Erythrocyte Incorporation of Ingested ^{58}Fe by 56-Day-Old Breast-Fed and Formula-Fed Infants

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ABSTRACT. As an index of iron absorption, erythrocyte incorporation of the stable isotope, ^{58}Fe , was determined 14 and 42 d after the administration of ^{58}Fe -labeled ferrous sulfate to 56-d-old exclusively breast-fed infants and to infants fed a low-iron (1.8 mg/L) formula. Each infant received a dose of 0.6 to 1.0 mg of iron with ascorbic acid between feedings on each of 3 consecutive d. Fourteen d after administration of the ^{58}Fe -labeled iron doses (age 70 d), arithmetic mean incorporation of the ^{58}Fe label into erythrocytes by breast-fed infants was 20.0% of the dose—only slightly less than that reported for erythrocyte incorporation of iron by fasting adults fed small quantities of ^{59}Fe -labeled human milk. Erythrocyte incorporation of iron by the formula-fed infants was 6.9% of the dose, suggesting that, even between feedings, components of infant formula exerted a major inhibitory effect on iron absorption. For reasons that are not clear, the percentage of the administered label incorporated into erythrocytes was significantly greater in breast-fed (but not in formula-fed infants) at 112 d of age than at 70 d of age. (*Pediatr Res* 33: 573–576, 1993)

Abbreviations

MIR_{58/57}, mass isotope ratio $^{58}\text{Fe}/^{57}\text{Fe}$

The body iron content at term birth of most infants is sufficient to support hematopoiesis until about 4 mo of age. Administration of iron before 4 mo of age might enhance iron stores and therefore decrease the quantity of absorbed iron required subsequently. Few physicians recommend iron supplements for breast-fed infants during the early months of life, and many physicians prefer low-iron formulas to "iron-fortified formulas," at least during the early months of life. The reasons that many physicians prefer to avoid iron supplementation during early infancy are poorly defined. Fear of adverse reactions is often cited, but two studies have failed to demonstrate a relation between iron administration and adverse reactions (1, 2).

If the extent of iron absorption is seriously limited during the early months of life, there would be little point in recommending use of iron supplements or iron-fortified formulas until an age at which iron could be shown to be reasonably well absorbed. The extent of iron absorption during the first few months of life is therefore an important bit of information that enters into the recommendation about iron supplementation of infants.

To obtain such information, we have determined the extent of erythrocyte incorporation of the stable isotope, ^{58}Fe , administered between feedings to normal 56-d-old breast-fed and formula-fed infants. Although the quantity of administered iron

promptly (*i.e.* within 2 wk) incorporated into erythrocytes is generally somewhat less than the amount absorbed, incorporation of an iron isotope into erythrocytes has been widely used as a index of the extent of iron absorption by adults and by term (3–6) and preterm (7, 8) infants.

SUBJECTS AND METHODS

The protocol for the study was reviewed and approved by the University of Iowa Committee on Research Involving Human Subjects. Informed consent was obtained from at least one parent of each infant.

Subjects, feedings, and administration of ^{58}Fe -enriched ferrous sulfate. Fourteen normal term breast-fed infants (seven males and seven females) and 20 formula-fed infants (10 males and 10 females), were studied. Gestational ages were 38 wk or more, and birth weights were 2685 to 4055 g. The infants were enrolled at 6 to 9 d of age, and until at least 62 d of age were either exclusively breast-fed with a daily supplement of vitamins A, C, and D and fluoride (Mead Johnson Nutritionals, Evansville, IN) or were fed a milk-based formula (Similac, Ross Laboratories) with a low iron concentration. The formula was from a single lot. Concentration of iron was determined by atomic absorption spectrophotometry in duplicate with three cans of formula from different cases. Concentrations ranged from 1.8 to 1.9 mg/L with a mean value of 1.8 mg/L.

