Iron absorption in breast-fed infants: effects of age, iron status, iron supplements, and complementary foods[1–3]

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ABSTRACT

Background: Iron supplements are often recommended for older breast-fed infants, but little is known about factors affecting iron absorption from human milk or supplements.

Objective: We investigated the effects of age, iron status, and iron intake on iron absorption in healthy, term, breast-fed infants.

Design: Twenty-five infants were randomly assigned to receive either *1*) iron supplements (1 $mg \cdot kg^{-1} \cdot d^{-1}$) from 4 to 9 mo of age, *2*) placebo from 4 to 6 mo and iron supplements from 6 to 9 mo, or *3*) placebo from 4 to 9 mo. Infants were exclusively breast-fed to 6 mo and partially breast-fed to 9 mo of age. Iron absorption was assessed by giving ^{58}Fe with mother's milk at 6 and 9 mo. Blood samples were obtained at 4, 6, and 9 mo, and complementary food intake was recorded at 9 mo.

Results: At 6 mo, mean (±SD) fractional iron absorption from human milk was relatively low (16.4 ± 11.4%), with no significant difference between iron-supplemented and unsupplemented infants. At 9 mo, iron absorption from human milk remained low in iron-supplemented infants (16.9 ± 9.3%) but was higher ($P = 0.01$) in unsupplemented infants (36.7 ± 18.9%). Unexpectedly, iron absorption at 9 mo was not correlated with iron status but was significantly correlated with intake of dietary iron, including supplemental iron.

Conclusions: Changes in the regulation of iron absorption between 6 and 9 mo enhance the infant's ability to adapt to a low-iron diet and provide a mechanism by which some, but not all, infants avoid iron deficiency despite low iron intakes in late infancy. *Am J Clin Nutr* 2002;76:198–204.

KEY WORDS Infants, human milk, breast milk, nonheme-iron absorption, stable isotopes, iron status, dietary iron intake, complementary food, iron supplements, dietary regulator, adaptation

INTRODUCTION

Rapid growth of infants during the first year of life requires an adequate supply of iron for synthesis of blood, muscle, and other tissues. Most health authorities recommend exclusive breast-feeding for 4–6 mo (1), a practice thought to prevent development of iron deficiency anemia in term, healthy infants (2). However, if infants are exclusively breast-fed beyond that age, they are at increasing risk of developing iron deficiency anemia (3–5). To prevent this, iron supplements (in the form of liquid drops) are often recommended for breast-fed infants after 4–6 mo of age if they do not consume adequate amounts of iron-rich complementary foods (6–8).

Although there is much interindividual variation in iron absorption, estimates of iron requirements in infancy and recommendations regarding the quantity of iron to be used to fortify infant foods are often derived from absorption data (9, 10). In healthy adults, absorption of iron increases in states of iron depletion, but it is not known if the same regulating mechanism functions during infancy, a period characterized by dramatic changes in the size of iron stores and the rate of erythropoiesis. Recently, we found different hemoglobin responses to iron supplementation before and after 6 mo of age, suggesting a developmental change in the regulation of iron metabolism during infancy (11). Other authors also suggested that iron absorption differs depending on infant age (12), but longitudinal studies are lacking.

Iron concentrations in human milk are low (0.2–0.4 mg/L), but it is thought that the high bioavailability of iron in human milk partly compensates for its low concentrations. In the 1970s, radioisotopes were used for absorption studies in infants; the mean fractional absorption of iron from human milk at 6 mo of age was found to be 49% in a frequently cited study (13). However, stable-isotope studies published in the 1990s suggested lower fractional absorption, ranging from 16% to 25% (14, 15).

The present study was designed to measure iron absorption from human milk in healthy, term, breast-fed infants at 6 mo of age and to repeat the measurement at 9 mo of age in the same infants. To ensure wide ranges of dietary iron intake and iron status, infants received iron supplements or placebo for ≥6 wk before the measurements. The primary aim was to investigate

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the influence of iron supplementation on the absorption of iron from human milk. The secondary aim was to study the effects of infant age, iron status, and complementary food intake on iron absorption.

