%)		
Total sample	16.3 (8/49)	7.0 (6/86) ³
Excluding high CRP	17.4 (8/46)	7.3 (6/82) ³
< 15 µg/L (%)		
Total sample	20.4 (10/49)	7.0 (6/86) ³
Excluding high CRP	21.7 (10/46)	7.3 (6/82) ³

¹ $\bar{x} \pm SD$.

² Significantly different from exclusively breast-fed, $P < 0.05$ (Student's *t* test).

³ Significantly different from exclusively breast-fed, $P < 0.05$ (logistic regression controlling for birth weight).

⁴ C-reactive protein concentration > 10 mg/L.

⁵ *n* in brackets.

seven infants had elevated C-reactive protein concentrations. Ferritin values for these infants were significantly greater than those of the other infants ($P < 0.05$), but exclusion of these values did not alter the results by treatment group. Excluding infants with elevated C-reactive protein concentration, the percentage of infants with ferritin concentration <12 µg/L was 17% (95% CI: 6%, 29%) in the EBF group and 7% (95% CI: 2%, 13%) in the BF+SF group (difference not significant in chi-square tests, but significant in logistic regression when birth weight was controlled for; $P < 0.05$). Results were similar when a ferritin cutoff of <15 µg/L was used. These percentages were only one-third to one-half the percentages of infants with low hemoglobin or hematocrit. Of the infants with hemoglobin concentrations <103 g/L, only 22% had a low (<12 µg/L) ferritin concentration (excluding infants with elevated C-reactive protein concentrations). Of the 14 infants with ferritin concentrations < 12 µg/L, 8 had a hemoglobin concentration <103 g/L.

The association between birth weight and low hemoglobin (<103 g/L), hematocrit, or ferritin (<12 µg/L) at 6 mo, by feeding group, is shown in **Figure 1**. Among infants weighing <2500 g at birth, 45–50% had low hemoglobin concentrations, 50% had a low hematocrit, and 15–43% had low ferritin concentrations, depending on the feeding group. By contrast, among infants weighing >3000 g at birth, these proportions were 5–18%, 9–11%, and 0%, respectively ($P < 0.01$ for the birth-weight differences for all three outcomes). Even with a ferritin cutoff of <15 µg/L, only one infant (in the EBF group) with a birth weight >3000 g showed evidence of low iron stores. Within the birth-weight category of 2500–3000 g, the percentage of infants with low hemoglobin, hematocrit, and ferritin values in Figure 1 appears greater in the EBF group than in the BF+SF group. However, the interaction between birth weight and feeding group was not significant for all three outcomes when continuous variables

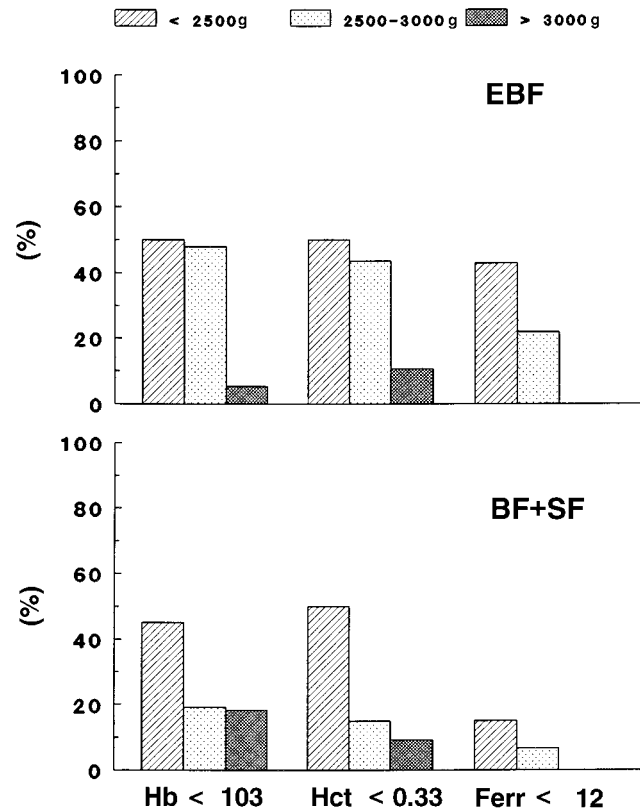


FIGURE 1. Percentage of infants with low hemoglobin (Hb < 103 g/L), hematocrit (Hct < 0.33), and plasma ferritin (Ferr < 12 µg/L) at 6 mo, by birth-weight category and feeding group. Sample sizes for the birth-weight categories of <2500, 2500–3000, and >3000 g were 8, 23, and 19 in the exclusively breast-fed (EBF) group and 20, 47, and 22 in the breast-fed + solid foods (BF+SF) group, respectively. Differences among birth-weight groups were significant ($P < 0.01$) using chi-squared tests for all three outcomes. Results were similar when a ferritin cutoff of <15 µg/L was used. The interaction between feeding mode and birth weight was not significant for the proportion with low hematocrit or ferritin values; for the proportion with low hemoglobin values, it was marginally significant ($P = 0.09$).

