

Iron supplementation of breast-fed Honduran and Swedish infants from 4 to 9 months of age

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Objective: The objective was to study the effects of iron supplementation on hemoglobin and iron status in 2 different populations.

Study design: In a randomized, placebo-controlled, masked clinical trial, we assigned term Swedish (n = 101) and Honduran (n = 131) infants to 3 groups at 4 months of age: (1) iron supplements, 1 mg/kg/d, from 4 to 9 months, (2) placebo, 4 to 6 months and iron, 6 to 9 months, and (3) placebo, 4 to 9 months. All infants were breast-fed exclusively to 6 months and partially to 9 months.

Results: From 4 to 6 months, the effect of iron (group 1 vs 2 + 3) was significant and similar in both populations for hemoglobin, ferritin, and zinc protoporphyrin. From 6 to 9 months, the effect (group 2 vs group 3) was significant and similar at both sites for all iron status variables except hemoglobin, for which there was a significant effect only in Honduras. In Honduras, the prevalence of iron deficiency anemia at 9 months was 29% in the placebo group and 9% in the supplemented groups. In Sweden, iron supplements caused no reduction in the already low prevalence of iron deficiency anemia at 9 months (<3%).

Conclusion: Iron supplementation from 4 to 9 months or 6 to 9 months significantly reduced iron deficiency anemia in Honduran breast-fed infants. The unexpected hemoglobin response at 4 to 6 months in both populations suggests that regulation of hemoglobin synthesis is immature at this age. (J Pediatr 2001;138:679-87)

Iron deficiency anemia is the most common micronutrient deficiency, with approximately 600 million individuals affected worldwide.¹ The healthy term infant is born with sufficient iron stores

to meet the needs during the first 4 to 6 months of life.² Breast milk is low in iron (0.2 to 0.4 mg/L), and even though the bioavailability is high,^{3,4} infants breast-fed for longer than 4 to 6 months

without receiving iron supplements or iron-fortified complementary foods are at risk of developing IDA.^{5,6} The association between IDA and impaired psychomotor development in infants is well documented.⁷

Hb Hemoglobin
IDA Iron deficiency anemia
MCV Mean-cell volume
TfR Transferrin receptor
ZPP Zinc protoporphyrin

Strategies for prevention of IDA in infants include education, iron fortification of common foods, and supplementation in the form of iron drops. It is practically impossible to supply enough iron from unmodified complementary foods to meet recommended intakes at 6 to 12 months.⁸ Iron drops have been recommended for infants who are exclusively breast-fed between 4 and 6 months⁹ and for breast-fed infants after 6 months of age if they do not consume adequate amounts of iron-fortified complementary foods.¹⁰⁻¹² However, the effect of prophylactic iron supplementation of healthy, term, breast-fed infants starting at 4 to 6 months of age is not known. Because there are potential adverse effects of iron supplementation, the efficacy and safety of this approach should be evaluated before large-scale prevention programs for IDA are implemented.

The aim of this randomized, double-blind, placebo-controlled trial was to investigate the effects of daily iron supplementation to breast-fed infants on hemoglobin and other measures of iron status. Because the optimal timing of iron supplementation of breast-fed infants is not known, we chose to

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study the effects starting at either 4 or 6 months and continuing to 9 months of age.

METHODS

Subjects

This study was conducted at 2 sites: San Pedro Sula, Honduras, and Umeå, Sweden. These sites were chosen because both populations have a high proportion of infants breast-fed for 9 months or longer. Including 2 very different sites permitted assessment of the effect of iron supplementation across a wide range of iron status and complementary feeding patterns. The average dietary iron intake of 6- to 12-month-old Swedish infants is relatively high (10 to 12 mg/d), and the main sources are iron-fortified formula or cereals.¹³ In contrast, the complementary diet in Honduras typically does not include iron-fortified products and is low in meat, poultry, and fish. Non-nutritional anemias, caused by malaria, thalassemia, or sickle cell anemia, are rare in both populations. Hookworm does not exist in Sweden but is found in Honduras, although the prevalence of infestation is likely to be low in breast-fed infants.

San Pedro Sula (population ~350,000) is an industrial city in Honduras. Umeå (population ~100,000) is a university town in northern Sweden. For the sake of simplicity, San Pedro Sula will be referred to as "Honduras" and Umeå as "Sweden." Term infants were recruited directly after delivery (Honduras) or at approximately 3 months of age (Sweden). All mothers giving birth during February and March 1997 at the public maternity hospital in San Pedro Sula were approached personally at the maternity ward. All parents of children born at Umeå University Hospital between February 1997 and July 1998 who were living within 20 km from the hospital were sent an information leaflet by mail, followed up by a telephone call. Eligibility criteria were as follows: (1) gestational age ≥ 37

weeks, (2) birth weight >2500 g, (3) no chronic illness, (4) maternal age ≥ 16 years, (5) infant exclusively breast-fed at 4 months (and not >90 mL/d of formula during any period since birth), (6) mother intended to exclusively breast-feed until 6 months (ie, ≤ 1 tablespoon per day of foods or fluids other than breast milk, and no iron-fortified foods), and (7) mother intended to continue breast-feeding to at least 9 months. Infants with Hb <90 g/L at any time were to be referred to a pediatrician for iron treatment, but no such cases occurred at 4 or 6 months. The study was approved by the Human Subjects Review Committees of the University of California, Davis, and Umeå University, Sweden. All participating mothers/parents gave written informed consent.

