Iron Deficiency, but Not Anemia, Upregulates Iron Absorption in Breast-Fed Peruvian Infants

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Abstract

Iron absorption in adults is regulated by homeostatic mechanisms that decrease absorption when iron status is high. There are few data, however, regarding the existence of similar homeostatic regulation in infants. We studied 2 groups of human milk-fed infants using ⁵⁷Fe (given as ferrous sulfate without any milk) and ⁵⁸Fe (given at the time of a breast-milk feeding) stable isotopes to determine whether healthy infants at risk for iron deficiency would regulate their iron absorption based on their iron status. We studied 20 Peruvian infants at 5–6 mo of age and 18 infants at 9–10 mo of age. We found no effect of infant hemoglobin concentration on iron absorption with 5–6 mo–old infants absorbing 19.2 ± 2.1% and 9- to 10-mo–old infants absorbing 25.8 ± 2.6% of the ⁵⁷Fe dose. For ⁵⁸Fe, 5- to 6-mo–old infants absorbed 42.6 ± 5.0% and 9 to 10-mo–old infants absorbed 51.9 ± 10.3%. Following log transformation, iron absorption from ⁵⁷Fe (r = −0.61, P < 0.001) and ⁵⁸Fe (r = −0.61, P = < 0.001) were inversely correlated to serum ferritin (S-Ft). For both the ⁵⁷Fe and ⁵⁸Fe doses, infants with S-Ft <12 mg/L (n = 11) had significantly higher iron absorption than those with S-Ft >12 mg/L. We concluded that iron absorption in infants is related to iron status as assessed by serum ferritin but not hemoglobin concentration. Infants with low iron status upregulate iron absorption from breast milk at both 5–6 and 9–10 mo of age. J. Nutr. 136: 2435–2438, 2006.

Introduction

Iron absorption is inversely related to iron status in adults (1). This homeostatic regulation both compensates for iron deficiency and protects against iron overload. There are few data, however, regarding the existence of similar homeostatic regulation in infants and young children. A previous study in a low-risk population of Swedish infants administered iron supplements or a placebo suggested that homeostatic regulation of iron absorption may be present at 9 but not at 6 mo of age (2). Comparable data are not available for a high-risk population.

As iron deficiency is of varying prevalence in infants (3), we chose to evaluate iron absorption in a population where iron status is less likely to be adequate. According to a national survey conducted in 2000, Peru has a high prevalence of anemic infants. The survey reported a 59% incidence of anemia among infants at 6–9 mo of age and 72% among 10- to 12-mo–old infants. This can be explained in part by the effect maternal iron status has on the delivery of fetal iron (4) and by the low iron intake of the infants (5). The aim of the present study was to evaluate whether 2 groups of healthy term, breast-fed infants, ages 5–6 and 9–10 mo, at high risk for iron deficiency can attempt to compensate for their poor iron status by increasing their iron absorption.

Subjects and Methods

Subjects

Two groups of infants were recruited from Villa El Salvador, a low-income peri-urban area of Lima, Peru. Recruitment of subjects was done by clinic staff from the local population. One group consisted of 5- to 6-mo–old infants (150–195 d) and the other of infants at 9–10 mo of age (270–315 d). All infants were healthy, term infants (≥37 wk gestation), singleton infants, had a birth weight ≥2500 g, and were being breast-fed. The 5–6–mo–old infants (n = 20) were exclusively breast-fed. The 9- to 10-mo–old infants (n = 18) were breast-fed and allowed age-appropriate complementary foods at the mother’s discretion. None of these infants were being supplemented with iron.

The Investigational Review Board of the Baylor College of Medicine and Affiliated Hospitals and the Ethics Committee at the Instituto de Investigación Nutricional Lima, Peru approved the protocol. Informed written consent was obtained from the families prior to enrollment. Five to 6-mo–old infants were recruited based on a screening of their hemoglobin values. We targeted an enrollment of 10 infants with a hemoglobin <105 g/L and 10 with a hemoglobin ≥105 g/L. Similarly, 9- to 10-mo–old infants were recruited based on a screening of their hemoglobin. We targeted an enrollment of 10 infants with a hemoglobin <100 g/L and 10 with a hemoglobin ≥100 g/L. Twenty infants were recruited for each of the age groups. Two infants in the 9- to 10-mo–old age group were excluded from the study due to unsuccessful blood draws.