SUBJECTS AND METHODS

Subjects

Twenty-five term (≥37 wk gestation) Swedish infants of normal birth weight (>2500 g) were recruited at 4 mo of age from a larger cohort of infants participating in an iron supplementation trial (11). The infants were initially recruited with an information leaflet sent to all mothers who had given birth at Umeå University Hospital and were living within 20 km of the hospital. Mother-infant pairs were eligible if the mother intended to breast-feed exclusively for 6 mo and at least partially for 9 mo and the infant had no chronic illnesses. A total of 121 infants were included in the iron supplementation trial; they were randomly assigned to receive either *1*) iron supplements from 4 to 9 mo of age, *2*) placebo from 4 to 6 mo and iron supplements from 6 to 9 mo, or *3*) placebo from 4 to 9 mo. From each of these 3 groups, 12–14 families were asked to participate in the current study; a total of 25 families agreed (6 in group 1, 8 in group 2, and 11 in group 3). The study protocol was approved by the Ethical Committee, Faculty of Medicine and Odontology, Umeå University. All participating families gave their written, informed consent.

Iron supplements and diet

The iron supplement was a liquid formulation of ferrous sulfate (Fer-In-Sol; Mead Johnson, Evansville, IN). The placebo solution was prepared by the pharmacy at the UC Davis Medical Center, Sacramento, CA, on the basis of the original recipe for Fer-In-Sol, but with ferrous sulfate omitted and food coloring added to give the placebo an appearance similar to that of the iron supplement. The investigators and the parents were blinded to the intervention. The supplement was given in a dose corresponding to 1 mg elemental $Fe \cdot kg^{-1} \cdot d^{-1}$; the volume was adjusted monthly according to the infant's weight.

The supplement or placebo was given by the mother each morning, just before or after breast-feeding and ≥1 h before or after the infant consumed any other food. The infants were exclusively breast-fed until ≥6 mo of age. When the infants were 4–6 mo of age, the mothers were discouraged from giving any other foods or fluids except for so-called taste portions (≤1 tablespoon/d) of commercial baby foods (strained fruits or vegetables) that contained little or no iron and were provided by the investigators. When the infants were between 6 and 9 mo of age, the mothers continued breast-feeding and gave complementary foods at their own discretion; no attempt was made by the investigators to influence the choice of foods or the extent of breast-feeding.

Intake of complementary food (defined as all food, either fluid or solid, except for human milk) was recorded in two 5-d food diaries at 8 mo (243 ± 2 d) and 9 mo (272 ± 1 d) of age, respectively. Intakes of specific nutrients were calculated by using the Food Composition Tables from the Swedish National Food Administration combined with information from Swedish baby food manufacturers. Nutrient intakes at 9 mo were calculated as the averages of the 8- and 9-mo values. Body weight was measured at 6 and 9 mo of age with a Seca model 835 digital infant scale (Seca Corporation, Hamburg, Germany). Energy and iron intakes at 9 mo were calculated per kg body weight by using the weight at 9 mo.

To account for supplemental iron intake, dietary iron intake was calculated by adding supplemental iron intake (0 or 1 $mg \cdot kg^{-1} \cdot d^{-1}$) to complementary food iron intake. Breast-milk intake was not measured, so the contribution of iron from this source was not included in the calculation, but it should, theoretically, be <0.04 $mg \cdot kg^{-1} \cdot d^{-1}$.

Stable isotopes

The natural abundance values of ^{57}Fe and ^{58}Fe are 2.1% and 0.3%, respectively. Metallic stable isotopes of ^{57}Fe and ^{58}Fe, enriched to 94.7% and 93.1%, respectively (Russian origin, purchased from Penwood Chemicals, Great Neck, NY) were dissolved in a mixture of 1.4 mol HNO_3/L and 0.4 mol H_2SO_4/L, heated at 300°C until dryness, resuspended in 0.2 mol H_2SO_4/L, and heated at 60°C until dissolved. The ^{58}Fe solution was sterile filtered, diluted to 0.20 mg ^{58}Fe/mL, and divided into aliquots of 0.15 mg ^{58}Fe. Ascorbic acid was added at a 2:1 molar ratio to the ^{57}Fe solution, which was then adjusted to pH 5.6, sterile filtered, diluted to 1.54 mg ^{57}Fe/mL, and divided into aliquots of 2.85 mg ^{57}Fe. Both stable-isotope ferrous sulfate solutions were stored under nitrogen at 4°C until used.

Study procedure

Iron absorption was estimated twice in each subject by giving a test meal 2 wk before 6 mo of age ($\bar{x}$: 168 d of age; range: 164–174 d) and by giving an identical test meal 2 wk before 9 mo of age ($\bar{x}$: 259 d of age; range: 253–264 d). For simplicity, these ages are referred to as 6 and 9 mo, respectively. A venous blood sample was obtained at an average of 17 d after each test meal and was analyzed for isotope ratios. Just before the second test meal, a blood sample was obtained and analyzed to determine the baseline isotope ratio.