Study Design

Subjects were stratified by study site and sex and randomized to 3 intervention groups: (1) iron supplement from 4 to 9 months of age (Fe 4-9), (2) placebo from 4 to 6 months and iron from 6 to 9 months (Fe 6-9), and (3) placebo from 4 to 9 months.

The iron supplement was a commercially available formulation (Fer-In-Sol, Mead Johnson, Evansville, Ind) of ferrous sulfate containing 25 mg/mL of elemental iron. The placebo solution had similar appearance and taste. The investigators and the parents were blinded to the intervention. The supplement was given at a dose corresponding to 1.0 mg of elemental iron per kg per day, which is the recommended supplemental dose for prophylactic purposes, and the dose was adjusted monthly according to weight. The supplement or placebo was given by the mother each morning, just before or after breast-feeding and at least 1 hour before or after any other food intake.

Between 4 and 6 months, the mothers were discouraged from giving any other foods or fluids except for "taste portions" (≤ 1 tablespoon per day) of foods with little or no iron. Between 6

and 9 months, the mothers continued breast-feeding but were allowed to give complementary food at their own discretion. No attempt was made by the investigators to influence the choice of foods or the extent of breast-feeding.

Data Collection and Analysis

Venous blood (~5 mL) was obtained at 4, 6, and 9 months of age. Part of the sample was collected in an EDTA test tube and immediately analyzed in duplicate for Hb (HemoCue, Ångelholm, Sweden) and ZPP (Protofluor Z, Helena Labs, Beaumont, Tex). These 2 devices were checked weekly against standard solutions at both sites. Erythrocyte mean cell volume was measured in the remainder of the EDTA blood with automated blood counters in Honduras (Cell-Dyn 610, Abbot Diagnostics, Berkshire, UK) and Sweden (Sysmex SE 9000, Tillquist, Sweden). Part of the sample was collected in a lithium heparin tube and, after centrifugation, plasma was stored frozen at -20°C until analyzed for ferritin (Coat-A-Count Ferritin IRMA, Diagnostic Products Corp, Los Angeles, Calif), TfR (Ramco, Houston, Tex), and C-reactive protein (Nanorid, The Binding Site, Birmingham, England).

Complementary food intake between 6 and 9 months was estimated in Honduras by a bi-weekly 24-hour recall and a food frequency questionnaire and in Sweden by a monthly 5-day food diary. Intakes of specific nutrients in Honduras were calculated with the FoodProcessor program (ESHA Research, Salem, Ore) and in Sweden with the Food Composition Tables from the National Food Administration combined with information from Swedish baby food manufacturers.

Birth weights were measured by the investigators in Honduras and excerpted from delivery charts in Sweden. From 4 to 9 months of age, infant weight was measured monthly by the investigators at both study sites.

Statistical Methods and Definitions

A prestudy analysis showed that a sample size of 39 infants per intervention group was required ($\alpha = .05$, 80% power) to detect a difference in Hb of 5 g/L among treatment groups. To compensate for possible differences in the effects of iron supplementation in Honduras and Sweden, the sample size was increased by 50% to 60 infants per group, or 30 infants per group per site. For these analyses anemia was defined as Hb <110 g/L.^{10,14} Iron deficiency was defined as 2 of 3 iron status indexes (ferritin, MCV, and ZPP) being abnormal, with the following cutoff values: ferritin <12 $\mu\text{g/L}$,¹⁵ MCV <70 fL,¹⁵ and ZPP >80 $\mu\text{mol/mol heme}$.¹⁶ IDA was defined as anemia in combination with iron deficiency.

All statistical analyses were performed with SPSS software version 9.0 (SPSS Inc, Chicago, Ill). Statistical methods used were *t* test for comparing means and χ^2 for comparing proportions. The Fisher exact test was used when applicable. Analysis of variance was used for multivariate analyses and the Bonferroni technique for multiple comparisons. Binary logistic regression was used for multivariate analyses with dichotomous outcome variables. Because the distributions of ferritin and ZPP values were skewed, they were log transformed in all calculations and presented as the geometric means. Analysis of covariance was performed for each response variable, with group and site as categorical factors and baseline value of the response variable as the covariate. An interaction term was fit to determine whether the relationship between the baseline value and the response variable was different between groups and sites; in all cases this interaction was not significant. Intervention effect was defined as the difference between the adjusted mean values of the intervention group and the placebo group at 6 or 9 months, controlling for baseline value. For analyses of intervention effects

from 4 to 6 months, the Fe 4-9 group was compared with the 2 unsupplemented groups. For analyses of intervention effects from 6 to 9 months, the Fe 6-9 group was compared with the placebo group, excluding the Fe 4-9 group because of their different baseline iron status at 6 months.