Hemoglobin cut-offs were selected based on the levels determined by Donnellof et al. (2) and Emond et al. (6) to be more appropriate for children of these ages.

Study procedure

All study visits took place at a community clinic in Villa El Salvador. Each infant had 2 iron absorption measurements made and the study required 4 visits.

Visit 1. Infants were screened by a finger stick for group assignment, and 120 mL of breast milk was collected from each mother. All milk collections took place 2–3 d prior to the absorption measurement and the milk was kept refrigerated at the clinic until the time of the study.

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Visit 2. In the morning of the absorption experiment, 60 mL of mother’s milk mixed with 150 μg of ⁵⁸Fe was given to the infant after a 2-h fast. The milk was heated to 37°C in a water bath prior to being fed to the infant in a preweighed feeding bottle. The bottle was weighed after the feeding and all milk losses (e.g., spitting up, spilling) were determined by collection in preweighed napkins. The bottle was rinsed with an additional 60 mL of mother’s milk, and fed to the infant to ensure the entire amount of isotope was consumed. No food or drink was given for 2 h after the feeding. Anthropometric data, dietary, and health records were collected.

Visit 3. The following day, 2 mg of ⁵⁷Fe as the sulfate was given in juice containing 50 mg ascorbic acid in a preweighed feeding bottle. Again, the bottle was weighed after the feeding, and all losses were determined by collection in preweighed napkins. No food or drink was given for 2 h before and after the feeding.

Visit 4. Fourteen days after Visit 2, the infants returned to the clinic and a venous blood sample (3 mL) was drawn for isotope ratio measurement and other biochemical indicators. Anthropometric data, dietary, and health records were collected.

Blood samples and laboratory analyses
Each sample was centrifuged at 2000 X g for 10 min at room temperature and separated into serum, plasma, and RBCs. The RBCs were saved for isotope ratio analysis on a thermal ionization mass spectrometer. Serum ferritin (S-Ft) (Coat-A-Count Ferritin IRMA, DPC), C-reactive protein (CRP) (C-Reactive Protein RID, The Binding Site), and plasma folate and vitamin B-12 (Quantaphase II, BioRad) were also measured.

Stable isotope methods
Iron isotopes of ⁷⁷Fe (93.9%) and ⁵⁸Fe enriched (93.1%) were purchased in the elemental form from Trace Sciences International. Iron isotope solutions were prepared as the sulfate by dissolving the metals in 30 μL of 7 mol/L nitric acid and 125 μL of 0.5 mol/L sulfuric acid for every mg of elemental iron. The solution was dried at 120°C, 230°C, and 500°C for 30 min each. The following day the powdered product was reconstituted with 240 μL of 0.2 mol/L sulfuric acid for every mg of elemental iron. Deionized water was added to produce a solution of 0.02 g/L for ⁵⁷Fe and 0.005 g/L for ⁵⁸Fe.

Iron isotope ratios were measured from red blood cells 14 d after dosing. Briefly, 0.3–0.5 mL of RBCs were digested with 10 mL 15 mol/L nitric acid until dry. After cooling, the digest was reconstituted with 0.6 mL 6 mol/L hydrochloric acid (HCl). The solution was then put through an anion exchange column for separation. A filter was placed in an 8 cm × 0.4 cm column and loaded with 2 mL of anion exchange resin. The column was prewashed with 4 mL 6 mol/L HCl, 4 mL of deionized water, and 1 mL of 6 mol/L HCl again. The sample was then loaded into the column and followed by 6 mL 6 mol/L HCl and 0.5 mL 0.5 mol/L HCl. The iron was collected from the column (1 mL 0.5 mol/L HCl) into a Teflon vial and dried on a hotplate. The sample was then reconstituted with 40 μL of 3% ultrapure HCl and 10 μL of sample was placed onto a rhenium filament with 2 μL of 0.7 mol/L phosphoric acid and 6 μg silica gel. The iron isotope ratios were measured with a thermal ionization magnetic sector mass spectrometer (MAT 261; Finnigan ThermoQuest). The results were expressed as the ratio of ⁷⁷Fe:⁵⁸Fe and ⁵⁷Fe:⁵⁸Fe.

