α-Lactalbumin and Casein-Glycomacropeptide Do Not Affect Iron Absorption from Formula in Healthy Term Infants1–3

Ewa A. Szymlek-Gay,4,5,6 Bo Lönnerdal,6 Steven A. Abrams,7 Anne S. Kvistgaard,8 Magnus Domellöf,5 and Olle Hernell5

4Centre for Physical Activity and Nutrition Research, School of Exercise and Nutrition Sciences, Deakin University, Burwood, Victoria, Australia; 5Department of Clinical Sciences, Pediatrics, Umeå University, Umeå, Sweden; 6Department of Nutrition, University of California, Davis, CA; 7USDA Agricultural Research Service, Children’s Nutrition Research Center, Department of Pediatrics, Baylor College of Medicine, Houston, TX; and 8Arla Foods Ingredients Group P/S, Viby, Denmark

Abstract

Iron absorption from infant formula is relatively low. α-Lactalbumin and casein-glycomacropeptide have been suggested to enhance mineral absorption. We therefore assessed the effect of α-lactalbumin and casein-glycomacropeptide on iron absorption from infant formula in healthy term infants. Thirty-one infants were randomly assigned to receive 1 of 3 formulas (4 mg iron/L, 13.1 g protein/L) from 4–8 wk to 6 mo of age: commercially available whey-predominant standard infant formula (standard formula), α-lactalbumin–enriched infant formula (α-LAC), or α-lactalbumin–enriched/casein-glycomacropeptide–reduced infant formula (α-LAC/RGMP). Nine breast-fed infants served as a reference. At 5.5 mo of age, 56Fe was administered to all infants in a meal. Blood samples were collected 14 d later for iron absorption and iron status indices. Iron deficiency was defined as depleted iron stores, iron-deficient erythropoiesis, or iron deficiency anemia. Iron absorption (mean ± SD) was 10.3 ± 7.0% from standard formula, 8.6 ± 3.8% from α-LAC, 9.2 ± 6.5% from α-LAC/RGMP, and 12.9 ± 6.5% from breast milk, with no difference between the formula groups (P = 0.79) or all groups (P = 0.44). In the formula-fed infants only, iron absorption was negatively correlated with serum ferritin (r = −0.49; P = 0.005) and was higher (P = 0.023) in iron-deficient infants (16.4 ± 12.4%) compared with those with adequate iron status (8.6 ± 4.4%). Our findings indicate that α-lactalbumin and casein-glycomacropeptide do not affect iron absorption from infant formula in infants. Low serum ferritin concentrations are correlated with increased iron absorption from infant formula. J. Nutr. 142: 1226–1231, 2012.

Introduction

Iron deficiency continues to be the most common nutritional deficiency worldwide (1). Infants are at particular risk, primarily due to increased physiological requirements during rapid growth that exceed their dietary iron intakes. In early childhood, iron deficiency anemia has been associated with impaired cognitive function, developmental delay, and behavioral problems (2). These negative effects may persist into adulthood and be irreversible despite iron therapy (2). Iron deficiency without anemia has also been suggested to impair child development, although the evidence for this remains limited (3). Minimizing iron deficiency in infancy is therefore important to prevent its negative effects.

To prevent iron deficiency states, it is recommended that healthy term infants are exclusively breast-fed for 4–6 mo, after which iron-rich complementary foods should be introduced in addition to breast milk (4). When breast feeding is not possible, or when the infant is partially breast fed, an iron-fortified infant formula should be used (4). However, the optimal concentration of iron in an infant formula for preventing iron deficiency is still under debate (4) and country-specific recommendations vary from 2 (5,6) to as high as 12 mg/L of iron (4). The high iron content of many formulas is of concern, because excessive iron intakes in iron-sufficient children may adversely affect their health and development (7,8). Reduction of iron concentration in formula may therefore protect infants from the potential adverse effects of excess dietary intakes.

