

Erythrocyte incorporation of iron by infants: iron bioavailability from a low-iron infant formula and an evaluation of the usefulness of correcting erythrocyte incorporation values, using a reference dose or plasma ferritin concentrations

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Bioavailability of iron (Fe) from a low-Fe infant formula was determined by erythrocyte incorporation of ⁵⁸Fe 14 d after administration in ten healthy, non-Fe-deficient infants. Two feeding protocols were compared, with each infant acting as his/her own control. At 140 and 154 d of age, infants were fed 1000 g of ⁵⁸Fe-labelled formula (1.44 mg total Fe/1000 g) as six feeds over 24 h (Protocol A) or as two feeds/day on three consecutive days (Protocol B). A water solution with ⁵⁷Fe and ascorbic acid was given separately as a reference dose in both study protocols. Erythrocyte incorporation of ⁵⁸Fe and ⁵⁷Fe was determined by thermal ionisation mass spectrometry. Geometric mean ⁵⁸Fe incorporation was 7.6 % (range 3.3–13.5 %) with Protocol A as compared to 10.6 % (range 6.7–18.6 %) with Protocol B ($P = 0.05$); paired t test. Inter-individual variability of ⁵⁸Fe was not reduced by correcting for the incorporation of ⁵⁷Fe from the reference dose, or by correcting for plasma ferritin concentration. Fractional erythrocyte incorporation of Fe from low-Fe infant formula was in the same range as our earlier published data on erythrocyte incorporation of Fe from human milk extrinsically labelled with ⁵⁸Fe (Davidsson *et al.* 1994a). The methodological evaluations included in this study clearly indicate the importance of using standardised study protocols when evaluating Fe bioavailability in infants. Corrections of erythrocyte incorporation data based on plasma ferritin or erythrocyte incorporation of Fe from a reference dose were not found to be useful.

Iron: Infants: Infant formula: Stable isotopes

Iron (Fe) deficiency is a major public health problem during early life, in particular in developing countries but also in infants and young children in industrialised countries (Innis *et al.* 1997, Looker *et al.* 1997). At birth, healthy term infants are endowed with substantial amounts of Fe, predominantly in haemoglobin, but also as tissue and storage Fe (Dallman, 1992). During the first months of life, body Fe present at birth, together with absorbed dietary Fe, are used to support the rapid expansion of the red blood cell mass as the infant grows. Dietary intake of Fe during the first months of life varies considerably, depending on feeding practices. Exclusive breast-feeding is strongly recommended during the first 4–6 months of life for infants in both developing and developed countries; however, human milk contains little Fe (Siimes *et al.*

1979, Lönnerdal, 1984). Depending on socio-economic factors, availability and local feeding practices, infants who are not breast-fed, or are partially breast-fed, are fed infant formulas either industrially produced or home made. Most commercial infant formulas are fortified with Fe, although Fe concentrations vary widely between countries. For example, Fe concentration in European infant formulas are typically in the range 4 to 7 mg/l while in the USA Fe-fortified infant formulas contain about 12 mg Fe/l. In addition, although Fe-fortified infant formulas are recommended by the US Academy of Pediatrics, commercial infant formulas with low Fe concentration (about 1.1–4.5 mg/l) are commonly used in the USA (Committee on Nutrition, American Academy of Pediatrics, 1999).

Only very limited information is available on the

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bioavailability of Fe to infants from formulas with different Fe concentrations and no evaluation of the efficacy of low-Fe formula to supply Fe to infants has been reported. Therefore, the present study was designed to investigate Fe bioavailability from low-Fe infant formula in healthy infants, using a stable isotope technique based on erythrocyte incorporation of ^{58}Fe 14 d after intake of labelled test meals. In addition, an evaluation of the mode of administration of labelled test meals on erythrocyte incorporation of ^{58}Fe was included in the study. The third objective of the study was to investigate the usefulness of correcting erythrocyte incorporation values in infants using either the erythrocyte incorporation of a separately administered reference dose of ^{57}Fe , or using plasma ferritin values. In adult Fe absorption studies, corrections using ferritin have been found more useful in reducing the inter-individual variation in Fe bioavailability than corrections based on Fe absorption from a reference dose (Cook *et al.* 1991).