Iron absorption was calculated as iron incorporated into the erythrocytes at 14 d based on the assumption that 90% of the absorbed iron was incorporated into RBCs (7). There is evidence that <80% of absorbed iron is promptly incorporated into erythrocytes of infants (8). Fomon et al. (9) reported that erythrocyte incorporation of ⁵⁸Fe was significantly greater in older infants (168 d) at 52% than in younger infants (56 d) at 23%. All of our subjects were >56 d old, and a large proportion were older than 168 d; thus, the 90% value may be more suitable for our subjects, however, we realized that using Fomon’s 52% value would increase our results. Most previous studies have used the 90% (10,11) value, so there is consistency among studies.

Sample size
We hypothesized that we would find a 50% greater absorption in anemic children relative to non-anemic children. Using previous iron absorption data (2), a group size of 8 infants in each group would give a power of 80% to detect a 50% difference in iron absorption between groups when testing at an alpha level of 0.05. We chose a group size of 10 infants for each group (40 infants total) to account for a 20% attrition rate.

Statistical analysis
The difference in iron absorption between each age group was compared by a 2-sample t test, by ANCOVA, and by linear regression. Data were analyzed by ANCOVA with hemoglobin, serum ferritin, age, gender, and anemia each as a covariant. Data are presented as means ± SEM.

Results
Baseline characteristics. Body weights and lengths did not differ between the anemic and non-anemic infants in each age group (Table 1). No subject had a serum CRP concentration >11.0 mg/L, indicating no evidence of acute infection.

Iron absorption from human milk. There was no significant age effect on iron absorption when ⁵⁸Fe was given with human milk. Absorption of ⁵⁸Fe was 42.6 ± 5.0% at 5–6 mo and 51.9 ± 10.3% at 9–10 mo (P = 0.4). There was no significant difference between anemic and non-anemic infants in absorption of ⁵⁸Fe given in human milk at 5–6 mo 36.8 ± 9.3% and 41.8 ± 7.9%, respectively (P = 0.8) and at 9–10 mo [42.6 ± 5.0% and 51.9 ± 10.9%, respectively (P = 0.7)] (Fig. 1).

Iron absorption from the non-milk iron dose. There was no significant age effect on iron absorption from ⁵⁷Fe as ferrous sulfate, given with ascorbic acid. Absorption of ⁵⁷Fe at 5–6 mo was 19.2 ± 2.1% and at 9–10 mo 25.8 ± 2.6% (P = 0.2). Absorption of ⁵⁷Fe did not differ between anemic and non-anemic groups at 5–6 mo [18.6 ± 3.4% and 19.7 ± 3.9%, respectively (P = 0.8)] and 9–10 mo [27.4 ± 6.7% and 24.2 ± 4.4%, respectively (P = 0.4) (Fig. 1).

Correlation between serum ferritin and iron absorption. Following log-transformation, iron absorption from both the labeled breast milk (⁵⁷Fe: r = −0.61, P = < 0.001) and from the reference dose (⁵⁸Fe: r = −0.44, P < 0.01) were each inversely correlated with serum ferritin (Fig. 2). For both the non-milk dose (⁵⁷Fe) and breast-milk dose (⁵⁸Fe), infants with S-Ft <12 μg/L (n = 11) had significantly higher iron absorption than those whose S-Ft was ≥12 μg/L (n = 27). ⁵⁷Fe absorption was 36.4 ± 4.5% for infants with S-Ft <12 μg/L and 16.7 ± 1.6% for infants with S-Ft ≥12 μg/L (P < 0.001). ⁵⁸Fe absorption was 56.4 ± 4.7% for infants with S-Ft <12 μg/L and ⁵⁸Fe absorption was 38.0 ± 5.3% for S-Ft ≥12 μg/L (P = 0.04). The Hb concentration did not differ between the groups with S-Ft < or ≥12 μg/L, (P = 0.45).

