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Erythrocyte Incorporation of Iron by 56-Day-Old Infants Fed a ^{58}Fe-Labeled Supplement

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ABSTRACT

In an effort to obtain information about absorption of supplemental iron by breast-fed infants during the early months of life, we determined erythrocyte incorporation of a stable iron isotope, administered to 56-d-old breast-fed infants in the form of a ^{58}Fe-labeled vitamin-iron supplement. Infants of similar age fed a milk-based formula low in iron (approximately 4 mg/L) were also studied. The ^{58}Fe-labeled vitamin-iron supplement was given between feedings. Fourteen days after administration of ^{58}Fe, mean erythrocyte incorporation of the isotope was 7.8% of the dose by breast-fed infants and 4.4% of the dose by formula-fed infants. The feeding-related difference was statistically significant, probably reflecting the greater quantities of inhibitors of iron absorption in the intestines of formula-fed infants. With mean iron intake from the ^{58}Fe-labeled vitamin-iron supplement of 7.99 mg for the breast-fed infants, erythrocyte incorporation of 7.8% of the dose corresponded to 0.62 mg, a value in the range of the estimated requirement for absorbed iron. We conclude that 2-mo-old breast-fed infants are able to absorb nutritionally significant amounts of iron from an iron supplement. (***Pediatr Res* 38: 373–378, 1995**)

Abbreviations

BV, blood volume
IR, isotope ratio

The term infant is born with a substantial amount of iron in storage sites, and iron stores are increased as the result of decrease in circulating erythrocytes during the early weeks of life. The quantity of storage iron is then sufficient to permit the nearly doubling of erythrocyte mass and muscle mass during the first 4 mo of life, but the transfer of iron from storage sites to active tissues largely depletes storage sites of iron unless exogenous iron is provided. We therefore consider it desirable to supplement the infant's diet with iron beginning soon after birth. This suggestion is not new. More more than 25 y ago the Committee on Nutrition of the American Academy of Pediatrics (1), stated that ". . . the early use of fortified formula results in augmentation of iron stores which helps to prevent later development of iron deficiency." This recommendation for early feeding of iron-fortified formulas has been reaffirmed more recently (2, 3). We believe that for breast-fed infants as well as for formula-fed infants augmentation of iron stores should be promoted by early supplementation of iron—a view that is in conflict with other recommendations. The prevailing recommendation is that iron supplementation of breast-fed infant be begun at 4 to 6 mo of age (3, 4).

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Although we believe that it is important to augment iron stores, little is known about the extent to which iron is absorbed during the early months of life. The present study was undertaken to obtain information on this point. We studied 56-d-old breast-fed infants given a daily supplement of iron (approximately 7.5 mg/d), and, as a surrogate for iron absorption, determined erythrocyte incorporation of iron using the stable isotope, ^{58}Fe, as a label. To investigate further the substantially greater erythrocyte incorporation of ^{58}Fe by breast-fed than by formula-fed infants given a small dose of ^{58}Fe between feedings (5), we included in the present study a group of formula-fed infants.

METHODS

Subjects

Normal infants of either sex with gestational age 37 wk or more and birth weight 2500 g or more were enrolled during the first 30 d of life. The study procedures were explained to one or both parents, and written consent was obtained. The study protocol was reviewed and approved by the University of Iowa Committee on Research Involving Human Subjects.

We wished to study 15 breast-fed and 15 formula-fed infants. Sixteen breast-fed infants (8 male and 8 female) and 19 formula-fed infants (10 male and 9 female) were enrolled in the study. For various reasons that we consider to be irrelevant to

this report, two infants in the breast-fed group and three in the formula-fed group dropped out before receiving the first ^{58}Fe dose. We had anticipated one or two dropouts after administration of ^{58}Fe and had therefore decided to administer ^{58}Fe to 16 infants per group. However, when 30 infants (14 breast-fed and 16 formula-fed) had received their ^{58}Fe doses and most had completed the study, the supply of ^{58}Fe-labeled supplement was exhausted, and rather than preparing a new batch, we elected to discontinue enrollment.