RESULTS

Subjects

At 4 months of age, 263 infants were randomized into the 3 intervention groups. Remaining in the study were 232 infants at 6 months and 214 at 9 months (Fig 1). All infants remaining in the study at 6 months were included in the statistical analyses. The total dropout rate was not significantly dif-

ferent between Honduras and Sweden. Considering both study sites separately, there were no significant differences between dropouts and non-dropouts with respect to intervention group, maternal height, weight or parity, infant sex, birth weight or weight, length, Hb, MCV, ZPP, or ferritin at 4 months. Compliance with the intervention was based on the mother's daily checklist or the amount of fluid remaining in the bottles each month. When these indicated that the infant had been given the study drops <75% of the days during either of the time intervals (4 to 6 months or 6 to 9 months), we considered these cases as noncompliant. With this definition, we found 24 noncompliers (16 in Honduras and 8 in Sweden). Noncompliers

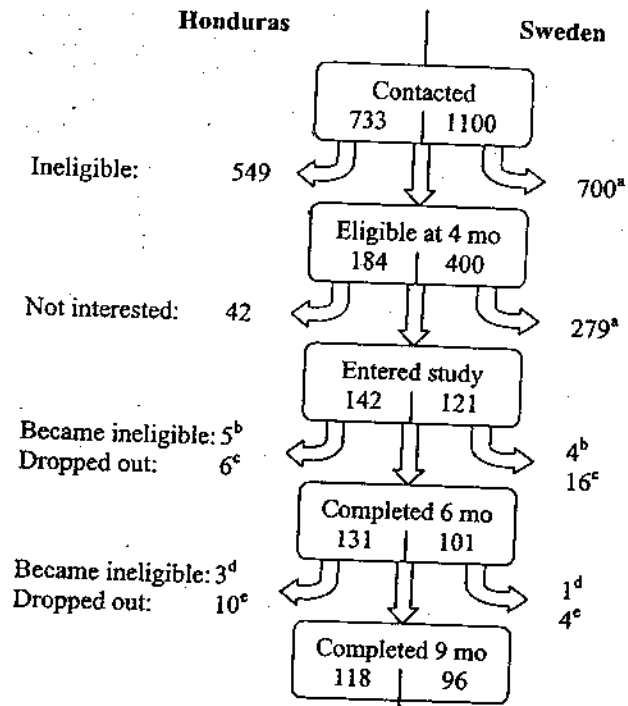


Fig 1. Schematic representation of recruitment and dropout of subjects. ^aAlso includes those who could not be reached. Approximation is based on telephone interviews in Sweden during part of the study. ^bBecause of introduction of food/fluid other than breast milk (Honduras: 2 in placebo group, 3 in Fe 6-9 group; Sweden: 1 in each intervention group) or because of occurrence of chronic disease (idiopathic thrombocytopenic purpura, n = 1, in Swedish Fe 6-9 group). ^cMoved away or refused to continue. Honduras: 2 in each group; Sweden: 3 in placebo group, 5 in Fe 6-9 group, 8 in Fe 4-9 group. ^dBecause of termination of breast-feeding. Honduras: 1 in Fe 4-9 group, 2 in Fe 6-9 group; Sweden: 1 in Fe 6-9 group. ^eMoved away or refused to continue. Honduras: 2 in placebo group and 4 in each of the 2 iron-supplemented groups; Sweden: 1 in Fe 4-9 group, 3 in Fe 6-9 group.

Table I. Subject characteristics

Characteristic	Honduras				Sweden			
	Placebo (n = 44)	Fe 4-9 mo (n = 45)	Fe 6-9 mo (n = 42)	P value	Placebo (n = 36)	Fe 4-9 mo (n = 31)	Fe 6-9 mo (n = 34)	P value
Maternal age (y)	26.0 (7.2)	24.7 (6.0)	26.1 (5.5)	.52	31.2 (4.4)	30.7 (3.4)	30.8 (5.1)	.90
Parity (No.)	3.1 (2.4)	2.7 (1.7)	2.6 (1.2)	.36	2.0 (1.3)	1.7 (1.2)	1.9 (1.0)	.62
Maternal height (cm)	149.4 (6.3)	151.4 (6.1)	151.3 (5.7)	.23	166.5 (6.2)	165.5 (5.6)	167.3 (6.7)	.50
Maternal weight (kg)	58.3 (13.6)	55.0 (9.9)	54.9 (9.4)	.27	64.3 (9.6)	65.1 (10.2)	66.7 (10.7)	.62
Sex (% male)	52.3	51.1	64.3	.40	41.7	48.4	50.0	.76
Birth weight (kg)	3.11 (0.38)	3.26 (0.39)	3.26 (0.41)	.13	3.53 (0.41)	3.61 (0.40)	3.75 (0.51)	.11
Birth length (cm)	48.9 (1.9)	49.4 (1.8)	49.4 (2.2)	.47	50.4 (1.9)	51.1 (1.9)	51.2 (2.4)	.26
Weight at 4 mo (kg)	6.50 (0.75)	6.60 (0.80)	6.84 (0.68)	.11	6.76 (0.75)	6.96 (0.89)	6.88 (0.78)	.59
Weight at 9 mo (kg)	8.05 (0.95)	8.10 (1.06)	8.15 (0.86)	.90	8.90 (1.09)	8.82 (1.09)	8.90 (0.88)	.93
Mean (SD)								

