abstract

OBJECTIVE: To determine if iron supplementation of 2 mg/kg per day, in addition to routine iron-fortified formula or mother’s milk, increased the hematocrit (Hct) at 36 weeks’ postmenstrual age (PMA).

METHODS: Infants with a birth weight <1500 g who reached 120 mL/kg per day of feedings before 32 weeks’ PMA were randomly assigned to iron (multivitamin with iron) or control (multivitamin) from enrollment until 36 weeks’ PMA (or discharge, if sooner). Investigators and caregivers were masked. Transfusion guidelines were used. The primary outcome was Hct at 36 weeks’ PMA. A nonparametric rank sum analysis was performed so that infants who died before 36 weeks and infants who were transfused could be included in an intention-to-treat analysis. Infants were ranked by death (lowest rank) then by number of transfusions (next lowest ranks). For infants who survived and were not transfused, the 36-week PMA Hct was used for the rank.

RESULTS: One hundred fifty infants were enrolled (76 iron, 74 controls). There were 2 deaths (1 in each group). One hundred other infants (47 iron, 53 controls) received transfusion(s). There was no significant difference in the primary outcome ranking (P = .59), in the number of transfusions per subject (P = .64), or in 36-week Hct (iron mean ± SD, 29.2% ± 4.0%; control, 28.3% ± 4.5%; mean difference and 95% confidence interval 0.9 [−0.5 to 2.3]; P = .21) or reticulocyte count among survivors. No short-term adverse effects of iron supplementation were observed.

CONCLUSIONS: Among infants <1500 g birth weight, iron supplementation, in addition to routine iron intake, did not significantly increase the 36-week Hct or the decrease number of transfusions. Pediatrics 2013;131:e433–e438
All infants have a decline in hemoglobin (Hgb) after birth because of increasing PaO₂ and Hgb saturation after birth. In very low birth weight (VLBW) infants (birth weight <1500 g), the nadir at 1 to 3 months after birth is lower than in term infants because of (1) greater phlebotomy losses for blood tests, (2) shortened red blood cell survival, and (3) rapid growth.1 Based on a study done in the 1970s with relatively stable preterm infants, the Hgb nadir does not depend on iron intake, but the recovery from this nadir is more rapid with iron supplementation in comparison with non-supplemented human milk or formula.2

Iron deficiency anemia is anemia resulting from the lack of sufficient iron for synthesis of Hgb. In term infants without sufficient dietary intake of iron, iron deficiency anemia occurs 3 months after iron stores are depleted, which is normally at 6 to 9 months of age. VLBW infants may be at increased risk of developing iron deficiency anemia because of (1) low iron stores at birth, (2) rapid depletion of iron stores owing to phlebotomy losses, and (3) inability to regulate iron absorption by the gastrointestinal tract.3 On the other hand, if VLBW infants are frequently transfused with red blood cells to maintain a specified hematocrit (Hct), as is common practice, iron deficiency anemia may be delayed or prevented. In VLBW infants, it has not been determined when iron stores typically become depleted and when iron deficiency anemia usually develops. It likely varies among infants, depending on phlebotomy losses and transfusion practices. Notwithstanding this lack of evidence, the American Academy of Pediatrics recommends that preterm infants should receive a total iron intake of 2 mg/kg per day for infants between 1500 and 2500 g birth weight and 4 mg/kg per day for infants weighing <1500.4

A careful literature review identified 2 interventional studies that addressed the need for iron intake above that provided in iron-containing formula or fortified mother’s milk in VLBW infants. One randomized trial enrolled 204 infants with birth weights <1301 g and assigned them to early iron intake (once they reached 100 mL/kg per day of feedings) versus late (61 days of age) iron intake.5 All infants received iron-fortified formula or mother’s milk without iron fortification. Iron status was assessed with ferritin at 61 days of age.5 This study showed no difference in the primary outcome of iron deficiency anemia (based on ferritin). There was a difference between the groups in transfusions, but transfusion decisions could have been biased in this unmasked study. There was also a high loss to follow-up.