Study Design

Beginning at the time of enrollment (on or before 30 d of age), parents of breast-fed and formula-fed infants were provided a supply of Vi-Daylin ADC Vitamins + Iron Drops (Ross Laboratories, Columbus, OH) and were instructed to give the infant 0.75 mL daily, providing (based on label claim) approximately 7.5 mg of iron in the form of ferrous sulfate, 1125 IU of vitamin A, 300 IU of vitamin D, and 26 mg of ascorbic acid. This preparation will be referred to as the vitamin-iron supplement. The vitamin-iron supplement was measured with a 2-mL syringe, which we provided, and administered directly into the infant's mouth. To provide an estimate of iron intake from the vitamin-iron supplement, we determined the iron concentration of the supplement and weighed back the bottles at 56 d of age.

Beginning at 56 d of age (±2 d), the vitamin-iron supplement was withheld, and each infant visited the Lora N. Thomas Pediatric Metabolic Unit on three consecutive days. On each day the infant was given a dose of the vitamin-iron supplement labeled with ^{58}Fe. Beginning on the day after the third dose of ^{58}Fe-labeled vitamin-iron supplement, a supply of Vi-Daylin ADC Vitamins Drops (Ross Laboratories) (without iron) was given to breast-fed infants, and parents were instructed to give 0.75 mL daily at least until the end of the study at 84 d of age. After the last of the three doses of ^{58}Fe-labeled vitamin-iron supplement, mothers of breast-fed infants were permitted to give supplemental feedings of a milk-based formula (Similac, Ross Laboratories) with low iron concentration, or to substitute feeding of this formula for breast feeding. Formula-fed infants continued to be formula fed and received no further supplement.

A baseline sample of blood was obtained by heel-stick at 56 d of age before the first dose of ^{58}Fe-labeled supplement, and follow-up blood samples were obtained at 70 and 84 d. Blood was collected in heparinized Microvette tubes (CB 1000 S, Sarstedt, Inc., Newton, NC), and plasma was separated from cells within 30 min of blood collection.

Feedings

Breast-fed infants. Before enrollment in the study, infants were exclusively breast-fed, except for one infant who had received two small formula feedings (about 10 mL) while in the hospital after birth. From the time of enrollment until 59 d of age all were exclusively breast-fed.

Formula-fed infants. From birth until the time of enrollment, all but four infants were fed various formulas. These four infants had been breast-fed for 3, 7, 10, and 26 d, respectively, and then fed various formulas. From the time of enrollment until completion of the study at 84 d of age, infants were fed Similac with low iron content. Two lots of formula were used, one providing 4.0 mg of iron per liter and the other providing 3.2 mg of iron per liter.

Administration of ^{58}Fe

On each of 3 consecutive days beginning at 56 d of age (±2 d), infants were given a dose of Vi-Daylin ADC Vitamins + Fe Drops labeled with ^{58}Fe. Enriched ^{58}Fe was obtained as elemental iron from U.S. Services Inc. (Summit, NJ). Its isotopic composition was (weight %): 93.243% ^{58}Fe, 6.593% ^{57}Fe, 0.155% ^{56}Fe, and 0.009% ^{54}Fe. It was converted to ferrous sulfate as described previously (6). Preparation of ^{58}Fe-enriched Vi-Daylin ADC Vitamins + Iron Drops was as follows: 52.68 mg of ^{58}Fe-enriched iron as ferrous sulfate were dissolved in the vitamin-iron supplement (8.48 mg of iron per g of solution) to produce 84.97 g of a solution containing (per g) 9.03 mg of iron, including 0.58 mg of ^{58}Fe and (label claim) 32 mg of ascorbic acid. The solution was stored in a nitrogen-purged rubber-stoppered vial.

The ^{58}Fe-labeled supplement was given between 0900 and 1300 h, at least 3 h after the last feeding and at least 1 h before the subsequent feeding. Approximately 0.8 mL of the labeled supplement was drawn up into a tared syringe and weighed. The mean amount of labeled iron administered to breast-fed infants was 7.99 (SD 0.39) mg per dose, and the mean amount administered to formula-fed infants was 8.10 (SD 0.18) mg per dose. The contents of the syringe were delivered directly into the infant's mouth and followed immediately by two 5-mL rinses of the syringe with a 5% solution of glucose in water, delivered from the syringe directly into the infant's mouth. The infants were closely observed to detect drooling from the mouth immediately after administration. When drooling of brown-colored supplement occurred, the amount was quantitated by weighing the preweighed cellulose bib. Drooling of (clear) rinse fluid occurred not infrequently, but the amount was not quantitated because we considered it unlikely to involve appreciable loss of label. Any regurgitation during the hour after administration of ^{58}Fe was recorded by the mother.

