Effects of iron supplementation on serum hepcidin and serum erythropoietin in low-birth-weight infants

Staffan Berglund, Bo Lönnertal, Björn Westrup, and Magnus Domellöf

ABSTRACT

Background: The iron-regulatory hormone hepcidin has not been studied in infants, who experience large physiologic changes in iron status.

Objective: The objective was to study hepcidin and erythropoietin and their correlation with iron status in iron-replete and iron-deficient low-birth-weight (LBW) infants—a group at particular risk of iron deficiency (ID).

Design: We randomly assigned 285 otherwise healthy LBW infants to receive, from 6 wk to 6 mo of age, 3 doses of iron supplements: 0 (placebo), 1, or 2 mg/kg daily. Hepcidin, erythropoietin, hemoglobin, and variables of iron status were analyzed.

Results: Serum hepcidin did not change over time in the placebo group, despite a rapid decrease in serum ferritin. In iron-supplemented infants, hepcidin increased significantly, reaching a mean (±SD) concentration of 19.2 ± 2.5 ng/mL in the 2-mg/kg group compared with 13.0 ± 2.6 ng/mL in the placebo group at age 6 mo (P < 0.001). The difference was even larger between iron-deficient and iron-replete infants. Hepcidin was independently positively correlated with ferritin at all ages and was negatively correlated with the transferrin receptor concentration at age 6 wk and with transferrin at age 6 mo. Erythropoietin was initially similar between groups but decreased significantly in iron-supplemented infants. In addition to being negatively correlated with hemoglobin, it was also independently negatively correlated with indicators of iron status.

Conclusions: Hepcidin is closely associated with iron status and may be a useful indicator of iron stores and ID in infants. Erythropoietin is negatively correlated with iron status, which suggests a feedback mechanism that needs further study. This trial is registered at clinicaltrials.gov as NCT00558454.

INTRODUCTION

ID is the most common micronutrient deficiency and, because of their rapid growth, infants and young children are at particular risk. This risk is even higher in preterm and LBW infants who have smaller iron stores at birth. During the past decade, there has been rapid progress in research on iron metabolism and its regulation. The discovery of the iron-regulating peptide hepcidin has caused a major breakthrough in the field. Iron transport across membranes, acting as a downregulator of the transport protein ferroportin. This effect is observed in duodenal enterocytes, the cells responsible for iron uptake in the intestine, but also in hepatocytes and macrophages, the major iron storage cells. Hepcidin, produced in the liver, is increased in iron overload and inflammation. Conversely, it is downregulated in ID, hypoxia, and conditions with increased erythropoiesis. In animal models, hepcidin deficiency leads to iron overload, and increased hepcidin leads to ID. The exact mechanisms of hepcidin expression are not yet fully known, but the research in this field is progressing rapidly. It has recently been suggested that erythropoietin could act directly or indirectly on hepatocytes to regulate hepcidin expression. In iron-loading anemias, such as thalassemias, hepcidin is decreased despite iron overload, which suggests that active erythropoiesis has a stronger effect on hepcidin than does iron overload.

During early infancy, large physiologic changes in iron status occur. Because of the pro-oxidative properties of iron, iron overload is potentially harmful and it has been suggested that iron supplementation of IR infants could have adverse effects on growth, infections, and possibly on neurodevelopment. It has been shown that iron metabolism is differently regulated during this early phase of life, possible explaining an increased risk of iron overload. The mechanisms behind the immature iron regulation in infants have not been delineated, but the involvement of hepcidin has been suggested. To date, few studies on hepcidin have been conducted in iron-deficient humans and none have been conducted in infants.