One approach to ensure that infants are not exposed to unnecessarily high levels of dietary iron yet are protected against developing iron deficiency is to improve the bioavailability of iron from current infant formulas. Ascorbic acid is one of several

1 Supported by Arla Foods Ingredients Group P/S, Aarhus, Denmark. E.A. Szymlek-Gay was in receipt of a New Zealand Science and Technology Postdoctoral Fellowship from the Foundation for Research, Science and Technology (UMEA0901).
2 Author disclosures: A. S. Kvistgaard is an employee of Arla Foods Ingredients Group P/S, Aarhus, Denmark. Arla Foods Ingredients Group P/S had no role in the conduct of the study, or in the collection, management, analysis, or interpretation of the data. E. A. Szymiek-Gay, B. Lönnerdal, S. A. Abrams, M. Domellöf, and O. Hernell, no conflicts of interest.
3 Supplemental Table 1 and Figure 1 are available from the “Online Supporting Material” link in the online posting of the article and from the same link in the online table of contents at jn.nutrition.org.
4 To whom correspondence should be addressed. E-mail: ewa.szymlekgay@deakin.edu.au.

First published online May 23, 2012; doi:10.3945/jn.111.153890.

© 2012 American Society for Nutrition.
constituents that have been identified to promote the absorption of fortification iron from infant formula (9) and is now added to virtually all commercially available formulas. Despite this, the absorption of iron from infant formula is still relatively low, ranging between 3 and 12% (9–12). Two milk proteins, α-lactalbumin and casein-glycomacropeptide, have been suggested to enhance mineral absorption and improve mineral status. α-Lactalbumin is present in the milk of nearly all mammals, although its concentration varies and is higher in human milk (2–3 g/L) compared with bovine milk (1.2 g/L) (13,14). α-Lactalbumin is able to bind minerals (15,16), which, due to its negative charge, may bind and transport minerals (18). Whether these bovine milk protein fractions positively affect mineral absorption from infant formula in infants has not been evaluated. Thus, the aim of this study was to determine the effect of α-lactalbumin and casein-glycomacropeptide on iron absorption from infant formula in healthy term, 6-mo-old infants.

**Participants and Methods**

**Participants.** One hundred and four healthy term (36–42 wk gestation) 4- to 8-wk-old infants were recruited between February 2001 and March 2004 from well-baby clinics in Umeå, Sweden to participate in a double-blind, randomized controlled trial that investigated the effects of α-lactalbumin-supplemented infant formula containing different concentrations of casein-glycomacropeptide on health outcomes (19–21). Infants who had commenced formula feeding prior to enrollment (n = 68) were randomly assigned to receive 1 of 3 formulas: commercially available whey-predominant standard infant formula (standard formula group); n = 22), α-lactalbumin-enriched infant formula (α-LAC group; n = 23), or α-lactalbumin-enriched/casein-glycomacropeptide-reduced infant formula (α-LAC/RGMP group; n = 23). Thirty-six infants whose mothers intended to exclusively breast feed until 6 mo served as a reference. From this cohort of infants, 42 families were invited to take part in the current iron absorption study (standard formula group, n = 10; α-LAC group, n = 11; α-LAC/RGMP group, n = 11; and breast-fed group, n = 10). The study was approved by the Ethics Committee on Research Involving Human Subjects of the Faculty of Medicine, Umeå University, Umeå, Sweden. Written informed consent was obtained from the parents of all infants.

**Study formulas.** The formulas (Arla Foods Ingredients Group P/S) were manufactured as previously described (22,23). The nutritional composition of the formulas has been described in detail elsewhere (19,21). Briefly, each formula provided 669 kcal/L, 13.1 g protein/L, and 4.0 mg iron as FeSO₄/L. All formulas were fortified with vitamin C (range: 95–96 mg/L) (13,14). α-Lactalbumin accounted for 11% of the total protein content in the standard formula and 25% in both the α-LAC and α-LAC/RGMP. Casein-glycomacropeptide constituted 14% of the total protein content in the standard formula, 15% in the α-LAC, and 10% in the α-LAC/RGMP.

**Infant feeding.** Infants received a formula (formula groups) or were breast fed (breast-fed group) ad libitum from 4–8 wk (baseline) to 6 mo of age. No other food or iron medications were permitted during the study apart from 15–30 mL of fruit and/or vegetable puree each day from 4 mo of age. The purees were provided by the investigators and contained no iron.

Abbreviations used: α-LAC, α-lactalbumin-enriched infant formula group; α-LAC/RGMP, α-lactalbumin-enriched/casein-glycomacropeptide-reduced infant formula group; Standard formula, commercially available whey-predominant standard infant formula group.

