Prevalence and predictors of iron deficiency in fully breastfed infants at 6 mo of age: comparison of data from 6 studies\textsuperscript{1–3}

Zhenyu Yang, Bo Lönnertal, Seth Adu-Afarwaa, Kenneth H Brown, Camila M Chaparro, Roberta J Cohen, Magnus Domellöf, Olle Hernell, Anna Larthe, and Kathryn G Dewey

ABSTRACT

Background: Iron deficiency (ID) can occur among exclusively breastfed infants before 6 mo of age. Objective: The objective was to determine which subgroups of fully breastfed infants are at highest risk of ID.

Design: We assessed the prevalence of ID (ferritin < 12 µg/L and iron deficiency anemia (IDA; ferritin < 12 µg/L and hemoglobin < 105 g/L) and risk factors associated with ID and IDA at 6 mo among 404 fully breastfed infants with a birth weight >2500 g from 6 studies in Ghana, Honduras, Mexico, and Sweden. Infants with an elevated C-reactive protein concentration (8%) were excluded.

Results: The percentages of infants with ID were 6% in Sweden, 17% in Mexico, 13–25% in Honduras, and 12–37% in Ghana. The percentages with IDA were 2% in Sweden, 4% in Mexico, 5–11% in Honduras, and 8–16% in Ghana. With data pooled, the key predictors of ID (20%) were male sex (adjusted odds ratio [AOR]: 4.6; 95% CI: 2.5, 8.5) and birth weight 2500–2999 g (AOR: 2.4; 95% CI: 1.4, 4.3). The predictors of IDA (8%) were male sex (AOR: 7.6; 95% CI: 2.5, 23.0), birth weight of 2500–2999 g (AOR: 3.4; 1.5, 7.5), and weight gain above the median since birth (AOR: 3.4; 95% CI: 1.3, 8.6). The combination of birth weight 2500–2999 g or male sex had a sensitivity of 91% for identifying ID and of 97% for identifying IDA.

Conclusions: Among fully breastfed infants with a birth weight >2500 g, IDA is uncommon before 6 mo, but male infants and those with a birth weight of 2500–2999 g are at higher risk of ID and IDA.

INTRODUCTION

The World Health Organization (WHO) recommends exclusive breastfeeding for 6 mo (1). However, iron deficiency (ID) is a concern for some exclusively breastfed (EBF) infants before 6 mo of age, especially in susceptible populations (1–5). ID during infancy has been linked to impaired psychomotor development (6, 7) and may compromise immune function (8). Combating ID during infancy is therefore a high priority, but it is challenging to identify feasible, effective, and safe intervention strategies for implementation during the first 6 mo. The introduction of complementary foods is not recommended before 6 mo and, in any case, may not prevent ID (9). Iron supplementation is permitted within the WHO definition of “exclusive breastfeeding,” but may have adverse consequences for infants who are not iron deficient (10–12). In many settings, it is not feasible to draw blood samples universally to screen for ID during infancy. Thus, a screening tool to identify infants for targeted iron supplementation may be useful.

During the first 6 mo of life, the main source of iron is fetal iron storage at birth and iron released from fetal hemoglobin during the first wk of life (13). Birth weight, umbilical cord clamping time, and prenatal iron supplementation influence total body iron at birth (14). During the postnatal period, weight gain, which is associated with expanding hemoglobin and myoglobin mass, influences iron requirements (14).

The purpose of these analyses was to 1) determine which subgroups of EBF infants are at risk of ID and iron deficiency anemia (IDA) before 6 mo and 2) evaluate the sensitivity and specificity of potential predictors of ID and IDA at 6 mo.