were used, nor for hematocrit and ferritin when the proportion “low” was used; the interaction was of marginal significance ($P = 0.09$) for hemoglobin <103 g/L.

Results of the multiple regression analyses were consistent with these findings: birth weight was the factor most strongly associated with ferritin concentration and with the likelihood of low hemoglobin or hematocrit values at 6 mo of age. When birth weight was controlled for, infant weight gain from birth to 6 mo was inversely related to ferritin concentration at 6 mo ($P < 0.05$), but not to the likelihood of low hemoglobin or hematocrit values. Maternal prenatal iron supplementation was not significantly related to any of the outcomes.

Infants with hemoglobin concentrations <110 g/L at 6 mo ($n = 82$) were given iron supplements for 3 mo; for 55 of these infants, follow-up hematologic data were obtained at 9 mo. Shown in **Table 4** is the hemoglobin response to iron supplementation in two subgroups: infants whose initial hemoglobin concentration was <103 g/L and those whose initial hemoglobin concentration was >103 but <110 g/L. The average change in hemoglobin from 6 to 9 mo was significantly greater than zero in

TABLE 4
Hemoglobin (Hb) response to 3 mo of iron supplementation (5 mg Fe · kg⁻¹ · d⁻¹) in two infant subgroups

g/L	Subgroup 1: initial Hb < 103 g/L	Subgroup 2: initial Hb > 103 but < 110
	(n = 30)	(n = 25)
Hemoglobin (g/L)		
6 mo	95 ± 6 ¹	106 ± 2
9 mo	104 ± 9	110 ± 10
Change from 6 to 9 mo	9 ± 8	4 ± 10 ²
Percentage with hemoglobin response (change from 6 to 9 mo) of: (%)		
≥ 10 g/L	40 (12/30)	28 (7/25)
< 5 g/L	33 (10/30)	36 (9/25)

¹ $\bar{x} \pm SD$.

² $P = 0.06$ for the difference between subgroups.

both subgroups, but was twice as great in the former (9 g/L) as in the latter (4 g/L; $P = 0.06$). In both subgroups, ≈35% of the infants showed changes in hemoglobin < 5 g/L.

Data on changes in hemoglobin and hematocrit from 6 to 12 mo for those infants from whom blood samples could be obtained at 12 mo are shown in **Table 5**. These results are presented separately for the subgroups who were or were not given iron supplements at 6 mo (those whose hemoglobin was < 110 g/L, which was more than half of the sample). In those not given iron supplements, hemoglobin concentrations declined significantly from 6 to 12 mo in both the EBF and BF+SF groups; the magnitude of the decline did not differ significantly between groups. The decline in hematocrit between 6 and 12 mo was marginally significant in both feeding groups. Among infants who were given iron supplements, hemoglobin and hematocrit both increased significantly from 6 to 12 mo, but the magnitude of this change did not differ significantly between feeding groups.

DISCUSSION

To our knowledge, this is the first randomized intervention trial to examine the effect of complementary foods on the iron status of breast-fed infants during the critical age of 4–6 mo. One limitation of the study was that iron status was not assessed at baseline, but only after the intervention. Nonetheless, because we used a randomized design and controlled for variables that are known to be related to iron status (such as birth weight), it is reasonable to draw causal inferences from the data. Infants who received iron-fortified complementary foods from 4 to 6 mo had higher hemoglobin, hematocrit, and ferritin values at 6 mo than infants who were exclusively breast-fed during the first 6 mo. However, the differences in hemoglobin and hematocrit, although statistically significant, were relatively small (≈4%), and there was still a considerable proportion (20–25%) of infants in the solid food group who had low hemoglobin or hematocrit values. Thus, although the BF+SF group consumed far more iron