Table II. Mean values of main study variables at 4, 6, and 9 months

Variable	Age (mo)	Honduras			P value†	Sweden			P value†
		Placebo (n = 44*)	Fe 6-9 mo (n = 42*)	Fe 4-9 mo (n = 45*)		Placebo (n = 36*)	Fe 6-9 mo (n = 34*)	Fe 4-9 mo (n = 31*)	
Hb (g/L)	4	113.9 (6.4)	109.9 (6.9)	112.4 (9.1)	.051	117.4 (7.7)	118.5 (6.5)	121.3 (7.2)	.083
	6	110.5 (7.2)	107.8 (7.6)	114.8 (9.7)	.001	117.7 (6.1)	119.3 (7.1)	125.9 (7.0)	<.001
	9	108.7 (8.8)	113.9 (8.3)	115.9 (10.9)	.002	114.4 (7.3)	117.1 (7.1)	121.3 (6.5)	.001
MCV (fL)	4	73.9 (4.5)	75.1 (5.1)	74.4 (4.8)	.509	80.1 (4.3)	79.7 (2.8)	78.9 (3.1)	.382
	6	69.2 (4.9)	69.4 (3.9)	70.6 (4.2)	.245	77.0 (4.1)	77.6 (3.6)	76.9 (2.9)	.714
	9	67.1 (5.5)	70.7 (4.3)	71.8 (4.6)	<.001	76.7 (4.3)	79.0 (3.9)	78.0 (3.3)	.069
Ferritin (µg/L)*	4	67.4 (45-127)	65.8 (36-119)	69.3 (37-130)	.962	114 (57-208)	110 (65-211)	107 (62-163)	.949
	6	32.5 (17-54)	30.5 (12-67)	55.0 (32-92)	.012	56.4 (25-120)	56.2 (28-110)	90.6 (61-131)	.056
	9	15.8 (8-31)	31.9 (18-57)	42.9 (24-74)	<.001	22.9 (12-51)	47.3 (21-81)	66.9 (47-93)	<.001
ZPP (µmol/mol heme)*	4	50.4 (40-66)	49.6 (40-62)	52.0 (44-63)	.802	46.3 (38-55)	43.7 (39-50)	46.4 (40-51)	.511
	6	57.4 (46-73)	57.8 (41-65)	52.7 (46-76)	.391	47.0 (38-56)	44.0 (39-50)	44.6 (40-53)	.484
	9	73.2 (56-88)	58.8 (47-76)	53.3 (41-67)	.002	51.3 (40-62)	42.9 (37-49)	41.6 (36-49)	.004
TfR (mg/L)	4	6.81 (2.0)	6.23 (1.8)	6.32 (2.0)	.328	6.74 (1.5)	6.82 (1.5)	7.31 (3.3)	.548
	6	7.54 (2.7)	7.11 (2.1)	6.24 (2.2)	.032	6.68 (1.9)	7.21 (1.6)	7.35 (3.1)	.436
	9	9.31 (3.4)	6.83 (1.9)	6.54 (2.3)	<.001	8.08 (2.5)	7.45 (1.6)	7.47 (2.8)	.467

Numbers in parentheses are standard deviations. Ranges in parentheses are 25th to 75th percentiles.

*Number of infants at 4 and 6 months. Corresponding numbers for 9 months, after late dropouts, were 35, 30, 30, 42, 36, and 40.

†P value for intervention (3 groups).

*Geometrical means (based on log values).

were included in all statistical analyses according to the "intention-to-treat" principle.

Baseline Values

The Honduran and Swedish subjects differed significantly in maternal age, height, weight, parity, and infant birth

weight and length (Table I), but there were no significant differences among intervention groups within either site. There were large socioeconomic differences between sites: 91% of the Honduran mothers had an education of ≤ 9 years, whereas 97% of the Swedish mothers had an education of > 9 years.