**Dietary and anthropometric assessment.** Formula intakes were recorded on 3 consecutive days at baseline and at 2, 3, 4, 5, and 6 mo of age. To assist in the recording of intakes, the formulas were fed to infants using graduated bottles accurate to ±5 mL. The formula intake data were used to estimate the mean daily intakes of iron at each time point. Iron intakes in the breast-fed group were not determined, because data on the amount and composition of breast milk were not collected.

Anthropometric measurements were taken at baseline and at 2, 3, 4, 5, and 6 mo of age. Nude weight was measured to the nearest 5 g with Seca 835 digital baby scales. Recumbent length was measured to the nearest 0.1 cm with a Harpenden infantometer (CMS Weighing Equipment). Weight-for-age and length-for-age Z-scores were calculated using the WHO child growth standards (24).

**Isotope preparation and administration.** ⁵⁸Fe (93.1% enrichment) was purchased from Penwood Chemicals. The isotope was converted to [⁵⁸Fe]Ferrous sulfate as previously described (25).

The isotope was administered to each infant at 5.5 mo of age (range: 5.3–5.7 mo). Two weeks before that, breast milk was collected from the mothers of infants in the breast-fed group. The milk was expressed with an electric breast pump and stored at −20°C in sterile containers until used. A day before isotope administration, 0.15 mg of ⁵⁸Fe was added to a preweighed feeding bottle containing 104 g (range: 47–131 g) of either the thawed breast milk obtained from infants’ own mothers or 1 of the 3 freshly prepared study formulas, depending on the infants’ group assignment. Following that, the bottle was slowly rotated at 4°C overnight to allow equilibration. In the morning, the isotopically labeled meal was heated in a water bath to −5°C then fed to each infant after a 2-h fast. The bottle was immediately weighed before and after feeding. All spills, spitting up, vomit, and other accidental milk losses were wiped with preweighed napkins. The amount of ⁵⁸Fe consumed by the infant was calculated by subtracting the amount of ⁵⁸Fe corresponding to the amount of meal left in the bottle and absorbed in the napkins from the original dose of 0.15 mg. No food or drink was given for 2 h after the feeding. A venous blood sample was collected 14 d following isotope administration to determine the enrichment of the erythrocytes with ⁵⁸Fe.

**Laboratory analyses.** Venous blood samples were drawn at baseline and at 4 and 6 mo of age into an EDTA-containing tube and a serum separation tube (Becton Dickinson). Samples were collected after a 2- to 4-h fast and were analyzed within 2–4 h of collection. Whole blood from the EDTA-containing tube was analyzed for hemoglobin and mean corpuscular volume with a Sysmex SE-9000 Automated Hematology Analyzer (Tillquist). For the 6-mo sample, the remaining whole blood was centrifuged at 1360 × g for 10 min at room temperature to obtain erythrocytes for isotope ratio analysis. Iron isotope ratios were measured as previously described (25).

Iron absorption was calculated as iron incorporated into the erythrocytes 14–28 d after isotope administration (26) assuming that 90% of the absorbed iron was incorporated into erythrocytes (10). Circulated iron was estimated by using an assumed blood volume of 80 mL/kg, the measured hemoglobin concentration, and the known concentration of iron in hemoglobin of 3.47 mg/g. Total absorbed iron was calculated by multiplying the percent iron absorption for each infant by the mean daily amount of iron consumed at 6 mo of age.

Blood in the serum separation tube was centrifuged at 1360 × g for 10 min at room temperature and the separated serum was analyzed for ferritin, iron, and total iron-binding capacity with a Boehringer Mannheim Hitachi 704/717/911 Automated Chemistry Analyzer (Roche Diagnostics Scandinavia, Boehringer Mannheim Scandinavia). Ferritin was determined by an immunonutriturabonomic assay calibrated against WHO Human Liver Ferritin First International Standard 80/602. Iron and total iron-binding capacity were analyzed by a ferrozine-based colorimetric assay (Iron kit 1533712 and Unsaturated Iron-Binding Capacity kit 1030600; Boehringer Mannheim Scandinavia, Boehringer Mannheim Scandinavia). Ferritin was determined by an immunonutriturabonomic assay calibrated against WHO Human Liver Ferritin First International Standard 80/602. Iron and total iron-binding capacity were analyzed by a ferrozine-based colorimetric assay (Iron kit 1533712 and Unsaturated Iron-Binding Capacity kit 1030600; Boehringer Mannheim Scandinavia) and were used to calculate transferrin saturation. Iron status indicators were determined at the Department of Clinical Chemistry, Umeå University Hospital, Sweden; values for the controls were within the certified ranges for all analyses. Iron isotope ratios were measured in the mineral MS laboratory of the USDA, Agricultural Research Service, Children’s Nutrition Research Center, Houston, TX.
Iron absorption was successfully determined in 40 infants (55% boys) at 6 mo of age. The infants’ mean birth weight (3522 ± 526 g), birth length (50.3 ± 2.2 cm), and gestational age (40.1 ± 1.4 wk) were similar between groups (all \( P ≥ 0.09 \)). At study entry, the infants were 1.2 ± 0.3 mo old (range: 25–59 d) and had anthropometric indices indicative of normal growth (Table 1). Weight-for-age and length-for-age Z-scores were also within the normal range at 2, 3, 4, 5, and 6 mo. Baseline characteristics did not differ among the formula groups except for sex distribution and weight. Between all groups, there were significant differences in sex distribution and anthropometric indices.