1 From the Department of Clinical Sciences, Pediatrics, Umeå University, Umeå, Sweden (SB and MD); the Department of Nutrition, University of California, Davis, CA (BL); and the Department of Women and Child Health, Division of Neonatology, Karolinska Institute, Stockholm, Sweden (BW).
2 Supported by grants from the Swedish Research Council (2005-1894), Visterbotten County Council, the Jerring Foundation, and the Medical Faculty, Umeå University. The iron drops were unconditionally provided by Astra Zeneca, Sweden.
3 Address correspondence to S Berglund, Department of Clinical Sciences, Pediatrics, Umeå University, SE-901 85 Umeå, Sweden. E-mail: staffan.berglund@pediatri.umu.se.
4 Abbreviations used: CRP, C-reactive protein; ID, iron deficiency; IR, iron replete; LBW, low birth weight; MCV, mean cell volume; TIR, transferrin receptor; TS, transferrin saturation.

Received February 14, 2011. Accepted for publication September 2, 2011.
In a randomized controlled trial, we recently showed that well-nourished, healthy, marginally LBW Swedish infants (2000–2500 g) are at risk of ID, a risk that was effectively decreased by iron supplementation with 2 mg/kg per day between 6 wk and 6 mo of age. The prevalence of ID at 6 mo was 36% in the placebo group compared with 4% in supplemented infants (25). The participants in this recent intervention trial constitute an excellent human model of ID in which to further explore regulators of iron homeostasis and the involvement of hepcidin. The aims of the current trial were to study the response of hepcidin, erythropoietin, and iron status to iron supplementation and their correlations and to evaluate changes in laboratory measures in iron-deficient and IR infants.

SUBJECTS AND METHODS

Study design

This was originally a randomized, double-blinded, controlled trial of iron supplementation from 6 wk to 6 mo of age in marginally LBW infants. The intervention outcomes with regard to iron status were presented elsewhere (25). The current study is based on further laboratory measures from this trial.

Subjects and intervention

The trial was performed between March 2004 and June 2007 at 2 Swedish tertiary hospitals: Umeå University Hospital (Umeå, Sweden) and Karolinska University Hospital (Stockholm, Sweden). Eligible participants were identified through delivery records and contacted at the delivery ward, the neonatal ward, or at home after discharge. We included 285 infants at 6 wk of age on the basis of the following inclusion criteria: 1) birth weight 2000–2500 g, 2) no disease symptoms at inclusion, 3) no chronic disease, 4) no previous blood transfusion, and 5) no history of iron supplementation.

Included infants were stratified by sex and study center and randomly assigned into 3 intervention groups to receive the following doses of iron supplementation: 0 (placebo), 1, or 2 mg/kg per day. Placebo and iron supplements were divided into 2 daily doses. The 1-mg group received one dose of iron supplements and one dose of placebo (in random order for each infant). To keep the randomization blinded, all participants received 2 bottles: 1 for the morning dose and 1 for the evening dose. The iron supplement was a ferrous succinate mixture (Ferrumyn S; Astra Zeneca) containing 3.7 mg Fe/mL. All investigators and parents were blinded to the intervention assignment as described in detail elsewhere (25). The dose was adjusted for actual weight at 12 and 19 wk. Compliance with the intervention was monitored by using a daily checklist, where parents were asked to register all doses given, and by weighing the bottles of iron or placebo before and after use. Poor compliance was defined as consumption of <70% of doses given.

Data collection

At inclusion, background data were collected from parents and from delivery records. The infants visited the study center at the following ages: 6 wk, 12 wk, 19 wk, and 6 mo. Before each visit, parents were asked to complete a 3-d food diary, which was used to calculate the mean total daily iron intake during the intervention, taking compliance and estimated iron intake from breast milk into account. These calculations were described in detail elsewhere (25).