At baseline (4 months), the Honduran infants (vs the Swedish) had significantly lower Hb (112 vs 119 g/L, $P < .001$), MCV (74.5 vs 79.6 fL, $P < .001$), and ferritin (68 vs 110 µg/L, $P < .001$) and higher ZPP (51 vs 45 µmol/mol heme, $P = .007$), whereas TfR was not significantly different between sites

Honduras	Sweden	P value
All groups (n = 131)	All groups (n = 101)	
25.6 (6.3)	30.9 (4.3)	<.001
2.8 (1.8)	1.9 (1.2)	<.001
150.7 (6.1)	166.5 (6.2)	<.001
56.1 (11.2)	65.3 (10.1)	<.001
55.7	46.5	.165
3.21 (0.39)	3.63 (0.45)	<.001
49.2 (2.0)	50.9 (2.1)	<.001
6.64 (0.74)	6.86 (0.80)	.033
8.09 (0.95)	8.88 (1.02)	<.001

(Table II). Part of the difference between sites in baseline iron status was explained by lower birth weight and larger weight gain during the first 4 months of life in the Honduran infants. When adjusting for these 2 factors, the difference in baseline ferritin between sites was reduced by 47%, and the difference in ZPP was reduced by 45%; however, the differences in Hb and MCV between sites were not appreciably reduced. During pregnancy 85% of the Honduran and 40% of the Swedish mothers received iron (or iron and multivitamin) supplements. There was no correlation between maternal iron supplementation and infant iron status at 4 months. There was no significant difference among the 3 intervention groups with respect to infant sex or baseline values for any of the main study variables (Hb, MCV, ferritin, ZPP, and TfR), analyzed separately for each site.

Effects of Iron Supplementation on Iron Status

The mean values of the main study variables for all groups at 4, 6, and 9 months are shown in Table II. The effects of iron supplementation on iron status during the time intervals 4 to 6, 6 to 9, and 4 to 9 months, controlling for baseline values, are shown in Tables III, IV, and V. The distribution of

Table III. Effect of iron supplementation from 4 to 6 months on iron status at 6 months

Variable	4 mo		6 mo*			
	Baseline (n = 232)	Placebo (n = 156)	Iron (n = 76)	Interv P value†	Site P value‡	Interv site P value§
Hb (g/L)	115.1	113.9	119.1	<.001	<.001	.913
MCV (fL)	76.7	73.0	73.6	.204	<.001	.130
Ferritin (mg/L)	83.9	40.4	69.0	<.001	.153	.995
ZPP (μmol/mol heme)	48.4	52.0	47.8	<.001	<.001	.090
TfR (mg/L)	6.67	(7.12)	(6.68)	(.062)	(.567)	.038

Interv, Intervention.
Numbers in parentheses are not interpretable because of significant interaction between intervention and site.
*Controlling for baseline value.
†P value for the intervention effect (iron compared with placebo).
‡P value for site (Honduras compared with Sweden).
§P value for the interaction between intervention and site (different effect of iron at the 2 sites).

Table IV. Effect of iron supplementation from 6 to 9 months on iron status at 9 months

Variable	6 mo		9 mo*			
	Baseline (n = 144)†	Placebo (n = 77)	Fe 6-9 mo (n = 67)	Interv P value†	Site P value‡	Interv site P value§
Hb (g/L)	113.3	(111.1)	(115.4)	<.001	(.173)	.011
MCV (fL)	72.9	71.8	74.1	<.001	.025	.310
Ferritin (μg/L)	40.7	18.7	38.9	<.001	.610	.870
ZPP (μmol/mol heme)	51.7	60.6	52.0	<.001	.005	.294
TfR (mg/L)	7.16	8.66	7.20	.2001	.947	.235

Interv, Intervention.
Numbers in parentheses are not interpretable because of significant interaction between intervention and site.
*Controlling for baseline value.
†Fe 4 to 9 months group excluded. All dropouts excluded.
‡P value for the intervention effect (iron compared with placebo).
§P value for site (Honduras compared with Sweden).
¶P value for the interaction between intervention and site (different effect of iron at the 2 sites).

Hb values was normal in all groups both before and after iron supplementation. C-reactive protein was elevated (>10 mg/L) in 39 of 671 blood samples at both sites (8.6% in Honduras, 3.2% in Sweden). When the corresponding ferritin values were excluded from the analyses, all the significant intervention effects on ferritin (Tables III, IV, and V) remained unchanged.

EFFECTS FROM 4 TO 6 MONTHS. The supplemented group (Fe 4-9) was compared with the 2 unsupplemented

groups (placebo and Fe 6-9). The effect of iron supplementation was similar at both sites: Hb increased by 5 g/L and ferritin by 29 μg/L, and ZPP decreased by 4 μmol/mol heme relative to changes in the unsupplemented infants (Table III). There was a significant effect on TfR (0.96 mg/L decrease compared with placebo) in Honduras but not in Sweden (not shown).

