Iron deficiency in infancy: applying a physiologic framework for prediction[1–3]

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ABSTRACT

Background: Infants aged 6–24 mo are at high risk of iron deficiency. Numerous studies worldwide have sought to identify predictors of iron deficiency in this age group.

Objective: The objectives of the study were to apply a physiologic model to identify risk factors for iron deficiency and to consider those risk factors under different conditions of iron supplementation. We predicted that factors related to iron status at birth (lower gestational age and lower birth weight), postnatal needs for iron (more rapid growth), and bioavailable iron (more cow milk) would be major risk factors.

Design: The physiologic framework was assessed in 1657 Chilean infants (aged 12 mo) with birth weights ≥3 kg who were randomly assigned at age 6 mo to high or low iron supplementation or no added iron. Based on venous blood, the analysis used mean corpuscular volume and concentrations of hemoglobin, free erythrocyte protoporphyrin, and ferritin. Logistic regression models were used to identify predictors of iron deficiency anemia and iron deficiency without anemia.

Results: The prevalence of iron deficiency (≥2 abnormal iron measures) was 34.9% at age 12 mo. Of 186 infants with hemoglobin concentrations <110 g/L, 158 (84.9%) were iron deficient. The only consistent (and the strongest) predictor of iron deficiency or iron deficiency anemia was lower 6-mo hemoglobin. Factors related to poorer iron status at birth (lower birth weight, shorter gestation though full-term, or both) were predictors in the no-added-iron and high-iron groups. Otherwise, predictors varied by iron supplementation.

Conclusion: Variations in predictors of iron deficiency or iron deficiency anemia according to iron supplementation suggest that direct comparisons across studies are tenuous at best without data on early iron status and certainty that specific conditions are comparable. *Am J Clin Nutr* 2006;84:1412–21.

KEY WORDS Iron deficiency, anemia, infants, iron supplementation, iron status, logistic regression, structural equation modeling

INTRODUCTION

Infants aged 6–24 mo constitute one of the groups at highest risk of iron deficiency (1). Several studies around the world have sought to identify predictors of iron deficiency in this age group, by considering factors such as those related to birth, growth, diet, sex, and socioeconomic status (SES). The physiology of iron balance has been implicit in the selection of variables or the discussion of results in previous studies. Building on this work, we used current understanding of the physiology of infant iron status to propose a conceptual model of relevant influences, and then we applied that model explicitly. This physiologic model (**Figure 1**) postulates that iron status in infancy should be determined in large part by 4 factors (1, 2): the iron the infant is born with (which is related to maternal iron status), the infant's postnatal needs for iron, the external sources of bioavailable iron, and iron losses (1, 3–11). Other factors may complicate the interpretation of iron status indicators and, thus, the assessment of infant iron status. Such factors include inflammation and infection (2, 12–14); hemoglobinopathies, chronic illness, and high blood lead concentrations (12); diurnal variation (12); and ethnic and sex differences (15–19).

On the basis of previous research, we expected that lower gestational age, lower birth weight, more rapid growth, and more cow-milk consumption would be major risk factors, but we did not have specific predictions about the importance of other physiologic factors. A secondary but related hypothesis was that some other influences reported in the literature, such as sex or socioeconomic status, would be mediated by physiologic factors. For example, poorer iron status in male infants may be explained by their greater iron needs due to more rapid growth. Iron deficiency in infants from poorer, less educated families may be accounted for by less iron in the infant diet or worse maternal iron status, such as that due to poor diet or a greater number of children (20).

We assessed this physiologic framework by using data from a study of the behavioral and developmental effects of preventing iron deficiency anemia among healthy Chilean infants who weighed ≥3 kg at birth (21). Hemoglobinopathies, malaria, parasites, other infectious diseases causing blood loss, and high lead concentrations were virtually absent. The analysis focused on predictors of iron status at age 12 mo in these infants, who had been randomly assigned at age 6 mo to receive high or low doses of supplemental iron or no added iron.

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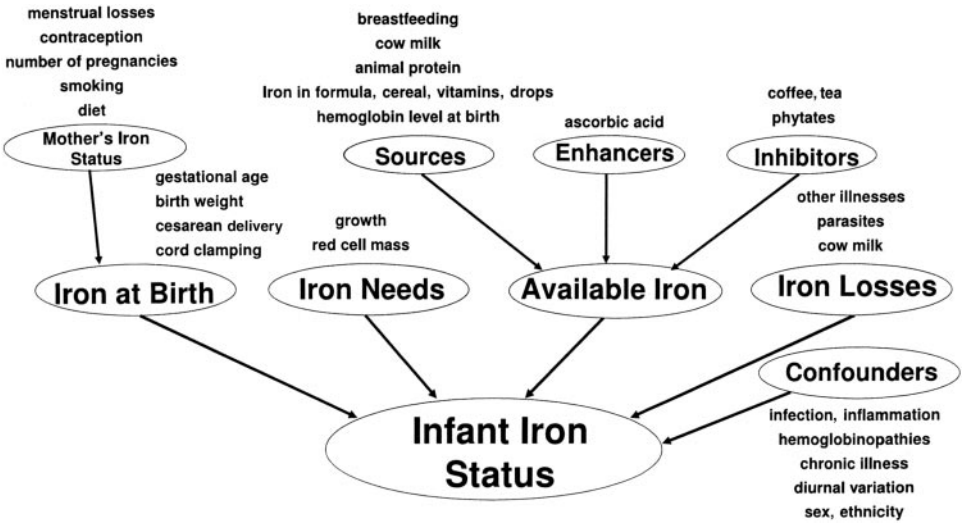

FIGURE 1. Physiologic model of iron status in infancy.