Laboratory Analyses

Blood was analyzed for Hb concentration by Coulter Counter, model M430 (Coulter Electronics, Inc., Hialeah, FL), and plasma for ferritin concentration by RIA using the RAINEN Assay System kit (catalog no. NEA-078, DuPont, Billerica, MA) in the first 40 determinations and, when the RAINEN assay was no longer available, the Quantimune kit (catalog no. 190-2001, Bio-Rad Laboratories, Hercules, CA) in the remaining 49 determinations. Because the RAINEN assay gave systematically lower values in parallel determinations, values obtained with the RAINEN assay were multiplied by a factor of 1.15.

The $^{58}Fe/^{57}Fe$ IR was determined by inductively coupled plasma mass spectrometry. Briefly, aliquots of packed erythrocytes that contained between 20 and 100 μg of iron were decomposed with concentrated (16 mol/L) nitric acid and

hydrogen peroxide in closed vessels in a microwave digestion apparatus (Milestone mls 1200 mega, Buck Scientific, Inc., East Norwalk, CT). Four milliliters of nitric acid (10 mol/L) were added to the digestate, and iron was selectively extracted with two 4-mL aliquots of xylene that contained 0.5 mol/L thenoyltrifluoroacetone (7). The xylene was stripped of its iron by addition of 3 mL of concentrated (12 mol/L) hydrochloric acid, and the acid was evaporated at a temperature below 150°C. The iron chloride that remained was brought to an iron concentration of 4 mg/L by dissolution in the appropriate volume of dilute (0.16 mol/L) nitric acid. Aliquots of this solution were introduced into an inductively coupled plasma mass spectrometer (Finnigan Sola, Finnigan MAT, Ltd., Hemel Hempstead, UK) in alternation with a reference solution at similar concentration prepared from certified isotopic reference iron (IRRM-014, Institute for Reference Materials and Measurements, Geel, Belgium). Mass channels that encompassed 57 and 58 nominal atomic mass units were monitored alternately, and measured ratios of ^{58}Fe to ^{57}Fe were calculated by the instrument software as specified in the application program for the IR mode of operation. The mean of the measured ratios for the reference solution was used to calculate a normalization factor (N) for each instrumental run:

$$N = 0.1330/\text{mean of measured ratios for reference solution,}$$

where 0.1330 is the certified $^{58}Fe/^{57}Fe$ ratio in the isotopic reference iron. Normalized ratios were calculated as follows:

$$\text{Normalized sample ratio} = N \times \text{measured sample ratio.}$$

Normalization factors varied from day to day and were between 0.9900 and 1.0100. The precision of measurement for a series of 10 consecutive ratio acquisitions on each sample was between 0.2 and 0.5% relative SD.

Calculation of Quantity of Administered ^{58}Fe Incorporated into Erythrocytes

Calculations differ slightly from those used previously (5, 8, 9) because atom IR rather than mass IR has been used. The quantity of administered ^{58}Fe label incorporated into erythrocytes ($^{58}Fe^{*}_{inc}$) at a specified time t after administration of the dose was calculated as follows:

$$^{58}Fe^{*}_{inc} = \frac{IR_t - IR_o}{IR_o} \times Fe_{circ} \times \frac{0.2819 \times 57.933}{100 \times 55.845,}$$

where $^{58}Fe^{*}_{inc}$ is expressed in mg, IR_t is the determined $^{58}Fe/^{57}Fe$ IR at time t after dosing, IR_o is the determined baseline ratio, Fe_{circ} is the quantity of total circulating iron (mg) at time t, and 0.2819 is the natural abundance (atom %) of ^{58}Fe, 57.933 is the atomic weight of ^{58}Fe, and 55.845 is the atomic weight of natural Fe.

The quantity of total circulating iron (mg) was estimated as follows:

$$Fe_{circ} = BV \times Hb \times 3.47,$$

where BV is blood volume in liters, Hb is Hb concentration in g/L, and 3.47 is the concentration of iron in Hb (mg/g). We assumed blood volume to be 65 mL per kg of body weight, a value almost identical to the mean value of 66 mL/kg reported by Bratteby (10) for eight normal infants between 100 and 138 d of age.

