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## Sex Differences in Iron Status During Infancy

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**ABSTRACT.** *Background.* It is commonly assumed that there is no difference in iron status between male and female infants, despite a lack of studies addressing this question.

*Objective.* To study sex differences in different measures of iron status in infants.

*Methods.* At 4 months of age, 263 term, breastfed infants (121 Swedish and 142 Honduran) were randomized to receive iron supplements or placebo until 9 months of age. Blood samples at 4, 6, and 9 months of age were analyzed for hemoglobin (Hb), mean cell volume (MCV), zinc protoporphyrin (ZPP), plasma ferritin, and transferrin receptors (TfR).

*Results.* At 4, 6, and 9 months, boys had significantly lower Hb, MCV, and ferritin and higher ZPP and TfR than girls. At 9 months, boys had a 10-fold higher risk of being classified as having iron deficiency anemia. The differences at 9 months in MCV (71.6 vs 75.1 fL) and ZPP (59 vs 49  $\mu\text{mol/mol}$  heme) remained significant after controlling for iron supplementation, site, growth variables, and other possible confounders. For ferritin, there was a remaining sex difference at 9 months among Swedish (29 vs 53  $\mu\text{g/L}$ ) but not Honduran infants. For Hb and TfR, sex differences at 9 months were larger in unsupplemented infants, especially in those with a birth weight of <3500 g.

*Conclusions.* There are substantial sex differences in Hb and other indicators of iron status during infancy. Some of these may be genetically determined, whereas others seem to reflect an increased incidence of true iron deficiency in boys. *Pediatrics* 2002;110:545–552; sex differences, gender, hemoglobin, MCV, ferritin, transferrin receptors, zinc protoporphyrin, breastfeeding, human infant, iron deficiency anemia.

ABBREVIATIONS. Hb, hemoglobin; MCV, mean cell volume; ZPP, zinc protoporphyrin; TfR, transferrin receptors.

It is well known that there are differences in iron status between males and females in adolescence and adulthood.<sup>1</sup> These differences are considered to be primarily attributable to menstrual losses of iron in fertile women, and they can be moderated by dietary factors and iron supplements.<sup>2,3</sup> However, recent studies in adolescents and adults also suggest that there may be other sex-related differences, such

as hormonal changes or differences in growth, that cause some indicators of iron status to vary between sexes.<sup>4,5</sup> To date, little has been reported about sex-related differences in indicators of iron status in infants, although iron deficiency in infants and toddlers is a significant public health problem that may adversely affect psychomotor development at this crucial age.<sup>1,6–8</sup>

Hemoglobin (Hb) and erythrocyte mean cell volume (MCV) are affected by the rate of erythropoiesis, but our knowledge about factors regulating erythropoiesis is still limited. Erythropoietin is known to induce reticulocytosis and can be used clinically to stimulate erythropoiesis in premature infants.<sup>9</sup> Zinc protoporphyrin (ZPP) forms as a metabolic by-product of iron-deficient erythropoiesis, and the ZPP/heme ratio is used clinically to detect states of pre-anemic iron depletion.<sup>10</sup>

Serum or plasma ferritin concentrations are correlated with body iron stores<sup>11</sup> but are also increased during inflammation and infection.<sup>12</sup> Infants are born with very high ferritin concentrations, which decrease during the first 9 to 12 months of life.<sup>13</sup> This change is believed to reflect the substantial iron stores of term infants at birth, which with time are depleted because of infant growth in combination with limited iron intake and poor bioavailability of dietary iron. However, little is known about other factors affecting ferritin concentrations during infancy. The concentration of soluble transferrin receptors (TfR) in serum or plasma has been suggested to correlate well with cellular iron needs and may therefore be a good indicator of iron status.<sup>14</sup> This notion was reinforced by the finding that, unlike serum ferritin, TfR is not affected by inflammation or infection.<sup>15</sup> Thus, TfR may be used to assess iron status in situations when infection is common. However, there have been few studies exploring factors other than infection that may affect TfR.

The overall aim of this randomized, placebo-controlled trial was to investigate the effects of daily iron supplementation on iron deficiency anemia in Swedish and Honduran breastfed infants. These results have been published elsewhere<sup>16</sup>: briefly, iron supplementation reduced the prevalence of iron deficiency anemia in Honduras at 9 months to 9% compared with 29% in the placebo group ( $P = .006$ ), whereas in Sweden, the proportion of iron deficiency anemia was <3% at 9 months in both unsupplemented and supplemented infants. The aim of the current study was to investigate whether there were any differences between male and female infants in

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Hb and other measures of iron status. The complete study group, consisting of iron-supplemented and unsupplemented Swedish and Honduran infants, represented a large number of subjects with highly variable iron status at baseline, as well as at completion of the intervention. This allowed us to study not only sex differences among infants in general at 4 to 9 months of age, but also the potential effect of iron supplementation on such differences.