SUBJECTS AND METHODS

Subjects

The data were obtained from 6 randomized clinical trials that were conducted by the co-authors in 4 countries, as summarized in Table 1. In all studies, venous blood was obtained from the infants at 6 mo of age (±1 wk) to assess hemoglobin and iron status. In the 2 studies in Ghana (Ghana 1 and Ghana 2), baseline blood samples were available at 6 mo of age for infants who were subsequently enrolled in the intervention trials conducted in 1994 and 2004, respectively (15, 16). In the first study in Honduras (Honduras 1; conducted in 1994) (17), EBF infants at 4 mo of age were randomly assigned to 1 of 2 groups: 1) continued exclusive breastfeeding until 6 mo of age (EBF group) or 2) introduction of iron-fortified complementary foods at 4 mo of age while continuing to breastfeed. For these analyses, only infants in the EBF group were included. In the combined study of infants in Sweden and Honduras, conducted in 1997 (18),

\textsuperscript{1}From the Program in International and Community Nutrition, Department of Nutrition, University of California, Davis, CA (ZY, BL, SA-A, KHB, CMC, RJC, and KGD); the Department of Clinical Sciences, Pediatrics, Umeå University, Umeå, Sweden (MD and OH); and the Department of Nutrition and Food Science, University of Ghana, Legon, Ghana (AL).

\textsuperscript{2}No funding source was involved in this study.

\textsuperscript{3}Reprints not available. Address correspondence to KG Dewey, Program in International and Community Nutrition, Department of Nutrition, University of California, One Shields Avenue, Davis, CA 95616-8669. E-mail: kgdewey@ucdavis.edu.

EBF infants 4 mo of age were randomly assigned to receive 1) iron supplements from 4 to 9 mo of age, 2) placebo from 4 to 6 mo of age and iron supplements from 6 to 9 mo of age, or 3) placebo from 4 to 9 mo of age. For these analyses, only infants who received placebo from 4 to 6 mo of age were included. In the Mexican study, conducted in 2003 (19), mother-infant pairs were randomly assigned to have delayed or early umbilical cord clamping (≈2 min or ≈10 s after delivery, respectively).

Only infants who were fully breastfed (ie, breast milk was the only source of milk) were included in these analyses. In some of the studies (Mexico, Honduras 2, Ghana 2, and Sweden), children were not excluded if they consumed small amounts of non-iron-fortified solid foods. Iron supplementation is recommended for all low-birth-weight infants (birth weight <2500 g) at ≈2 mo of age (20). Thus, infants with a birth weight <2500 g or with missing data for birth weight were excluded from these analyses. Because plasma ferritin is an acute phase protein that becomes elevated during infection and systemic inflammation (21), we only included subjects with a C-reactive protein concentration <10 mg/L in the analyses that included ferritin (22).

Potential predictor measurements

Birth weight was obtained from birth certificates or from the parents. Infants were weighed at 6 mo of age by using a standardized suspended spring balance or electronic scale. Information on maternal prenatal iron supplementation was obtained by using a structured questionnaire in all of the studies except Ghana 1. To explore the influence of variation in timing of umbilical cord clamping, alternative statistical models incorporating this information were constructed (see below). Actual time of umbilical cord clamping was recorded in the Mexican study. Early umbilical cord clamping was assumed for Honduras 1, Honduras 2, Ghana 1, and Ghana 2 on the basis of usual hospital/clinic practice. The usual practice for the study hospital in Sweden is late umbilical cord clamping.

Sample collection and laboratory analysis

Venous blood was collected into tubes containing lithium heparin or EDTA, and the plasma was separated and stored at -20°C until analyzed. Hemoglobin was analyzed by using a Hemocue meter (Hemocue Inc, Mission Viejo, CA) or an automated system (Cell-Dyne 610; Abbott Laboratories, Abbott Park, IL) at the local laboratory. The plasma ferritin concentration was analyzed by using immunoradiometric assay kits (Coat-A-Count Ferritin IRMA; Diagnostic Products Corp, Los Angeles, CA). Ferritin was analyzed in duplicate, and any sample with a high CV (CV >15%) was reanalyzed. C-reactive protein was analyzed by using radial immunodiffusion kits (The Binding Site, Birmingham, United Kingdom). Control materials were included in the C-reactive protein kits. Ferritin controls were obtained from Diagnostic Products Corp.