Statistical Analysis

Descriptive statistics and parametric and nonparametric statistical analyses were performed using SAS version 6.08 (SAS Institute, Cary, NC). Erythrocyte incorporation values were compared by analysis of variance and Wilcoxson rank sum test. The effect of feeding on ^{58}Fe erythrocyte incorporation was also examined by covariate analysis, using plasma ferritin as the covariate. Results of the applicable parametric and nonparametric tests were consistent. Only the results of the parametric tests are presented. Similarly, we carried out the analyses both with arithmetic and geometric means and found similar levels of significance. Only data concerning arithmetic means are presented. Gender-related differences were not significant and gender was eliminated as a factor in the statistical analyses. Statistical significance was set at $\alpha = 0.05$.

RESULTS

Thirty infants received the three doses of ^{58}Fe-labeled supplement. One infant (formula-fed) had mild diarrhea and temperature elevation on the first day of ^{58}Fe administration and regurgitated an unquantified amount of labeled supplement after the second ^{58}Fe administration. Data for this infant are not presented. For the remaining 29 infants (14 breast-fed and 15 formula-fed), body weight, Hb, and plasma ferritin concentrations and the $^{58}Fe/^{57}Fe$ IR at ages 56 (baseline), 70, and 84 d of age are presented in Table 1. Percent erythrocyte incorporation of iron 14 and 28 d after administration (at ages 70 and 84 d, respectively) is also presented.

After administration of the ^{58}Fe-labeled dose of supplement, drooling of measurable amounts of brown-stained supplement, presumably containing ^{58}Fe, occurred on one or more occasions in 8 breast-fed and 10 formula-fed infants. In two of the formula-fed infants drooling occurred on each of 3 d totaling 1.1 g (subject 56371) and 0.8 g (subject 5638), respectively. In the other 16 infants, total measured losses of brown-stained fluid totaled 0.4 g or less (mean 0.24 g).

Because the concentration of ^{58}Fe in the brown-stained fluid lost by the infants was likely to have been less than the concentration in the supplement as administered, we have presented in the table data based on quantity of ^{58}Fe administered, but have excluded data from subjects 5637 and 5638 in calculating the means and carrying out the statistical calculations. An estimate of the possible error introduced by failing to correct for the measured losses will be presented.

For the 27 infants whose data are included in the mean values in the table, mean birth weight was 3680 g (range 3345–4420 g) for breast-fed male infants, 3487 g (3060–4155 g) for breast-fed female infants, 3574 g (2850–4080 g) for formula-fed male infants, and 3267 g (2705–3940 g) for formula-fed female infants. Mean age at the time of enrollment was 17.6 d for breast-fed infants and 12.4 d for formula-fed infants. On a gender- and feeding-specific basis, gains in