## MATERIALS AND METHODS

### Participants

This study was a randomized, double-blind, placebo-controlled iron supplementation trial conducted at 2 sites: San Pedro Sula, Honduras, and Umeå, Sweden. Term infants were recruited directly after delivery (Honduras), or at ~3 months of age (Sweden). Eligibility criteria were as follows: 1) gestational age  $\geq 37$  weeks, 2) birth weight  $> 2500$  g, 3) no chronic illness, 4) maternal age  $\geq 16$  years, 5) infant exclusively breastfed at 4 months (and not  $> 90$  mL/d of formula during any period since birth), 6) mother intended to exclusively breastfeed until 6 months (ie,  $\leq 1$  tablespoon/day of foods or fluids other than breast milk, and no iron-fortified foods), and 7) mother intended to continue breastfeeding to at least 9 months. Infants with Hb  $< 90$  g/L at any time were to have been 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 Committee of the University of California, Davis, and the Ethical Committee, Faculty of Medicine and Odontology, Umeå University, Sweden. All participating mothers/parents gave written informed consent.

### Study Design

At 4 months of age, infants were stratified by study site and sex and randomized to 3 intervention groups: 1) iron supplements from 4 to 9 months old; 2) placebo from 4 to 6 months and iron from 6 to 9 months; and 3) placebo from 4 to 9 months.

The iron supplement was a commercially available formulation (Fer-In-Sol, Mead Johnson, Evansville, IN) of ferrous sulfate containing 25 mg/mL of elemental iron. The placebo solution had similar appearance and taste. The dose was 1 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 each infant's weight. The supplement or placebo was given by the mother each morning, just before or after breastfeeding 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" ( $\leq 1$  tablespoon per day) of foods with little or no iron. Between 6 and 9 months, the mothers continued breastfeeding 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 breastfeeding.

### 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 ethylenediamine tetraacetic acid test tube and immediately analyzed in duplicate for Hb (HemoCue, Ängelholm, Sweden) and ZPP (Protofluor Z, Helena Labs, Beaumont, TX). These 2 devices were checked weekly against standard solutions at both sites. MCV was measured in the remainder of the EDTA blood, using 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  $-20^{\circ}\text{C}$  until analyzed for ferritin (Coat-A-Count Ferritin IRMA, Diagnostic Products Corp, Los Angeles, CA), TfR (Ramco, Houston, TX), and C-reactive protein (Nanorid, The Binding Site, Birmingham, England). Iron deficiency anemia was defined as anemia (Hb  $< 110$  g/L) in combination with at least 2 of 3 of the following: MCV  $< 70$  fL, ZPP  $> 80$   $\mu\text{mol/mol}$  heme, and ferritin  $< 12$   $\mu\text{g/L}$ .<sup>16</sup>

Nutrient intake from complementary food was assessed between 6 and 9 months, as described elsewhere.<sup>16</sup> Birth weights were measured by the study team in Honduras and recorded from delivery charts in Sweden. From 4 to 9 months old, infant weight

was measured monthly by the investigators at both study sites. Compliance with the intervention was monitored by asking the mothers to keep a daily checklist indicating whether the drops were given and by collecting the used bottles each month and measuring the amount of fluid remaining. Compliance was defined as taking the study drops for  $> 75\%$  of the time during both of the 2 time intervals (4–6 months and 6–9 months).

### Statistics

All statistical analyses were performed using SPSS software version 10.0 (SPSS Inc, Chicago IL). Statistical methods used were multiple linear regression, analysis of covariance, *t* test,  $\chi^2$  test, and Fisher exact test. Stepwise multiple linear regression was used to test the significance of interactions. Because the distributions of ferritin and ZPP values were skewed, they were log-transformed in all calculations. For presentation, these 2 variables were transformed back to the original scale and presented as the geometric means and geometric standard deviations. Because differences between means for log values are equivalent to ratios between the corresponding geometrical means, mean differences in these 2 variables (ZPP and ferritin) were expressed as percentages.

## RESULTS

At 4 months old, 263 infants (142 in Honduras, 121 in Sweden) were randomized into the 3 intervention groups. Remaining in the study were 232 infants at 6 months and 214 at 9 months. All infants remaining in the study at 6 months were included in the statistical analyses. Total dropout rate was not significantly different between Honduras and Sweden or between male and female infants. Boys, compared with girls, had nonsignificantly higher birth weight and significantly larger postnatal weight gain (Table 1). There were no significant differences between sexes in energy or iron intake from complementary food (Table 1). There was no significant interaction between infant sex and randomization group for any of the iron status variables at 4 months.