TABLE 5
Change in hemoglobin (Hb) and hematocrit (Hct) from 6 to 12 mo

	Exclusively breast-fed	Breast-fed + solid foods
Percentage given supplements from 6 to 9 mo (%)	66 (33/50)	55 (49/89)
Number with data at 12 mo	29	68
Infants not given iron supplements		
Hb (g/L)		
6 mo	114.4 ± 4.9 [11] ¹	118.4 ± 6.7 [30] ²
12 mo	108.7 ± 7.4 [11]	111.0 ± 11.1 [30]
Change from 6 to 12 mo	-5.6 ± 7.9 [11] ³	-7.4 ± 10.0 [30] ³
Hct (l)		
6 mo	0.364 ± 0.016 [11]	0.363 ± 0.02 [30]
12 mo	0.350 ± 0.017 [11]	0.355 ± 0.031 [30]
Change from 6 to 12 mo	-0.014 ± 0.015 [11] ⁴	-0.009 ± 0.03 [30] ⁴
Infants given iron supplements		
Hb (g/L)		
6 mo	98.9 ± 9.4 [18]	102.2 ± 6.2 [38]
12 mo	111.7 ± 7.2 [18]	111.6 ± 6.6 [38]
Change from 6 to 12 mo	12.7 ± 8.6 [18] ³	9.5 ± 8.8 [38] ³
Hct (l)		
6 mo	0.324 ± 0.026 [18]	0.336 ± 0.02 [38]
12 mo	0.346 ± 0.022 [18]	0.350 ± 0.019 [38]
Change from 6 to 12 mo	0.023 ± 0.023 [18] ³	0.014 ± 0.022 [38] ³

¹ $\bar{x} \pm SD$; n in brackets.

² Significantly different from exclusively breast-fed, $P < 0.05$.

³ Significant change from 6 to 12 mo, $P < 0.05$.

⁴ Marginally significant change from 6 to 12 mo, $P < 0.10$.

than the EBF group, this did not fully eliminate the risk of developing anemia by 6 mo of age. The follow-up data at 12 mo of age did not show any longer-term effect of the intervention, although the provision of iron supplements to infants with low hemoglobin concentrations at 6 mo makes interpretation of these data difficult.

Although absorption of iron from ferrous sulfate is better than from most other inorganic fortifiers, the results of this study may have been different if a source of heme iron (which is better absorbed) had been used. However, the addition of vitamin C to the rice cereal products and the consumption of vitamin C-fortified fruit with the cereal at the morning meal were likely to have enhanced iron absorption from the solid foods (16). Absorption of iron from egg yolk with rice is estimated to be $\approx 72\%$ of that from ferrous sulfate (17). Thus, the total bioavailability of iron from the solid foods was likely to have been relatively high compared with that of most other iron-fortified complementary foods for infants. An intervention of longer duration may have had a greater effect, given that an average intake of ≈ 3 mg electrolytic iron/d in infant cereal was sufficient to prevent iron deficiency anemia in breast-fed infants during later infancy in Chile (18). However, the purpose of our study was to evaluate whether complementary foods have any effect during the specific interval of 4–6 mo, not whether iron-fortified foods continued thereafter are effective in preventing anemia.

Few other studies have directly compared iron status or anemia rates of breast-fed infants given complementary foods beginning at different ages. Among Italian infants who were breast-fed throughout the first year of life and who never received cow milk, iron-enriched formula, or iron supplements, the prevalence of anemia (hemoglobin < 110 g/L) at 12 mo was 0% among those who were exclusively breast-fed for ≥ 7 mo, but 43% among those who were given other foods before 7 mo (19). This implied that a longer duration of exclusive breast-feeding was protective against anemia. By contrast, Calvo et al (20) found that 44% of exclusively breast-fed infants in Argentina had a hemoglobin concentration < 110 g/L at 6 mo, compared with 14% of infants fed iron-fortified formulas. Interestingly, few of the infants in either group had low serum ferritin concentrations at 6 mo. In affluent countries, most comparisons of breast-fed infants with those fed iron-fortified formulas have found no significant difference in iron status at 6 mo of age, but at 9–12 mo the risk of iron deficiency among breast-fed infants increases unless foods rich in iron are included in the diet (21–23).

Birth weight is a critical explanatory factor for iron status during infancy. In the Honduran cohort, birth weight was strongly related to the risk of low hemoglobin, hematocrit, or ferritin values at 6 mo. Among infants with a birth weight > 3000 g, none had a ferritin concentration < 12 $\mu\text{g/L}$ at 6 mo, even in the EBF group. Other investigators also found a significant association between birth weight and ferritin concentration (14). Because of the well-known relation between birth weight and iron reserves, it is commonly recommended that low-birth-weight infants (< 2500 g) receive iron supplements in the form of medicinal drops beginning at 2–3 mo of age (24).