The second randomized trial enrolled 42 infants with birth weights <1500 g who reached 100 mL/kg per day of feedings by 14 days of age.6 Infants were randomly assigned to 3 to 4 mg/kg per day of iron or no iron supplementation.6 Infants were predominantly given mother’s milk with fortifier not containing iron.6 Iron status was assessed with ferritin at 60 days of age.6 This study also showed no difference, possibly because of the low power to demonstrate a clinically important difference.

Another more recent randomized trial enrolled 285 infants with birth weights of 2000 to 2500 g.2 Infants were allocated to 3 different doses of iron supplementation (no iron, 1 mg/kg per day iron, or 2 mg/kg per day iron) starting at 6 weeks and ending at 6 months.7 Anemia was defined as Hgb (g/L) <90 at 6 weeks, <95 at 12 weeks, <105 at 6 months; iron deficiency at 6 months was defined when ≥2 of 4 indicators were present (ferritin <12 μg/L, mean corpuscular volume <71 fl, transferritin saturation <10%, or transferrin receptor level >11 μg/L).7 This study showed a difference in iron deficiency anemia at 6 months in a dose-dependent manner.

One might assume that lower-birthweight infants would be more susceptible to iron deficiency anemia, but, if smaller infants are more likely to be transfused, they might be less susceptible to iron deficiency anemia. In addition to the problems with lack of masking, loss to follow-up, and low power, the previous studies may not be generalizable to current practice. Use of restrictive transfusion guidelines and mother’s milk without iron supplementation could have made iron deficiency more common than it would be under current practice conditions. In our NICU, relatively liberal transfusion policies have been adopted because of recent randomized trials suggesting long-term benefits of maintaining higher hemoglobin levels in extremely low birth weight (<1000 g birth weight) infants.8,9 Mother’s milk is typically fortified with iron-containing fortifier when a volume of 120 mL/kg per day is tolerated.

A problem with the diagnosis and treatment of iron deficiency anemia in VLBW infants is that there are no valid diagnostic tests. In term infants, serum ferritin and mean corpuscular volume can be used to diagnose iron deficiency anemia, but, in preterm infants, mean corpuscular volume and ferritin are typically higher than in term infants; they are therefore not reliable measures for the diagnosis of iron deficiency anemia. In addition, ferritin can be affected by inflammation. For these reasons, the previous studies leave the unanswered question of whether it is beneficial to give additional iron intake above that of iron-fortified formula or iron-fortified human milk to prevent iron deficiency anemia in a setting of relatively liberal transfusions. We planned the following masked randomized trial to determine if iron supplementation (2 mg/kg per day), in addition to feeding with routine iron-fortified milk (formula or fortified
mother's milk), increased the Hct at 36 weeks' postmenstrual age (PMA) in preterm infants with birth weights <1500 g.

**METHODS**

The study was approved by the Institutional Review Board of The University of Texas Health Science Center at Houston Medical School and conducted at Children's Memorial Hermann Hospital. In-born or outborn infants with a birth weight of <1500 g admitted between January 2010 and December 2011 who reached 120 mL/kg per day of feedings before 32 weeks' PMA were eligible for the trial. Infants with bowel resection or cyanotic heart disease were excluded. All enrolled infants had written informed parental consent before enrollment.

Infants were assigned to 1 of 2 strata according to gestational age (GA) by dates at birth (<27 weeks' GA and ≥27 weeks' GA). Once infants reached 120 mL/kg per day of feedings, they were randomly allocated (computer-generated randomization table with variable block size) by the research pharmacy to intervention (multivitamin with iron) or control group (multivitamin without iron) in a 1:1 ratio. The enrolling investigators were masked to the allocation sequence; the study investigators, clinicians, and parents were masked to group assignment until the study data collection was complete. It is possible that bedside nurses who administered the medication could have identified differences in the appearance or smell of the preparations with and without iron, but there were no known episodes of unmasking of physicians or nurse practitioners. Multiple births were randomly assigned separately.