Laboratory measures

At 6 wk, 12 wk, and 6 mo, phlebotomy was performed. From each blood sample, EDTA-treated blood was sent for a complete blood count, including hemoglobin and MCV, by using an automated blood counter in each hospital laboratory. The blood was drawn into serum separator tubes and centrifuged, and serum was frozen at −70°C until analyzed for ferritin by ELISA (Dxi; Beckman Coulter), transferrin by turbidimetry (Synchron LX; Beckman Coulter), iron by a colorimetric ferrozine-based assay (Synchron LX), TfR by ELISA (Ramco), CRP by turbidimetry (Synchron LX), and erythropoietin by ELISA (R&D Systems Inc). TS was calculated from serum iron and transferrin (26). Hepcidin was assessed in serum by using a commercial peptide enzyme immunoassay (S-1337; Bachem) with a typical sensitivity (concentration of compound that results in 50% inhibition of maximal activity) of 1.5 ng/mL. Samples with a result high above the typical sensitivity were diluted and re-analyzed. A lower limit of detection has not been defined by the manufacturer, but they report a detection range of 0 to 25 ng/mL.

ID was indicated when ≥2 of 4 indicators of iron status were outside the reference range, and infants were defined as IR when none of the indicators were outside the same reference range (Table 1) (27–30).

Dropouts and discontinued intervention

Infants with anemia at 6 wk [defined as a hemoglobin concentration <90 g/L (27)] or 12 wk [<95 g/L (28)] were called back to the study center for a confirmative blood sample and analysis of serum ferritin (via standard hospital routines and methods) and were then referred to a pediatrician for evaluation. In 22 cases (13 at 6 wk and 9 at 12 wk), the pediatrician suggested iron supplementation. Those infants discontinued the intervention and were prescribed unblinded iron supplementation; however, they continued the trial as unblinded cases. This differs from the previously published study from this trial, in which the 16 infants with confirmed anemia at 6 wk were excluded from the analyses. Twenty-four infants (8.5%) discontinued the trial as dropouts (Figure 1).

Statistical analyses

Sample size was based on estimated differences in the main outcomes, as described elsewhere (25). Because ferritin,

<table>
<thead>
<tr>
<th>TABLE 1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cutoffs for iron-status indicators at ages 12 wk and 6 mo 1</td>
</tr>
<tr>
<td>12 wk</td>
</tr>
<tr>
<td><strong>Ferritin (µg/L)</strong></td>
</tr>
<tr>
<td><strong>MCV (fL)</strong></td>
</tr>
<tr>
<td><strong>TS (%)</strong></td>
</tr>
<tr>
<td><strong>TfR (µg/L)</strong></td>
</tr>
</tbody>
</table>

1 Iron deficiency was indicated when ≥2 of 4 indicators were outside these cutoffs. Infants with no indicators outside the cutoffs were considered iron replete. MCV, mean cell volume; TfR, transferrin receptor; TS, transferrin saturation.
hepcidin, and erythropoietin values showed skewed distributions, they were log transformed in all calculations and transformed back for presentation as geometric means and SD.

Group comparison was performed by using ANOVA and a Bonferroni post hoc test whenever applicable. Possible confounding baseline and background characteristics were explored with ANCOVA. Comparisons over time within groups were performed by using paired \( t \) test. Relations between hemoglobin and iron status (TfR, ferritin, MCV, serum iron, transferrin, and TS) with hepcidin and erythropoietin, respectively, were explored with scatter plots and stepwise univariate and multivariate linear regression models.

All measures with a corresponding CRP \( \geq 8 \) mg/L were excluded (1 at 6 wk, 9 at 12 wk, and 9 at 6 mo). Infants given unblinded supplementation at 6 or 12 wk and infants from iron-supplemented groups (1 and 2 mg/kg daily) with poor compliance were excluded, which resulted in the lower number in each group.

**RESULTS**

**Baseline values and characteristics**

Of the 285 infants included and randomly assigned, one infant with a diagnosis of \( \beta \)-thalassemia at 6 wk and one with ABO immunization at birth were excluded from all analyses because they did not fulfill the inclusion criteria (Figure 1). Analyses of baseline and background characteristics for included infants showed no significant differences between the intervention groups, as presented in detail elsewhere (25). Of the included infants, 49% were boys and 56% were preterm (gestational age, 37 wk). The mean (\( \pm \)SD) gestational age was 36.5 \( \pm \)1.9 wk and birth weight was 2.3 \( \pm \)0.1 kg.