EFFECTS FROM 6 TO 9 MONTHS. Infants starting iron supplementation at 6 months (Fe 6-9) were compared with

Table V. Effect of iron supplementation from 4 to 9 or 6 to 9 months on iron status at 9 months

Variable	4 mo		9 mo*				
	Baseline (n = 214) [†]	Placebo (n = 77)	Fe 6-9 mo (n = 67)	Fe 4-9 mo (n = 70)	Interv P value [‡]	Site P value [§]	Interv site P value [¶]
Hb (g/L)	115.1	(111)	(116)	(117)	(<.001)	(.942)	.017
MCV (fL)	76.8	71.9	74.2 [‡]	74.9 [‡]	<.001	<.001	.097
Ferritin (μg/L)	83.5	18.7	38.4 [‡]	51.2 [‡]	<.001	.316	.693
ZPP (μmol/mol heme)	48.7	61.3	52.1 [‡]	46.2 [‡]	<.001	<.001	.354
TfR (mg/L)	6.68	8.59	7.24 [‡]	6.90 [‡]	<.001	.300	.060

Interv: Intervention

Numbers in parentheses are not interpretable because of significant interaction between intervention and site.

[†]Controlling for baseline value.

[‡]All dropouts excluded.

[§]P value for the intervention effect (all 3 groups).

[¶]P value for site (Honduras compared with Sweden).

[¶]P value for the interaction between intervention and site (different intervention effect at the 2 sites).

[‡]Significantly different from placebo.

[‡]Significantly different from placebo and from Fe 6-9 mo.

the placebo group. The effect of iron supplementation, relative to changes in the placebo group, was significant and similar at both sites for MCV (+2.3 fL), ferritin (+20 μg/L), ZPP (-8.6 μmol/mol heme), and TfR (-1.5 mg/L) (Table IV). The effect of iron supplementation on Hb at 6 to 9 months differed between sites, with no significant effect in Sweden but a significant increase of 7.0 g/L in Honduras compared with placebo (Fig 2). Controlling for iron intake from complementary food did not significantly change these results.

To investigate whether the different effect of iron on Hb at 6 to 9 months in Honduras and Sweden could be explained by differences in baseline iron status at 6 months, the interactions between intervention and the baseline values of all the individual indexes of iron status on Hb at 9 months were studied, controlling for baseline Hb and site. These analyses showed that the effect of iron supplementation on Hb from 6 to 9 months was significantly modulated by the 6-month levels of MCV ($P = .020$), ferritin ($P = .023$), ZPP ($P = .048$), and TfR ($P = .004$). In contrast, the effect of iron supplementation from 4 to 6 months was not significantly modulated by any of the in-

dexes of iron status at 4 months ($P = .321$ to $.917$).

EFFECTS FROM 4 TO 9 MONTHS. All 3 intervention groups were compared. The effect of iron supplements was similar at the 2 sites for MCV, ferritin, ZPP, and TfR (Table V). Iron supplementation from either 4 or 6 months had similar and significant effects on MCV (+2.7 fL) and TfR (-1.5 mg/L) compared with placebo. There was a significantly more pronounced effect of iron supplementation from 4 to 9 months compared with 6 to 9 months, on ferritin (+32 vs +20 μg/L, $P = .044$) and ZPP (-15 vs -9.3 μmol/mol heme, $P = .016$). The effect of iron supplementation on Hb was different at the 2 sites: in Honduras, supplementation had a significant effect on Hb (+8.6 g/L, both groups combined), and there was no difference in effect depending on whether supplementation was started at 4 or 6 months. In Sweden, supplementation from 4 to 9 months had a significant effect on Hb (+4.5 g/L, $P = .002$), whereas the effect of iron from 6 to 9 months was not significant (+2.0 g/L, $P = .132$). However, when the effects of 4- to 9-month and 6- to 9-month iron supplementation on Hb were compared with each other,

they were not significantly different ($P = .104$).

Effect on IDA

At 4 months, there was a low proportion of IDA (<3%) in all groups at both sites (Fig 3). The proportion of IDA in Honduras at 6 months was 8.9% in the supplemented compared with 18.8% in the 2 unsupplemented groups ($P = .135$). The reduction in IDA at 9 months by supplementation from either 4 or 6 months was significant ($P = .006$), and at 9 months there was no significant difference between the 2 supplemented Honduran groups ($P = .70$). In Sweden the proportion of IDA was still <3% in the placebo group at 9 months, and there was no significant effect of iron supplementation on the prevalence of IDA at 6 or 9 months.

Influence of Complementary Food

The mean intake of energy and iron from complementary food between 6 and 9 months of age was calculated for each infant. Divided by mean weight at 6 to 9 months, mean energy intake was 22.3 kcal/kg/d in Honduras and 38.6 kcal/kg/d in Sweden. Iron intake (mean ± SD) was 0.14 ± 0.11 mg/kg/d in Honduras and 0.44 ± 0.38 mg/kg/d

in Sweden. Associations of daily energy and iron intake from complementary food with the outcome variables at 6 to 9 months were studied separately in each intervention group at each site. We calculated correlations between iron intake and each outcome variable, controlling for energy intake, baseline outcome value at 6 months, and weight gain at 6 to 9 months. In a similar manner, we calculated correlations between energy intake and each outcome variable, controlling for iron intake, baseline outcome value, and weight gain. The only significant result was a positive correlation between energy intake and ZPP ($P = .001$) in the Honduran placebo group. In the combined sample, no correlation was seen between food iron density (mg Fe/kcal) and any of the outcome variables, controlling for site, intervention, baseline outcome value, and interactions among site, intervention, and iron density.