Materials and methods

Infant formulas

A whey-predominant infant formula with low Fe content (Study Formula) was produced especially for the study according to the specifications for a commercial infant formula (Nan[®], Nestlé S.A., Vevey, Switzerland) except for the Fe content. Ferrous sulphate was added to a final concentration of 1.55 mg Fe/l during manufacture (Nestlé Food Company, Eau Claire, WI, USA). All other nutrients met the requirements specified by the Food and Drug Administration (1985). Vitamins and minerals were purchased separately from Fortitech Inc. (Schenectady, NY, USA) and added during manufacture according to the quantities normally added to the commercial product.

A separate batch of formula was produced for the administration of stable isotopes with identical composition as the Study Formula but without added ferrous sulphate or ascorbic acid. After addition of the ^{58}Fe label (see 'Formula labelling'), the total Fe content was 1.44 mg Fe/1000 g formula.

Both batches of formula were produced as 'Ready-to-Feed' liquid formula and filled aseptically into 960 ml cans. The nutritional composition of both products was analysed and the microbiological safety of the formulas was ensured before release from the factory.

Infants and feeding regimen

Ten term infants (four girls, six boys) were enrolled within 4 d of age 112 d. Infants were fed Fe-fortified infant formulas (12 mg Fe/l) from birth. All infants were apparently healthy, with no known diseases or disorders. From enrolment at 112 d until completion of the study, infants were fed (on demand) the specially prepared Study Formula with Fe content of 1.55 mg Fe/l. After 140 d of age, infants were permitted limited amounts of complementary foods such as fruit and vegetable purées. Fe-fortified cereals were not allowed and Fe supplements were

not given during the entire study. No solids were permitted during the days when ^{58}Fe -labelled infant formula was fed.

Study design

Using a crossover design, each infant was studied twice with the first study performed within 4 d of age 140 d and the second study within 4 d of age 154 d. Each study consisted of the administration of a precisely known amount of ^{58}Fe -sulphate in 1000 g infant formula which was given over approximately 24 h (Protocol A) or in equal portions on each of three consecutive days (Protocol B). Three hours after completing the ingestion of ^{58}Fe -labelled infant formula, the infants received a small volume of an aqueous solution of ^{57}Fe -sulphate containing ascorbic acid (reference dose) under standardised conditions. Five infants started with Protocol A while the other five infants started with Protocol B. The order of study protocols was assigned on an alternating basis. A total of three capillary blood samples were obtained by heel-stick using disposable blades (Tenderfoot, International Technidyne Corp., Edison, NJ, USA) and collected in heparinised Microvette tubes (CB 1000 S, Sarstedt, Newton, NC, USA). The first sample was drawn immediately before ingestion of ^{58}Fe -labelled infant formula and the second and third samples 14 d after the start of ^{58}Fe ingestion in Protocols A and B respectively. The first enriched blood sample (drawn on day 14) was used as the baseline value for the second part of the study. Blood samples were analysed for Fe status indices and stable isotope enrichment.

Stable isotopes

Stable isotopes of Fe with high enrichments of ^{58}Fe (>91 % ^{58}Fe ; Isotec, St. Quentin, France) and ^{57}Fe (>95 % ^{57}Fe ; Medgenix, Ratingen, Germany) were purchased as metals and converted to $^{57}\text{FeSO}_4$ and $^{58}\text{FeSO}_4$ by dissolution in H_2SO_4 (0.5 mol/l) according to Kastenmayer *et al.* (1994). Individual portions of isotope solutions containing ^{58}Fe (approximately 1.18 mg) or ^{57}Fe (approximately 3.00 mg) were prepared by precisely weighing aliquots into acid-washed Reacti vials (Pierce, Rockford, IL, USA). The vials were purged with N_2 and kept refrigerated until used. Isotopic composition of the stable isotope solutions was determined by thermal ionisation mass spectrometry (TIMS; Kastenmayer *et al.* 1994). The exact amount of stable isotope administered to the infants was used in the calculation of fractional Fe incorporation into erythrocytes.