**Effect of iron intake on hepcidin and erythropoietin**

The results of hepcidin and erythropoietin at 6 wk, 12 wk, and 6 mo together with other iron-status indicators are illustrated in Figure 2. At baseline, the mean (\( \pm \)SD) hepcidin concentration was 12.2 \( \pm \)1.8 ng/mL, and no significant difference was observed between the groups (\( P = 0.298 \)). The mean (\( \pm \)SD) baseline erythropoietin concentration was 8.6 \( \pm \)1.5 mU/mL, and no significant difference was observed between groups (\( P = 0.342 \)). At 6 mo, significant differences in hepcidin (\( P = 0.007 \)), erythropoietin (\( P < 0.001 \)), and all other iron-status indicators were observed between groups (Figure 2). Furthermore, a significant age \( \times \) group interaction was observed for all variables except MCV. Post hoc analyses showed that the 2 iron-supplemented groups at 6 mo had similar concentrations of both hepcidin (\( P = 1.000 \)) and erythropoietin (\( P = 1.000 \)), but that the iron groups had significantly higher hepcidin (19.9 \( \pm \)2.1 compared with 13.0 \( \pm \)2.6 ng/mL; \( P = 0.002 \)) and lower erythropoietin (5.8 \( \pm \)1.5 compared with 7.9 \( \pm \)1.6 mU/mL; \( P < 0.001 \)) concentrations than did the placebo group. Already at 12 wk, a significant group effect for hepcidin (\( P = 0.005 \)), ferritin (\( P = 0.007 \)), serum iron (\( P < 0.001 \)), transferrin (\( P = 0.007 \)), and TS (\( P < 0.001 \)) was observed (Figure 2). Multivariate analyses of possible confounders showed no significant confounding or interacting effect when the following baseline and background variables were included in the models of group
effect on hepcidin and erythropoietin at 6 mo: sex, gestational age at birth, baseline weight, and baseline length.

To further explore the association between hepcidin and iron intake, the hepcidin results at 6 mo were regressed on the calculated daily iron intake between 6 wk and 6 mo (Figure 3). A significant positive linear correlation was found ($R^2 = 0.076, P = 0.001$). Of infants with hepcidin values below the 10th percentile (6.55 ng/mL) at 6 mo, 16 of 20 subjects had a preceding estimated iron intake of $0.4$ mg/kg per day and 15 were iron deficient.

Hepcidin and erythropoietin in iron-deficient and IR infants

At 6 mo of age, 31 (13%) of the 234 infants with complete iron-status analyses and no instance of a CRP concentration $>8$ mg/L at any time point were excluded. Infants with an elevated C-reactive protein concentration ($>8$ mg/L) at any time point were excluded. $n = 61-71$ in the placebo group, 46-52 in the 1-mg/kg group, and 50-57 in the 2-mg/kg group. $P$ values for the age × group interaction were 0.005 for hepcidin, 0.137 for MCV, 0.002 for iron and TfR, and <0.001 for EPO, ferritin, hemoglobin, transferrin, and TS. EPO, erythropoietin; MCV, mean cell volume; TfR, transferrin receptor; TS, transferrin saturation.

Correlations between hepcidin, erythropoietin, and iron-status variables

Linear univariate regression analyses of correlations between iron-status indicators and hepcidin and erythropoietin are presented in Table 3 and Table 4, respectively. All indicators of iron status (ferritin, MCV, TfR, TS, transferrin, and serum iron) were significantly correlated with hepcidin at 6 mo, and some iron-status indicators (always including ferritin) were correlated at 6 and 12 wk. In multivariate analyses, ferritin was always included in the best predicting model of hepcidin. An independent correlation of erythropoietin with at least some iron-status variables was observed at all ages. Furthermore, hepcidin
DISCUSSION