Milk from each mother was collected ≈2 wk before the test meal by using a manual or electric breast pump; milk was stored at −20°C in a sterile container until used. For each test meal, a portion of milk (76–150 mL) obtained from the infant's own mother was thawed in a preweighed plastic feeding bottle and mixed with 150 μg ^{58}Fe. The bottle was then slowly rotated at 4°C overnight to allow equilibration. The test meal was given in the morning during a home visit by one of the investigators (MD) and a research nurse (MB). The ^{58}Fe-labeled milk was heated in a water bath to ≈37°C before it was fed to the infant. To accurately assess the dose given, all vials were weighed before and after use and all small losses of milk (from spitting up or spilling) were determined by absorbing the losses into preweighed napkins; the corresponding stable-isotope content was subtracted from the original total dose. The dose of ^{58}Fe added to the milk corresponds to ≈50% of the normal daily amount of iron obtained from breast milk (15, 16).

A reference dose of 2.85 mg ^{57}Fe was also given; it was mixed with a small amount of apple juice and was given orally to the infant with a 2-mL syringe. To avoid any interaction between the absorption of human milk iron and the absorption of the several-fold-larger reference dose of iron, the latter was given ≥6 h after completion of the test meal. No food or fluid was given for a period of ≥2 h before and after the reference dose. After the reference dose was given, the syringe was rinsed once with ultrapure water and the water was given to the infant. The full

TABLE 1
Subject characteristics at baseline (4 mo of age)[1]

Characteristic	Value
Male sex (%)	52
Body weight (kg)	6.7 ± 1.4[2]
Hemoglobin (g/L)	117 ± 5
Ferritin (μg/L)	151 ± 110
ZPP (μmol/mol heme)	45 ± 10
TfR (mg/L)	6.6 ± 1.4

[1] n = 25. ZPP, zinc protoporphyrin; TfR, transferrin receptor. There were no significant differences between groups for infant sex distribution, body weight, or concentrations of hemoglobin, ferritin, ZPP, or TfR.
[2] $\bar{x}$ ± SD.

reference dose was given in all cases. No iron supplement or placebo was given on the day of the test meal. No food other than human milk was given from 6 h before the test meal until 2 h after the reference dose.

Laboratory analyses

Venous blood samples (≈5 mL) were obtained at 4, 6, 8.5, and 9 mo of age. Each blood sample was divided between one tube containing lithium heparin and one tube containing EDTA. Blood from the heparin-containing tube was centrifuged at 1750 × g for 10 min at room temperature, and the washed erythrocytes from ≥1 mL of blood were saved for analysis of isotope ratios. Plasma from the heparin-containing tube was analyzed for ferritin content by using an immunoradiometric assay (Coat-A-Count; Diagnostic Products Corp, Los Angeles) and for transferrin receptors by using an enzyme immunoassay (TfR; Ramco, Houston) at the Department of Nutrition, University of California, Davis. Blood from the tube containing EDTA was analyzed for hemoglobin by using an automated blood counter (Sysmex SE 9000; Tillquist, Stockholm, Sweden) at the Department of Clinical Chemistry, Umeå University Hospital, Sweden and was analyzed for zinc protoporphyrin (ZPP) by using a fluorometer (Protofluor Z; Helena Labs, Beaumont, TX) at the Department of Clinical Sciences, Pediatrics, Umeå University. Inter- and intraassay CVs for the ferritin, transferrin receptor, and ZPP kits were all <10%.

Iron isotope ratios were determined in the mineral mass spectrometry laboratory of the US Department of Agriculture, Agricultural Research Service, Children's Nutrition Research Center, Houston. Erythrocytes obtained at 6, 8.5, and 9 mo were digested in concentrated HNO_3 in a titration flask on a hot plate at sub-boiling temperature for 24 h. The sample was then dried and redissolved in 6 mol HCl/L and iron was extracted with an ion-exchange column as described previously (15). Extracted iron was resuspended in 30–50 μL of 3% HNO_3 and loaded onto the filament of a magnetic sector thermal ionization mass spectrometer (MAT 261; Finnigan, Bremen, Germany) and the ratios of ^{57}Fe to ^{56}Fe and ^{58}Fe to ^{56}Fe were determined as described previously (15).