Definitions used for the analyses

ID was defined as a plasma ferritin concentration <12 μg/L (23). Anemia was defined as a hemoglobin concentration <105 g/L (24); in Mexico the hemoglobin cutoff was 117 g/L to adjust for the altitude (19). IDA was defined as both ID and anemia. “Lower” birth weight was defined as birth weight between 2500 and 3000 g; infants with a birth weight <2500 g were excluded from the analyses. High weight gain was defined as >4320 g between birth and 6 mo of age, which was the median of weight gain in the combined group of infants.

Statistical methods

SAS (release 9.0; SAS Institute Inc, Cary, NC) was used for all of the analyses. A univariate procedure was used to check the central tendency and variation of all continuous variables (ferritin, hemoglobin, birth weight, and weight gain during the first 6 mo). Because ferritin is not normally distributed, logarithm transformation was done. Means (or medians) and SDs (or interquartile ranges) were calculated for all continuous variables. Correlation coefficients were calculated between log ferritin, hemoglobin, and potential predictors that were continuous variables. Cross-tabulation tables were created between the dichotomous outcomes (ID, anemia, or IDA) and each of the potential predictors that were categorical variables. The sensitivity and specificity of the predictors were calculated for both ID and IDA. Chi-square test or Fisher’s exact test statistics were computed for each predictor. Multiple linear regression models...
were used to predict log ferritin and hemoglobin. Smallest Mallow Cp and greatest adjusted $R^2$ were used to select the best model; residual plots and tolerance were used to diagnose the fit of the models. Logistic regression was used to predict ID and IDA with the same set of covariates used in the linear regression models. The $R^2$ and area under the curve (AUC) for each receiver operating characteristic (ROC) curve were calculated for each logistic regression model. For the multiple linear regression and logistic regression analyses, study site was included in the first set of models as a covariate. In a second set of models, the variable for estimated timing of cord clamping was substituted for the study site variable to determine whether it would improve the explanatory power. (These 2 variables could not be used in the same model because of multicollinearity.)

RESULTS

The number of infants excluded because birth weight was missing or <2500 g was 32, 169, and 8 in Ghana 1, Ghana 2, and Honduras 1, respectively. In addition, 98, 14, and 206 infants were not fully breastfed in Ghana 1, Ghana 2, and Mexico, respectively, and were also excluded from these analyses. The final sample size is shown in Figure 1.

As shown in Table 1, ferritin, hemoglobin, birth weight, and weight gain in the first 6 mo of life varied widely within and across studies. The median plasma ferritin concentration was lowest in Ghana 2 (20.3 μg/L); intermediate in Ghana 1, Honduras 1, and Mexico (27.6–35.7 μg/L); and highest in Sweden (61.3 μg/L). After altitude was adjusted for in the Mexican study, the mean hemoglobin concentration was highest in the Swedish study (118 g/L), intermediate in the Mexican study (114 g/L), and lowest in the Ghanaian and Honduras studies (106–109 g/L). The mean birth weights were very similar (3.1–3.2 kg), except in the Swedish study (3.6 kg). In contrast, weight gain during the first 6 mo of life ranged from 4.1 kg (Sweden and Mexico) to 4.3–4.7 kg (Ghana and Honduras). The percentages of infants with ID, anemia, IDA, lower birth weight (2.5–3.0 kg), and high weight gain are shown in Table 2 by study site. Overall, 19.7% were iron deficient, 28.0% were anemic, and 7.9% had IDA.

Birth weight, weight gain, and sex were significantly associated with ferritin in the multiple linear regression models (Table 3). Because estimated cord clamping time was highly related to study site, the statistical models included either study site (model 1) or cord clamping time (model 2), but not both. The coefficients for birth weight, weight gain, and sex were similar between the 2 models. Study site was associated with log ferritin in model 1, and late cord clamping was positively associated with log ferritin in model 2. After birth weight, weight gain, and sex were controlled for, log ferritin was significantly lower only in Ghana 2 when compared with the study in Sweden ($P < 0.001$). Prenatal iron supplementation was inversely related to log ferritin in model 2 but not in model 1. After the prenatal iron supplementation variable was excluded, the models showed little change (data not shown).