Infants in the study received iron-fortified formula or iron-fortified mother’s milk (both achieve an intake of at least 2mg/kg per day of iron when the infant is receiving at least 133 mL/kg per day of feedings). Infants in the intervention group received an additional 2 mg/kg per day of iron from the multivitamin, and infants in the control group received no additional iron. Study drug was administered as a timed medication (not always given with a feeding). Transfusion guidelines were based on birth weight, level of respiratory support, and day of age (see Table 1). A standard transfusion volume of 20 mL/kg packed red blood cells was used. During the intervention period, infants received the study drug by mouth or feeding tube once daily. The study drug dose was adjusted weekly for the infant's current weight. If feedings were held, the study drug was held, and once feedings reached 60 mL/kg per day, the study drug was resumed. The study intervention was continued until 36 weeks' PMA or discharge, if the infant was discharged sooner. Compliance with the study intervention and transfusion guideline was monitored during the intervention period.

The primary outcome was Hct at ≥36 weeks' PMA. The hematocrit could be obtained by venous or capillary sampling. For infants discharged from the hospital before 36 weeks' PMA, the last Hct before discharge was used. For infants transferred before 36 weeks' PMA, the Hct at 36 weeks was sought from the receiving hospital and used if available. For infants transferred before 36 weeks with no available Hct at 36 weeks, the last Hct before transfer was used. For those who died before 36 weeks' PMA, the Hct at 36 weeks was considered to be missing.

A nonparametric rank sum analysis was planned as follows, so that infants who died before 36 weeks and infants who were transfused could be included in an intention-to-treat analysis. Infants were ranked by death (lowest rank) then by number of transfusions (next lowest ranks). For infants who survived and were not transfused, the 36 weeks’ PMA Hct was used as the primary outcome. Analyses were performed with Stata Version 11 (StataCorp, College Station, TX).

The predetermined sample size of 150 was based on a retrospective observational pilot study from the 2008 calendar year. A sample size of 75 per group was calculated to achieve 80% power to detect a difference in Hct of 2% between groups, assuming a mean Hct of 25.6% in the control group, a SD 4.4%, with a significance level (α) of 0.05 by using a 2-sided 2-sample t test. We used this method for the sample size determination because we were unable to predict how many infants would be transfused, and we expected the intervention to have a greater effect on hematocrit than on transfusion.

Predefined secondary outcomes were as follows: (1) number of blood transfusions received between enrollment and 36 weeks’ PMA, (2) bronchopulmonary

<table>
<thead>
<tr>
<th>Infant Status</th>
<th>Hematocrit (Peripheral) Threshold for Transfusion, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Birth Weight, ≤1000 g</td>
<td>Birth Weight, 1000–1500 g</td>
</tr>
<tr>
<td>Respiratory support,* ≤2 wk old</td>
<td>≥38</td>
</tr>
<tr>
<td>No respiratory support; ≤2 wk old</td>
<td>≥35</td>
</tr>
<tr>
<td>Respiratory support,* &gt;2 wk old</td>
<td>≥30</td>
</tr>
<tr>
<td>No respiratory support; &gt;2 wk old</td>
<td>≥25</td>
</tr>
<tr>
<td>Ventilator, &gt;2 wk old</td>
<td>≥30–35</td>
</tr>
<tr>
<td>CPAP or unstable on oxygen, &gt;2 wk old</td>
<td>≥25%–30%</td>
</tr>
<tr>
<td>No respiratory support or stable on oxygen</td>
<td>≥20% (and retic count &lt;5%)</td>
</tr>
<tr>
<td>Plan for discharge in 1–2 wk</td>
<td>Do not transfuse</td>
</tr>
</tbody>
</table>

* Respiratory support includes ventilator or CPAP or supplemental oxygen.