This was the first study of hepcidin in iron-supplemented and iron-deficient infants and the first to explore correlations between hepcidin, erythropoietin, and iron-status indicators in children. Increased gene expression of hepcidin as a response to iron loading was first suggested by Pigeon et al (7). Hepcidin production is regulated by multiple signals, including systemic iron availability and hepatic iron stores. The molecular mechanisms were recently described in part (5, 6, 31). In brief, it is suggested that hepatocytes sense the iron saturation of transferrin in a membrane-bound complex of TfR1, TfR2, and the HFE protein. The signal is integrated with signals of hepatocyte iron stores in a not yet fully understood pathway. This dual input to the induction of hepcidin production suggests that hepcidin could change both as a response to short-term changes in serum iron and also to slower changes in iron stores.

Clinical trials of the hepcidin response to iron supplementation are few and we found none in infants. In the current trial, iron supplements resulted in increased serum hepcidin at 12 wk and 6 mo as compared with placebo. This finding is similar to the results of Malyszko et al (32), who studied serum prohepcidin and hepcidin in hemodialyzed patients undergoing iron therapy and found a significant positive effect of iron on hepcidin concentration. The association between iron loading and hepcidin concentration is further supported by the regression analyses, which showed a significant dose-dependent effect of iron intake on hepcidin when all iron sources were taken into account.

Blood samples of the current trial were collected at different times during the day. This possibly contributed to an increased variance because there is diurnal variation in hepcidin (33). Furthermore, because the supplements in the current trial were administered in the morning (except in half of the 1-mg/kg group, who received placebo in the morning, as described above) and blood samples were collected during the day at different times, the increased hepcidin concentrations found may theoretically not only reflect iron stores but also temporary daily peaks caused by absorption of the supplements, as previously suggested by others (33–35). However, our regression analyses showed that the

![FIGURE 3. Association between mean iron intake during the intervention and hepcidin at age 6 mo in marginally low-birth-weight infants with various iron intakes at the ages of 6 wk and 6 mo. ○, infants with iron deficiency at 6 mo (n = 31); ○, non-iron-deficient infants (n = 158). Iron intake was calculated from weighted means of the sum of all estimated iron sources (supplements, complementary food, infant formula, and breast milk). Infants with an elevated C-reactive protein concentration (≥ 8 mg/L) at any time point were excluded.](image_url)
best predictor of hepcidin was ferritin, which suggests that hepcidin correlated with iron stores rather than with circulating iron. The strong correlation between hepcidin and ferritin compared with other iron-status indicators was observed previously by others (33, 35–38), which suggests that hepcidin may be a useful indicator of iron stores.

Decreased gene expression of hepcidin as a response to ID was first shown by Nicolas et al (9) in mouse models, and this has been confirmed in human studies (31, 33, 37, 39). Cherian et al (37) examined pediatric refugees in whom the prevalence of ID was 19%. Those children (n = 181), 16 y of age and were predominantly male. Significantly lower concentrations of urinary hepcidin were found in children with ID than in children without ID (0.3 compared with 2.9 nmol/mmol Cr; P = 0.004). The 31 infants who developed ID at 6 mo in the current trial showed a trend of decreasing serum hepcidin concentrations from 6 wk

### TABLE 3
Univariate and multivariate linear regression models assessing the relation of hepcidin with erythropoietin, hemoglobin, and iron-status variables in marginally low-birth-weight infants with various iron intakes