Formula labelling

Labelled formula was prepared during the 24 h immediately preceding administration by adding the contents of one vial of $^{58}\text{FeSO}_4$ to 1000 g infant formula without added Fe. Ascorbic acid (75 mg as sodium ascorbate, Fortitech Inc.) was added at the same time. The formula was stirred gently for 24 h at 4°C to ensure equilibration between added isotope and native Fe according to the method described earlier (Davidsson *et al.* 1994a). The final Fe content was 1.44 mg Fe/1000 g formula. Equal portions of

the labelled formula (approximately 167 g each) were weighed into six pre-weighed feeding bottles and kept refrigerated until used.

Administration of labelled formula

In Protocol A, infants were fed the first two to three bottles of labelled formula by a research nurse at the Lora N. Thomas Metabolism Ward, Department of Pediatrics, University of Iowa, Iowa City, IA, USA. The remaining labelled formula was fed by the parents at home. Parents were instructed orally and in writing about the procedure for administration of labelled formula. The next morning the infant returned to the Metabolism Ward and, if there was still labelled formula left, it was fed. The exact quantity of labelled formula ingested was calculated by weighing feeding bottles after feeding. Pre-weighed bibs and wipes were used to quantify losses of labelled formula during and after feeding. Three hours after the last feeding of labelled formula, the reference dose of ^{57}Fe was given by a research nurse.

During protocol B, the infants were fed two bottles of labelled formula on each of three consecutive days in the Metabolism Ward. Research nurses administered all labelled formula during Protocol B. The reference dose was given 3 h after the consumption of the last bottle of labelled formula on day 3. Procedures were otherwise as during Protocol A.

Reference dose

To the precisely weighed content of one vial of $^{57}\text{FeSO}_4$ solution were added sodium ascorbate (123 mg) and sucrose (630 mg) and the volume was brought to 5 ml with deionised water (Davidsson *et al.* 1994a). The solution was given directly into the mouth of the infant by using a plastic syringe. No food or fluid was given for 1 h after ingestion of the reference dose.

Analysis

Haemoglobin was determined using a Coulter Counter (Model M430, Coulter Electronics Inc., Hialeah, FL, USA). Plasma was separated from blood cells within 30 min of collection and stored at -20°C until ferritin was analysed by radioimmunoassay using the Quantimune kit (catalogue no. 190-2001, Bio-Rad Laboratories, Hercules, CA, USA).

Infant formulas were analysed for Fe content by atomic absorption spectrometry (Varian Techtron, model 975, Mulgrave, Australia). Duplicate samples of freeze-dried formulas were ashed in silica Ehrlenmeyer flasks in a muffle furnace at 510°C for 48 h. Ash was dissolved in concentrated HCl and diluted to 25 ml with ultra-pure water. Fe was measured by the standard addition technique (Van de Castele & Block, 1993). A reference material; Wheat Flour 1567a (National Institute of Standards and Technology, Gaithersburg, MD, USA) was analysed together with the formulas. The analysed value ($13.8 \pm 0.6 \mu\text{g/g}$) was found to be within the certified range for Fe ($14.1 \pm 0.5 \mu\text{g/g}$). The nutritional composition of the two

formulas was analysed by standard techniques in a quality control laboratory (Nestlé Food Company).

Thermal ionisation mass spectrometry was used for measurements of isotopic ratios in whole blood samples according to the method described in detail earlier (Kastenmayer *et al.* 1994).