If a central Hct (from a catheter) is used, the transfusion threshold is 3% lower than the peripheral (capillary or venous) Hct threshold. CPAP, continuous positive airway pressure.
dysplasia (defined as use of supplemental oxygen at 36 weeks’ PMA to maintain oxygen saturation of 85%–95%), (3) sepsis (defined as a positive blood or cerebrospinal fluid culture that was treated with antibiotics for at least 7 days), (4) necrotizing enterocolitis (NEC)/gastrointestinal perforation (medical NEC defined as ≥stage II Bell’s criteria, or surgical NEC; gastrointestinal perforation defined as exploratory laparotomy or surgical drain for perforation or suspected NEC), (5) apnea of prematurity (operationally defined as receiving caffeine at 36 weeks’ PMA), and (6) growth (assessed by weight at 36 weeks’ PMA or at discharge if discharged before 36 weeks).

RESULTS
Among the 253 eligible infants screened for enrollment between January 2010 and December 2011, 150 infants were enrolled (76 iron, 74 control) (see Fig 1). Baseline characteristics were similar among the groups (see Table 2). There were 2 deaths (1 in each group) before 36 weeks’ PMA; both deaths were attributed to NEC. Two infants (1 in each group) were withdrawn from the study before 36 weeks’ PMA per parental request. One hundred of 150 infants (47 iron, 53 control) received transfusion(s) during the intervention period.

All infants were analyzed according to their assigned groups. Compliance with the study drug administration was 98% (operationally defined as receiving caffeine at 36 weeks’ PMA), and growth (assessed by weight at 36 weeks’ PMA or at discharge if discharged before 36 weeks).

FIGURE 1
Enrollment flow diagram. CPS, Child Protective Services.
TABLE 2 Baseline Characteristics of the Enrolled Infants by Study Group

<table>
<thead>
<tr>
<th></th>
<th>Control (n = 74)</th>
<th>Iron Supplement (n = 76)</th>
<th>P</th>
<th>95% CI for Mean or Median Difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Birth weight, g, mean ± SD</td>
<td>851 ± 252</td>
<td>976 ± 266</td>
<td>0.21</td>
<td>(0.09 to 0.43)</td>
</tr>
<tr>
<td>Gestational age, wk, mean ± SD</td>
<td>27.3 ± 1.3</td>
<td>27.3 ± 1.3</td>
<td>1.00</td>
<td>(0.89 to 1.11)</td>
</tr>
<tr>
<td>Days of age at enrollment/intervention, mean ± SD</td>
<td>23.0–31.0</td>
<td>22.9–30.7</td>
<td>0.89</td>
<td>(0.71 to 1.17)</td>
</tr>
<tr>
<td>Respiratory support at enrollment, %</td>
<td>17 ± 10</td>
<td>17 ± 8</td>
<td>0.60</td>
<td>(0.26 to 1.0)</td>
</tr>
<tr>
<td>Ventilator</td>
<td>15</td>
<td>17</td>
<td>0.40</td>
<td>(0.17 to 0.75)</td>
</tr>
<tr>
<td>NCPAP</td>
<td>41</td>
<td>38</td>
<td>0.68</td>
<td>(0.31 to 1.1)</td>
</tr>
<tr>
<td>Nasal cannula</td>
<td>27</td>
<td>24</td>
<td>0.68</td>
<td>(0.31 to 1.1)</td>
</tr>
<tr>
<td>None</td>
<td>18</td>
<td>21</td>
<td>0.68</td>
<td>(0.31 to 1.1)</td>
</tr>
<tr>
<td>Oxygen at enrollment, %</td>
<td>&gt;21%</td>
<td>69</td>
<td>0.09</td>
<td>(0.04 to 0.22)</td>
</tr>
<tr>
<td>&lt;21%</td>
<td>31</td>
<td>37</td>
<td>0.09</td>
<td>(0.04 to 0.22)</td>
</tr>
</tbody>
</table>

NCPAP: nasal continuous positive airway pressure.

least 28 doses of study drug (n = 61 for iron, and n = 59 for control). We again found no difference in the hematocrit (iron mean ± SD 29.0% ± 3.6%, control 28.0% ± 4.1%, P = .16 by t test) or reticulocyte count (iron mean ± SD 6.0 ± 2.2%, control 5.5% ± 2.0%; P = .17 by t test).