### Sex Differences at 4 and 6 Months

At 4 months, boys (compared with girls) had 2 g/L lower mean Hb ( $P = .013$ ), 2.7 fL lower MCV ( $P < .001$ ), 11% higher ZPP ( $P = .007$ ), 40% lower ferritin ( $P < .001$ ), and 1.1 mg/L higher TfR ( $P < .001$ ; Table 2). When adjusting for site, birth weight, and postnatal weight gain, all sex differences remained sig-

TABLE 1. Participant Characteristics

	Boys	Girls	<i>P</i>
<i>n</i>	120*	112*	
Birth weight (kg)	3.4 (0.5)	3.3 (0.4)	.065
Weight gain 0–4 mo (kg)	3.5 (0.7)	3.2 (0.6)	$< .001$
Weight gain 0–9 mo (kg)*	5.3 (0.9)	4.8 (0.9)	$< .001$
Complementary food energy (kcal/kg/d)*	33 (22)	33 (18)	.805
Complementary food Fe (mg/kg/d)*	0.3 (0.3)	0.3 (0.3)	.901
Honduran (%)	61	52	.186†
Honduras–placebo ( <i>n</i> )	23	21	
Honduras–Fe 4–9 mo ( <i>n</i> )	23	22	.397‡
Honduras–Fe 6–9 mo ( <i>n</i> )	27	15	
Sweden–placebo ( <i>n</i> )	15	21	
Sweden–Fe 4–9 mo ( <i>n</i> )	15	16	.760‡
Sweden–Fe 6–9 mo ( <i>n</i> )	17	17	

Mean (standard deviation). *P* values are 2-tailed. The lower part of the table shows the sample sizes cross-tabulated by site, randomization group, and sex.

\* At 9 months, *n* = 108 (boys) and 106 (girls).

† Fisher exact test was used.

‡  $\chi^2$  test was used.

**TABLE 2.** Sex Differences in Iron Status at 4 and 6 Months of Age

	4 Months					6 Months				
	Boys*	Girls*	Sex Difference†	P‡	P§	Boys*	Girls*	Sex Difference†	P‡	
Hb (g/L)	114 (8)	116 (8)	-2	.013	.095	113 (9)	117 (10)	-4	.001	
MCV (fL)	75.4 (4.7)	78.1 (4.9)	-2.7	<.001	.002	71.3 (5.4)	74.7 (4.9)	-3.4	<.001	
ZPP (μmol/mol heme)	51 (1.4)	46 (1.3)	+11%	.007	.025	55 (1.4)	47 (1.3)	+19%	<.001	
Ferritin (μg/L)	65 (2.5)	110 (2.0)	-40%	<.001	<.001	37 (2.8)	64 (2.5)	-42%	<.001	
TfR (mg/L)	7.2 (2.3)	6.2 (1.6)	+1.1	<.001	.001	7.6 (2.7)	6.4 (1.6)	+1.2	<.001	

N = 232 (120 boys, 112 girls).

\* Mean (standard deviation) for Hb, MCV, and TfR. Geometric mean (standard deviation) for ferritin and ZPP. Note that, whereas the arithmetic standard deviation is added (subtracted) to the mean, the geometric standard deviation is multiplied (divided) with the geometric mean.

† Sex difference: Mean difference (boys – girls) for Hb, MCV, and TfR. Mean difference as percentage of girls’ mean for ZPP and ferritin.

‡ Bivariate P value for sex.

§ Multivariate P value for sex, model including the variables study site, birth weight, and postnatal weight gain (birth to 4 months). All interactions between sex and each of the other 3 variables were tested in the model, and none were significant.

nificant except Hb ( $P = .095$ ). None of the possible interactions between sex and other variables (study site, birth weight, or postnatal weight gain) were significant for any of the outcome variables (Table 2). The proportion of infants with iron deficiency anemia at 4 months was low (2%) with no significant sex difference ( $P = .123$ ).

At 6 months, the sex differences remained and were significant for all variables in a bivariate analysis (Table 2). Because similar sex differences in iron status existed at 4, 6, and 9 months of age (see below), we chose to simplify the presentation by not including multivariate analyses for 6 months.

**Sex Differences in Response to Iron Supplementation**

If the sex differences in iron status are caused by a “true” iron deficiency, iron supplementation would theoretically cause a larger response in boys than in girls, thus reducing the sex difference. To investigate this, the interaction between sex and iron supplementation was calculated for the change in each variable between 4 and 9 months of age (Table 3). The interaction was significant for Hb and TfR, even when controlling for birth weight, weight gain from 4 to 9 months, complementary food energy intake, study site, compliance, and baseline value (Table 3).

In contrast, for MCV, ZPP, and ferritin, the interaction between sex and iron supplementation was not significant (Table 3).