Variables associated with hemoglobin are shown in Table 4. Hemoglobin concentration in Mexican children was adjusted for altitude. As described above, 2 separate models were built, with either study site or cord clamping time as one of the potential predictors. Birth weight and sex, but not weight gain, were significantly associated with hemoglobin concentration at 6 mo of age. The coefficients for birth weight, sex, and weight gain were comparable between the 2 models. In model 1, hemoglobin concentration was significantly higher in the Swedish infants than in the other groups, although the difference was only marginally significant compared with the Mexican infants ($P =$...
odds of ID than did those with late cord clamping. For ID was significantly higher only in infants in Ghana 2 and female infants. Compared with the Swedish infants, the odds ratio for ID was significantly higher in the Mexican infants (even those with early cord clamping) than in those in the Ghanian and Honduras studies (P < 0.05; data not shown). The hemoglobin concentration was significantly higher in the Mexican infants (even those with late cord clamping) than in those in the Mexican infants with late cord clamping (P = 0.07, data not shown). In model 2, late cord clamping was positively related to hemoglobin concentration.

In the logistic regression models for ID, the odds ratios for birth weight category and male sex were similar between the 2 models (Table 5). The odds of ID were 2.3–2.4 higher among infants who had a birth weight between 2500 and 2999 g than in those who had a birth weight >2999 g and were 4.0–4.6 higher in male than in female infants. Compared with the Swedish infants, the odds ratio for ID was significantly higher only in infants in Ghana 2 and Honduras 2. Infants with early cord clamping had a 4.5 higher odds of ID than did those with late cord clamping.

Birth weight (2500–2999 g), high weight gain, and male sex were significantly associated with IDA (Table 6). The odds of IDA were 3.0–3.4 times higher among infants who had a birth weight between 2500 and 2999 g than in those who had a birth weight >2999 g, 3.1–3.4 times higher among infants whose weight gain was >4320 g between birth and 6 mo of age than in those whose weight gain was less than that, and 7.2–7.6 times higher in male than in female infants. Study site and cord clamping time were not significantly associated with IDA when birth weight, weight gain, and sex were controlled for.

The sensitivities of predicting ID were 0.45, 0.66, and 0.76 for lower birth weight (2500–2999 g), high weight gain, and male sex, respectively (Table 7). The respective specificities were 0.72, 0.54, and 0.55. The multiple combination of birth weight (2500–2999 g) or male sex yielded a sensitivity of 0.91 and 0.6), even in the Mexican infants with late cord clamping (P = 0.07, data not shown). The hemoglobin concentration was significantly higher in the Mexican infants (even those with early cord clamping) than in those in the Ghanian and Honduras studies (P < 0.05; data not shown). The hemoglobin concentration in Mexican children was adjusted for altitude.

Table 3
Predictors of ferritin in infants fully breastfed for 6 mo

<table>
<thead>
<tr>
<th>Variable</th>
<th>Model 1 ± SE</th>
<th>P value</th>
<th>Model 2 ± SE</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Birth weight (kg)</td>
<td>β (SE)</td>
<td></td>
<td>β (SE)</td>
<td></td>
</tr>
<tr>
<td>Weight gain, 0–6 mo (kg)</td>
<td>−0.3 ± 0.6</td>
<td>0.62</td>
<td>−0.6 ± 0.6</td>
<td>0.29</td>
</tr>
<tr>
<td>Sex, female vs male</td>
<td>3.5 ± 1.0</td>
<td>0.0005</td>
<td>3.6 ± 1.0</td>
<td>0.0004</td>
</tr>
<tr>
<td>Prenatal iron supplement, yes vs no</td>
<td>0.8 ± 1.2</td>
<td>0.54</td>
<td>−0.3 ± 1.2</td>
<td>0.81</td>
</tr>
<tr>
<td>Cord clamping time, late vs early</td>
<td>—</td>
<td>—</td>
<td>6.3 ± 1.1</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Study site</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ghana 2 vs Sweden</td>
<td>−9.9 ± 1.7</td>
<td>&lt;0.0001</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Honduras 1 vs Sweden</td>
<td>−9.9 ± 2.0</td>
<td>&lt;0.0001</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Honduras 2 vs Sweden</td>
<td>−7.4 ± 1.7</td>
<td>&lt;0.0001</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Mexico vs Sweden</td>
<td>−3.2 ± 1.7</td>
<td>0.06</td>
<td>—</td>
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</table>