Calculations

Incorporation of iron into erythrocytes was determined as described previously (16) by evaluating the recovery of the orally administered isotopes in blood obtained 2–3 wk after isotope administration. Circulating iron was estimated by using an assumed blood volume of 80 mL/kg (17), the measured hemoglobin concentration, and the known concentration of iron in hemoglobin (3.47 mg/g). Fractional absorption was calculated, assuming that 90% of the absorbed isotope was incorporated into erythrocytes (18). The amount of incorporated ^{58}Fe was corrected for the content of ^{58}Fe in the ^{57}Fe tracer (0.16%) (19). At the second measurement for each infant, the baseline isotope ratio was measured and corrected for in the calculation.

All statistical analyses were performed with SPSS software, version 10.0 (SPSS Inc, Chicago). The statistical methods used were the paired t test, independent sample t test, analysis of variance, and linear regression. The level of significance was set at $P < 0.05$. With the use of previous variability data (14), a prestudy analysis showed that a group size of 10 would give a power of 80% to find a 20% difference in iron absorption between groups. The actual number of infants recruited was 25, which limited the statistical power. However, because absorption from the reference dose was found to be strongly correlated with absorption from breast milk, we had 2 independent measurements of iron absorption in each infant at each point in time, which increased the reliability of the method. Analyses were done to check for normality of iron-status variables at baseline, dietary iron intake, and residuals of absorption variables at 6 and 9 mo. One variable (absorption from the reference dose) was slightly skewed and this could be corrected by log transformation. However, all significant results remained significant after log transformation, and therefore we used the original values for ease of presentation.

RESULTS

Subjects, test meals, and blood sampling

Iron absorption was measured in 25 infants at 6 mo of age, and the measurement was repeated in 18 of them at 9 mo of age. All 7 families that dropped out of the study did so because of parental refusal to continue with the demanding study procedure. Characteristics of the subjects at 4 mo of age are shown in **Table 1**. There were no significant differences between groups in baseline concentrations of hemoglobin, ferritin, ZPP, or transferrin receptors; body weight; or infant sex distribution.

After correcting for the small amount of milk ($\bar{x}$: 4 mL) remaining in the bottle and collected in napkins, a mean of 103 mL human milk (range: 76–156 mL) containing a mean of 144 μg ^{58}Fe (range: 125–149 μg) was given to the infants during the test meals. In 7 of 43 test meals, the infant refused to take the bottle; the milk was given by nasogastric tube in those instances.

Iron absorption

Human milk

At 6 mo, the mean (±SD) fractional absorption of ^{58}Fe from human milk was 16.4 ± 11.4%, with no significant difference between iron-supplemented infants (11.9 ± 7.4%) and unsupplemented infants (17.8 ± 12.2%) (**Table 2**). At 9 mo, mean fractional iron absorption was significantly higher in unsupplemented infants (36.7 ± 18.9%) than in iron-supplemented infants (16.9 ± 9.3%). There was also a significant increase in fractional iron absorption from 6 to 9 mo in the unsupplemented group (P = 0.009) but not in the 2 groups of iron-supplemented infants.

Reference dose

At 6 mo, the mean fractional absorption of ^{57}Fe from the reference dose was 12.3 ± 6.7%, with no significant difference

TABLE 2
Fractional iron absorption from human milk and a reference dose in infants of different ages

Group	6 mo (all infants)			6 mo (excluding dropouts)[1]			9 mo		
	n	Human milk	Reference dose	*n*	Human milk	Reference dose	*n*	Human milk	Reference dose
		%			%			%	
Iron-supplemented from 4 to 9 mo	6	11.9 ± 7.4[2]	8.5 ± 2.8	6	11.9 ± 7.4	8.5 ± 2.8	6	17.8 ± 12.0	6.0 ± 4.2
Iron-supplemented from 6 to 9 mo	8	14.7 ± 11.3	12.1 ± 3.4	4	18.6 ± 15.3	11.2 ± 4.7	4	15.6 ± 3.9	7.4 ± 3.1
Placebo	11	20.0 ± 12.9	14.4 ± 9.1	8	14.3 ± 8.7[3]	13.2 ± 9.3[4]	8	36.7 ± 18.9	19.8 ± 6.4[4]
Combined groups									
Iron-supplemented[5]	6	11.9 ± 7.4	8.5 ± 2.8	6	11.9 ± 7.4	8.5 ± 2.8	10	16.9 ± 9.3	6.6 ± 3.7
Unsupplemented[6]	19	17.8 ± 12.2	13.5 ± 7.2	12	15.7 ± 10.9	12.5 ± 7.9	8	36.7 ± 18.9[7]	19.8 ± 6.4[8]
All subjects	25	16.4 ± 11.4	12.3 ± 6.7	18	14.5 ± 9.8	11.2 ± 6.8	18	25.7 ± 17.2	12.4 ± 8.3