Infants with a relatively high rate of weight gain are also at greater risk of iron deficiency. In the Honduran cohort, ferritin concentrations at 6 mo were inversely related to weight gain during the first 6 mo of life, when birth weight was controlled for. Other investigators reported the same association (13, 14). In

some developing countries, infants who are exclusively breast-fed are reported to gain weight more rapidly than their counterparts who are partially breast-fed (3, 25), which may lead to a more rapid depletion of iron reserves.

The results of this study support the conclusion of other investigators (13, 14) that the cutoff values for defining anemia and iron deficiency at 6 mo of age need to be reevaluated. First, there was a discrepancy between the prevalences of low hemoglobin and low ferritin values: 59% of infants had a hemoglobin concentration below the standard cutoff of 110 g/L, yet only 10% of the sample had a ferritin concentration < 12 $\mu\text{g/L}$ (11.5% had a concentration < 15 $\mu\text{g/L}$). Even among the 34 infants with a hemoglobin concentration below our cutoff of 103 g/L, 26 (76%) had a ferritin concentration ≥ 12 $\mu\text{g/L}$ (excluding those with elevated C-reactive protein concentrations), which is inconsistent with the assumption that low ferritin values precede the development of anemia when iron deficiency is the primary causal factor. It may be that this assumption is not valid during infancy. There was no evidence of other nutrient deficiencies (eg, vitamin A, vitamin B-12, or folate; data not shown) that could have accounted for the discrepancy between hemoglobin and ferritin values. It is possible that some of the infants with normal C-reactive protein concentrations still had elevated ferritin concentrations as a result of past infections, because C-reactive protein may normalize before ferritin does. However, this seems unlikely to account for much of the discrepancy because morbidity rates were low in the first 6 mo among these fully breast-fed infants (8). Other investigators also found that at 6 mo the prevalence of low ferritin concentrations is much lower than the prevalence of low hemoglobin (13, 19). Second, the subsequent hemoglobin response to iron supplementation was not as much as we had expected. Among the 57 infants given iron supplements at 6 mo, the average change in hemoglobin by 9 mo was 9 g/L in those with an initial hemoglobin concentration < 103 g/L, compared with 4 g/L in those with an initial hemoglobin concentration ≥ 103 but < 110 g/L. A difference in hemoglobin response by initial status was expected, but it is noteworthy that about one-third of these 57 infants showed little or no change (< 5 g/L) in hemoglobin concentrations after iron supplementation. Some of this lack of change could be explained by noncompliance and individual variation in iron requirements, but another possibility is that the standard hemoglobin cutoff of 110 g/L is too high.

In affluent populations, hemoglobin and hematocrit values of breast-fed infants generally remain stable or increase between 6 and 12 mo, whereas ferritin concentrations decline (13). This appears to be a normal phenomenon reflecting the shift in iron from body reserves to erythrocytes, and implies that the appropriate cutoff values for hemoglobin and hematocrit may change with age. In the Honduran cohort, however, hemoglobin and hematocrit values generally declined after 6 mo in those not receiving iron supplements, which probably reflected the low iron content or bioavailability of the foods consumed after the intervention period.

To conclude, the introduction of iron-fortified complementary foods to breast-fed infants in this population at 4 mo of age increased hemoglobin, hematocrit, and ferritin concentrations, but a considerable proportion of infants became anemic by 6 mo nonetheless. Thus, complementary feeding before 6 mo of age (with the level of iron fortification used in this study) is not likely to be an adequate mechanism for preventing infant anemia in developing countries. Birth weight is a key factor in predict-

ing which infants are at risk of iron deficiency. Assuming that plasma ferritin is an adequate index of iron deficiency during infancy, the data suggest that exclusively breast-fed infants with a birth weight >3000 g do not need an additional source of iron before 6 mo. In contrast, those with a birth weight <2500 g are clearly at risk for iron deficiency and should receive medicinal iron drops beginning at 2–3 mo of age (24). In those with birth weights between 2500 and 3000 g, the situation is less clear. For this subgroup, potential options include use of medicinal iron drops or complementary foods that provide more iron than those used in this study. It is unknown whether the latter option is feasible or effective, and it may entail risks such as increased diarrheal morbidity in populations where food contamination is common. Medicinal iron drops may therefore be a safer and more effective choice for this subgroup, although the risks and benefits of iron supplementation also require further evaluation. For all infants, other strategies for prevention of iron deficiency, such as delayed clamping of the umbilical cord at birth (26), should also be kept in mind. Further research is needed on the definition, causes, and prevention of iron deficiency anemia among breast-fed infants in both disadvantaged and affluent populations. 

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