<table>
<thead>
<tr>
<th>Variable</th>
<th>log10 Heparicin (ng/mL)</th>
<th>6 wk</th>
<th>12 wk</th>
<th>6 mo</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>β</td>
<td>R²</td>
<td>P</td>
<td>β</td>
</tr>
<tr>
<td>Univariate regression</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>log₁₀ Erythropoietin (mU/mL)</td>
<td>-0.054</td>
<td>0.003</td>
<td>0.402</td>
<td>-0.066</td>
</tr>
<tr>
<td>Hemoglobin (g/L)</td>
<td>0.071</td>
<td>0.005</td>
<td>0.261</td>
<td>-0.066</td>
</tr>
<tr>
<td>log₁₀ Ferritin (µg/L)</td>
<td>0.366</td>
<td>0.134</td>
<td>&lt;0.001</td>
<td>0.422</td>
</tr>
<tr>
<td>MCV (µL)</td>
<td>0.015</td>
<td>0.000</td>
<td>0.810</td>
<td>0.115</td>
</tr>
<tr>
<td>Transferrin (g/L)</td>
<td>-0.143</td>
<td>0.020</td>
<td>0.023</td>
<td>0.192</td>
</tr>
<tr>
<td>TIR (µg/L)</td>
<td>-0.199</td>
<td>0.040</td>
<td>0.001</td>
<td>0.018</td>
</tr>
<tr>
<td>TS (%)</td>
<td>0.121</td>
<td>0.015</td>
<td>0.055</td>
<td>0.122</td>
</tr>
<tr>
<td>Serum iron (µmol/L)</td>
<td>0.055</td>
<td>0.003</td>
<td>0.383</td>
<td>0.046</td>
</tr>
<tr>
<td>Multivariate regression</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>log₁₀ Ferritin and TIR</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>log₁₀ Ferritin</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Serum iron (µmol/L)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hemoglobin and TS</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Infants with an elevated C-reactive protein concentration (≥8 mg/L) at any time point were excluded. R² and P values were unadjusted. MCV, mean cell volume; TIR, transferrin receptor; TS, transferrin saturation.

### TABLE 4
Univariate and multivariate linear regression models assessing the relation of erythropoietin with hepcidin, hemoglobin, and iron-status variables in marginally low-birth-weight infants with various iron intakes

<table>
<thead>
<tr>
<th>Variable</th>
<th>log₁₀ Erythropoietin (mU/mL)</th>
<th>6 wk</th>
<th>12 wk</th>
<th>6 mo</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>β</td>
<td>R²</td>
<td>P</td>
<td>β</td>
</tr>
<tr>
<td>Univariate regression</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>log₁₀ Heparicin (ng/mL)</td>
<td>-0.054</td>
<td>0.003</td>
<td>0.402</td>
<td>-0.066</td>
</tr>
<tr>
<td>Hemoglobin (g/L)</td>
<td>-0.499</td>
<td>0.249</td>
<td>&lt;0.001</td>
<td>-0.154</td>
</tr>
<tr>
<td>log₁₀ Ferritin (µg/L)</td>
<td>-0.062</td>
<td>0.004</td>
<td>0.340</td>
<td>-0.174</td>
</tr>
<tr>
<td>MCV (µL)</td>
<td>-0.099</td>
<td>0.010</td>
<td>0.121</td>
<td>-0.061</td>
</tr>
<tr>
<td>Transferrin (g/L)</td>
<td>0.237</td>
<td>0.056</td>
<td>&lt;0.001</td>
<td>0.207</td>
</tr>
<tr>
<td>TIR (µg/L)</td>
<td>0.273</td>
<td>0.075</td>
<td>&lt;0.001</td>
<td>0.202</td>
</tr>
<tr>
<td>TS (%)</td>
<td>-0.423</td>
<td>0.179</td>
<td>&lt;0.001</td>
<td>-0.301</td>
</tr>
<tr>
<td>Serum iron (µmol/L)</td>
<td>-0.336</td>
<td>0.113</td>
<td>&lt;0.001</td>
<td>-0.248</td>
</tr>
<tr>
<td>Multivariate regression</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hemoglobin and TS</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TS</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Infants with an elevated C-reactive protein concentration (≥8 mg/L) at any time point were excluded. R² and P values were unadjusted. MCV, mean cell volume; TIR, transferrin receptor; TS, transferrin saturation.