SUBJECTS AND METHODS

Subjects

The study was conducted between September 1991 and August 1996 in 4 contiguous working-class communities on the outskirts of Santiago, Chile. The overall project consisted of 2 components: a preventive trial and a neurophysiologic study. The findings reported here are from the preventive trial only and do not pertain to the infants (<7% of the total cohort) who entered the neurophysiologic study at age 6 mo (*see* Procedures).

Infants were enrolled in conjunction with routine health care visits at community clinics. The following entrance criteria were used to identify healthy infants: birth weight ≥3.0 kg, singleton term birth, routine vaginal delivery, no major congenital anomalies, no major perinatal complications, no phototherapy, no hospitalization for >5 d, no acute or chronic illness, and no iron therapy. The 3.0-kg birth-weight cutoff was used because some clinics had a preexisting program providing iron to infants weighing <3 kg. Exclusion criteria were residence outside the 4 communities; another infant <12 mo old in the household; infant in day care; illiterate or psychotic caregiver or no stable caregiver available to accompany the child for appointments; and, until mid-1994, "exclusive" breastfeeding, defined as <250 mL cow milk or formula/d. All but 8 infants were initially breastfed. Refusal was 6.0%, and attrition was 7.8%. A total of 1657 infants completed the preventive trial (21). A flow chart of the study's stages and number of subjects at each stage is provided in **Figure 2**; background characteristics of the sample completing the preventive trial are given in **Table 1**.

Written informed consent was obtained from the parent(s) or legal guardian of each child. The study was approved by the institutional review boards of the University of Michigan, University of Chile, and the Office for Protection from Research Risks at the National Institutes of Health.

Procedures

Hematologic tests and supplementation

Screening infants for anemia was not a regular part of pediatric care in Chile, and routine iron supplementation was not the policy there at the time of the current study. Clinics distributed unmodified powdered milk as part of a legally required and highly effective program for preventing generalized undernutrition (23). Iron supplementation and testing for anemia and iron deficiency were part of the research study.

A screening hemoglobin determination was obtained at age 5–6 mo by finger stick (HemoCue; Leo Diagnostics, Helsingborg, Sweden) to ensure that no infant with iron deficiency anemia (IDA) entered the preventive trial (Figure 2). A venipuncture was performed in infants with HemoCue concentrations <103 g/L and in the next child seen after a child with hemoglobin ≥115 g/L. Seventy-three infants with IDA that was confirmed in venous blood, together with 62 randomly selected nonanemic infants, were invited to participate in the neurophysiologic study and were treated with oral iron. The prevalence of iron deficiency with or without anemia in the preventive trial would probably have been higher without the exclusion. However, exclusion of the few nonanemic infants likely had little effect because almost 800 infants with equal or higher 6-mo hemoglobin concentrations entered the supplementation study.

In the supplementation component (preventive trial), the remaining 93% of the infants were randomly assigned to high- or low-iron or no-added-iron regimens, starting at age 6 mo (21). In the high-iron group, the infants taking ≥250 mL/d by bottle at age 6 mo received formula averaging 12 mg Fe/L, and those taking <250 mL/d by bottle at age 6 mo received vitamins with iron (15 mg elemental iron as ferrous sulfate) for those taking less by bottle. The reason for the 2 approaches to giving iron was that we did not want to interfere with breastfeeding by giving formula or cow milk to infants who were taking <1 bottle/d (250 mL) at age 6 mo. The low-iron group received formula averaging 2.3 mg of iron/L; by design (21), all were taking ≥250 mL/d by bottle at age 6 mo. In the no-added-iron group, infants taking ≥250 mL/d by bottle at age 6 mo received unmodified powdered cow milk (the routine feeding in Chile at the time); infants taking <250 mL/d by bottle at age 6 mo received vitamins without iron. Consumption was verified at weekly home visits and monthly clinic appointments to monitor health and growth. A venous blood sample (7–10 mL) was obtained at age 12 mo from all 1657 infants who completed the supplementation study (n = 534, 405,

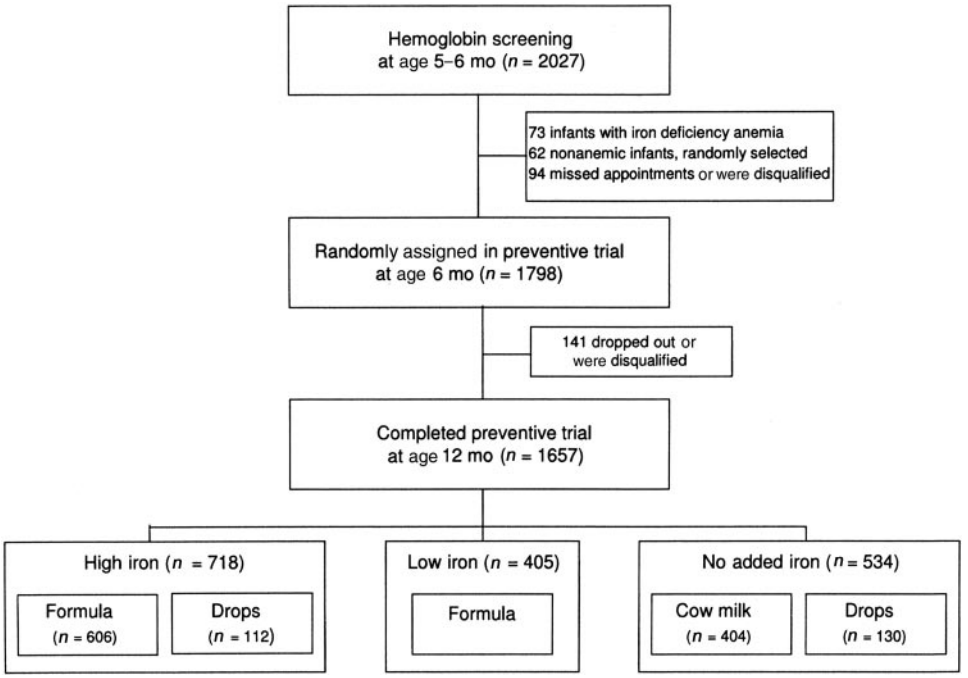

FIGURE 2. Flow chart of stages of the study and number of subjects at each stage. High- or low-iron formula was given to infants taking ≥250 mL/d by bottle at age 6 mo; drops (vitamin drops ± iron) were given to infants taking <250 mL/d by bottle at age 6 mo; cow milk was given to infants taking ≥250 mL/d by bottle at age 6 mo.

and 718 for no-added-iron, low-iron, and high-iron groups, respectively). Infants with IDA were treated with oral iron (ferrous sulfate, 30 mg elemental iron/d). Details of the supplementation study have been previously published (21).