**Sex Differences in Iron Status at 9 Months**

At 9 months, boys (compared with girls) had 4 g/L lower mean Hb ( $P < .001$ ), 3.5 fL lower MCV ( $P < .001$ ), 20% higher ZPP ( $P = .007$ ), 30% lower ferritin ( $P = .007$ ), and 1.1 mg/L higher TfR ( $P = .002$ ; Table 4). Because the sex differences in Hb and TfR were different in iron-supplemented and unsupplemented infants (Table 3) and because there was a significant interaction between sex and study site for ferritin at 9 months (Table 4), these 3 variables were studied separately in subgroups of infants (see below). For MCV and ZPP, however, there were no significant interactions between sex and any other variable (iron supplementation, duration of iron supplementation, compliance, birth weight, postnatal weight gain, complementary food energy intake, or study site). Furthermore, the sex differences for MCV and ZPP at 9 months remained when controlling for all these potential confounders in a multivariate analysis ( $P < .001$  and  $P = .002$ , respectively; Table 4). Even when restricting the population sample to Swedish, iron-supplemented infants ( $n = 60$ ), sex differences re-

**TABLE 3.** Sex Differences in the Change in Iron Status Among Iron-Supplemented and Unsupplemented Infants

	Iron-Supplemented					Unsupplemented					Interaction Sex* Fe	
	Boys †	Girls †	Sex Difference‡	P§	P	Boys †	Girls †	Sex Difference‡	P‡	P	P¶	P#
Hb (g/L)	+2 (8)	+2 (8)	0	.937	.749	-7 (8)	-2 (6)	-5	.003	<.001	.016*	.031*
MCV (fL)	-2.7 (4.3)	-2.3 (3.8)	-0.4	.563	.059	-6.1 (4.0)	-4.2 (3.6)	-1.9	.032	.022	.192	.187
ZPP (%)	+5 (1.3)	-1 (1.3)	+6	.188	.232	+35 (1.4)	+21 (1.3)	+14	.130	.050	.556	.226
Ferritin (%)	-41 (2.4)	-54 (0.8)	+13	.070	.544	-77 (2.1)	-78 (0.8)	+1	.677	.646	.402	.232
TfR (mg/L)	+0.3 (2.0)	+0.5 (1.5)	-0.2	.450	.945	+2.4 (2.8)	+1.5 (1.8)	+0.9	.090	.129	.046*	.010*

n = 213 (108 boys, 105 girls; 136 iron-supplemented, 77 unsupplemented).

\* Significant interaction between sex and iron supplementation.

† Mean (standard deviation) change (9 months–4 months) for Hb, MCV, and TfR. Geometric mean (standard deviation) difference as percentage of mean at 4 months for ZPP and ferritin. Note that, whereas the arithmetic standard deviation is added (subtracted) to the mean, the geometric standard deviation is multiplied (divided) with the geometric mean.

‡ Sex difference in change (boys – girls).

§ Bivariate P value for sex.

|| Multivariate P value for sex, model including the variables study site, duration of iron supplementation (4–9 or 6–9 months), compliance, birth weight, weight gain (4–9 months), complementary food energy intake (kcal/kg/d), and baseline value of the studied variable at 4 months.

¶ P value for the interaction between sex and iron supplementation (yes or no) with only those 2 variables included in the model.

# P value for the interaction between sex and iron supplementation, with the following variables also included in the model: site, duration of iron supplementation, compliance, birth weight, weight gain, complementary food energy intake, and baseline value at 4 months.

**TABLE 4.** Sex Differences in Iron Status at 9 Months of Age

	Boys*	Girls*	Sex Difference†	P‡	P§
Hb (g/L)	113 (9)	117 (8)	-4	<.001	
MCV (fL)	71.6 (6.2)	75.1 (5.6)	-3.5	<.001	<.001¶
ZPP ( $\mu\text{mol/mol heme}$ )	59 (1.5)	49 (1.7)	+20%	.001	.002¶
Ferritin ( $\mu\text{g/L}$ )	27 (2.7)	39 (2.4)	-30%	.007	#
TfR (mg/L)	8.2 (3.0)	7.1 (2.1)	+1.1	.002	**

*N* = 213 (108 boys, 105 girls).

\* Mean (standard deviation) for Hb, MCV, and TfR. Geometric mean (standard deviation) for ferritin and ZPP.

Note that, whereas the arithmetic standard deviation is added (subtracted) to the mean, the geometric standard deviation is multiplied (divided) with the geometric mean.

† Sex difference: mean difference (boys - girls) for Hb, MCV, and TfR. Mean difference as percentage of girls' mean for ZPP and ferritin.

‡ Bivariate *P* value for sex.

§ Multivariate *P* value for sex, model including the variables study site, iron supplementation (yes or no), duration of supplementation (4-9 or 6-9 months), compliance (yes or no), birth weight, postnatal weight gain (birth to 9 months), and complementary food energy intake (kcal/kg/d).

|| Multivariate *P* value for sex not presented because there was a significant sex difference in Hb response to iron supplementation (*P* = .031; Table 3).