Table 4
Predictors of hemoglobin (g/L) in infants fully breastfed for 6 mo

<table>
<thead>
<tr>
<th>Variable</th>
<th>Model 1 ± SE</th>
<th>P value</th>
<th>Model 2 ± SE</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Birth weight (kg)</td>
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<td></td>
<td>β (SE)</td>
<td></td>
</tr>
<tr>
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<td>0.62</td>
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</tr>
<tr>
<td>Sex, female vs male</td>
<td>3.5 ± 1.0</td>
<td>0.0005</td>
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<td>0.0004</td>
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<td>−0.3 ± 1.2</td>
<td>0.81</td>
</tr>
<tr>
<td>Cord clamping time, late vs early</td>
<td>—</td>
<td>—</td>
<td>6.3 ± 1.1</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Study site</td>
<td></td>
<td></td>
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<td>Ghana 2 vs Sweden</td>
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<td>Honduras 1 vs Sweden</td>
<td>−9.9 ± 2.0</td>
<td>&lt;0.0001</td>
<td>—</td>
<td>—</td>
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<tr>
<td>Honduras 2 vs Sweden</td>
<td>−7.4 ± 1.7</td>
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<td>—</td>
</tr>
<tr>
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<td>−3.2 ± 1.7</td>
<td>0.06</td>
<td>—</td>
<td>—</td>
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</tbody>
</table>

1 n = 344 excluding Ghana study 1 because of the lack of prenatal iron supplement information.
2 Geometric regression coefficients based on multiple linear regression. This means that the mean ferritin changes β-fold for a 1-unit increase in the predictor variable, with the other covariates held constant. For example, if the ferritin concentration of a boy with a birth weight of 3.0 kg is 20 μg/L at 6 mo of age, the ferritin concentration would be 32.4 μg/L (20 μg/L × 1.62) if the birth weight was 4.0 kg.
3 Model 1 included study site but not cord clamping time; R² = 0.30.
4 Model 2 included cord clamping time but not study site; R² = 0.25.
5 The percentages with prenatal iron supplementation were 90.2%, 57.9%, 81.4%, 87.6%, and 37.9% for the Ghana study 2, Honduras study 1, Honduras study 2, Mexican study, and Swedish study, respectively.
a specificity of 0.36, with the AUC of the ROC curve being 0.76 (P < 0.001) (Figure 2). Using the additive combination of lower birth weight (2500–2999 g) and male sex yielded a sensitivity of 0.30 and a specificity of 0.91 (Table 7).

As shown in Table 8, the sensitivities of predicting IDA were 0.53, 0.81, and 0.88 for lower birth weight (2500–2999 g), high weight gain, and male sex, respectively. The respective specificities were 0.71, 0.53, and 0.52. The combination of birth weight (2500–2999 g) or male sex yielded a sensitivity of 0.97 and a specificity of 0.36, with the AUC of the ROC curve being 0.76 (P < 0.001) (Figure 3). Using the additive combination of lower birth weight (2500–2999 g) and male sex yielded a sensitivity of 0.44 and a specificity of 0.90 (Table 8).

**DISCUSSION**

In these samples of fully breastfed infants with birth weights >2500 g, the prevalence of ID at 6 mo of age was 6–37%.