The appropriate cutoffs for iron deficiency in infancy are subject to debate (24–26), and the prevalence varies according to the cutoffs used. On the basis of the cutoff recommended by the World Health Organization (27) and the Centers for Disease Control and Prevention (12), we defined anemia at age 12 mo as a hemoglobin concentration <110 g/L. Following a commonly used approach (28), we defined iron deficiency as 2 of 3 abnormal iron measures: mean corpuscular volume (MCV) <70 fL, free erythrocyte protoporphyrin (FEP) >100 μg/dL red blood cells (87.2 μmol/mol), or serum ferritin <12 μg/L. These specific cutoffs were used in previous studies by our group (29–33) and are more stringent for MCV and FEP than are those often recommended (12, 34–36). Blood lead concentrations were measured in the last 331 infants enrolled in the study. The mean ± SE lead concentration was 7.8 ± 0.2 μg/dL, and we found no statistically significant differences between supplement groups and no significant correlation with any measure of iron status.

Other aspects

Information on family background included (but was not limited to) household composition, parental education and occupation, and other indicators of SES. Data on parental education was available for almost every infant, but lack of funds toward the end of the study meant that the complete socioeconomic interview could be obtained only for a randomly selected 10% of subjects. Even for measures subjected to sampling, the number of subjects averaged well over 1000 (range: 995–1379). No significant differences were found in family background between infants with and without complete background data, which confirmed that the missing-data process was completely at random.

Dietary data collection focused on milk feeding. The age at the first bottle was recorded in days, as was the age at complete weaning (if weaned) from the breast. Of the sample, 34.7% nursed for ≥12 mo. The amount of formula or cow milk ingested was recorded at weekly home visits, which yielded measures of average daily intake both at a given age and as cumulative measures. At study entry, the median volume of cow-milk intake was 364 mL/d, and it increased (formula or cow milk, depending on supplementation group) to 432 mL/d at age 12 mo. (With more than one-third of infants still nursing at the conclusion of the current study, formula or cow milk was not the only milk source.) Information on solid foods and other liquids was not obtained. However, a survey in the same communities in the same period (G Pena, F Pizarro, A Letelier, unpublished observations, 1992) found that most families followed local pediatric society recommendations: solid foods (fruit and cereal) to start at ≈4 mo of age, meat and vegetables at age 6 mo, and legumes and eggs at age 9 mo. This diet provided ≈5 mg Fe/d, mostly of vegetable origin with poor bioavailability.

Continuity of pediatric care, monthly check-ups, and testing for IDA before and after the supplementation trial provided infants with considerably closer monitoring than they would otherwise have received. Formula or milk, vitamins, pediatric care, study tests and evaluations, transportation, and iron therapy (if indicated) were provided free of charge.

Data analysis

We used logistic regression to determine the factors that predicted anemia, IDA, iron deficiency without anemia, and iron deficiency total (ie, iron deficiency with or without anemia). The

TABLE 1
Background characteristics of the sample of Chilean 12-mo-old infants[1]

Characteristic	Value[2]
Infant	
Male [*n* (%)]	872 (52.6)
Gestational age (wk)	39.4 ± 1.0 (37.0–42.0)
Birth weight (kg)	3.54 ± 0.4 (3.00–5.04)
Length at birth (cm)	50.7 ± 1.7 (42.0–59.0)
Weight-for-age at 12 mo (*z* score)	−0.05 ± 0.9 (−2.98–3.53)
Length-for-age at 12 mo (*z* score)	−0.06 ± 0.8 (−2.59–3.21)
Age at first bottle (mo)	3.5 ± 3.0 (0–12.5)
Nursing at 1 y [*n* (%)][3]	555 (34.7)
Hemoglobin at 6 mo (g/L)	114.0 ± 9.3 (84–147)
Family	
Maternal age (y)	26.4 ± 6.0 (14.1–46.4)
No. of children for mother	2.1 ± 1.1 (1–9)
Maternal education (y)	9.4 ± 2.6 (1–17)
Father absent [*n* (%)][4]	208 (15.2)
Socioeconomic status index[5]	27.9 ± 6.5 (14–47)
Maternal smoking [*n* (%)][6,7]	260 (24.9)

[1] $n = 1657$. Sample size was smaller for some family variables because of reduced administration of family measures toward the end of the study due to a lack of funding.

[2] All values are $\bar{x} \pm$ SD; range in parentheses, unless indicated otherwise.

[3] $n = 1599$.

[4] $n = 1369$.

[5] Socioeconomic status index consisted of items from the Graffar measure (47), a measure of socioeconomic status that is sensitive to differences at the lower range. Items included were the number of persons in household "eating from one pot," father's presence in the household, the highest education level of the head of household, property ownership, type of house construction, characteristics of the kitchen, sewage, running water, the number of garbage collections per week, and total count of 6 household goods (car, refrigerator, washing machine, stereo system, black-and-white television, and color television). Index values indicate that the sample had a uniformly low socioeconomic status.

[6] $n = 1044$.