[1] Dropouts were excluded to allow direct comparison with the same infants at 9 mo.
[2] $\bar{x}$ ± SD.
[3] Significantly different from 9 mo, $P = 0.009$ (paired t test).
[4] The increase in iron absorption from 6 to 9 mo was not significant ($P = 0.066$) when original values were used but was significant when log-transformed absorption values were used ($P = 0.018$).
[5] At 6 mo, iron from 4 to 9 mo group only; at 9 mo, iron from 4 to 9 mo and iron from 6 to 9 mo groups combined.
[6] At 6 mo, placebo and iron from 6 to 9 mo groups combined; at 9 mo, placebo group only.
[7,8] Significantly different from iron-supplemented group (independent-sample two-tailed t test): [7] $P = 0.01$, [8] $P < 0.001$.

between iron-supplemented infants (8.5 ± 2.8%) and unsupplemented infants (13.5 ± 7.2%) (Table 2). At 9 mo, mean fractional iron absorption was significantly higher in unsupplemented infants (19.8 ± 6.4%) than in iron-supplemented infants (6.6 ± 3.7%). The increase in iron absorption from 6 to 9 mo in the placebo group was not significant ($P = 0.066$) when original values were used but was significant when log-transformed absorption values were used ($P = 0.018$).

Fractional iron absorption from the reference dose was significantly correlated with iron absorption from human milk measured simultaneously in the same infant (**Figure 1**), even though the latter absorption value was significantly higher at both 6 and 9 mo of age ($P = 0.027$ and $P < 0.001$, respectively). However, because the amount of iron in the reference dose was several times higher than the amount of iron in the human milk test meal, the absolute amount of iron absorbed from the reference dose was 10-fold higher. Thus, an average of 370 μg Fe was absorbed from the reference dose while an average of 35 μg Fe was absorbed from the human milk test meal, assuming an iron concentration in human milk of 0.3 mg/L.

Influence of iron status

Iron supplementation had significant effects on iron status. Iron-supplemented infants had higher mean (±SD) plasma ferritin concentrations than did unsupplemented infants at 6 mo (116 ± 27 and 69 ± 27 μg/L, respectively, $P = 0.002$) and at 9 mo (77 ± 29 and 27 ± 29 μg/L, respectively, $P < 0.001$), controlling for baseline values at 4 mo.

At both 6 and 9 mo of age, we did not find any significant correlations between different indexes of iron status and iron absorption. Scatterplots of the associations between plasma ferritin concentrations and iron absorption from human milk and from the reference dose at 9 mo are shown in **Figure 2**. The corresponding correlations for hemoglobin, ZPP, and transferrin receptors were not significant. At 9 mo, 3 of the 18 infants had ferritin values <12 μg/L (11) and 1 of the 18 had serum transferrin receptors >11 mg/L (20), but none had hemoglobin <110 g/L or ZPP >80 μmol/mol heme (11). In the 4 infants with indications of poor iron status (ferritin <12 μg/L or transferrin receptors >11 mg/L), mean absorption of iron from human milk and from the reference dose was not significantly different from that in infants with normal iron status. No infant met the criteria for iron deficiency, defined as having 2 of the following: ferritin <12 μg/L, ZPP >80 μmol/mol heme, and transferrin receptors >11 mg/L.

Influence of dietary iron intake

Complementary food intake at 9 mo was recorded for an average of 9 d per infant. The mean (±SD) daily energy intake was 180 ± 88 kJ/kg (range: 21–355 kJ/kg) and the mean iron intake was 0.53 ± 0.50 $mg \cdot kg^{-1} \cdot d^{-1}$ (range: 0.08–2.33 $mg \cdot kg^{-1} \cdot d^{-1}$). Infants with the lowest iron intakes from complementary foods were still almost exclusively breast-fed at 9 mo, whereas infants with the highest iron intakes consumed significant amounts of iron-fortified complementary foods, such as fruit drinks and cereals.