### Iron status

Dietary iron intakes across the formula groups were 3.3 ± 0.6 mg/d at baseline, 3.6 ± 0.6 mg/d at 2 mo, 3.7 ± 0.5 mg/d at 3 mo, 4.0 ± 0.8 mg/d at 4 mo, 4.0 ± 0.8 mg/d at 5 mo, and 3.6 ± 0.7 mg/d at 6 mo. There was no evidence that iron intakes differed among the 3 groups at baseline or at any time during the study (all \( P ≥ 0.21 \)). Based on previous data on iron intakes of breast-fed infants (30), the breast-fed group consumed <10% of the amount of iron received by the formula groups.

### Iron absorption

There was no evidence that mean fractional iron absorption (Table 2) differed among the 3 formula groups (\( P = 0.79 \)), all groups (\( P = 0.44 \)), or between the formula-fed and breast-fed infants (\( P = 0.13 \)). Adjustments for serum ferritin concentration, sex, baseline weight, and baseline length did not alter these findings (all adjusted \( P ≥ 0.76 \)) or among all groups (\( P = 0.54 \)).

### Sample size determination

Based on previous iron absorption data (12), we estimated that 10 children/group would be sufficient to detect a 5.5% unit difference in iron absorption between groups with 80% power and \( α = 0.05 \).

### Statistical analysis

Group differences in baseline characteristics listed in Table 1 were tested by ANOVA for continuous variables and Fisher’s exact tests for categorical variables; pairwise comparisons were made where appropriate. Differences in iron intakes and total absorbed iron between the formula groups were tested by ANCOVA while controlling for serum ferritin concentration (total absorbed iron only), sex, and baseline weight. Iron status indices were compared between groups by ANOVA and ANCOVA was then used to adjust for sex, baseline weight, and baseline length (only when the breast-fed group was included). Comparisons over time within groups were performed by repeated-measures ANOVA with pairwise comparisons made where appropriate. Multiple logistic regression analysis was used to compare the prevalence of iron deficiency between groups at 6 mo while controlling for baseline status. Iron absorption was compared between groups by Student’s \( t \) tests for independent samples or ANOVA as appropriate; ANCOVA was subsequently used to adjust for serum ferritin concentration, sex, baseline weight, and baseline length (only when the breast-fed group was included). Linear regression analyses were used to examine the relation between iron absorption and iron status indices (all groups), iron absorption and dietary iron intakes (formula groups only), and between serum ferritin concentration and dietary iron intakes (formula groups only).

All analyses were conducted in Stata 10.1 for Macintosh (Stata Corp.) and SPSS 18.0.3 for Macintosh. All tests were 2-sided with significance determined by \( P < 0.05 \). Serum ferritin and total absorbed iron were log-transformed before analysis to compensate for non-normal distributions and are presented as geometric means (95% CI). Iron absorption is presented as means ± SD and also as geometric means (95% CI) to facilitate comparisons with published data expressed as such.