Calculations and statistical methods

The quantity of ^{58}Fe and ^{57}Fe incorporated into erythrocytes was calculated as previously described (Kastenmayer *et al.* 1994) and was expressed as a percentage of the amount administered. Correction of cross-contamination of stable isotopes was made by assuming that the contribution of ^{57}Fe from the ^{58}Fe label was negligible (Kastenmayer *et al.* 1994). The amount of ^{58}Fe ingested with the ^{57}Fe label, which was incorporated into erythrocytes, was then calculated, assuming an identical absorption for both isotopes present in the ^{57}Fe label. Isotopic enrichments determined 2 weeks after the first administration of isotopes served as baseline values for calculations of erythrocyte incorporation after the second isotope administration.

Values for erythrocyte incorporation of ^{58}Fe were corrected to the geometric mean incorporation of ^{57}Fe from the reference dose and to the geometric mean plasma ferritin concentration according to Cook *et al.* (1991), using for each subject the mean of all available plasma ferritin analyses (two to three analyses per infant).

Student's paired *t* test was used for comparisons of erythrocyte incorporation of ^{58}Fe and ^{57}Fe between Protocols A and B. Values were converted to logarithms before statistical analysis and reconverted to antilogarithms to recover the original values (Cook *et al.* 1969). Order effect (Protocol A *v.* Protocol B) was tested by using two-sample *t* tests. Comparisons were also made by randomized block analysis of variance using ^{57}Fe incorporation from the reference dose as a covariate. Correlations between erythrocyte incorporation of ^{57}Fe from the reference doses administered on two separate occasions as well as between plasma ferritin concentrations and erythrocyte incorporation of ^{58}Fe and ^{57}Fe were evaluated by Spearman's rank correlation.

Ethical considerations

The study protocol was reviewed and approved by the University of Iowa Committee on Research Involving Human Subjects. Parents were informed about the aims and procedures of the study and informed consent was obtained from at least one parent of each infant.

Results

The Study Formula provided (per litre) 2733 kJ, protein 1.68 g, Fe 1.55 mg, zinc 6.2 mg, calcium 532 mg, phosphorus 313 mg and ascorbic acid 109 mg. Corresponding values for the infant formula manufactured without added Fe or ascorbic acid were 2724 kJ, protein 1.66 g, Fe 0.13 mg, zinc 5.9 mg, calcium 550 mg and phosphorus 319 mg. No ascorbic acid was detected in this product.

Table 1. Age, sex, body weight and iron status indices (haemoglobin and plasma ferritin) of participating infants

Infant	Sex	Age (d)	Body weight (g)	Haemoglobin (g/l)	Plasma-ferritin ($\mu\text{g/l}$)
5685	M	138	8580	140	ND
		152	8790	146	51
		166	9060	140	48
5941	M	143	6810	128	58
		157	7160	128	47
		170	7530	122	39
5946	F	142	7150	116	55
		156	7210	122	42
		169	7870	112	46
5947	M	144	6790	111	27
		158	7020	116	20
		172	7200	116	30
5949	M	141	7820	122	84
		154	8070	124	57
		169	8230	126	50
5696	M	136	7190	126	33
		150	7340	126	24
		164	7450	126	27
5272	F	136	8140	128	112
		150	8290	125	86
		169	9010	116	39
5697	M	140	6690	106	25
		154	6990	111	ND
		168	7290	106	18
6050	F	141	8040	117	80
		155	8410	117	66
		168	8670	111	72
6052	F	138	6660	130	61
		152	6740	128	51
		166	7320	140	79

ND, not determined.

Individual data on body weight and Fe status are given in Table 1. One child was anaemic (haemoglobin $< 110 \text{ g/l}$) while none of the infants was Fe deficient (plasma ferritin $< 12 \mu\text{g/l}$). Erythrocyte incorporation data are presented in Table 2. Geometric mean ^{58}Fe incorporation was 7.6 % (range 3.3–13.5 %) from formula given over approximately 24 h (Protocol A) and 10.6 % (range 6.7–18.6 %) from formula fed on three consecutive days (Protocol B).