For 3 consecutive d beginning at 56 d of age (± 4 d), each infant visited the Lora N. Thomas Pediatric Metabolic Unit of the University of Iowa and was given a dose of ^{58}Fe -enriched ferrous sulfate. Formula-fed infants were more readily recruited for the study than were breast-fed infants, and all of the formula-fed infants were given the test doses from the same batch of ^{58}Fe -enriched ferrous sulfate, providing 0.5 mg of ^{58}Fe in a total of 0.6 mg of iron in each test dose. Some of the breast-fed infants were given iron from this batch, whereas other breast-fed infants were given iron from other batches in which the ^{58}Fe enrichment was different. The breast-fed infants received 0.5 to 0.6 mg of ^{58}Fe and a total quantity of iron in each test dose of 0.6 to 1.0 mg.

For all the infants, each dose consisted of a solution of ^{58}Fe -enriched ferrous sulfate in 1.9 mL of a 10% solution of glucose in water with 10 mg of ascorbic acid. The solution was drawn up into a 5-mL syringe, weighed, and delivered from the syringe directly into the infant's mouth. This dose was 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 dose of iron was given between 0900 and 1300 h. Feedings were not permitted for 2 h before or for 1 h after ingestion of the iron dose. The infants were closely observed to detect regurgitation during the hour after ingestion of the dose.

At approximately 62 d of age (*i.e.* 4 d after the third test dose of iron had been administered), the parents of the breast-fed infants were instructed to give a daily supplement of 7.5 mg of iron in the form of ferrous sulfate (Fer-in-Sol, Mead Johnson, Evansville, IN) and were permitted to feed iron-fortified formula

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(Similac with Iron, Ross Laboratories) as a supplement to breast feeding. No effort was made to ensure compliance with the instructions to give the daily supplement of iron. At approximately 62 d of age, the low-iron formula previously fed to the formula-fed infants was changed to an iron-fortified formula (Similac with Iron), providing 12 mg of iron/L (label claim) in the form of ferrous sulfate. Women who elected to discontinue breast feeding fed their infants the iron-fortified formula.

Laboratory analyses. Venous or capillary blood was obtained at ages 56, 70, and 112 d of age (± 4 d). The sample at 56 d of age was obtained before administration of the first of the three doses of ^{58}Fe . Blood was analyzed for Hb concentration by Coulter Counter, model M430 (Coulter Electronics, Inc., Hialeah, FL), for ferritin concentration by RIA using the Rainen Ferritin [^{125}I] RIA Kit, (catalog no. NEA-078, E.I. DuPont de Nemours and Co., New England Nuclear Research Products, Boston, MA), and for the mass isotope ratio, $^{58}\text{Fe}/^{57}\text{Fe}$ (MIR_{58/57}), by inductively coupled plasma mass spectrometry as described previously (9).

Calculations. The quantity of ^{58}Fe label incorporated into erythrocytes at ages 70 and 112 d was calculated as described previously (6). Statistical analyses were performed using SAS version 5.18 (SAS Institute Inc., Cary, NC).

RESULTS

No infant was observed to regurgitate during the hour after administration of the test dose of ^{58}Fe . Data on body weight, Hb concentration, serum ferritin concentration, and MIR_{58/57} are presented for ages 56, 70, and 112 d in Table 1. Also included in the table is the percentage of the administered ^{58}Fe label that was incorporated into erythrocytes at 70 and 112 d of age.

Hb concentrations were in the anticipated range for infants from 2 to 4 mo of age. Serum concentrations of ferritin of most of the infants were relatively high, as has been observed previously (5, 10).

As calculated from the increase in MIR_{58/57} in blood, the arithmetic mean incorporation into erythrocytes of the ^{58}Fe label in breast-fed infants was 20.0% of the dose at 70 d of age and 22.7% of the dose at 112 d of age (geometric means, 16.0 and 19.1%, respectively). In the 12 infants for whom a value for MIR_{58/57} was obtained both at 70 d and at 112 d of age, the difference in incorporation at 70 and 112 d of age was significant (paired *t* test, $p < 0.001$).