### Results

#### Participants

Iron absorption was successfully determined in 40 infants (55% boys) at 6 mo of age. The infants’ mean birth weight

**TABLE 1** Characteristics of infants at baseline

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Standard formula</th>
<th>( α )-LAC</th>
<th>( α )-LAC/RGMP</th>
<th>( P ) value</th>
<th>Breast-fed</th>
<th>( P ) value</th>
</tr>
</thead>
<tbody>
<tr>
<td>( n )</td>
<td>10</td>
<td>10</td>
<td>11</td>
<td>9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male, %</td>
<td>70.0(^a)</td>
<td>10.0(^b)</td>
<td>63.6(^a)</td>
<td>0.013</td>
<td>77.8(^a)</td>
<td>0.010</td>
</tr>
<tr>
<td>Age, mo</td>
<td>1.3 ± 0.3</td>
<td>1.1 ± 0.2</td>
<td>1.3 ± 0.3</td>
<td>0.16</td>
<td>1.2 ± 0.3</td>
<td>0.27</td>
</tr>
<tr>
<td>Weight, g</td>
<td>4722 ± 279(^a)</td>
<td>4073 ± 458(^b)</td>
<td>4680 ± 537(^ab)</td>
<td>0.006</td>
<td>5267 ± 620(^a)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Length, cm</td>
<td>55.9 ± 1.3(^ab)</td>
<td>54.5 ± 2.2(^a)</td>
<td>56.2 ± 2.2(^ab)</td>
<td>0.17</td>
<td>58.6 ± 3.0(^a)</td>
<td>0.005</td>
</tr>
<tr>
<td>Weight-for-age Z-score</td>
<td>0.2 ± 0.4(^a)</td>
<td>−0.4 ± 0.6(^b)</td>
<td>0.1 ± 0.9(^ab)</td>
<td>0.16</td>
<td>0.9 ± 1.1(^b)</td>
<td>0.007</td>
</tr>
<tr>
<td>Length-for-age Z-score</td>
<td>0.4 ± 0.7(^a)</td>
<td>0.3 ± 0.8(^b)</td>
<td>0.5 ± 1.0(^ab)</td>
<td>0.80</td>
<td>1.7 ± 1.5(^b)</td>
<td>0.025</td>
</tr>
</tbody>
</table>

\(^{1}\) Values are mean ± SD. Means in a row with superscripts without a common letter differ, \( P < 0.05 \). \( α \)-LAC, \( α \)-lactalbumin–enriched infant formula group; \( α \)-LAC/RGMP, \( α \)-lactalbumin–enriched/casein-glycomacropeptide–reduced infant formula group; Standard formula, commercially available whey-predominant standard infant formula group.

\(^{2}\) For the difference between the formula groups.

\(^{3}\) For the difference between all groups.
iron did not differ among the formula groups ($P = 0.70$), and was 0.29 mg/d (95% CI: 0.22, 0.37 mg/d) across the 3 groups.

Relation between iron absorption and iron status. Iron absorption was inversely correlated with serum ferritin concentration (natural logarithm) in the formula-fed infants (Supplemental Fig. 1). The associations between iron absorption and hemoglobin concentration, mean corpuscular volume, and transferrin saturation were not significant (all $P \geq 0.07$). There was no close correlation between iron absorption and any of the iron status indices in the breast-fed group (all $P \geq 0.51$).

In the formula-fed infants, iron absorption was higher in iron-deficient infants compared with those with adequate iron status [mean difference: 7.8% (95% CI: 1.1, 14.5%) ] (Table 2). This difference remained significant after adjustment for sex and baseline weight [mean adjusted difference: 9.2% (95% CI: 2.2, 16.3%); $P = 0.013$]. In the breast-fed group, we found no evidence that iron absorption differed between infants with iron deficiency and those with adequate iron status, although our study was not sufficiently powered to detect this [mean difference: 3.3% (95% CI: −7.1, 17.7%); $P = 0.38$; absorption data not shown in table].

Relation between iron absorption, dietary iron intakes, and serum ferritin. Iron absorption and dietary iron intakes were not closely associated in the formula-fed infants (fractional iron absorption $\alpha = 0.20 \times$ dietary iron intake mg/d + 8.74; $r = 0.02; P = 0.90$). The weak inverse correlation between serum ferritin concentration (natural logarithm) and dietary iron intakes was also not significant [ln (serum ferritin mg/dL) $= -0.31 \times$ dietary iron intake mg/d + 4.78; $r = -0.26; P = 0.16$].

### Discussion

Modification of the protein composition of infant formula by increasing its proportion of $\alpha$-lactalbumin and altering its casein-glycomacropeptide proportion did not affect iron absorption in healthy 6-mo-old infants. We also found no evidence that iron absorption differed between formula-fed and breast-fed infants.