<table>
<thead>
<tr>
<th>TABLE 1</th>
<th>Characteristics of infants¹</th>
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<tr>
<td></td>
<td>Anemic</td>
</tr>
<tr>
<td>5–6 mo</td>
<td></td>
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<tr>
<td>Age, mo</td>
<td>5.9 ± 0.2</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>8.6 ± 0.3</td>
</tr>
<tr>
<td>Length, cm</td>
<td>66.3 ± 0.7</td>
</tr>
<tr>
<td>9–10 mo</td>
<td></td>
</tr>
<tr>
<td>Age, mo</td>
<td>9.1 ± 0.2</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>9.3 ± 0.2</td>
</tr>
<tr>
<td>Length, cm</td>
<td>70.6 ± 0.5</td>
</tr>
</tbody>
</table>

¹ Values are means ± SEM, n = 10 (5–6 mo) or n = 9 (9–10 mo).
Biochemical findings. Plasma folate and vitamin B-12 concentrations did not differ between the anemic and non-anemic infants in each age group (Table 2). Plasma folate concentrations were 28.6 ± 3.2 nmol/L and 26.1 ± 2.5 nmol/L for the 5–6 mo group and 9–10 mo group, respectively. Serum ferritin was negatively correlated with plasma folate \((r = -0.37, P = 0.001)\). Plasma folate was positively related to \(^{58}\)Fe absorption \((r = 0.23, P = 0.035)\), however, when S-Ft was added to the analysis the relation was not significant \((P = 0.13)\), implying that the correlation involved S-Ft. Plasma folate was not related to \(^{57}\)Fe absorption \((P = 0.46)\), including when S-Ft was included in the analysis \((P = 0.65)\). Plasma vitamin B-12 values were 226 ± 22 pmol/L and 219 ± 38 pmol/L for the 5–6 mo group and 9–10 mo group, respectively.

Iron status did not differ due to gender. The hemoglobin (Hb) concentration in males \((n = 21)\) was 102.4 ± 2.1 g/L and for females, 100.5 ± 2.5 g/L \((P = 0.56)\). Serum ferritin for males was 36.5 ± 9.6 and for females 22.8 ± 3.8 \(\mu\)g/L \((P = 0.16)\). Absorption of both \(^{57}\)Fe and \(^{58}\)Fe did not differ between males and females at 5–6 and 9–10 mo. Anemia status did not affect \(^{57}\)Fe absorption \((P = 0.96)\), \(^{58}\)Fe absorption \((P = 0.51)\), S-Ft \((P = 0.43)\), or plasma folate \((P = 0.80)\).

Discussion

We found that iron absorption was not affected by Hb concentration in this at-risk population of Peruvian infants. There were no differences in iron absorption between anemic and non-anemic groups at either 6 or 9 mo of age. This finding was unexpected based on previous data referred to as the “iron stores regulator theory.” This theory is based on the inverse relation between iron stores and iron absorption in adults (12), but has not been validated in infants. The mechanisms regulating iron absorption may be immature at this age, which has been supported by Domellöf et al. (2) who studied iron absorption in 6 and 9-mo-old breast-fed infants who were given iron supplements (iron sufficient) or a placebo. At 6 mo of age, iron-supplemented infants were not able to downregulate iron absorption (as a consequence of higher iron stores). However, at 9 mo of age, a significant downregulation of iron absorption occurred in the supplemented infants. In our study, infants with lower iron status, as measured by serum ferritin, exhibited an upregulation of iron absorption at both 6 and 9 mo. Results from the latter study suggest that infants with low iron status are able to compensate for their impaired status by increasing iron absorption, whereas infants with adequate iron status and who are given additional iron are not able to decrease iron absorption to protect against fortification or supplementation strategies that may lead to iron intakes above recommendations or that might have unexpected negative implications (13). Our data also suggest that Hb may not be a reliable marker of iron status in healthy infants (14,15). Iron absorption from both labeled breast milk and from the reference dose were inversely correlated with serum ferritin, indicating that, in terms of regulation of absorption, ferritin may be more indicative of iron needs of infants than Hb.