The arithmetic mean incorporation into erythrocytes of the ^{58}Fe label in formula-fed infants was 6.9% of the dose at 70 d of age and 6.9% of the dose at 112 d of age (geometric means, 6.0 and 6.3%, respectively). Whether calculated for 70 d or for 112 d of age, incorporation of the ^{58}Fe tag into erythrocytes was significantly less for formula-fed infants than for breast-fed infants (*t* test, $p < 0.001$).

The difference in erythrocyte incorporation of the ^{58}Fe label was also examined in relation to the serum concentration of ferritin. As was to be anticipated with infants from 56 to 112 d of age, many of the serum concentrations of ferritin were quite high. The geometric mean serum ferritin concentration at 56 d of age was 152 $\mu\text{g/L}$ in the breast-fed infants and 125 $\mu\text{g/L}$ in the formula-fed infants. The difference was not significant (*t* test, $p = 0.58$). Corresponding concentrations at 70 d of age were 187 and 143 $\mu\text{g/L}$ (*t* test, $p = 0.45$). Serum concentrations of ferritin were determined in 31 of the 36 infants at 56 and/or 70 d of age. Based on the concentration of ferritin at 56 or 70 d of age or the mean of the two values when both were available, the relation between percentage of incorporation of the ^{58}Fe label into erythrocytes at 70 d of age was inversely correlated with serum ferritin concentration (partial correlation analysis adjusted for type of feeding, $r = -0.61$, $p < 0.001$). Significant inverse correlation of serum ferritin and erythrocyte incorporation of iron was also observed in our previous studies of 126- (5) and 154-d-old (6) infants.

The difference in incorporation of the ^{58}Fe label at 70 and 112 d of age was significantly greater by breast-fed than by formula-fed infants (analysis of covariance with serum ferritin concentration as the covariate, $p < 0.001$).

DISCUSSION

When a quite small dose of iron (0.6 to 1.0 mg) accompanied by a relatively large dose of an enhancer of iron absorption (10 mg of ascorbic acid) was administered between feedings to 56-d-old, exclusively breast-fed infants, arithmetic mean incorporation of the ^{58}Fe label into erythrocytes 14 d after administration of the iron label was 20.0% of the dose (geometric mean 16.0%). The arithmetic mean value for erythrocyte incorporation of the stable iron isotope by 56-d-old breast-fed infants may be compared with results obtained with fasting adult subjects given small quantities of human milk (86 to 100 mL) tagged with the radioisotope, ^{59}Fe . In three studies, arithmetic mean values for erythrocyte incorporation of the radioisotope were 20.8% of intake for 10 subjects (11), 15.4% of intake for eight subjects (12), and 23.7% of intake for five subjects (13).

In normal adults, a high percentage of an orally administered iron isotope is promptly incorporated into erythrocytes (14), and it is generally assumed that 80% or more of absorbed iron is incorporated into erythrocytes within 14 d (15). The percentage of absorbed iron promptly incorporated into erythrocytes is probably less in 56-d-old infants than in adults. Garby *et al.* (16) administered ^{59}Fe i.v. to infants of various ages. Incorporation of the ^{59}Fe label into erythrocytes 12 to 14 d after administration of the radioiron was (judging from graphic data) 30 to 50% of the dose for three infants 25 to 33 d of age, 65% for an infant 65 d of age, 85% for three infants from 81 to 129 d of age, and 70 to 90% for three infants from 223 to 273 d of age. If, in our study, incorporation of iron into Hb was 65% of the amount absorbed, arithmetic mean absorption (calculated from the MIR_{58/57} at 70 d of age) was 30.8% of the dose by the breast-fed infants and 10.6% of the dose by the formula-fed infants.

Previous studies of the erythrocyte incorporation of an iron isotope by breast-fed infants have not been reported. However, by whole-body counting for the radioisotope, ^{59}Fe , 2 wk after its oral administration, data on *absorption* of iron from human milk has been studied in 5.9- to 7.3-mo-old breast-fed infants (17) and in adult subjects fed human milk (18). In breast-fed infants (17), arithmetic mean absorption of iron was 38.1% of the dose when the tracer was given with a feeding and 48.8% of the dose when the tracer was given 3 h after a feeding. Thus, results obtained by whole-body counting after administration of small doses of ^{59}Fe iron to 6- to 7-mo-old breast-fed infants are in quite good agreement with results obtained by determining erythrocyte incorporation of ^{58}Fe by 2-mo-old breast-fed infants.