At 9 mo, there was a significant inverse correlation between dietary iron intake and iron absorption from human milk

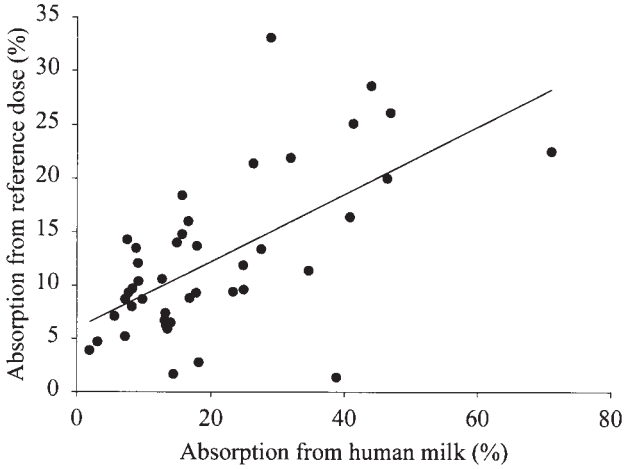

FIGURE 1. Correlation between fractional iron absorption from human milk and fractional iron absorption from a reference dose of ferrous sulfate; $n = 43$ ($r = 0.63$, $P < 0.001$).

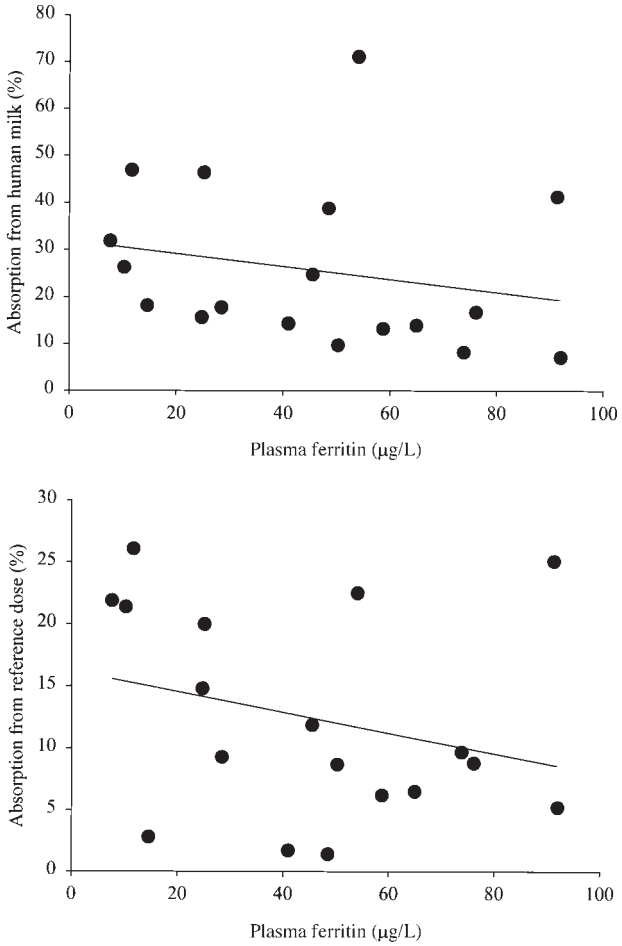

FIGURE 2. Associations between plasma ferritin concentration and fractional iron absorption from human milk and from a reference dose at 9 mo of age (n = 18); both of these associations were not significant.

(P = 0.031) and from the reference dose (P = 0.001, **Figure 3**). In a multivariate analysis, the effect of supplemental iron on iron absorption (from human milk or the reference dose) was highly significant, whereas the effect of complementary food iron was not significant, showing that supplemental iron had a larger effect on iron absorption than did complementary food iron (data not shown). However, in the subgroup of unsupplemented infants (n = 8), there was a significant inverse correlation between complementary food iron intake and iron absorption from the reference dose ($r = -0.75$, $P = 0.031$). In contrast to the results for dietary iron intake, there was no significant correlation between complementary food energy intake and iron absorption from human milk or from the reference dose.

DISCUSSION

Mean fractional iron absorption from human milk at 9 mo was significantly higher in unsupplemented infants (36.7 ± 18.9%) than in iron-supplemented infants (16.9 ± 9.3%). This was expected, because the unsupplemented, breast-fed infants had significantly smaller iron stores at 9 mo than did the iron-supplemented infants, as assessed by plasma ferritin concentrations; low iron stores are thought to increase intestinal iron absorption. However, we found that iron absorption was directly associated with dietary iron intake but not with indexes of iron status, including plasma ferritin. These findings were unexpected and suggest that in infants of this age, dietary iron intake per se is an important regulator of iron absorption and has a direct effect rather than an effect that is secondary to its influence on body iron stores.