[7] Of mothers who smoked, 81% reported smoking only 1–5 cigarettes/d; 14% smoked 5–10 cigarettes/d, and 5% smoked >10 cigarettes/d. No mother reported using illicit drugs; 30 mothers reported consuming alcoholic beverages, with 3 reporting >1 drink/wk. Data on these behaviors during pregnancy were not available.

independent variables (potential predictors) considered for inclusion in the models were measures of the factors influencing iron status in the physiologic framework. For instance, measures related to iron at birth were gestational age, birth weight, and the number of children (maternal iron status may be worse with a greater number of births). Hemoglobin at age 6 mo may reflect iron status at birth, early growth and diet, or both. Growth characteristics were used as measures of iron needs. The measures related to available iron were average daily intake (mL/d) of cow milk or formula. On the basis of previous studies, sex and family characteristics (eg, maternal education and SES) were additional predictors. Missing values for family background were imputed by using multiple imputation techniques (37) with IVEWARE software (38). Ten data sets were imputed to account for the uncertainty related to the imputation process. Each of the imputed data sets was analyzed separately, and the results were combined by using PROC MIANALYZE in SAS software (version 9.1; SAS Institute Inc, Cary, NC).

The likelihood score (chi-square) test statistic was used as the selection criterion to derive the best logistic regression model for the iron status outcomes. Sets of variables that significantly increased the likelihood score function were included in the models. Among several similarly parsimonious models, we selected the one that was most uniform in predicting the several outcomes, as described later in this section. With the exception of formula or cow-milk intake in which the results varied, the direction of factors was coded to show increased risk of iron deficiency, IDA, or anemia. The resulting partial estimates of regression coefficients were converted to odds ratios (ORs), together with their 95% CIs. Variables with ORs that are significantly different from 1 (ie, the 95% CIs did not include 1) were considered predictors of iron status. Each predictor was independently statistically significant—that is, after control for all other predictors. These procedures were used to construct the best logistic models for each iron supplementation group at age 12 mo. In light of findings in previous studies and emphasis in the literature, sex and intake of milk or formula were included in the final models, regardless of statistical significance. For each model, the overall *P* value, chi-square statistic, df, and the area under the receiver operating characteristic (ROC) curve [(AUC_{ROC}) also called the *c*-statistic] were calculated. The *c*-statistic measures the discrimination power of the model (39). In this case, discrimination power relates to the ability of the model to correctly classify infants for each iron status outcome. Values <0.6 indicate poor discrimination power, and values in the range of ≥0.8 to 0.9 indicate excellent predictive power.

ORs were calculated for several planned iron status outcomes within supplement conditions. To determine whether predictors of iron deficiency without anemia were similar or different from predictors of iron deficiency with anemia—a more severe and chronic condition—we created mutually exclusive and exhaustive groupings: IDA, iron deficiency without anemia, and no iron deficiency. The ORs were calculated for anemia, IDA, iron deficiency without anemia, and iron deficiency total (iron deficiency with or without anemia).

Structural equation modeling was used to test our secondary hypothesis that physiologic factors would mediate the effects of sex and family background. For these analyses, a latent variable—"iron status"—was created by using principal component factor analysis of MCV and concentrations of hemoglobin, FEP (reverse-coded), and ferritin, with the last 2 being log transformed. This weighted average composite index used the loading weights on the single resulting factor; a higher value on this continuous variable indicates better iron status. The structural equation model was fitted by using AMOS software (version 5.0; Small Waters Corp, Chicago, IL) with highly nonsignificant estimates excluded. The goodness of fit of the model was tested on the basis of several statistics: Bentler's Comparative Index, chi-square, and standardized root mean-square residual. Separate models were created for each iron supplementation group.

RESULTS

Iron status depending on iron supplementation

Iron deficiency was clearly a problem in this population at the time. At the conclusion of the preventive trial, 571 infants (34.9%) met the criterion of ≥2 abnormal iron measures. Almost all of the anemia was due to iron deficiency. Of 186 infants with hemoglobin <110 g/L, 158 (84.9%) had ≥2 abnormal iron measures.

TABLE 2
Iron status of Chilean 12-mo-old infants by iron-supplementation condition[1]

	No added iron (n = 534)	Low iron (n = 405)	High iron (n = 718)
Anemia [n (%)]	138 (25.8)[a]	20 (4.9)[b]	28 (3.9)[b]
Iron deficiency anemia [n (%)]	120 (22.5)[a]	17 (4.2)[b]	21 (2.9)[b]
Iron deficiency without anemia [n (%)][2]	157 (29.5)[a]	145 (36.7)[b]	111 (15.6)[c]
Iron deficiency total [n (%)][2]	277 (52.1)[a]	162 (41.0)[b]	132 (18.6)[c]

[1] Statistical significance of group comparisons was based on Fisher's exact test. Values in a row with different superscript letters are significantly different.
[2] n = 532, 395, and 710 for no-added-iron, low-iron, and high-iron groups, respectively, because iron status could not be classified in the nonanemic infants who had ≥1 missing iron measure.

The prevalence of anemia, IDA, iron deficiency without anemia, and iron deficiency total at age 12 mo by supplementation group is shown in **Table 2**. Iron status varied substantially depending on supplementation. For all outcomes, infants in the no-added-iron group had significantly poorer iron status than did those in the low- and high-iron groups. The prevalence of iron deficiency without anemia and iron deficiency total differed significantly between the 3 groups. The low-iron group had the highest prevalence of iron deficiency without anemia, but the no-added-iron group had the highest prevalence of iron deficiency total; the high-iron group had the lowest prevalence of both outcomes. There were no statistically significant differences between low- and high-iron groups in the prevalence of anemia or IDA (*see* also reference 40).