When fasting adult subjects were fed 200 mL of human milk labeled with ^{59}Fe , mean absorption of iron was 37.3% of intake by four males and five females in one portion of a study and 32.9% of intake by nine females in another portion of a study (18). These values are not remarkably different from those reported by Saarinen *et al.* (17) for 6- to 7-mo-old breast-fed infants.

In our study, arithmetic mean erythrocyte incorporation of the ^{58}Fe label given with a small dose of iron 2 h after a formula feeding was 6.9% of intake. Assuming that, at 56 d of age, 65% of absorbed iron is promptly incorporated into erythrocytes, iron absorption was 10.6% of intake. This estimate of absorption is less than the 19.5% of the dose reported by Saarinen *et al.* (17) to be absorbed by 6- to 7-mo-old infants given a small dose of ^{59}Fe 3 h after a formula feeding. However, 6- to 7-mo-old infants would be expected to empty the stomach more rapidly than 2-mo-old infants (19), and more of the formula feeding would be expected to have left the stomach in 3 h than in 2 h. The extent of stomach emptying is an important consideration because cow

Table 1. Erythrocyte incorporation of iron from test feedings

Table 1. Erythropoietic incorporation of iron from test feedings																
Subject	Sex	Iron test dose (μg/ dose)	56 d				70 d					112 d				
			Wt (g)	Hb (g/L)	Ferritin (μg/L)	MIR _{58/57}	Wt (g)	Hb (g/L)	Ferritin (μg/L)	MIR _{58/57}	% Incorp*	Wt (g)	Hb (g/L)	Ferritin (μg/L)	MIR _{58/57}	% Incorp
Breast-fed																
4095	F	608	4275	119	103	0.1463	4440	112	430	0.2014	8.7	5250	110		0.2032	10.5
4388	M	1007	5185	122	22	0.1462	5420	120	30	0.3420	34.2	6050	114	10	0.3513	38.0
4391	F	767	5010	112	274	0.1464	5300	100		0.2159	9.4	6000	102	232	0.2264	12.5
5001	M	608	6635	108	145	0.1473	7075	101		0.2643	26.9	7825	111		0.2631	32.4
5006	F	604	5010	114		0.1484	5270	106		0.2192	13.1	6195	115	135	0.2214	17.3
5008	M	604	5640				5905	117	1000	0.2639	25.9	6665	120	620	0.2523	27.0
5010	F	1010	4815			0.1463	4890				5785	114	39		0.2044	10.2
5012	M	1004	6165	103	135	0.1462	6655	110	106	0.3204	34.1	7870	111		0.2966	35.1
5014	M	987	4555	117	401	0.1473	4865	118	218	0.1931	7.3	5505	123	84	0.1914	8.3
5015	M	739	6395				7010	104	69	0.3798	45.8	8290	112	19	0.3511	51.2
5016	F	738	4920	135		0.1466	5225	122	148	0.2118	11.3	5860	98	154	0.2206	11.5
5019	M	781	5510	114	632	0.1496	5875	113	484	0.1726	4.5	6910	113			
5209	F	603	4850	116	72	0.1462	5120	120		0.3008	28.0	5790	108		0.3127	30.7
5211	F	617	5430	140	196	0.1458	5730	137		0.1926	10.5	6455	126		0.1937	11.1
Mean		778	5291	118	220	0.1469	5606	114	311	0.2521	20.0	6447	113	162	0.2529	22.7
SD		174	675	11	192	0.0011	781	10	324	0.0656	13.1	909	7	200	0.0576	13.8
Formula-fed																
4083	F	616	5600	112		0.1444	6005	113		0.1657	4.5	6975	122		0.1595	4.0
4084	M	600	5485	109		0.1491	6000	112	365	0.1564	2.4	6850	112	57	0.1661	5.2
4085	F	603	4975	103		0.1418	5190	120		0.1627	3.1	6090	120	290	0.1604	3.1
4086	M	605	4510	120		0.1476	5300	130	40	0.2289	18.7					
4087	M	611	5505	111	210	0.1476	5975	116	275	0.1630	3.9	6975	121	87	0.1576	3.3
4088	M	595	5790	107		0.1470	5975	111		0.1881	9.1	7735	114	39	0.1741	8.1
4089	M	604	5645	101	69	0.1470	6005	104	113	0.2075	11.8	7630			0.1868	
4090	M	605	5900	125	260	0.1459	6450	120	115	0.1697	6.3	7545	122	76	0.1654	6.2
4091	M	606	6015	107	113	0.1453	6280	106	105	0.1845	8.1	6810	113	53	0.1786	7.9
4093	M	620	5775				6285	104	167	0.1580	2.6	7995			0.1569	
4094	M	607	4645	109	150	0.1439	5280	111	118	0.2020	11.7	6415	121		0.1888	12.1
4096	F	608	5400			0.1446	5800	106	117	0.1660	3.9	7565	119		0.1596	3.8
4097	F	614	4605	101		0.1430	4920	101	273	0.1752	4.8	5905	118		0.1665	4.8
4098	M	617	5605	107	108	0.1470	5980		192	0.1992		7015	121		0.1910	11.6
4099	F	608	5125	127	95	0.1449	5425	118	215	0.1826	7.6	6400	133		0.1838	10.4
5000	F	611	4830	109		0.1438	5235	111	138	0.1863	7.5	6000	115		0.1847	8.6
5003	F	597	4800	108	70	0.1459	5005	111	108	0.1723	4.9	5900	110		0.1696	5.2
5004	F	603	4585	106	108	0.1433	4885	103	60	0.1848	6.5	5975	113		0.1788	7.4
5005	F	609	4965	117	192	0.1482	5305	116		0.1868	7.8	6265	125		0.1827	9.0
5007	F	619	6410			0.1483	7000	122	240	0.1673	5.6	8200	120	50	0.1658	6.0
Mean		608	5309	111	138	0.1457	5715	112	165	0.1803	6.9	6855	119	107	0.1725	6.9
SD		7	549	8	64	0.0021	573	8	88	0.0186	4.0	752	6	88	0.0115	2.8