The closer correlation between iron status and absorption from the dose given separately from breast milk implies that absorption of nonbreast-milk iron (such as that given with a

<table>
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<tr>
<th>TABLE 2</th>
<th>Plasma and serum biochemistry of anemic and nonanemic infants at 2 ages(^1)</th>
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<tbody>
<tr>
<td></td>
<td>Anemic</td>
</tr>
<tr>
<td>5–6 mo</td>
<td></td>
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<tr>
<td>Hemoglobin, g/L</td>
<td>90.3 ± 0.1* (88.4–99.0)</td>
</tr>
<tr>
<td>Serum Ferritin, (\mu)g/L</td>
<td>32.5 ± 6.3 (7.9–53.5)</td>
</tr>
<tr>
<td>Plasma Folate, nmol/L</td>
<td>28.6 ± 13.4 (10.2–55.1)</td>
</tr>
<tr>
<td>Plasma Vitamin B-12, pmol/L</td>
<td>223 ± 123 (121.8–524.1)</td>
</tr>
<tr>
<td>B-12, pmol/L</td>
<td></td>
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<tr>
<td>9–10 mo</td>
<td></td>
</tr>
<tr>
<td>Hemoglobin, g/L</td>
<td>93.3 ± 0.1* (88.7–99.1)</td>
</tr>
<tr>
<td>Serum ferritin, (\mu)g/L</td>
<td>11.9 ± 2.6 (3.0–25.4)</td>
</tr>
<tr>
<td>Plasma ferritin, nmol/L</td>
<td>25.8 ± 7.3 (18.6–37.4)</td>
</tr>
<tr>
<td>Plasma vitamin B-12, pmol/L</td>
<td>243 ± 111 (144.9–438.6)</td>
</tr>
</tbody>
</table>

\(^1\) Values are means ± SEM (range), \(n = 10\) (5–6 mo A), \(n = 10\) (5–6 mo NA), \(n = 9\) (9–10 mo A), \(n = 9\) (9–10 mo NA).

\(^{*}\) Different from non-anemic, \(P < 0.0001\).
supplement) may more readily respond to iron status changes. A major part of iron in breast milk is bound to lactoferrin (16). The presence of lactoferrin receptors in the small intestine of infants (17) and the fact that a large proportion of breast-milk lactoferrin can survive proteolysis in the gut and is found intact in the stool of breast-fed infants (18), strongly suggest that part of breast-milk iron is taken up by lactoferrin receptors (19). Because there is no evidence of upregulation of the lactoferrin receptor during iron deficiency, breast-milk iron may not be regulated as tightly as iron from supplements.

Studies have reported that iron supplementation before 6 mo of age prevents the physiological fall in Hb that occurs from 1 to 6 mo (20,21) and that this may have beneficial hematologic and developmental outcomes for some infants (22). Thus, early introduction of highly bioavailable iron sources, before 6 mo of age, may be beneficial in this high-risk population. However, some studies have reported detrimental effects such as decreased growth and increased morbidity (23), decreased zinc absorption (24), and altered vitamin A metabolism (25) when iron supplements are provided to infants who do not need them. As described above, it is possible that young infants may lack the capacity to downregulate iron absorption, either due to immaturity or to other micronutrient deficiencies, and that iron given to such infants may cause adverse effects. Population-based consideration of iron status on individual outcomes should be considered when deciding to give an early iron supplement.

We conclude that the regulation of iron absorption is mature by 6 mo but with a relatively modest responsiveness to iron deficiency from iron absorption in breast milk and with a greater response of iron absorption from nonbreast-milk sources. The use of Hb to assess iron status and to thereby identify likely responders to iron supplementation may not be effective in this population.

Acknowledgment

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Literature Cited