¶ All interactions between sex and each of the other 7 variables were tested in the model, and none were significant.

# Multivariate *P* value for sex not presented because there was a significant interaction between sex and site in this model (*P* = .002).

\*\* Multivariate *P* value for sex not presented because there was a significant sex difference in TfR response to iron supplementation (*P* = .010; Table 3).

mained: 2 fL in MCV (boys 77.5 vs girls 79.5 fL, *P* = .038) and 10% in ZPP (boys 44 vs girls 40  $\mu\text{mol/mol heme}$ , *P* = .132).

#### Sex Differences in Hb at 9 Months

At 9 months, iron-supplemented boys (vs iron-supplemented girls) had 4 g/L lower Hb (115 vs 119 g/L, Table 5). This difference remained significant when controlling for study site, duration of iron supplementation, compliance, birth weight, postnatal weight gain, and complementary food intake, and there were no significant interactions between sex and any of these factors among iron-supplemented infants. However, among unsupplemented infants at 9 months, there were significant interactions between sex and birth weight (*P* = .028) and between sex and complementary food energy intake (*P* = .014). The latter was in the direction of a larger sex difference in

infants with a higher complementary food energy intake. However, the number of infants with a higher intake (>50 kcal/kg/d) was low (*n* = 14), and when a single outlier was excluded, the interaction was no longer significant. Because of the interaction between sex and birth weight, 2 birth weight groups were studied separately. Among unsupplemented infants with a birth weight of <3500 g, boys (vs girls) had 9 g/L lower Hb (105 vs 114 g/L), the difference being significant even when controlling for other possible explanatory factors (*P* = .002), whereas among unsupplemented infants with a birth weight of  $\geq 3500$  g, boys and girls did not have significantly different Hb (113 vs 114 g/L; Table 5).

#### Sex Differences in Ferritin at 9 Months

For ferritin at 9 months, there was a significant interaction between sex and site (*P* = .002), indicat-

**TABLE 5.** Sex Differences in Hb at 9 Months of Age

Population Sample	<i>n</i>	Boys	Girls	Sex Difference*	P†	P‡
Iron-supplemented	136	115 (9)	119 (9)	-4	.004	.004§
Unsupplemented	77	108 (9)	114 (7)	-6	.003	
Unsupplemented, birth weight $\geq 3500$ g¶	29	113 (4)	114 (6)	-1	.613	.769#
Unsupplemented birth weight <3500 g**	48	105 (10)	114 (7)	-9	.002	.002#

Mean (standard deviation) Hb (g/L).

\* Sex difference: mean difference (boys - girls).

† Bivariate *P* value for sex.

‡ Multivariate *P* value for sex.

§ Model including the variables study site, duration of iron supplementation (4-9 or 6-9 months), compliance, birth weight, postnatal weight gain (0-9 months), and complementary food energy intake (kcal/kg/d). Interactions between sex and each of the other 6 variables were tested in the model, and none were significant.

|| Model as in footnote §. *P* value for sex not presented because of significant interactions between sex and birth weight (*P* = .028) as well as between sex and complementary food energy intake (*P* = .014). Hence, birth weight groups were studied separately.

¶ Unsupplemented infants with a birth weight of  $\geq 3500$  g.

# Model including the variables study site, postnatal weight gain (4-9 months), and complementary food energy intake (kcal/kg/d). Interactions between sex and each of the other 3 variables were tested in the model, and none were significant.

\*\* Unsupplemented infants with a birth weight of <3500 g.

ing that the sex difference in ferritin was different in Honduras and Sweden (Table 6). When studying the 2 sites separately, we found that there was no significant sex difference in Honduras (9% difference,  $P = .875$  controlling for confounders, Table 6), whereas in Sweden there was a significant interaction between sex and birth weight ( $P = .001$ ), reflecting a larger sex difference in Swedish infants with lower birth weight. Among Swedish infants with a birth weight of <3500 g, boys had 68% lower ferritin than girls ( $P = .018$ ), whereas among those with a birth weight of  $\geq 3500$  g, boys had 40% lower ferritin than girls ( $P = .033$ ). These sex differences in Swedish infants remained when controlling for iron supplementation, duration of iron supplementation, compliance, postnatal weight gain, and complementary food energy intake, and there were no significant interactions between sex and any of these variables. To evaluate whether the site-dependent sex difference observed at 9 months was present already at 4 months, the study sites were examined separately. At 4 months, the interaction between sex and site was not significant ( $P = .10$ ), but the tendency was the same: Swedish boys at 4 months had lower ferritin than girls (79 vs 150  $\mu\text{g/L}$ ) whereas the difference was smaller in Honduras (58 vs 82  $\mu\text{g/L}$ ).