Best predicting model achieved by stepwise multivariate linear regression, including log₁₀ hepcidin, hemoglobin, and the 2 best predicting iron-status variables.
were excluded. EPO, erythropoietin.

The discovery of hepcidin has resulted in further evidence of an association between erythropoietin and iron metabolism, because it was shown early by Nicolas et al (44) that erythropoiesis stimulates hepcidin downregulation. This observation has been reproduced, but the exact mechanism is not known (13). Furthermore, it has been suggested and confirmed that the effect of erythropoiesis on hepcidin can inhibit and overrule opposite signaling from inflammation or iron loading, which explains the paradoxic hepcidin downregulation in iron-loading anemias, such as thalassemia (13). However, in the current trial, hepcidin production seemed more strongly associated with iron status than with erythropoietin, which is similar to the results of Schulze et al (38), who also compared erythropoietin and iron-status effects on hepcidin in iron-deficient adult women.

Iron status undergoes rapid changes during the first 6 mo of life and we have suggested that infants have an impaired ability to downregulate iron absorption during this period, which causes an increased risk of iron overload (22, 23). The differences in hepcidin and erythropoietin observed between iron-deficient and IR infants in this trial, already at 12 wk, suggest that such an inability could not be explained by an impaired ability to produce those hormones. However, even though hormone expression seems to be adequate, the absolute concentrations may still be low or there could be poor sensitivity at receptor sites. In the placebo group of this trial, only small changes were observed over time in hepcidin, which possibly suggests a relatively constant physiologic reference range compared with the large and rapid changes observed in ferritin, MCV, TS, and TIR in this and previous trials (27–30). A constant concentration of hepcidin during the first 6 mo of life would make it a promising indicator of iron status in this age group. However, no group in the current trial represented truly “healthy” infants, so we concluded that there is an urgent need to establish reference ranges for hepcidin in healthy term infants. Recently, several validated kits have become commercially available, and we used one of them. However, results from the different kits are not yet completely comparable because of a lack of an international standard for hepcidin. Kling et al (42) found a slow decrease in erythropoietin from 2 to 6 mo of age, similar to the findings in IR infants in the current trial.
In conclusion, the current trial showed that serum hepcidin in LBW infants responds to iron supplementation, iron repletion, and iron deficiency in a manner similar to what has been theoretically hypothesized and similar to the response observed in a few previous trials performed in adults. Hepcidin concentrations increased significantly as a response to iron loading and were significantly lower in iron-deficient infants. Hepcidin was closely correlated with serum ferritin, but also with other indicators of iron status. Our results suggest that hepcidin could be useful as an indicator of iron status in infants. However, more studies on physiologic changes in healthy term infants are needed to construct reference ranges. Furthermore, we have shown a close negative correlation between iron supplementation and erythropoiesis—a correlation that is not sufficiently accounted for by the effect of iron on hemoglobin concentrations. Instead, it suggests a close interplay between iron stores and erythropoiesis, prompting further mechanistic studies.

We thank the following staff for dedicated fieldwork, and data collection: Kerstin Andersson in Stockholm and Ruth-Gerd Larsson, Åsa Sundström, and Margareta Bäckman in Umeå. We also thank Olle Hernell for scientific advice and support; Yvonne Andersson, Carina Lagerqvist, and Xiaou Du for help with the laboratory analyses; and Hugo Lagercrantz for support of the Stockholm part of the study.

The authors’ responsibilities were as follows—MD and BW: designed the research; BW, MD, and SB: conducted the research; BL: performed the laboratory analyses; and SB, BL, and MD: analyzed the data and wrote the manuscript. All authors read and approved the final manuscript. The authors declared no competing financial interests.

REFERENCES

35. Zimmermann MB, Troesch B, Biebinger R, Egli I, Zeder C, Hurrell RF. Plasma hepcidin is a modest predictor of dietary iron bioavailability in humans, whereas oral iron loading, measured by...