Table 2. Erythrocyte incorporation (% of dose) of ^{58}Fe from infant formula administered over 24 h (Protocol A) or in three equal portions (Protocol B) and of ^{57}Fe given as a reference dose

Infant	Protocol A		Protocol B	
	Infant formula	Reference dose	Infant formula	Reference dose
5685	8.0	23.3	6.9	22.1
5941	11.8	16.1	18.6	20.8
5946	13.3	21.6	17.0	14.1
5947	13.5	27.6	10.2	20.2
5949	3.4	19.3	7.4	26.6
5696	3.3	10.8	8.6	25.2
5272	4.6	20.1	14.1	32.9
5697	10.6	24.0	15.0	45.4
6050	7.3	16.8	6.7	20.5
6052	8.8	27.0	9.3	22.3
Mean*	7.6	20.0	10.6	23.9
+ 1 SD	12.9	26.5	15.6	32.7
- 1 SD	4.5	15.1	7.3	17.4

* Geometric mean.

The difference just failed to reach statistical significance ($P 0.05$). After analysis of covariance, using incorporation of ^{57}Fe from the reference dose as a covariate, the difference was clearly not statistically significant ($P 0.146$). Five infants were fed labelled infant formula in the order Protocol A then Protocol B and the other five infants in the order Protocol B then Protocol A. No statistically significant order effect was found ($P 0.066$). As expected, erythrocyte incorporation of ^{57}Fe from the water solution with added ascorbic acid (reference dose) was higher than incorporation of ^{58}Fe given with infant formula. Geometric mean ^{57}Fe incorporation was 20.0 % (range 10.8–27.6 %) following Protocol A and 23.9 % (range 14.1–45.4 %) following Protocol B. The difference was not statistically significant ($P 0.21$). CVs for the incorporation of ^{57}Fe were 9.4 and 9.9 % and for incorporation of ^{58}Fe 26.4 and 16.2 % for Protocols A and B respectively.

Data on erythrocyte ^{58}Fe incorporation from infant formula corrected for plasma-ferritin and ^{57}Fe incorporation from the reference dose are given in Table 3. It is evident that neither correction significantly changed the data. The CV changed from 26.4 % (uncorrected; Protocol A) to 21.5 % when corrected for ^{57}Fe incorporation and to 29.8 % when corrected for plasma ferritin values. The CV for Protocol B changed from 16.2 % (uncorrected) to 20.4 % and 22.1 % when corrected for the incorporation of ^{57}Fe and plasma ferritin values, respectively.

No statistically significant correlation ($r 0.079$, $P 0.83$) was found between the erythrocyte incorporation of ^{57}Fe from reference doses administered during Protocols A and

Table 3. Corrected values for erythrocyte incorporation (% of dose) of ^{58}Fe from infant formula (Protocols A and B)

(Corrections were made to geometric mean plasma ferritin (46 $\mu\text{g/l}$) and geometric mean erythrocyte incorporation from the reference dose (20.0 %, Protocol A; 23.9 %, Protocol B)

Infant	Protocol A		Protocol B	
	Plasma ferritin	Correction for Reference dose	Plasma ferritin	Correction for Reference dose
5685	7.8	6.9	7.4	7.5
5941	12.2	14.7	19.2	21.4
5946	13.7	12.3	17.5	28.8
5947	7.4	9.8	5.6	12.1
5949	4.6	3.5	10.0	6.6
5696	2.0	6.1	5.2	8.2
5272	7.2	4.6	22.1	10.2
5697	4.9	8.8	6.9	7.9
6050	11.5	8.7	10.5	7.8
6052	12.0	6.5	12.6	10.0
Mean*	7.3	7.5	10.4	10.7
+ 1 SD	13.2	11.7	17.4	17.3
-1 SD	4.0	4.9	6.2	6.6

* Geometric mean.

B. Neither was erythrocyte incorporation of ^{58}Fe in Protocol A statistically significantly correlated with that in Protocol B (r 0.60, P 0.067). Within protocols, the relationship between incorporation of ^{58}Fe and ^{57}Fe was weak for Protocol A (r 0.588, P 0.074) and non-existent for Protocol B (r 0.042, P 0.907). No relationship was found in either protocol between plasma ferritin concentration and erythrocyte incorporation of ^{58}Fe or ^{57}Fe .