Table 1. *Subject characteristics and erythrocyte incorporation of iron*

Subject		56 days				70 days					84 days				
Number	Sex	Weight (g)	Hb (g/L)	Ferritin (μg/L)	IR_0*	Weight (g)	Hb (g/L)	Ferritin (μg/L)	IR_{14}*	Incorp. (%)	Weight (g)	Hb (g/L)	Ferritin (μg/L)	IR_{28}*	Incorp. (%)
Breast-fed															
5652	M	5300	115	118	0.1318	5800	111	210	0.1792	10.1	6210	112	115	0.1729	9.4
5653	F	5950		78	0.1332	6440	110	103	0.1680	7.9	6780	107	85	0.1627	6.9
5654	F	4980	110	83	0.1316	5430	107	69	0.1868	10.0	5950	102	53	0.1921	11.5
5655	F	4980	120	280	0.1341	5250	110	290	0.1554	4.3	5370	118	218	0.1643	6.5
5656	F	5680	110	281	0.1316	6120	112	425	0.1589	5.7	6600	113	345	0.1603	6.6
5657	M	5560	112	220	0.1324	5890	108		0.1694	7.5	6400	115	127	0.1634	7.2
5658	F	5550	122	235	0.1319	6190	124	265	0.1760	10.6	6560	122	182	0.1646	8.1
5659	M	5730	124	155	0.1330	6110	123	140	0.1641	7.5	6270	117	140		
5661	F	5100	108		0.1339	5480	112		0.1641	6.2	5780	115	100	0.1639	6.6
5662	F	5020	112	380	0.1339	5540	120		0.1466	2.9	5950	110	240	0.1472	3.0
5663	M	6120	111	132	0.1315	6540	106	178	0.1798	10.3	6770	118	138	0.1966	16.2
5664	F	5350	124	230	0.1317	5720	122	200	0.1739	9.1	6040	120	134	0.1781	10.4
5666	M	5900			0.1336	6520	100	103	0.1702	9.4	6790	100	56	0.1680	9.3
5667	M	5860	124	187	0.1336	6450	105	139	0.1657	7.1	6810	114	83	0.1653	8.0
Mean		5506	116	198	0.1327	5963	112	193	0.1684	7.8	6306	113	144	0.1692	8.4
SD		388	6	90	0.0010	439	7	103	0.0106	2.4	445	6	80	0.0132	3.1
Formula-fed															
5627	M	4785	105	37	0.1354	5410	106	37	0.2118	15.5	5880	110	17	0.2027	15.4
5630	M	6560	118	263	0.1330	6940	111	244	0.1444	2.5	7330	120	169	0.1442	2.8
5631	F	5220	110	36	0.1328	5570	112	28	0.1580	4.7	5790	112	39	0.1608	5.4
5632	M	6020	132	274	0.1349	6310	124	320	0.1438	2.4	6700	117	217	0.1430	2.2
5633	F	4200	108	146	0.1345	4560	104	102	0.1564	3.3	4820	108		0.1572	3.8
5634	F	4760	104	201	0.1339	5320	92	149	0.1576	3.6	5710	106			
5636	F	4070	94	144	0.1347	4370	100	101	0.1508	2.3	4600	106	101	0.1526	2.8
5637	F	4640	106	67	0.1320	5030	107	126	0.1443	1.7	5230	106	69	0.1455	2.0
5638	M	5170	109		0.1354	5380	112	155	0.1472	2.5	5650	110	148	0.1438	1.9
5639	F	4960	106	157	0.1355	5360	114	180	0.1730	7.4	5640	118	163	0.1735	8.2
5640	M	6550	96	108	0.1352	7120	95	93	0.1574	5.0	7660	97	100	0.1578	5.5
5642	M	5690	112	113	0.1323	6110	108	72	0.1483	2.9	6580	114	67	0.1458	2.8
5643	F	5060	119		0.1352	5360	104	345	0.1386	0.8	5810	108	210		
5644	M	5230	102	110	0.1331	5580	112	167			5940	112	76	0.1612	5.6
5645	M	6010	90	89	0.1331	6630	90	143	0.1481	2.6	7010	101	69	0.1450	2.4
Mean**		5317	107	140	0.1341	5742	106	152	0.1573	4.4	6113	110	112	0.1585	5.2
S.D.		805	11	76	0.0011	841	10	99	0.0194	3.9	907	7	68	0.0174	3.9

* IR_0 refers to normalized $^{58}Fe/^{57}Fe$ isotope ratio at 56 d (baseline); IR_{14} refers to the ratio at 70 d, and IR_{28} at 84 d.

** Mean values and SD values exclude data for subjects 5637 and 5638, who had large drooling losses.

weight and length from 28 to 84 d of age were similar to gains in weight and length of a larger group of infants studied previously (11). (Means for birth weight and gains in weight and length of the formula-fed infants are nearly the same if calculated without the exclusion of subjects 5637 and 5638).

Although not statistically significant, mean concentrations of Hb and serum ferritin at ages 56, 70, and 84 d were somewhat less for formula-fed than for breast-fed infants. However, Hb concentrations less than 100 g/L in three formula-fed infants (subjects 5636, 5640, and 5645) were not associated with low concentrations of serum ferritin and therefore do not suggest the presence of iron deficiency.

Mean erythrocyte incorporation of the ^{58}Fe label 14 d after administration of the isotope (*i.e.* at 70 d of age) was 7.8% (SD 2.4) of the dose by breast-fed infants and 4.4% (SD 3.9%) of the dose by formula-fed infants. The difference was statistically significant ($p = 0.012$). Twenty-eight days after administration of the ^{58}Fe label (*i.e.* at 84 d of age), mean erythrocyte incorporation of the isotope was 8.4% (SD 3.1%) of the dose by breast-fed infants and 5.2% (SD 3.9%) of the dose by formula-fed infants. The difference was statistically significant ($p = 0.033$).