Predictors of poor iron status at age 12 mo

The ORs for risk factors for anemia, IDA, iron deficiency without anemia, and iron deficiency total in the 3 groups are shown in **Table 3**. The discrimination power (*c*-statistic) for all models was in the acceptable range. The *c*-statistic for logistic models with IDA as the outcome was uniformly high (range: 0.79–0.84), which reflected excellent discrimination power in predicting IDA as compared with no iron deficiency. Smaller values of the *c*-statistic (range: 0.63–0.72) for the models with iron deficiency without anemia or iron deficiency total as the outcome indicate somewhat less discrimination power.

Anemia and iron deficiency anemia

Predictors of anemia and IDA were generally similar, but they varied substantially depending on iron supplementation. In the no-added-iron group, many factors related to IDA. Male sex, lower gestational age (even though all infants were born at ≥37 wk), lower birth weight (even though all infants weighed ≥3 kg), lower hemoglobin at age 6 mo (even though infants with IDA had been excluded and treated), more weight gain over the first year of life, lower maternal education, and lower SES were all independent risk factors. After control for these factors, the intake of cow milk (average mL/d between ages 6 and 12 mo) did not predict IDA. For infants in the low-iron group, the only significant predictor of IDA at age 12 mo was a lower hemoglobin at age 6 mo. Higher intake of the low-iron formula showed a suggestive trend as a risk factor for anemia. In the high-iron group, a lower hemoglobin at age 6 mo and accelerating weight gain in the second 6 mo of life were statistically significant risk factors. Lower intake of high-iron formula predicted anemia and showed a suggestive trend for IDA. Lower birth weight showed a suggestive trend for IDA, and weight gain in the first year showed a suggestive trend for anemia.

Iron deficiency without anemia and iron deficiency total

Only 3 factors predicted iron deficiency without anemia in the no-added-iron group: male sex, lower gestational age, and more weight gain in the first year of life. Lower maternal education showed a suggestive trend. Predictors of iron deficiency total were the same as those for IDA except SES, which was not significant. For infants in the low-iron group, a lower hemoglobin at age 6 mo predicted iron deficiency without anemia and iron deficiency total. Compared with results for iron deficiency with or without anemia as the outcome in the no-added-iron group, the direction of effects changed for sex. Male infants were at significantly lower risk of iron deficiency and iron deficiency total (but not of IDA) than were female infants. For infants in the high-iron group, lower birth weight and lower hemoglobin concentration at age 6 mo were risk factors for iron deficiency. The amount of milk or formula between ages 6 and 12 mo did not predict iron deficiency without anemia in any group but showed a suggestive trend for iron deficiency total in the high-iron group (less risk of iron deficiency with more high-iron formula intake).

To make the study's main findings readily apparent, we show the ORs graphically for IDA and iron deficiency without anemia under the different conditions (**Figure 3**). The only consistent (and the strongest) predictor of iron deficiency or IDA at age 12 mo was lower hemoglobin at age 6 mo. Factors related to poorer iron status at birth (lower birth weight, shorter gestation though full-term, or both) were predictors in the no-added-iron and high-iron groups. Predictors otherwise varied on the basis of iron supplementation. Figure 3 also shows that the predictors of iron deficiency without anemia were quite similar to those for IDA in the no-added-iron group but less so in the iron-supplemented groups.

To give some idea of the clinical meaning of the observed effects, we show how the ORs translate into per-unit effects, by using IDA in the no-added-iron group as the outcome. The odds of IDA at age 12 mo were 3 times as high for boys as for girls. Each week less in gestation at term birth (37–42 wk) was associated with a 40% increase in the odds of IDA at age 12 mo. Although all infants weighed ≥3 kg at birth, lower birth weight, even after control for gestational age and the other factors, also increased the odds of IDA. For each 100 g less in birth weight, the odds of IDA at age 12 mo increased by >100%. Poorer iron status at age 6 mo predicted later IDA, such that for each 10 g/L less hemoglobin concentration, the odds of IDA at age 12 mo increased 77%. With regard to postnatal growth, for each 1-kg weight gain from birth to age 12 mo, the odds of IDA increased 57%. For each year less maternal education, the odds of infant IDA increased 23%. For each 1-SD increase in the SES index

TABLE 3
Risk factors for poor iron status in Chilean infants at age 12 according to iron supplementation status[1]