#### Sex Differences in TfR at 9 Months

For TfR, in iron-supplemented infants ( $n = 136$ ), there was a significant interaction between sex and compliance, and because of this, iron-supplemented, compliant infants ( $n = 117$ ) were studied separately. Among these infants, there was no significant sex difference in TfR (boys 7.2 vs girls 6.9 mg/L; Table 7). However, among unsupplemented infants at 9 months, there was a significant interaction between sex and birth weight ( $P = .028$ ), as well as between sex and complementary food energy intake ( $P = .044$ ). The latter was in the direction of a larger sex difference in infants with a higher complementary food energy intake, but this interaction was not significant unless the interaction between sex and birth weight was included. Among unsupplemented in-

fants with a birth weight of <3500 g, boys (vs girls) had 3.3 mg/L higher TfR (11.0 vs 7.7 mg/L; Table 7), and among unsupplemented infants with a birth weight of  $\geq 3500$  g, boys (vs girls) had 1.6 mg/L higher TfR (8.6 vs 7.0 mg/L; Table 7), the differences in both weight groups being significant even when controlling for other possible explanatory factors ( $P = .006$  and  $P = .016$ , respectively).

#### Sex Differences in Prevalence of Iron Deficiency Anemia at 9 Months

At 9 months of age, there was a larger proportion of iron deficiency anemia among boys, compared with girls (17% vs 2%;  $P < .001$ ), using a multiple criteria definition of iron deficiency anemia based on conventional cutoff levels (defined above). Because our results suggest that there may be iron-independent sex differences in MCV, ZPP, and ferritin, we also used an alternative definition of iron deficiency anemia (Hb <110 g/L and TfR >11 mg/L),<sup>17</sup> which showed a similarly increased risk for boys (10% vs 1%;  $P = .003$ ).

#### DISCUSSION

At 4 months of age, we found lower Hb, MCV, and plasma ferritin and higher ZPP and TfR in boys than in girls; all of these seemingly suggesting greater iron deficiency in boys. We are not aware of any previous reports of sex differences in iron status in this age group, although Choi et al<sup>18</sup> recently reported higher TfR in male than in female infants both at birth (cord blood) and at 4 to 6 months of age. The observed sex differences at 4 months cannot be attributed simply to growth-related factors, because they remained when controlling for birth weight and postnatal weight gain and because the corresponding interactions were not significant. Furthermore, dietary factors are not likely to be responsible, because all infants were exclusively breastfed at this time.

At 9 months, the sex differences for MCV and ZPP were at least as large as at 4 months: boys had a mean of 3.5 fL lower MCV and 20% higher ZPP compared with girls. Even in a subgroup of infants, which

TABLE 6. Sex Differences in Ferritin at 9 Months of Age

Population Sample	<i>n</i>	Boys*	Girls*	Sex Difference†	<i>P</i> ‡	<i>P</i> §
Honduran infants	118	26 (2.8)	29 (2.3)	-9%	.573	.872
Swedish infants	96	29 (2.7)	53 (2.2)	-44%	.002	¶
Swedish, birth weight $\geq 3500$ g#	62	35 (2.4)	58 (2.0)	-40%	.017	.016**
Swedish, birth weight <3500 g††	34	15 (2.9)	47 (2.4)	-68%	.003	.048**

\* Geometric mean (standard deviation) ferritin ( $\mu\text{g/L}$ ).

† Sex difference: mean difference (boys - girls) as percent of girls' mean.

‡ Bivariate *P* value for sex.

§ Multivariate *P* value for sex.

|| Model including the variables iron supplementation, duration of supplementation, compliance, birth weight, postnatal weight gain (0-9 months), and complementary food energy intake (kcal/kg/d). Interactions between sex and each of the other 6 variables were tested in the model, and none were significant.

¶ Model as in footnote ||. *P* value for sex not presented because of significant interactions between sex and birth weight ( $P = .001$ ). Hence, birth weight groups were studied separately.

# Swedish infants with a birth weight of  $\geq 3500$  g.

\*\* Model including the variables iron supplementation, duration of supplementation, compliance, postnatal weight gain, and complementary food energy intake (kcal/kg/d). Interactions between sex and each of the other 5 variables were tested in the model, and none were significant.

†† Swedish infants with a birth weight of <3500 g.