We also calculated percent incorporation of ^{58}Fe with intakes corrected for drooling losses. These calculations were done with and without subjects 5637 and 5638. With the inclusion of subjects 5637 and 5638, corrected mean incorporation at 70 d was 8.3% (SD 2.5%) by breast-fed and 4.4% (SD 3.6%) by formula-fed infants. The difference was statistically significant ($p < 0.002$). Excluding subjects 5637 and 5638, corrected mean incorporation at 70 d of age by formula-fed infants was 4.5% (SD 3.9%). For the reason mentioned previously, the true percentages for incorporation of the ^{58}Fe label 14 d after administration of the isotope are likely to lie closer to the uncorrected values (7.8% for the breast-fed infants and 4.4% for the formula-fed infants) than to the corrected values (8.3 and 4.5%, respectively). However, it is evident that corrected and uncorrected results are quite similar.

The relation of percent erythrocyte incorporation of the ^{58}Fe label (uncorrected) at 70 d of age to plasma ferritin concentra-

tion (average of 56- and 70-d values) is presented in Figure 1 for the 25 subjects. Subject 5661 was excluded because there was no serum ferritin value and subject 5644 was excluded because erythrocyte incorporation of ^{58}Fe at 70 d of age was not determined. It is evident that the slopes of the regressions were similar for the two feeding groups. The correlation of ^{58}Fe erythrocyte incorporation on plasma ferritin concentration was statistically significant for breast-fed infants ($r = 0.488$, $p = 0.008$) but not for formula-fed infants ($r = 0.290$, $p = 0.071$). Adjusting ^{58}Fe incorporation for ferritin concentration by covariance analysis, the difference in incorporation between breast-fed and formula-fed infants was highly significant ($p < 0.001$).

Iron nutritional status and estimated iron intake. At 56 d of age, mean Hb concentration of the breast-fed infants was significantly greater than that of the formula-fed infants ($p = 0.03$). At 70 and 84 d of age the differences were not significant ($p = 0.055$ and 0.22, respectively). Mean plasma ferritin concentrations of the breast-fed infants were somewhat greater than those of the formula-fed infants, but the differences were not significant at 56 d of age ($p = 0.10$), nor at ages 70 and 84 d ($p = 0.34$ and 0.30, respectively). Concentrations of Hb and plasma ferritin were similar whether or not data on subjects 5637 and 5638 were included.

Weighing back of the bottles of the vitamin-iron supplement indicated that mean intake of iron from this source was 5.9 mg/d (range 2.7–8.1 mg/d) by the 14 breast-fed infants and 6.6 mg/d (range 1.2–9.7 mg/d) by the 13 formula-fed infants. Assuming an iron concentration of 0.45 mg/L for human milk and average milk consumption of 0.7 L/d, dietary iron intake before 56 d of age may have been about 0.3 mg/d. Mean total intake of iron by the breast-fed infants was therefore about 6.2 mg/d. Dietary iron intake by the formula-fed infants (from iron concentration of the formulas and the amount consumed, as determined by weighing of the formula bottles) between 28 and 56 d of age was 3.0 mg/d (range 2.2 to 3.9 mg/d). Thus, mean total iron intake by the formula-fed infants was about 9.6 mg/d (range 4.2 to 13.0). The greater availability of iron to the breast-fed infants (as indicated by greater erythrocyte incorporation) apparently more than compensated for their lower iron intake, leading to more satisfactory iron nutritional status of the breast-fed than of the formula-fed infants.

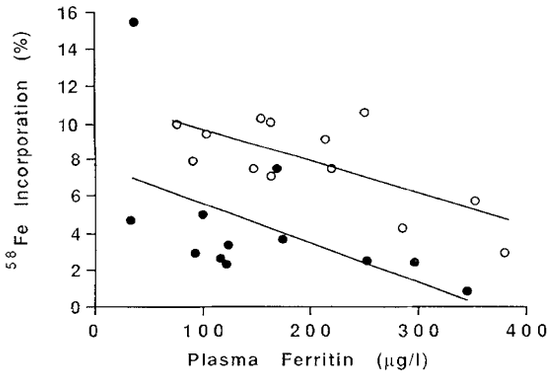

Figure 1. Relation between percent ^{58}Fe incorporation 14 d after administration of ^{58}Fe, and plasma ferritin concentration (average of 56 d and 70 d values) in breast-fed (○) and formula-fed (●) infants. The regression lines are indicated for breast-fed ($y = 11.40 - 0.01758 \cdot x$) and formula-fed ($y = 7.71 - 0.02121 \cdot x$) infants.