Risk factor	Anemia	Iron deficiency anemia	Iron deficiency without anemia	Iron deficiency total
No added iron				
Boys vs girls	2.46 (1.57, 3.86)[2,3]	3.16 (1.82, 5.47)[3]	1.55 (1.01, 2.39)[4]	1.99 (1.37, 2.89)[3]
Gestational age (1-wk decrease)	1.19 (0.97, 1.44)	1.40 (1.11, 1.78)[3]	1.25 (1.00, 1.55)[4]	1.31 (1.10, 1.56)[3]
Birth weight (100-g decrease)	1.75 (0.94, 3.28)[5]	2.23 (1.06, 4.69)[4]	1.42 (0.77, 2.61)	1.55 (0.91, 2.64)[5]
Hemoglobin at 6 mo (10-g/L decrease)	1.86 (1.47, 2.35)[3]	1.77 (1.34, 2.33)[3]	1.16 (0.92, 1.47)	1.38 (1.13, 1.68)[3]
Weight gain (1-kg/y increase)	1.07 (0.86, 1.33)	1.57 (1.17, 2.10)[3]	1.52 (1.23, 1.89)[3]	1.51 (1.24, 1.83)[3]
Maternal education (1-y decrease)	1.19 (1.10, 1.28)[3]	1.23 (1.12, 1.36)[3]	1.07 (0.97, 1.18)[5]	1.13 (1.04, 1.22)[3]
Socioeconomic status index (1-SD increase)	1.35 (1.07, 1.71)[3]	1.49 (1.11, 2.00)[3]	1.00 (0.76, 1.31)	1.17 (0.95, 1.46)
Cow milk intake (100-mL increase)	0.96 (0.85, 1.08)	0.96 (0.82, 1.12)	1.02 (0.91, 1.15)	1.01 (0.92, 1.11)
Discrimination power (*c*-statistic)[6]	0.74	0.80	0.66	0.72
Overall *P*	<0.0001	<0.0001	<0.0001	<0.0001
Abnormal/total (*n*)[7]	138/528	120/371	155/406	275/526
Low iron supplementation				
Boys vs girls	1.39 (0.54, 3.58)	0.97 (0.34, 2.77)	0.63 (0.41, 0.97)[4]	0.66 (0.44, 1.00)[4]
Hemoglobin at 6 mo (10-g/L decrease)	2.84 (1.56, 5.16)[3]	3.27 (1.70, 6.31)[3]	1.52 (1.19, 1.95)[3]	1.63 (1.28, 2.07)[3]
Formula intake (100-mL increase)	1.28 (0.97, 1.70)[5]	1.23 (0.90, 1.69)	1.03 (0.92, 1.17)	1.05 (0.93, 1.19)
Discrimination power (*c*-statistic)[6]	0.77	0.79	0.63	0.64
Overall *P*	0.001	0.001	0.001	<0.001
Abnormal/total (*n*)[7]	20/403	17/247	145/375	162/392
High iron supplementation				
Boys vs girls	1.80 (0.74, 4.38)	2.02 (0.71, 5.71)	1.31 (0.84, 2.05)	1.40 (0.91, 2.13)
Birth weight (100-g decrease)	1.75 (0.54, 5.68)	3.89 (0.93, 16.39)[5]	1.99 (1.06, 3.75)[4]	2.18 (1.20, 3.95)[3]
Hemoglobin at 6 mo (10-g/L decrease)	2.21 (1.36, 3.59)[3]	2.28 (1.31, 3.97)[3]	1.51 (1.18, 1.92)[3]	3.81 (0.56, 25.76)[3]
Weight gain (1-kg/y increase)	0.66 (0.41, 1.06)[5]	0.64 (0.37, 1.13)	1.07 (0.87, 1.30)	1.02 (0.83, 1.26)
Accelerating weight gain (1-SD difference)	1.82 (1.20, 2.75)[3]	2.10 (1.30, 3.38)[3]	0.88 (0.70, 1.11)	3.41 (0.59, 19.79)
Formula intake (100-mL increase)	0.76 (0.63, 0.93)[3]	0.83 (0.68, 1.03)[5]	0.95 (0.86, 1.04)	0.93 (0.85, 1.01)[5]
Discrimination power (*c*-statistic)[6]	0.83	0.84	0.63	0.65
Overall *P*	<0.0001	<0.0001	<0.001	<0.0001
Abnormal/total (*n*)[7]	28/715	21/595	110/684	131/705

[1] The odds ratios (ORs) for logistic regression coefficients were calculated to reflect the unit change shown in parentheses after each risk factor; 95% CIs that do not include 1 are statistically significant, according to the chi-square test.
[2] OR; 95% CI in parentheses (all such values).
[3] $P \leq 0.01$.
[4] $P \leq 0.05$.
[5] $P \leq 0.10$.
[6] Discrimination power (defined as ability of the model to correctly classify infants for a given iron status outcome) was measured by using the area under the receiver operating characteristic curve (41).
[7] The numbers are slightly low because of missing data for ≥1 predictors.

(higher scores indicate poorer SES), the odds of IDA increased 49%.

Structural equation modeling tested our secondary hypotheses that effects of sex and family background would be mediated by growth and dietary factors. The best model for the no-added-iron group, with the corresponding parameter estimates in bold, is shown in **Figure 4**. All goodness-of-fit statistics indicated a good fit for the model [Bentler's Comparative Index = 0.92, $\chi^2 = 2.8$, and standardized room mean-square residual = 0.058]. We applied the same model for the iron-supplemented groups. Parameter estimates for the high-iron group are shown in italics. Also as shown in Figure 4, the composite iron measure was highly related to functional indicators of iron status (hemoglobin and FEP concentrations and MCV) and less strongly but still significantly related to iron stores (ferritin).

By far the strongest effect on iron status at age 12 mo in each supplementation group was hemoglobin at age 6 mo. Sex was related to weight gain in the first year of life in the no-added-iron group (boys gained more weight), but there was an independent effect of sex on iron status such that being male was associated with poorer iron status even after more weight gain was taken into account. With high iron supplementation, the effect of sex was markedly attenuated but still statistically significant. The effects of family background on iron status in the no-added-iron group were apparently not exerted through cow-milk intake. The amount of cow milk was not significant in the model, whereas both maternal education and SES showed significant direct effects even after control for the interconnectedness of these 2 factors. In contrast, in the high-iron condition, the amount of formula consumed related to iron status (the greater the intake, the better the iron status), whereas SES did not and maternal education showed only a suggestive trend. For the low-iron group, 6-mo hemoglobin and SES were the only factors with a statistically significant relation to the iron status composite; there was a suggestive trend for a negative relation between iron status and the amount of formula consumed (data not shown).