DISCUSSION

At 4 months of age, the Honduran and Swedish infants already had significantly different iron status. This observation is not likely explained by differences in dietary intake of iron, because all infants were exclusively breast-fed, and breast milk iron concentration has been shown to be unaffected by maternal iron status.¹⁷ Even though only term infants with birth weights >2500 g were included, the Honduran infants had significantly lower birth weight and larger weight gain from birth to 4 months, suggesting catch-up growth. Both of these factors had a negative impact on ferritin and ZPP at 4 months, placing the Honduran infants at higher risk for iron deficiency. We do not have information on maternal nutritional status during pregnancy, maternal smoking, timing of umbilical cord clamping, or infections during the first 4 months of life, but these factors, possibly in combination with genetic factors, may also

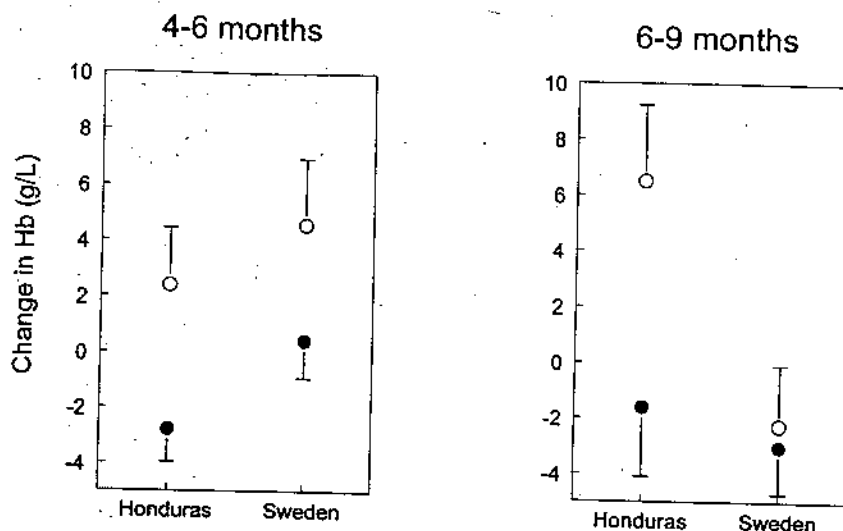


Fig 2. Change in Hb between 4 to 6 and 6 to 9 months of age (mean and 95% CI) in Swedish and Honduran infants receiving iron (O) or placebo (●).

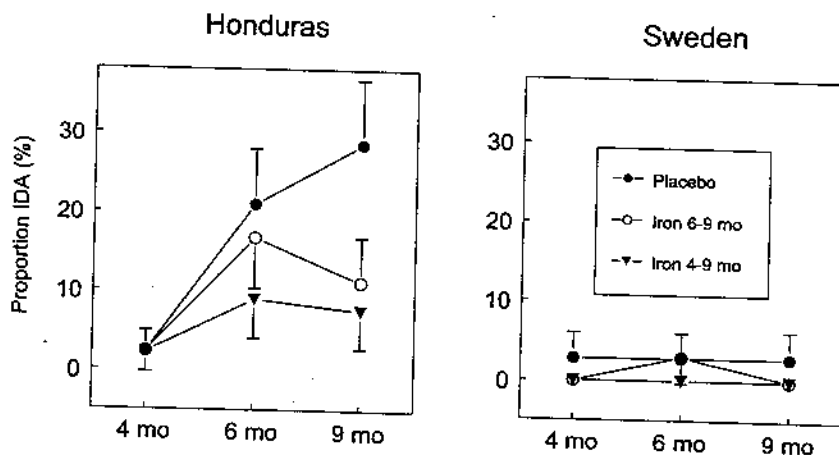


Fig 3. Prevalence of IDA (with 95% CI) in Honduran and Swedish infants at 4, 6, and 9 months of age in different intervention groups. IDA defined as Hb <110 g/L and at least 2 of following 3: MCV <70 fL, ferritin <12 µg/L, ZPP >80 µmol/mol heme.

be important in explaining the differences in baseline iron status between the Honduran and Swedish infants.

Between 4 and 6 months of age, iron supplementation had a significant positive effect on Hb, which was independent of study site, initial Hb, and initial iron status. This unexpected, uniform effect might reflect an immature regulation of iron metabolism at this age, so that iron was absorbed and used for Hb synthesis without any negative feedback mechanism, despite the fact that

our infants can be assumed to have been iron sufficient at 4 months of age.