**TABLE 6**

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Model 1&lt;sup&gt;2&lt;/sup&gt;</th>
<th>Model 2&lt;sup&gt;4&lt;/sup&gt;</th>
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</thead>
<tbody>
<tr>
<td>Birth weight, 2500–2999 g&lt;sup&gt;2&lt;/sup&gt;</td>
<td>2.4 (1.4, 4.3)&lt;sup&gt;2&lt;/sup&gt;</td>
<td>2.3 (1.3, 4.0)&lt;sup&gt;2&lt;/sup&gt;</td>
</tr>
<tr>
<td>High weight gain&lt;sup&gt;5&lt;/sup&gt;</td>
<td>—</td>
<td>1.5 (0.9, 2.6)</td>
</tr>
<tr>
<td>Male sex</td>
<td>4.6 (2.5, 8.5)&lt;sup&gt;5&lt;/sup&gt;</td>
<td>4.0 (2.2, 7.3)&lt;sup&gt;5&lt;/sup&gt;</td>
</tr>
<tr>
<td>Early cord clamping</td>
<td>—</td>
<td>4.5 (1.9, 11.0)&lt;sup&gt;5&lt;/sup&gt;</td>
</tr>
<tr>
<td>Ghana 1 vs Sweden</td>
<td>1.6 (0.4, 6.1)</td>
<td>—</td>
</tr>
<tr>
<td>Ghana 2 vs Sweden</td>
<td>7.4 (2.4, 23.2)&lt;sup&gt;5&lt;/sup&gt;</td>
<td>—</td>
</tr>
<tr>
<td>Honduras 1 vs Sweden</td>
<td>1.5 (0.4, 6.3)</td>
<td>—</td>
</tr>
<tr>
<td>Honduras 2 vs Sweden</td>
<td>3.6 (1.1, 11.7)&lt;sup&gt;5&lt;/sup&gt;</td>
<td>—</td>
</tr>
<tr>
<td>Mexico vs Sweden</td>
<td>2.7 (0.8, 8.9)</td>
<td>—</td>
</tr>
</tbody>
</table>

<sup>1</sup> Multiple logistic regression analysis; iron deficiency anemia was defined as a plasma ferritin concentration <12 μg/L. n = 404.

<sup>2</sup> Model 1 included study site but not cord clamping time.

<sup>3</sup> Model 2 included cord clamping time instead of study site.

<sup>4</sup> The reference group consisted of subjects with a birth weight >3000 g. All infants with a birth weight <2.5 kg or with missing birth weight were excluded from the study.

<sup>5</sup> P < 0.05.

<sup>6</sup> High weight gain was defined as >4320 g between birth and 6 mo of age.

and the prevalence of IDA was 1.5–15.7% across different countries. The key predictors of ID were birth weight between 2500 and 3000 g and male sex, and greater weight gain from birth to 6 mo was also a predictor of IDA. When these 3 variables were controlled for, there were no significant between-site differences in IDA, although it should be noted that because the prevalence of IDA was low, the statistical power to detect differences between sites was limited.

Total body iron is linearly associated with body weight during the fetal period and at birth (25). After birth, there is little change in body iron content during approximately the first 6 mo for EBF infants (13). We found that both plasma ferritin and hemoglobin concentrations at 6 mo of age were positively related to birth weight. Similar findings were previously reported for ferritin (26, 27) and hemoglobin (28, 29).

Substantial evidence indicates that ferritin and hemoglobin concentrations are lower in boys than in girls (29–32). Even after birth weight, weight gain during the first 6 mo, and cord clamping time were controlled for, a sex difference was still evident in our study and in other studies. It is possible that boys have lower body iron stores at birth or higher intestinal iron losses than do girls. Genetic or hormonal factors may also partially explain the difference, but further research is needed to clarify the reason.