DISCUSSION

In an effort to obtain information about absorption of iron by normal infants during the early months of life, we determined erythrocyte incorporation of the stable iron isotope, ^{58}Fe, administered in the form of a vitamin-iron supplement to 56-d-old infants. The ^{58}Fe-labeled vitamin-iron supplement was given 3 h after the previous feeding to breast-fed infants and to infants fed a milk-based formula low in iron (3–4 mg Fe/L). For at least 28 d before administration of the ^{58}Fe-labeled supplement, all parents were requested to give their infants approximately 7.5 mg/d of iron in the form of ferrous sulfate. With few exceptions, compliance with the instructions appeared to be good.

Fourteen days after administration of the supplement, mean erythrocyte incorporation of ^{58}Fe by breast-fed infants was 7.8% of the dose when uncorrected for drooling losses and 8.3% of the dose when corrected for drooling losses. With mean iron intake from the ^{58}Fe-labeled supplement of 7.99 mg for the breast-fed infants, erythrocyte incorporation of 7.8% of the dose corresponded to 0.62 mg. Even if one ignores the quantity of iron that is presumably absorbed but not promptly incorporated into erythrocytes, the amount incorporated into erythrocytes (0.62 mg) is in the range of 0.55 to 0.75 mg/d estimated to be the infant's mean requirement for absorbed iron (12). Thus, at least by 2 mo of age, administration of about 7.5 mg of supplemental iron to breast-fed infants can meet the requirement for absorbed iron.

Erythrocyte incorporation of the ^{58}Fe label by formula-fed infants was significantly less than that by breast-fed infants. Fourteen days after administration of the supplement, mean erythrocyte incorporation of ^{58}Fe by formula-fed infants was 4.4% of the dose when uncorrected for drooling losses and 4.5% of the dose when corrected for drooling losses. The present findings therefore confirm our previous findings (5) that incorporation of iron by breast-fed infants is greater than by formula-fed infants even when the dose is given 3 h after a feeding of milk or formula. The lesser extent of erythrocyte incorporation of iron by formula-fed infants presumably reflects difference in extent of absorption, resulting primarily from the presence of greater quantities of known inhibitors of iron absorption (calcium and bovine milk proteins) (13–20) in the intestinal tracts of the formula-fed infants.

For each feeding group, erythrocyte incorporation of iron was inversely correlated with plasma ferritin concentration. Nevertheless, although plasma ferritin concentration of the breast-fed infants was greater than that of formula-fed infants, erythrocyte incorporation of iron was greater by the breast-fed infants. Thus, to the extent that plasma ferritin concentration reflects iron stores and erythrocyte incorporation of iron reflects iron absorption, factors inhibiting iron absorption appeared to be more important than iron stores in determining the extent of absorption.

The percentage erythrocyte incorporation of iron from the ^{58}Fe-labeled vitamin-iron supplement providing approximately

8 mg of iron was less than from the small test dose (approximately 0.6 mg of iron) used in the previous study (7.8 *versus* 20.0% for breast-fed infants, and 4.4 *versus* 6.9% for formula-fed infants). This finding was anticipated because of the previously noted inverse relation between quantity of iron ingested and percentage absorption (21) or percentage erythrocyte incorporation (22) of iron.

It is generally assumed that in normal and iron-deficient adults in the absence of bone marrow failure, ineffective erythropoiesis, aplastic anemia, hemolytic anemia or iron overload, 80% (23) or 90% (20) of an absorbed iron isotope is incorporated into erythrocytes in 14 d. Few data are available concerning the extent of erythrocyte incorporation of absorbed iron by infants. Studies in which radioiron was administered i.v. to infants of various ages (24) indicated that administration to infants from 2 to 8 wk of age generally resulted in less than 80% erythrocyte incorporation of the iron isotope. Moreover, sequential studies of reticulocyte counts and erythropoietic activity of the bone marrow indicated that erythropoiesis in normal term infants falls to low levels by 9 d of age, has begun to increase by 29 d of age, and reaches relatively high levels by 59 d of age (25). It therefore seems likely that from 2 wk until nearly 8 wk of age, erythrocyte incorporation of an administered dose of iron will be less than was observed in the 8-wk-old infants in the present study. Whether absorption of iron as well as erythrocyte incorporation of iron is less before 8 wk of age, a period of relatively low hematopoietic activity, is unknown.

We conclude that administration of an iron supplement to breast-fed infants during the early months of life will appreciably augment body iron stores and decrease the likelihood that iron deficiency will develop later in the first year. A detailed consideration of the arguments for and against administration of iron to breast-fed infants before 4 mo of age is beyond the scope of the present communication.

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