**TABLE 7.** Sex Differences in TfR at 9 Months of Age

Population Sample	<i>n</i>	Boys*	Girls*	Sex Difference†	<i>P</i> ‡	<i>P</i> §
Iron-supplemented infants	136	7.2 (2.2)	6.9 (2.2)	+0.3	.438	
Iron-supplemented, compliant	117	6.9 (1.6)	7.1 (2.3)	-0.2	.644	.723¶
Unsupplemented infants	77	10.1 (3.5)	7.4 (1.8)	+2.7	<.001	#
Unsupplemented, birth weight ≥3500 g**	29	8.6 (1.9)	7.0 (1.8)	+1.6	.015	.016††
Unsupplemented, birth weight <3500 g‡‡	48	11.0 (3.9)	7.7 (2.0)	+3.3	.001	.006††

\* Mean (standard deviation) TfR (mg/L).

† Sex difference: mean difference (boys – girls).

‡ Bivariate *P* value for sex.

§ Multivariate *P* value for sex.

|| Model including the variables study site, duration of iron supplementation, compliance, birth weight, postnatal weight gain (0–9 months), and complementary food energy intake (kcal/kg/d). Interaction between sex and each of the other 6 variables were tested in the model. *P* value for sex not presented because of significant interactions between sex and compliance (*P* = .002). Hence, iron-supplemented compliant infants were studied separately.

¶ Model including the variables study site, duration of iron supplementation, postnatal weight gain, and complementary food energy intake (kcal/kg/d). Interactions between sex and each of the other 5 variables were tested in the model, and none were significant.

# Model including the variables study site, postnatal weight gain, and complementary food energy intake. *P* value for sex not presented because of significant interactions between sex and birth weight (*P* = .028) as well as between sex and complementary food energy intake (*P* = .044). Hence, birth weight groups were studied separately.

\*\* Unsupplemented infants with a birth weight of ≥3500 g.

†† Model including the variables study site, postnatal weight gain, and complementary food energy intake. Interactions between sex and each of the other 3 variables were tested in the model, and none were significant.

‡‡ Unsupplemented infants with a birth weight of <3500 g.

could be assumed to be iron-sufficient (Swedish, iron-supplemented infants), boys had 2 fL lower MCV and 10% higher ZPP. The sex differences in MCV and ZPP were significant when controlling for possible explanatory variables and were not affected by iron supplementation. At 9 months, Swedish boys had ~40% to 70% lower ferritin values than girls, the larger difference observed in infants with lower birth weight. This is compatible with our previous finding that Swedish 12 months old boys had 31% lower mean ferritin compared with girls.<sup>19</sup> Because iron supplementation did not affect the sex differences in these variables, we conclude that the lower MCV values and higher ZPP values in boys, as well as the lower ferritin in Swedish boys, were not caused by iron deficiency. One possible explanation might be hormone-mediated differences in metabolism. It is known, for example, that serum insulin and leptin concentrations are different in male and female infants during this period, even when correcting for body weight or body mass index.<sup>20</sup> Although the mechanism is not known, differences in lean versus fat body mass synthesis may indirectly affect the internal kinetics of iron metabolism. To our knowledge, such interactions have not been studied in experimental animals or human subjects.

In contrast to Swedish infants, Honduran infants showed no sex difference in ferritin at 9 months of age. This site-dependent sex difference was observed also at 4 months, suggesting that the sex difference in ferritin may be influenced by genetic or maternal factors differing between populations. This may explain the contradictory findings in previous studies of cord blood ferritin, some of which have shown significantly lower values in boys compared with girls,<sup>21</sup> and others which have not.<sup>22</sup>

In unsupplemented infants with a birth weight of <3500 g, boys had 9 g/L lower Hb than girls at 9

months, but there was no sex difference in Hb in infants with a birth weight of ≥3500 g. Because low birth weight is directly associated with low iron stores at birth,<sup>23</sup> this observation suggests that the Hb difference in unsupplemented infants at 9 months may be explained by iron deficiency in boys. This is further supported by our observation that the sex difference in Hb increased in unsupplemented but not in iron-supplemented infants between 4 and 9 months. These results are consistent with those from a study of 1175 healthy, British infants. In that study, no sex difference in Hb was seen at 8 months of age, but in a follow-up of the same infants at 18 months old, significantly lower Hb was observed in boys, suggesting a greater risk for boys to develop iron deficiency anemia.<sup>24,25</sup> Furthermore, among unsupplemented Swedish infants, there was no significant sex difference in Hb at 9 months (*P* = .841), reflecting the lower risk for iron deficiency anemia in Swedish infants. However, in contrast to these low-risk unsupplemented infants, we unexpectedly found in iron-supplemented infants at 9 months that boys had 4 g/L lower Hb compared with girls. This sex difference in iron-supplemented infants was similar in both birth weight groups and at both study sites (data not shown), and can possibly be explained by an immaturity in the regulation of Hb synthesis resulting in an unregulated Hb response to iron supplementation in infancy, as we have observed previously.<sup>16</sup>