At 6 to 9 months, the effect of iron supplementation on Hb was no longer uniform, because iron supplementation increased Hb significantly more in those infants who were iron deficient at 6 months. The positive effect of iron supplementation on Hb in Honduras but not in Sweden was explained by the larger proportion of pre-existing iron deficiency in Honduras at 6 months (25% vs 2% in Sweden). We

therefore speculate that there is a regulatory mechanism that increases iron absorption, or iron use for Hb synthesis, in infants with iron deficiency, a mechanism that seems not to be present at 4 to 6 months of age.

It is well known that large iron stores downregulate the absorption of iron in adults,¹⁸ but it is not known whether this occurs during infancy. Little is known about the mechanisms that regulate the extent to which absorbed iron is used for Hb synthesis or put into storage. In this study the effect of iron supplementation on ferritin was significant and independent of study site and pre-existing iron deficiency at both 4 to 6 and 6 to 9 months, indicating that infants might not be able to downregulate their iron absorption as effectively as adults. For example, the Swedish infants who received iron supplementation beginning at 4 months must have had generous iron stores at 6 months, yet continued iron supplementation of these infants from 6 to 9 months had a significant positive effect on ferritin compared with placebo (not shown), whereas Hb in the same infants actually decreased compared with placebo during the same period. This discrepancy suggests that iron absorption, as measured by ferritin response, is regulated independently from Hb synthesis, erythropoiesis, or both. It is also noteworthy that in the same infants, MCV increased, whereas ZPP and TfR decreased compared with placebo. The opposite direction of change in Hb compared with MCV, which was observed also between 4 and 6 months, indicates that red blood cell size and Hb synthesis are regulated differently at this age. Further studies are required to clarify the regulation of iron absorption, Hb synthesis, and erythropoiesis in term infants between 4 and 9 months of age. A better understanding of this regulation has implications for how to define anemia and iron deficiency in this age group.¹⁹

Neither in Sweden nor in Honduras did iron from complementary foods,

which in Sweden amounted to 44% of the iron supplement dose, have any effect on any of the main study variables or on the proportion of IDA at 9 months. This result is surprising, considering that the iron drops had such a significant influence on iron status between 6 and 9 months at both sites. This finding suggests that complementary food iron is poorly absorbed, even in Sweden, where commercially available infant food often is fortified with ferrous sulfate and ascorbic acid. However, it is likely that any effect of iron from complementary foods would be evident only in the placebo groups. No such effect was observed, but our statistical power to detect it was limited. Another limitation is that there was relatively little variation in iron intake, especially in Honduras, compared with the methodologic error inherent in dietary data, which would also make it difficult to detect such a relationship.

The proportion of iron deficiency and IDA in the Honduran placebo group at 9 months was 40.5% and 28.6%, respectively, indicating a significant public health problem. Iron supplementation starting at either 4 or 6 months significantly reduced the incidence of iron deficiency and IDA in the Honduran infants but, considering the outcomes at 9 months, there was no significant advantage of giving the iron supplement from 4 to 9 months compared with 6 to 9 months.

Of the Swedish unsupplemented infants, 20% had Hb <110 g/L, and 29% had ferritin <12 µg/L at 9 months (not shown), which is similar to previous results.^{20,21} However, with the present definitions, the prevalence of iron deficiency and IDA was only 2.9%. Because the prevalence was already low in the placebo group, it was not surprising that iron supplementation resulted in no further reduction in the incidence of iron deficiency or IDA in the Swedish infants. However, the discrepancy between the proportion of infants with low Hb or ferritin and the proportion with IDA suggests re-

evaluation of the commonly used cut-off levels for infants.¹⁹

The results of our study indicate that iron supplementation in the form of ferrous sulfate drops at a dose of 1.0 mg/kg/d, given from 6 to 9 months of age, significantly reduces the risk of IDA among term, breast-fed infants in a population with a high prevalence of IDA (Honduras) but not in a population with a low prevalence (Sweden). In Honduras there was no significant advantage of beginning the supplement at 4 months compared with 6 months with respect to Hb, MCV, or the prevalence of IDA. However, we did observe a significantly lower proportion of iron deficiency at 6 months in Honduran infants who began iron supplements at 4 months. Considering the possible deleterious effects of even preanemic iron deficiency on early infant development, this should be studied further in a larger cohort. Our results also suggest that the regulation of iron absorption is immature in infants before 6 to 9 months of age, and that iron absorption and Hb synthesis are independently regulated. Stable isotope studies of iron absorption in this age group support this interpretation.²² If iron-replete infants of this age group are not able to downregulate the absorption of supplemental iron, as our data suggest, then extended iron supplementation would increase the risk for iron overload, which may have adverse long-term effects. In this same cohort we observed that iron supplementation impaired linear growth.²³

In conclusion, iron supplementation of term, breast-fed infants from 4 to 6 months to at least 9 months of age can improve iron status and reduce anemia in socioeconomically disadvantaged populations where IDA is prevalent. However, similar supplementation in populations where IDA is uncommon is not beneficial and may even be harmful, particularly before 6 months of age.

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