Our finding that hemoglobin at age 6 mo was the best predictor of iron status at age 12 mo, regardless of supplementation condition, was unanticipated. To understand this finding better, we analyzed the relation between hemoglobin concentrations and

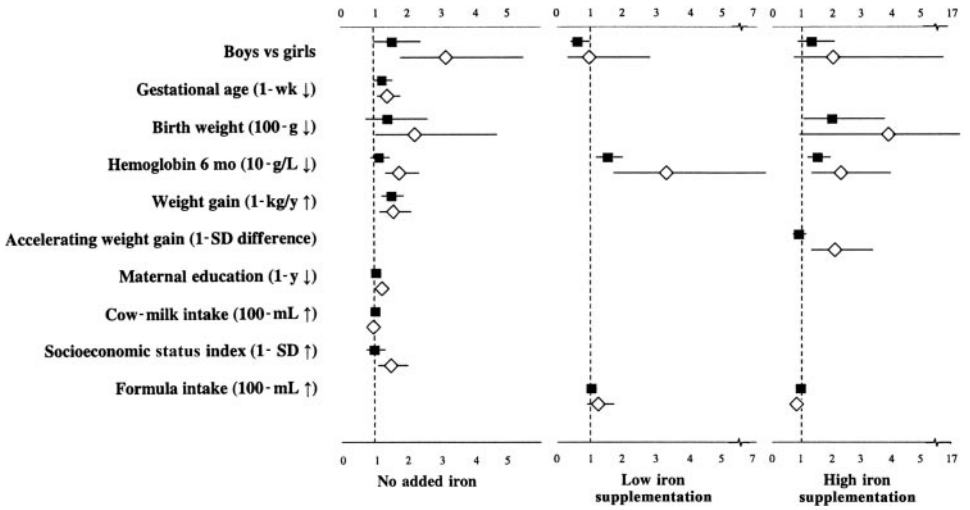

FIGURE 3. Predictors of iron deficiency with or without anemia in Chilean infants at age 12 mo depending on iron supplementation. Odds ratios and 95% CIs are shown for predictors of iron deficiency without anemia (■) or iron deficiency anemia (◇). ↑, increase; ↓, decrease.

cow-milk intake at age 6 mo (it should be noted that clinics distributed the cow milk free of charge and that iron supplementation did not start until age 6 mo). Although infants were largely breastfed, ≈25% consumed >500 mL cow milk/d at study entry. Hemoglobin at age 6 mo was not related to the amount of cow milk ($r = -0.03$, $P = 0.23$). Dietary factors other than breast or cow milk were not directly assessed but, according to Chilean practice at the time, most infants were unlikely to have received cereal or juice for >2 mo (G Pena, F Pizarro, A Letelier, unpublished observations, 1992). We also considered the possibility that lower hemoglobin at age 6 mo may relate to a large-for-gestational-age size: that is, a disordered glucose metabolism in the mother can cause excessive weight gain in the fetus and insufficient iron transfer across the placenta (41, 42). Infants weighing >4 kg at birth (11.5% of the sample) had a mean hemoglobin concentration of 115.2 ± 8.6 g/L at age 6 mo, which was significantly higher than the concentration seen in infants with birth weights ≤4 kg—113.8 ± 9.3 g hemoglobin/L ($t_{1,249} = -2.02$, $P = 0.04$).

Because early hemoglobin concentrations were not measured in other related studies, we also reran all logistic regression models without this factor. Significant predictors other than sex remained unchanged in the high- and low-iron groups. Sex became marginal in several models, rather than statistically significant, and became statistically significant for iron deficiency total in the high-iron group, rather than nonsignificant. In the no-added-iron group, only one major change was seen: weight gain became nonsignificant as a predictor of IDA. Other changes

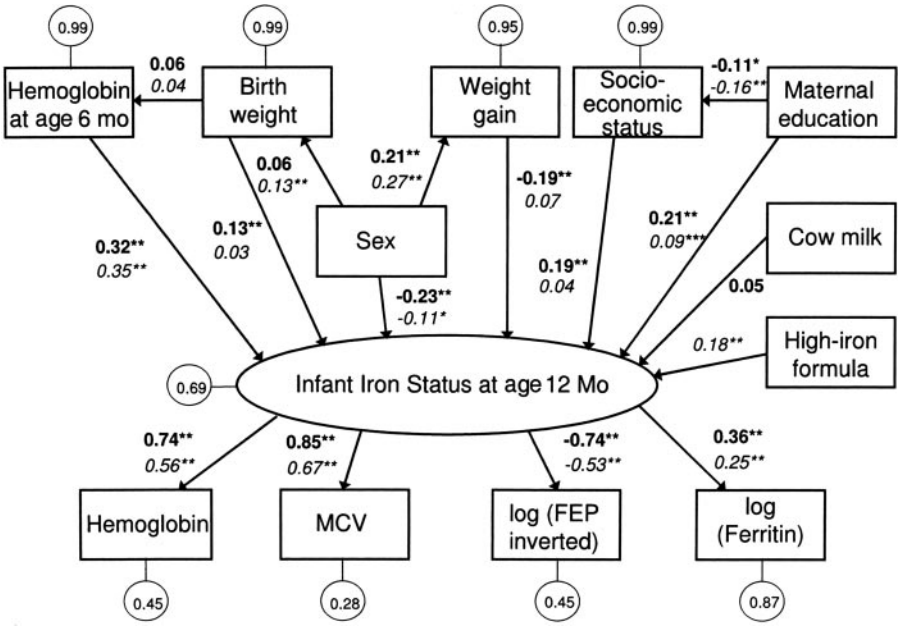

FIGURE 4. Structural equation model of iron status at age 12 mo in Chilean infants who did not receive supplemental iron. Parameter estimates for the no-added-iron group are shown in bold ($n = 534$) and those for the same model in the high-iron group are shown in italics ($n = 718$). Higher scores indicate lower socioeconomic status. MCV, mean corpuscular volume; FEP, free erythrocyte protoporphyrin. Residual errors are shown in circles. *$P \leq 0.05$, **$P \leq 0.01$, ***$P \leq 0.10$.

in models without 6-mo hemoglobin values consisted of statistically significant predictors that became marginal or marginal predictors that became statistically significant. Without the 6-mo hemoglobin value, the discrimination power of all models (*c*-statistic) was worse.