* % Incorp, percentage of ^{58}Fe label incorporated into erythrocytes.

milk contains calcium, caseins, and whey proteins that are potent inhibitors of iron absorption (20–28).

The greater incorporation of iron into erythrocytes by 2-mo-old breast-fed than by 2-mo-old formula-fed infants in our study, and, in the study by Saarinen *et al.* (17), the greater iron absorption by 6- to 7-mo-old breast-fed infants than by formula-fed infants of similar age are probably explained primarily by the lesser quantities of inhibitors of iron absorption in human milk than in infant formulas.

In the breast-fed infants, but not in the formula-fed infants, the amount of the administered ^{58}Fe label incorporated into erythrocytes was significantly greater 56 d after the test feedings (112 d of age) than 14 d after the test feedings (70 d of age). Thus, at least in the breast-fed infants, some ^{58}Fe label was apparently stored and subsequently withdrawn from stores for incorporation into newly formed erythrocytes.

Although it is generally believed that little erythropoiesis occurs during the first 2 mo of life, arithmetic mean erythrocyte incorporation of iron 14 d after administration of the ^{58}Fe label was 22.7% of the dose in the breast-fed infants and 6.9% of the dose

in the formula-fed infants. Geometric mean erythrocyte incorporation of the administered ^{58}Fe (6.9% of intake) in the 2-mo-old infants did not differ greatly from the geometric mean value of 7.9% of intake by 126-d-old formula-fed infants studied previously (5). However, only nine infants were included in the earlier study, and the quantity of iron given in the test doses was 1.95 mg rather than the 0.6 to 1.0 mg given in the present study.

Based on the extent of erythrocyte incorporation of small doses of ^{58}Fe , the data presented in this report do not support the widely held belief that iron absorption and utilization are extremely low during the first 2 mo of life.

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