Discussion

The results from this study showed that mean Fe erythrocyte incorporation was about 8–11 % from low-Fe formula when evaluated in healthy, non-Fe deficient infants. Although the difference in erythrocyte incorporation of ^{58}Fe between Protocols A (geometric mean 7.6 %) and B (geometric mean 10.6 %) was not statistically significant (P 0.05), the difference was not negligible. The smaller CV in erythrocyte incorporation of ^{58}Fe during Protocol B can be assumed to indicate that day-to-day differences are smoothed out by administration of labelled test meals over 3 d. Erythrocyte incorporation of ^{58}Fe was higher when administered in six equal portions over three consecutive days as compared with ingestion of 1000 g formula over 24 h. Thus, our data indicate agreement with the earlier observation of higher whole-body Fe retention from a smaller volume of labelled formula, as compared with a larger volume, in infant rhesus monkeys (Davidson *et al.* 1990). The adherence to strictly standardised experimental conditions, for example volume of labelled test meals, mode of feeding, etc., is therefore important in studies designed to evaluate the influence of dietary components on Fe bioavailability or to compare the efficiency of Fe absorption from different foods.

Comparisons between studies of Fe bioavailability are further complicated by the influence of Fe status on Fe

bioavailability. Fe absorption from a reference dose has been established as a useful tool to correct Fe absorption values for differences in Fe status in adults (Layrisse *et al.* 1969) and would be an interesting approach also in infants. So far, no information has been published on the usefulness of a reference dose for this purpose in infant studies. However, observations from earlier studies demonstrated statistically significant correlations between ^{58}Fe erythrocyte incorporation from ^{58}Fe administered between meals and plasma ferritin in formula fed (Fomon *et al.* 1988) and breast-fed infants (Fomon *et al.* 1995). These data indicate that the administration of a reference dose as a measure of Fe status could be useful also in infant studies. In the present study we therefore included measurements of ^{57}Fe erythrocyte incorporation from a reference dose. Our data demonstrated markedly less inter-individual variation in erythrocyte incorporation for ^{57}Fe given as a reference dose than for ^{58}Fe given with infant formula. However, no statistically significant correlation between erythrocyte incorporation of ^{57}Fe from the reference dose and plasma ferritin could be found (P 0.83). Furthermore, no statistically significant (P 0.21) correlation was found between the incorporation of ^{57}Fe administered on two separate occasions, 2 weeks apart, during which time only minor changes in Fe status parameters were observed. The lack of correlation between erythrocyte incorporation from the two separate reference doses as well as lack of correlation between plasma ferritin and ^{57}Fe incorporation from the reference dose indicate the limited usefulness of the inclusion of a reference dose in studies of Fe bioavailability in non-Fe-deficient infants. Nevertheless, using ^{57}Fe incorporation from the reference dose as a covariate, the difference in ^{58}Fe incorporation between the two protocols was diminished to some extent. The use of plasma ferritin as a covariate did not influence the variation in ^{58}Fe incorporation between study protocols.

In this study, Protocol A was chosen to mimic the administration of labelled feeds as used in our previous study with extrinsically-labelled human milk (Davidsson *et al.* 1994a). For comparison, a protocol with ingestion of labelled test meals over a period of 3 d was included (Protocol B). Although comparison of data on Fe bioavailability between separate studies must be made with great caution, it is interesting to note the similar geometric means in erythrocyte incorporation from low-Fe formula in the present study and from extrinsically-labelled human milk (Davidsson *et al.* 1994a). The geometric mean incorporation of ^{58}Fe from extrinsically-labelled human milk, prepared by the addition of ^{58}Fe (0.5 mg) to human milk (700–1000 g), was 10.6 % (range 3.1–33.7 %; erythrocyte incorporation re-calculated from absorption data of Davidsson *et al.* 1994a).