DISCUSSION

The physiologic model shown in Figure 1 helped identify predictors of infant iron status. It also helped interpret the results of this study by indicating which components of the model were more important. The most consistent finding across supplementation conditions was that a lower hemoglobin concentration at age 6 mo was the best predictor of iron deficiency with or without anemia at age 12 mo. Because hemoglobin at age 6 mo did not relate to cow-milk intake, it may reflect iron status at birth. This possibility should be assessed directly in future studies. Other factors related to poorer iron status at birth predicted iron deficiency, IDA, or both later on. Lower birth weight and shorter gestation, even within the full-term range, were risk factors for IDA in the no-added-iron group. Indicators of greater iron needs were significant primarily in the no-added-iron group. Specific predictors were male sex and more-rapid weight gain. Accelerating weight gain in the second half of the first year also predicted IDA in the high-iron supplementation group.

A surprising result was that indicators of available iron in the diet (formula or milk) were not strong predictors of iron status in this population. The average daily intake of cow milk between ages 6 and 12 mo showed no relation to iron status at age 12 mo after control for other factors, with or without adjustment for hemoglobin at age 6 mo. The lack of effect of cow milk may be due to the intensity of breastfeeding in the sample. Our only other measure of available iron in the current study in Chile was the intake of iron-fortified formula. Trends suggested that greater intake of low-iron formula increased the risk of anemia and poorer iron status. In contrast, more intake of high-iron formula reduced the risk of anemia at age 12 mo (only a suggestive trend in some other models); more intake also related to better iron status in the structural equation model. Nonetheless, the weak associations indicate that, in this largely breastfed sample, cow-milk or formula intake played a relatively minor role after adjustment for iron status in the first 6 mo of life and other factors. However, other sources of iron in the infant diet were not assessed and thus not accounted for in the models.

In our secondary hypotheses, we had predicted that the effects of sex would be accounted for by more rapid growth in male infants. Male sex was strongly related to more rapid growth, but, contrary to expectation, being male predicted poorer iron status in the no-added-iron group even after control for birth weight and growth. This finding seems to support the suggestion that there are sex differences in iron status independent of more rapid postnatal growth in males (17). However, male sex did not increase the risk of iron deficiency or IDA in the high-iron group and reduced the risk of iron deficiency in the low-iron group. Although few infants had IDA, the numbers were substantial for iron deficiency without anemia ($n = 145$ and 111 in low- and high-iron groups, respectively). These numbers are more than adequate to detect an effect of sex on iron deficiency, had such an effect been present. Furthermore, the structural equation models showed no effect of sex on iron status in the low-iron group and a substantially attenuated effect of male sex in the high-iron group compared with that in the no-added-iron group. These results showing that the effect of sex varied with iron supplementation cast doubt on the need for separate standards for male and female infants.

We had also predicted that socioeconomic factors would act through physiologic factors, such as a poorer diet for infant, mother, or both or more childbearing and hence poorer maternal iron status. Family factors did not enter the logistic regression models under conditions of iron supplementation, but they contributed independently in the no-added-iron group at age 12 mo. These findings may not negate the postulated physiologic mechanism, however, because we did not assess some relevant factors (eg, quality of the infant diet, infant intake of coffee or tea, iron content of the maternal diet, and iron status during pregnancy).

Our analytic approach was chosen to facilitate comparison with the Euro-Growth Study (43), which included ≈500 infants aged 12 mo from 11 countries and used entrance criteria and hematologic cutoffs quite similar to those in the current study in Chile. Because of the widespread use of iron-fortified infant foods in Europe, the high-iron group in the Chilean sample seems most appropriate for direct comparison. The only congruent finding was that greater formula intake protected against iron deficiency in Europe and against anemia in Chile. Otherwise, no overlap in significant predictors was found. Many similarities between the studies support comparison, but important differences also exist that may help account for the differing results. First, breastfeeding was much more extensive in the Chilean sample (universal initiation and widespread continuation past age 6 mo). Second, the intakes of cow milk and formula were measured differently—by the duration (in mo) in the Euro-Growth Study and by average daily intake throughout the second 6 mo of life in the Chilean study. Third, the study in Chile did not assess the intake of solid foods or juices, whereas hemoglobin concentrations at age 6 mo were available only for the current study. Fourth, the Chilean sample was >3 times as large as the Euro-Growth Study sample, and infant iron status was experimentally modified by random assignment to high-iron, low-iron, or no-added-iron group. Iron deficiency was much more common in Chile than in Europe, which accounted for a much higher proportion of anemia in Chile; only 24% of anemic infants in the Euro-Growth Study met the criterion for iron deficiency, whereas 85% of those in Chile did so. Although these differences between the studies may account for the different results, an additional aspect of the Euro-Growth Study should be considered: the very low number of infants with IDA ($n = 11$) and the only marginally higher number with iron deficiency ($n = 24$). With such small numbers, meaningful relations may not reach the level of statistical significance, or outliers may unduly influence the results. This same limitation applies to our analyses using IDA as the outcome in the low- and high-iron groups but not to the other models in the study in Chile, which had more than adequate numbers of subjects.

With a high prevalence of iron deficiency in the Chilean sample, it is possible to conclude that risk factors for IDA and iron deficiency without anemia differ to some degree and that the predictors of either outcome differ substantially depending on iron supplementation. These findings raise important cautions in interpreting the numerous studies that have analyzed factors related to IDA in infancy in a variety of populations. Predictors could also be expected to vary if malnutrition, infection, or both

were common or if breastfeeding was less universal and extensive. Our results suggest that, unless the specific ages and conditions are comparable, direct comparisons across studies are tenuous at best and meaningless at worst.

In conclusion, the physiologic model proved helpful in identifying relevant factors for analysis and in pointing to data that should be obtained in future studies. The model also helped interpret the significant findings and assess the relative contributions of different factors. Risk factors varied according to iron supplementation, which suggests the need for caution in generalizing results across studies. The underlying prevalence of iron deficiency and IDA in a population will also profoundly influence the relations that can be detected and interpreted with confidence. Finally, poor iron status in the first 6 mo, which was not measured in previous studies, may be the most important risk factor of all.

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