Infant formula supplemented with $\alpha$-lactalbumin or casein-glycomacropeptide has recently been shown to improve zinc absorption and increase plasma zinc concentration in infant rhesus monkeys (31). Iron bioavailability, however, was not affected in that study (31). Likewise, we found no evidence that enriching infant formula with $\alpha$-lactalbumin had an effect on iron absorption in 6-mo-old human infants. It is unclear why the addition of $\alpha$-lactalbumin to infant formula enhanced zinc bioavailability but did not improve iron absorption in infant rhesus monkeys (31) or human infants in the current study. This could be because $\alpha$-lactalbumin has a specific zinc-binding site (16,32), whereas iron binds to $\alpha$-lactalbumin via a nonspecific binding site (33). Because there was no casein-glycomacropeptide–supplemented group in the present study, we were not able to assess the effect of an increased concentration of casein-glycomacropeptide in infant formula on iron absorption in human infants. However, a modest reduction of casein-glycomacropeptide in infant formula did not appear to affect iron absorption in our study. Whether the addition of $\alpha$-lactalbumin or casein-glycomacropeptide to infant formula enhances zinc absorption and improves zinc status in human infants needs to be investigated.

Notwithstanding the growing interest in the role of minerals in child nutrition, there is limited information on the availability of iron to infants from infant formulas. Previous reports have shown the geometric mean fractional iron absorption from formulas fortified with ascorbic acid to vary between 6.6 and 11.8% from formulas containing $\leq 2.5$ mg iron/L (10,12), 4.2 and 5.9% from formulas with 8–10 mg iron/L (9,11), and between 2.5 and 11.3% from formulas containing $\geq 12$ mg iron/L (9,11). In comparison, the geometric mean fractional iron absorption across the 3 formulas in our study was 7.6%, which is within the previously reported ranges. Fomon et al. (11) reported fractional iron absorption values averaging $<3%$ from infant formula containing 12 mg iron/L, which are the lowest fractional iron absorption results reported in 5.5- to 6.5-mo-old infants. These values were recalculated based on the original report assuming that 90% of all iron absorbed from the formula was incorporated into erythrocytes and are considerably lower than that found in the current study. In both our and Fomon et al.’s (11) studies, the composition of formulas was similar, except for the 3-fold difference in iron concentration. Furthermore, the infants had comparable mean ferritin and hemoglobin concentrations, although 3 infants in our study had iron deficiency at 6 mo of age, whereas all infants in the study of Fomon et al. (11) were iron sufficient.

Notwithstanding the differences in iron content of the formulas and iron status of infants, the total absorbed iron (0.29 mg/d) or the quantity of iron incorporated into erythrocytes by the infants in our study [0.26 mg/d (95% CI: 0.20, 0.33

### Table 2: Fractional iron absorption from an isotopically labeled ($^{59}$Fe) test meal of standard formula, $\alpha$-LAC, $\alpha$-LAC/RGMP, or breast milk fed to 6-mo-old infants

<table>
<thead>
<tr>
<th>Group</th>
<th>$n$</th>
<th>Absorption</th>
</tr>
</thead>
<tbody>
<tr>
<td>Standard formula</td>
<td>10</td>
<td>10.3 ± 7.0</td>
</tr>
<tr>
<td>$\alpha$-LAC</td>
<td>10</td>
<td>8.6 ± 3.8</td>
</tr>
<tr>
<td>$\alpha$-LAC/RGMP</td>
<td>11</td>
<td>9.2 ± 6.5</td>
</tr>
<tr>
<td>All formula groups</td>
<td>31</td>
<td>9.4 ± 5.8</td>
</tr>
<tr>
<td>Formula-fed infants with adequate iron status</td>
<td>28</td>
<td>8.6 ± 4.4</td>
</tr>
<tr>
<td>Formula-fed infants with iron deficiency</td>
<td>3</td>
<td>16.4 ± 12.4$^*$</td>
</tr>
<tr>
<td>Breast-fed</td>
<td>9</td>
<td>12.9 ± 6.5</td>
</tr>
<tr>
<td>All infants</td>
<td>40</td>
<td>10.1 ± 6.1</td>
</tr>
</tbody>
</table>

$^*$ Values are mean ± SD or geometric mean (95% CI). *Different from the formula-fed infants with adequate iron status, $P = 0.023$. $\alpha$-LAC, $\alpha$-lactalbumin–enriched infant formula group; $\alpha$-LAC/RGMP, $\alpha$-lactalbumin–enriched/casein-glycomacropeptide–reduced infant formula group; Standard formula, commercially available whey-predominant standard infant formula group.