In unsupplemented infants at 9 months old, boys had 1.6 to 3.3 mg/L higher TfR than girls, with the larger difference observed in infants with lower birth weight (<3500 g). However, in iron-supplemented, compliant infants, there was no sex difference in TfR at 9 months. This suggests that boys—especially those with a lower than average birth weight—are at higher risk for iron deficiency, as measured by TfR,

and that this can be prevented by iron supplementation. Choi et al<sup>18,22</sup> found in a cross-sectional study that boys had ~1 mg/L lower TfR than girls at birth and also at 4 to 6 months of age ( $P < .01$ ), but this difference was not observed at 7 to 12 months of age. It should, however, be noted that infants with Hb <110 g/L were excluded from analysis in that study. Similarly, excluding infants with Hb <110 g/L from our study at 4 and 9 months, we found a significant difference in TfR at 4 months ( $P = .001$ ) but not in unsupplemented infants at 9 months ( $P = .551$ ), controlling for site, birth weight, and postnatal weight gain. This supports our conclusion that the sex difference in TfR at 9 months is resulting from true iron deficiency, because the exclusion of anemic infants is likely to significantly reduce the proportion of iron-deficient subjects in the analysis. We found higher TfR in boys compared with girls at 4 months of age, suggesting iron deficiency in male infants. At 9 months, the difference in TfR had increased in unsupplemented infants, but had disappeared in iron-supplemented, compliant infants, suggesting that the TfR difference at 9 months, and possibly also at 4 months, was attributable to "true" iron deficiency.

There are several possible reasons for this increased risk of iron deficiency in boys. 1) Larger postnatal weight gain in boys is not a sufficient explanation, because the difference remained when controlling for postnatal weight gain. 2) Sex differences in food intake cannot be excluded, but several findings make this an unlikely explanation: a) there were no significant differences between sexes in complementary food energy or iron intake, b) the sex difference in TfR remained when controlling for complementary food energy intake, and c) the sex difference in TfR was observed already at 4 months, when all infants were exclusively breastfed. 3) Increased erythropoietic activity in boys during fetal life was suggested by Choi et al<sup>22</sup> as an explanation, but we find this unlikely, because the sex difference persists long beyond birth in our study. Hb in boys remained lower than in girls, suggesting that boys did not have a postnatal erythropoietic rate exceeding that which can be explained by their larger weight gain (see point 1 above). 4) Boys may be born with smaller iron stores than girls, despite their higher birth weight. This would be consistent with the findings of Choi et al,<sup>22</sup> who found higher TfR in cord blood of newborn boys. 5) Boys may have larger intestinal iron losses than girls. This cannot be excluded, because to our knowledge, it has not been studied. 6) Boys may have lower iron absorption than girls. This is unlikely because, using stable isotopes, we found no significant difference between sexes in iron absorption from human milk or from a dose of ferrous sulfate in a study on iron absorption in 25 infants at 4 and 9 months old.<sup>26</sup> 7) Boys may have more infections, which may affect iron status. However, in the current study, we found no significant difference between sexes in proportion of days with diarrhea or days with fever at 4 to 6 or 6 to 9 months old, and there was no significant sex difference in the proportion of infants with C-reactive protein >10 mg/L at any time (data not shown).

## CONCLUSION

We found significant sex differences in Hb, MCV, ZPP, ferritin, and TfR at 4, 6, and 9 months, seemingly suggesting a relative iron deficiency in boys. At 9 months, boys had an ~10-fold increased risk for being diagnosed with iron deficiency anemia. We postulate that the sex differences in Hb and TfR reflect a truly increased risk for iron deficiency in boys, possibly because of sex differences in fetal iron accretion. However, for MCV and ZPP, the remaining sex differences at 9 months were independent of iron supplementation, growth variables, and complementary food intake, suggesting that other sex-related differences such as genetic or hormonal factors are responsible. A similar, iron-independent sex difference was observed for plasma ferritin in Swedish but not in Honduran infants, suggesting that ferritin is influenced not only by sex but also by genetic or maternal factors in different populations. Our observation that sex differences in 2 of the variables (Hb and TfR) were responsive to iron supplementation whereas sex differences in the 3 other variables (MCV, ferritin, ZPP) were not, may be attributable to the fact that each iron status variable represents a different aspect of iron metabolism and also changes in response to noniron-related factors. Furthermore, some iron status variables, compared with others, may more accurately reflect iron status in infants.

Our results imply that infant boys, compared with girls, have a significantly higher risk for iron deficiency. Furthermore, there may be a need to develop sex-specific cutoff levels for MCV, ZPP, and ferritin, which would have important implications for assessments of individuals as well as populations. However, before sex-specific cutoffs can be established for these indicators, there is a need for larger population-based studies in term infants, as well as additional iron supplementation trials in high-risk populations. In such studies, it will be desirable to include functional outcomes, such as neurodevelopment, because it is not known whether male and female infants are equally susceptible to the functional consequences of iron deficiency.

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