No adverse effects were noted in any infant enrolled in the study. Two parents withdrew their infants (1 in each group) because of concerns about constipation from the iron. At the time of withdrawal, the stools were documented as 1 to 2 loose or soft stools per day and 1 soft stool every 1 to 2 days, respectively. During the intervention period, 56 study infants (iron 26/76 infants, control 30/74) had feedings held for >24 hours for feeding intolerance (defined as increased residuals). There was no difference between groups in feedings held for 24 hours (P = .42 by \( \chi^2 \) test).

DISCUSSION

We found no difference in the hematocrit at 36 weeks’ PMA or in the number of transfusions with iron supplementation in addition to iron-containing formula or human milk. These findings are consistent with other similar studies in VLBW infants that found no difference in iron deficiency anemia. Our findings differed from those of Franz et al\(^\text{10}\) in that we found no effect of iron supplementation on transfusions. Potential explanations for this difference are that all our Control group infants were receiving at least 2 mg/kg per day of dietary iron intake and that our trial was masked and therefore less susceptible to bias in decisions regarding transfusions. The trial by Franz et al also used a higher dose of iron (range, 2–4 mg/kg per day) and a smaller proportion of the infants were transfused (39% in the early iron group and 53% in controls), possibly because more restrictive transfusion guidelines were used in that trial.

Iron deficiency may have deleterious effects on other organ systems and processes besides red blood cell production. The effects on neurodevelopment and immune function have been observed in older infants.\(^\text{10}\) The effect of iron...
supplementation on neurodevelopment in preterm infants has not been well studied.11 Our study did not have sufficient power or long enough follow-up to assess potential beneficial effects on other organ systems.

The findings from this study are best generalized to settings where similar relatively liberal transfusion guidelines are used. Liberal transfusion guidelines could mitigate the effect of iron intake on anemia in premature infants, because iron requirements for red blood cell synthesis may be lower when phlebotomy losses are replaced with transfused blood. A single red blood cell phlebotomy losses are replaced with cell synthesis may be lower when cause iron requirements for red blood cell iron.13 On the other hand, if our transfusion guidelines had been the explanation for our finding of no difference in 36-week Hct, we would have expected to see a difference in transfusions between the groups, and we did not observe this. By using a rank sum analysis to incorporate the difference in transfusions into the primary analysis of Hct, we expected to be able to identify important between-group differences in either transfusions or Hct; yet no difference was seen. Our study was powered to identify a difference of 2% in Hct at 36 weeks; we did not have adequate power to identify a smaller difference in Hct or a small difference in the proportion of infants transfused. It is also possible that a higher dose of iron or a longer duration of study treatment would have had a larger effect.

CONCLUSIONS

Among infants <1500 g birth weight, iron supplementation in addition to routine iron intake did not affect hematocrit at 36 weeks or the number of transfusions before 36 weeks’ PMA. No adverse effects of iron supplementation were observed in this trial. In the absence of any other randomized trials to address this question, there is no evidence from clinical trials to support giving >2 mg/kg per day of iron to these infants. If future trials are undertaken, a larger sample size or longer duration of treatment is recommended.

ACKNOWLEDGMENTS

The authors acknowledge the clinical research nurses, Peggy Robichaux, Georgia McDavid, Katrina Burson, and Pattie Tate, for all their contributions to this interventional trial.

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Randomized Trial of Iron Supplementation versus Routine Iron Intake in VLBW Infants

Tiffany A. Taylor and Kathleen A. Kennedy

Pediatrics; originally published online January 21, 2013;
DOI: 10.1542/peds.2012-1822
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/content/early/2013/01/15/peds.2012-1822