$^*$ Iron deficiency is defined as depleted iron stores, iron-deficient erythropoiesis, or iron deficiency anemia.

---

**alpha-Lactalbumin and glycomacropeptide in formula**
that found in the current or earlier studies (11.8–20.7%) that Peruvian infants has recently been reported to correlate to the U.S. infants (39). In contrast, iron absorption in 5- to 6-mo-old ferritin in 6-mo-old Swedish infants (41) or in 5- to 7-mo-old showed that iron absorption was not associated with serum confidence in our finding, this is in line with earlier reports that mined (42). Although the small sample size may limit the explain this discrepancy; however, this still remains undeter-

A possibility that iron absorbed from breast milk follows a between iron absorption from breast milk and serum ferritin. There was no evidence of a difference in iron status indicators from the formulas (9.4%). Previous studies on iron absorption which is 13 times less than the iron concentration of the infant milk formula (38). Recent studies using stable isotopes, however, from breast milk compared with infant formula (10–12,25,39–41); this may be due to improve-

Human milk has an iron concentration of ~0.3 mg/L (30), which is 13 times less than the iron concentration of the infant formulas used in this study. Despite this, we found that the absorption of iron from breast milk (12.9%) was similar to that from the formulas (9.4%). Previous studies on iron absorption in infancy using radioisotope methodology found substantial differences in iron bioavailability between breast milk and cow milk formula (38). Recent studies using stable isotopes, however, have shown smaller or no differences between breast milk and infant formula (10–12,25,39–41); this may be due to improve-

Although the infants absorbed ~0.04 mg of iron/L from breast milk compared with ~0.4 mg/L from infant formula, there was no evidence of a difference in iron status indicators between the breast-fed and formula-fed infants. Contrary to the formula-fed infants, we found no evidence of an association between iron absorption from breast milk and serum ferritin. A possibility that iron absorbed from breast milk follows a different metabolic pathway than that from infant formula may explain this discrepancy; however, this still remains undetermined (42). Although the small sample size may limit the confidence in our finding, this is in line with earlier reports that showed that iron absorption was not associated with serum ferritin in 6-mo-old Swedish infants (41) or in 5- to 7-mo-old U.S. infants (39). In contrast, iron absorption in 5- to 6-mo-old Peruvian infants has recently been reported to correlate to the infants’ iron status (25). The absorption of iron from breast milk in the Peruvian infants was considerably higher (42.6%) than that found in the current or earlier studies (11.8–20.7%) that used stable isotope tracers (39–41). The Peruvian infants were likely to be more iron deficient than the infants in Sweden, the U.S., or in the current study. Thus, it is possible that although iron absorption in 6-mo-old, breast-fed, generally iron-sufficient infants is not related to iron stores or iron status, as we and others have shown, predominantly breast-fed infants with poor iron status are able to compensate for their low iron stores by upregulating iron absorption from breast milk. However, the extent to which iron incorporation into erythrocytes is affected by iron status at this age is still little known (42).

In summary, we found no evidence that α-lactalbumin or casein-glycomacropeptide had an effect on iron absorption from infant formula in healthy term, 6-mo-old children. Furthermore, the absorption of iron in formula-fed infants in our study did not differ from that observed in breast-fed infants. Our results also demonstrate that low iron stores result in upregulation of iron absorption from infant formula but have no direct effect on iron absorption from breast milk, suggesting that at 6 mo of age, serum ferritin, within the range observed in our study, may be a useful biological marker for assessing iron absorption from infant formula but not from breast milk.

Acknowledgments
The authors thank Margareta Henriksson and Margareta Bäckman for participant recruitment, isotope administration, and data collection; and Dr. Zhensheng Chen for assistance in mass spectrometric analysis. O.H. and B.L. designed research; A.S.K. participated in development of formulas; O.H. and M.D. conducted research; S.A.A. provided the stable isotope and measured iron isotope ratios; E.A.S.-G. analyzed the data, wrote the paper, and had primary responsibility for final content; and E.A.S.-G., B.L., S.A.A., A.S.K., M.D., and O.H. reviewed the manuscript. All authors read and approved the final manuscript.

Literature Cited