**FIGURE 2.** The receiver operating characteristic curve for model one (birth weight between 2500 and 2999 g, high weight gain, male, and early cord clamping) in discriminating iron deficiency in fully breastfed infants at 6 mo of age (n = 404). The area under the curve is 0.76 (c-statistics) by using multiple logistic regression analyses.
Birth weight 2500–2999 g and Birth weight 2500–2999 g or Male sex 0.88 0.52, (c-statistics) by using multiple logistic regression analyses.

Weight gain moglobin cutoff was 117 g/L to adjust for altitude. Sensitivity and specificity for predicting iron deficiency anemia 1438 YANG ET AL in Ghana 2 infants than in Swedish infants. Hemoglobin concentration was similar between the Swedish infants and Mexican infants, but hemoglobin concentration was still higher in Swedish than in Mexican infants with late cord clamping (data not shown). It was previously shown that hemoglobin concentration varies among different ethnic groups and is related to genetic background, which may partially explain the differences in hemoglobin across the countries (35, 36).

Because the characteristics of the Swedish infants were so different from those of the infants in the other studies, the analyses were redone without the Swedish infants. The results for the main predictors (birth weight, sex, weight gain, and cord clamping time) were similar to the results found when the Swedish infants were included. After exclusion of the Swedish study, the prevalences of ID and IDA were 22% and 9%, respectively.

The proportion of anemia attributable to ID was only 28% in the pooled data, varying from ~14% in Honduras 1 to ~55% in the Swedish study (Table 2). In 5 of the 6 studies, <40% of anemia was due to ID at 6 mo of age, even though we used a lower cutoff for anemia (<105 g/L) than the current WHO cutoff (<110 g/L). Data from several trials in Southeast Asia also showed that only 13–43% of anemia among infants at ~6 mo was attributable to ID (37–40). These results are not consistent with the usual assumption that ~50% of anemia is attributable to ID in infants and young children. Possible explanations for this discrepancy are that 1) some of these trials excluded subjects with severe anemia or low birth weight, so the prevalence of anemia and IDA in the study populations may have been lower than in the general population and 2) a high prevalence of hemoglobinopathies or micronutrient deficiencies other than iron (e.g., vitamin B-12 and vitamin A) could have explained some of the anemia at 6 mo. Further research is warranted.

One limitation of this analysis was that the samples were not randomly selected from each study area, and subjects with missing data for birth weight were excluded, which restricted our ability to generalize these findings. Our finding of a prevalence of 6.1% ID for EBF Swedish infants at 6 mo of age was similar to the prevalence of 7.3% in another study in Sweden (41), and our prevalence of 18.3% anemia (with or without ID) for fully breastfed Mexican infants at 6 mo of age was slightly higher than the prevalence of 11% in another study in Mexico (42). We were unable to find any other studies in Ghana or Honduras with data on the prevalence of ID or anemia among EBF or fully breastfed infants at this age. Another limitation was that because information on cord clamping time of individual infants was available only for the study in Mexico, the results associated with cord clamping should be interpreted with caution.

An important policy issue is whether to screen for ID or IDA before 6 mo of age. In many settings, routine collection and analysis of blood samples is not feasible. We therefore examined whether a screening algorithm based on noninvasive predictors of ID and IDA could be developed. Our results indicated that the sensitivity of each individual predictor for identifying ID or IDA was not very high, but the sensitivity of the combination of lower birth weight (2500–2999 g) or male sex was >0.9 for both outcomes, and the AUC for the ROC curve was >0.70, which indicates moderate or good discrimination power (32). However, the specificity was only 0.36 for ID and 0.33 for IDA, which means that this indicator would result in 64–67% false-positive

<table>
<thead>
<tr>
<th>Predictor</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Birth weight 2500–2999 g</td>
<td>0.53</td>
<td>0.71</td>
<td>0.0052</td>
</tr>
<tr>
<td>Weight gain &gt;4320 g since birth</td>
<td>0.81</td>
<td>0.53</td>
<td>0.0002</td>
</tr>
<tr>
<td>Male sex</td>
<td>0.88</td>
<td>0.52</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Birth weight 2500–2999 g or</td>
<td>0.97</td>
<td>0.33</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>male sex</td>
<td>0.44</td>
<td>0.90</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

1 Iron deficiency anemia was defined as a plasma ferritin concentration <12 µg/L and a hemoglobin concentration <105 g/L; in Mexico, the hemoglobin cutoff was 117 g/L to adjust for altitude.