In humans, 3 regulators of nonheme-iron absorption have been identified. They are known as the stores regulator, the erythropoietic regulator, and the dietary regulator (21). Many studies have shown that iron absorption is inversely related to iron stores, a mechanism sometimes referred to as the stores regulator (22). Iron stores are most often assessed by measuring serum or plasma ferritin, which is thought to accurately predict iron absorption in healthy, adult men (23). In the present study, we found no significant correlation between plasma ferritin concentration and iron absorption.

In situations when the erythropoietic drive is high in relation to the iron supply, iron absorption is known to increase; this is sometimes called the erythropoietic regulator and is observed in various types of anemia characterized by iron-deficient erythropoiesis (22).

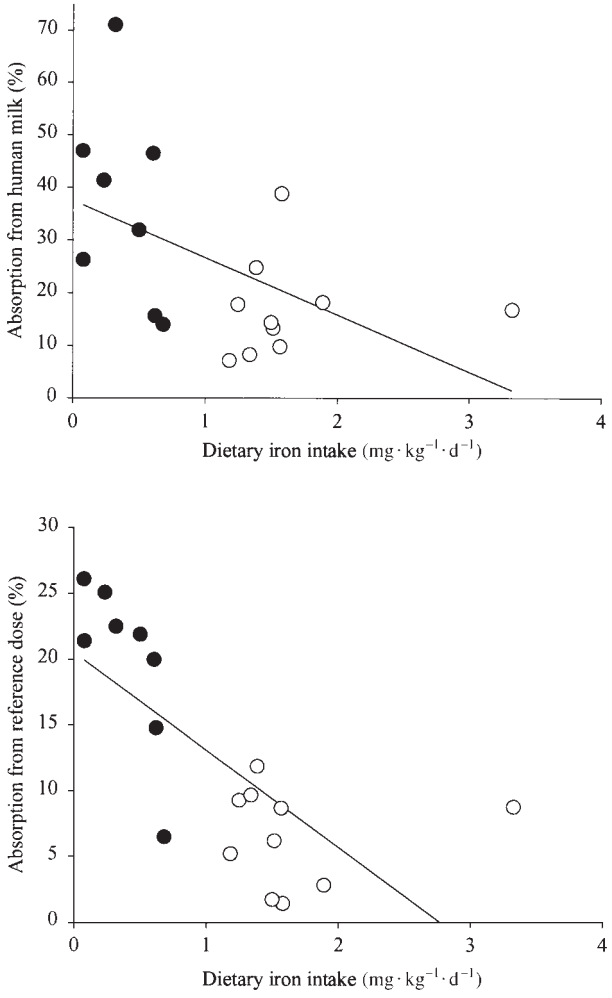

FIGURE 3. Associations between dietary iron intake and fractional iron absorption from human milk ($r = -0.51$, $P = 0.031$) and from a reference dose ($r = -0.72$, $P = 0.001$) at 9 mo of age (●, unsupplemented infants; ○, iron-supplemented infants); n = 18. Dietary iron included supplemental iron but excluded breast-milk iron.

ZPP and transferrin receptors can be used to assess functional iron deficiency of the erythron, and elevated concentrations may therefore be associated with increased iron absorption. However, in the present study, we found no significant correlation between iron absorption and these 2 variables.

Thus, we found no correlation between iron absorption and various measures of iron status in this study. However, we cannot exclude the possibility that such correlations might be found in a larger cohort of infants with a wider range of iron-status values.

Iron absorption is also regulated by recent dietary iron intake, independent of the size of iron stores and the rate of erythropoiesis. A bolus dose of iron given enterally renders enterocytes resistant to absorbing additional iron for several days, a phenomenon that has been referred to as mucosal block (24, 25). This dietary regulator of iron absorption has generally received less emphasis than the other 2 regulators, possibly because habitual dietary iron intake and the size of iron stores often are related in the nonexperimental setting. However, a recent study suggested that regulation of nonheme-iron absorption in adult men is correlated with recent intake of bioavailable iron rather than with iron stores measured in terms of ferritin concentrations (26).