Due to practical and ethical reasons, no direct comparisons have been made of Fe bioavailability from human milk and infant formula in infants. As already discussed, comparisons of Fe bioavailability between studies can only provide limited information. Bioavailability of Fe depends on the presence of enhancers and inhibitors in the diet and, obviously, there are numerous differences in the overall composition of the low-Fe formula used in the present study and the extrinsically-labelled human milk in our

previous study (Davidsson *et al.* 1994a). However, it is of importance to note that the low-Fe infant formula used in the present study was a whey-predominant formula with a low content of bovine casein, a relatively low calcium content and a high concentration of ascorbic acid. Bovine casein and calcium are known to inhibit (Hurrell *et al.* 1989; Hallberg *et al.* 1991, 1992) and ascorbic acid to enhance (Derman *et al.* 1980; Stekel *et al.* 1986; Gillooly *et al.* 1984; Davidsson *et al.* 1994b; Fairweather-Tait *et al.* 1996) Fe bioavailability.

Furthermore, the total Fe content of test meals is of importance since decreased fractional absorption of Fe has been demonstrated with increased Fe content of the test meal in adults (Bothwell *et al.* 1979). In our previous study (Davidsson *et al.* 1994a) the total Fe content of human milk was increased considerably by the addition of Fe stable isotopes, resulting in 0.6–0.7 mg Fe/1000 g. Very little is known about the effect of different Fe doses on Fe bioavailability in infants. A recent study reported mean ^{58}Fe incorporation of 4.3 % from a formula with 8 mg Fe/l as compared with incorporation of 2.6 % from a formula fortified with 12 mg Fe/l (Fomon *et al.* 1997). It is not known if erythrocyte incorporation could be influenced by small differences in Fe concentration; i.e., the difference between 0.6 mg Fe/kg labelled human milk and 1.4 mg Fe/kg of the infant formula used in this study. If indeed such small differences have a significant effect on fractional erythrocyte incorporation, it should have favoured human milk.

It should be noted that erythrocyte incorporation was used as a proxy for Fe bioavailability in the present study, as well as in previous studies by us (Davidsson *et al.* 1994a; Fomon *et al.* 1997). This methodology assumes that a large proportion of newly-absorbed Fe is readily incorporated into erythrocytes. Although some information is available on erythrocyte incorporation of injected Fe in very young infants (Garby *et al.* 1964) and in low-birth-weight infants (Gorten *et al.* 1963; Ehrenkranz *et al.* 1992; Zlotkin *et al.* 1995), no studies have been reported on erythrocyte incorporation rate of absorbed Fe in older, term infants. Recently, significant differences between Fe retention (based on 11 d faecal collections) and erythrocyte incorporation of ^{58}Fe were reported in term infants (Fomon *et al.* 2000). Geometric mean retention and erythrocyte incorporation were 26.9 and 5.2 % respectively in nine young infants (20–69 d) and 32.5 and 12.5 % respectively in nine older infants (165–215 d). The authors conclude that infants incorporate far less than 80 % of retained isotope into erythrocytes. Thus, estimating Fe retention based on the assumption that 80–100 % of absorbed Fe is promptly incorporated into erythrocytes will result in a several-fold underestimate of retention. However, these methodological uncertainties do not invalidate the use of erythrocyte incorporation for the purpose of comparing different diets or when evaluating the impact of dietary composition on erythrocyte incorporation of Fe.

Conclusion

Fractional erythrocyte incorporation of Fe from low-Fe infant formula was in the same range as our earlier

published data on erythrocyte incorporation of Fe from human milk extrinsically labelled with ^{58}Fe (Davidsson *et al.* 1994a). The methodological evaluations included in this study clearly indicate the importance of using standardised study protocols when evaluating Fe bioavailability in infants. Corrections of erythrocyte incorporation data based on plasma ferritin or Fe bioavailability from a reference dose were not found to be useful in the non-Fe-deficient infants included in this study.

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