2 Chi-square or Fisher’s exact test.

3 The reference group consisted of subjects with a birth weight ≥3000 g.

Greater weight gain since birth was associated with a significantly lower ferritin concentration at 6 mo, which is consistent with the results of other studies (33, 34). However, hemoglobin concentration was not related to weight gain in our analyses. Rapid growth in the first 0.5 y of life requires mobilization of body iron stores for synthesis of hemoglobin and other iron-containing molecules in body tissues, whereas hemoglobin should not be affected until iron stores have been depleted for some time.

Both ferritin and hemoglobin concentrations were significantly associated with the cord clamping variable, but it should be recognized that this variable was based on assumed clamping practices in each site, except Mexico. Thus, there was strong collinearity between the cord clamping variable and study site. Because of this, we constructed separate multiple regression models using either study site or cord clamping, but not both variables simultaneously. After birth weight, weight gain, and sex were controlled for, the ferritin concentration was similar between the sites, except for a significantly lower ferritin concentration in Ghana 2 infants than in Swedish infants. Hemoglobin concentration was significantly higher among the Swedish infants than in the infants of all other studies. After the infants in the Mexican study were stratified by cord clamping time, ferritin concentration was similar between the Swedish infants and Mexican infants, but hemoglobin concentration was still higher in Swedish than in Mexican infants with late cord clamping (data not shown). It was previously shown that hemoglobin concentration varies among different ethnic groups and is related to genetic background, which may partially explain the differences in hemoglobin across the countries (35, 36).

An important policy issue is whether to screen for ID or IDA before 6 mo of age. In many settings, routine collection and analysis of blood samples is not feasible. We therefore examined whether a screening algorithm based on noninvasive predictors of ID and IDA could be developed. Our results indicated that the sensitivity of each individual predictor for identifying ID or IDA was not very high, but the sensitivity of the combination of lower birth weight (2500–2999 g) or male sex was >0.9 for both outcomes, and the AUC for the ROC curve was >0.70, which indicates moderate or good discrimination power (32). However, the specificity was only 0.36 for ID and 0.33 for IDA, which means that this indicator would result in 64–67% false-positive
results. Providing iron treatment to all infants who test “positive” based on this screening algorithm could be risky, because iron supplementation of iron-replete infants has been associated with slower linear growth, greater diarrhea morbidity, and greater mortality (10, 11). Thus, the use of such a screening tool would require follow-up testing of those who are “positive” for either lower birth weight or male sex, which is a large proportion of all infants. This creates a dilemma in environments where resources for follow-up blood testing are unavailable. Adding to the complexity of this situation is the uncertainty over the consequences of an infant being iron-deficient or having IDA for a couple of months (eg, from 4 to 6 mo of age). If iron-rich or iron-fortified complementary foods are consumed beginning at 6 mo of age, there may not be any lasting consequences of a transitory state of ID or IDA, although evidence on this question is lacking. Because the percentage of infants with IDA before 6 mo was <10% in these 6 studies, it is debatable whether follow-up blood testing of all male infants and of female infants with birth weights of 2500–2999 g is cost-effective. To resolve this issue, further research is needed to determine the consequences of ID and IDA that occurs before 6 mo and that is of relatively short duration.

The authors’ responsibilities were as follows—ZY and KGD: study design, analysis and interpretation of data, and writing of the manuscript; and BL, SA-A, KHB, CMC, RJC, MD, OH, AL, and KGD: implementation and data management for the original clinical trials. All authors reviewed and edited the manuscript. There were no potential conflicts of interest for any of the authors.

REFERENCES