In the present study, the small difference in iron absorption between iron-supplemented and unsupplemented infants at 6 mo of age was not significant. Even if such a difference might be significant in a larger cohort, the small difference observed still suggests that the role of the dietary regulator of iron absorption is limited at this age. One explanation for this might be that the stores regulator effectively down-regulates intestinal iron absorption in these presumably iron-sufficient infants, regardless of dietary iron intake. Alternatively, it could be that neither of these 2 regulators is fully active at this early age. The latter view is supported by the fact that the observed iron absorption at 6 mo was not particularly low, and we showed previously that iron supplementation from 4 to 6 mo of age results in increased hemoglobin and ferritin concentrations regardless of initial iron status (11).

At 9 mo of age, there was an inverse correlation between iron absorption and dietary iron intake, but again we found no significant correlation between iron absorption and various indexes of iron status. This implies that the size of iron stores is not responsible for the significant increase in iron absorption that occurred in the unsupplemented infants between 6 and 9 mo of age. Rather, this observation supports the theory that the dietary regulator of iron absorption is immature in the 6-mo-old infant and is subject to developmental changes between 6 and 9 mo of age. Several new transporters of iron were found in enterocytes (27), but little is known as yet about their expression in human infants. However, recent data from animal studies suggest that the expression of one iron transporter, DMT1, is not affected by dietary iron during early infancy but is affected in late infancy (28), which would support our findings.

The observed increase in iron absorption from human milk in unsupplemented infants at 9 mo of age does not imply that infants need no source of iron other than breast milk during the second half of infancy. Rather, this adaptation may not be sufficient to prevent iron deficiency in exclusively breast-fed infants. Assuming a breast-milk intake of 1000 mL/d, even if the bioavailability of human milk iron reached an unattainable 100%, absorbed iron would amount to no more than 0.3 mg/d, which is insufficient to meet the estimated requirement of 280 mg absorbed iron during the first year of life (29). However, we found a similar increase in iron absorption from the reference dose in unsupplemented infants with a low complementary food iron intake. Thus, one can speculate that iron absorption from all dietary sources (excluding heme iron) is up-regulated in response to low dietary iron intake at 9 mo of age. This might prove to be a valuable compensatory mechanism in partially breast-fed infants with low-iron diets and might explain why we found no correlation between complementary food iron intake and iron status in 9-mo-old Swedish infants (11).

At both 6 and 9 mo of age, fractional absorption of iron from human milk correlated well with fractional absorption from the reference dose, even though the latter was significantly lower. The reason for this difference might be that some component of human milk facilitates iron absorption, but we cannot exclude the possibility that the larger amount of iron in the reference dose might saturate intestinal iron-binding proteins or receptors, resulting in lower fractional absorption. Both of these mechanisms would also apply to the supplemental iron drops. In the iron-supplemented infants, mean fractional absorption of iron from the reference dose was 7–9% at 6 and 9 mo of age, corresponding to 0.07–0.08 mg $Fe \cdot kg^{-1} \cdot d^{-1}$ absorbed from iron drops. This might be an adequate supplementation dose for infants in high-risk populations.

In comparison, unsupplemented infants in the present study would absorb 0.01 mg $Fe \cdot kg^{-1} \cdot d^{-1}$ from breast milk if we assume an intake of 700 mL milk/d (corrected for complementary food intake) at 9 mo of age. The average daily complementary food iron intake in the same infants was 3.4 mg. Assuming a fractional iron absorption of 10% from complementary food, the amount of absorbed iron from that source would be 0.04 $mg \cdot kg^{-1} \cdot d^{-1}$, showing that complementary food was the main source of iron in these partially breast-fed infants. By using strict criteria, we found no case of iron deficiency anemia in the unsupplemented infants at 9 mo of age. This confirms our previous findings (11) that breast-feeding in combination with adequately iron-fortified complementary food, possibly together with the ability to up-regulate iron absorption in response to low dietary iron intake as shown here, ensures a sufficient amount of absorbed iron, at least until 9 mo of age in this low-risk population.

In conclusion, we have for the first time shown the importance of the dietary regulator of nonheme-iron absorption in breast-fed infants. The effect of recent dietary iron intake needs to be taken into consideration when designing future studies of iron absorption in infants, because dietary differences may at least partly explain the inconsistent absorption rates found in previous studies. Moreover, our results suggest that the regulation of iron absorption undergoes developmental changes between 6 and 9 mo of age. These changes enhance the infant's ability to adapt to a low-iron diet and provide a mechanism by which some, but not all, infants may avoid developing iron deficiency despite low iron intakes in the second 6 mo of life.

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