Absorption and Loss of Iron in Toddlers Are Highly Correlated[1,2]

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ABSTRACT For estimating the requirements for dietary iron, it is important to know the amount of iron that is lost from the body. Inevitable losses of iron have been determined in adult humans but not in infants or children. We administered ^{58}Fe, the least abundant stable isotope of iron, to free-living infants at 168 d of age (5.6 mo) and followed them to age 26 mo. There was no dietary restriction after isotope administration. Blood was obtained at regular intervals for determination of isotopic enrichment and indices of iron status. We estimated the quantity of circulating iron, noncirculating active iron, and storage iron at each age. The administered isotope equilibrated with total body iron by 13 mo of age. From 13 to 26 mo of age, we estimated inevitable loss and absorption of iron from the change in tracer abundance in circulating iron. The rate of decrease of tracer abundance was proportional to addition of tracee, i.e., absorption of iron. Conversely, the rate of decrease in quantity of tracer was proportional to removal of tracee, i.e., loss of iron. From 13 to 26 mo of age, iron absorption was (mean ± SD) 0.49 ± 0.13 mg/d and inevitable iron loss was 0.25 ± 0.12 mg/d. Intersubject variability of iron loss and iron absorption was high, and iron loss and absorption were highly correlated ($r = 0.789$, $P < 0.001$). Iron stores were low throughout the study and decreased significantly from 13 to 26 mo of age, suggesting that iron absorption from the diet was inadequate to maintain or increase iron nutritional status. The data suggest that, in this cohort, which may be representative, the intake of bioavailable iron from 13 to 26 mo of age was insufficient to maintain iron nutritional status. J. Nutr. 135: 771–777, 2005.

KEY WORDS: • *iron absorption* • *iron loss* • *toddlers* • *stable isotope*

For an individual to remain in good iron nutritional status, the amount of iron absorbed must at least equal the amount of iron lost. For the growing individual, it is necessary not only to absorb enough iron to replace inevitable iron loss but, in addition, to absorb the amount of iron needed for growth. The iron needed for growth can be estimated with an approach that has been applied to infants (1) and to adolescents after the peak of the adolescent growth spurt (2) and that should be applicable to any age. Loss of iron, on the other hand, has been measured only in adult men (3) and in adolescents of either gender beyond the peak of the growth spurt (2).

Loss of iron occurs mainly from the gastrointestinal tract but also from the skin and in secretions. It is generally thought that endogenous fecal iron (in contradistinction to exogenous, i.e., nonabsorbed dietary iron) represents iron that is lost from the body and that its excretion is not regulated, i.e., is inevitable. The inevitable iron loss by adult men was determined by Green et al. (3), who administered the long-lived radioisotope, ^{55}Fe, and then allowed the isotope to reach equilibration with total body iron. Thereafter, the fractional disappearance of the isotope from the circulation (where it is present almost entirely in hemoglobin) is the same as the fractional loss of iron from the body. Green et al. (3) measured the disappearance of circulating isotope, beginning ~10 mo after isotope administration, over a period of several years and estimated the loss of iron by adult men to be ~1 mg/d. Evidence supporting the assumption that, after sufficient time, an iron isotope becomes completely distributed throughout the body iron pool was provided by the study of an individual who had been given ^{55}Fe 36 mo previously and subjected to repeated phlebotomy (4).

In a study of adolescents after the peak of the adolescent growth spurt (2), we labeled the subjects with the least abundant stable isotope of iron, ^{58}Fe. After an equilibration period of at least 1.56 y, we measured the abundance of the isotope in circulating erythrocytes during an observation period of >3 y in each subject. From the fractional disappearance of ^{58}Fe, we estimated inevitable iron loss to be 0.70 mg/d by men and 0.84 mg/d by women. These losses represented 48% of the estimated requirement for absorbed iron by men and 73% of the estimated requirement for absorbed iron by women.

Little information is available regarding iron loss by younger subjects. The present report concerns a study in which we administered ^{58}Fe to infants at age 168 d (5.6 mo). The administered isotope became distributed within total body iron by 13 mo of age, and we were able to determine inevitable iron loss and iron absorption during the interval from 13 to 26 mo of age.

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[2] Individual values for plasma ferritin concentration at 168d, erythrocyte incorporation of ^{58}Fe at 168d, and iron loss, iron absorption and iron gain from 13 to 26 mo are available in Supplemental Table 1 with the online posting of this paper at www.nutrition.org.

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SUBJECTS AND METHODS

Study design. Normal infants were enrolled by 154 d of age and were given ^{58}Fe orally within 4 d of 168 d of age. Blood was obtained just before isotope administration and within 4 d of each of the following ages: 182, 196, 224, 252, 280 d and 11, 13, 15, 17, 20, 23, and 26 mo. Intake of iron was restricted from d 154 until isotope administration but was unrestricted thereafter. The study protocol was reviewed and approved by the Institutional Review Board of the University of Iowa and a parent provided written informed consent.

Subjects. The subjects were term infants of either gender (n = 35) with birth weight >2500 g; they were considered normal by their physicians and the investigators. By chance, all were Caucasian. Many had participated in other studies in our unit. Two infants were breast-fed at the time of enrollment, with 1 receiving small amounts of supplemental formula. Of the infants fed formula at the time of enrollment (n = 33), 5 had been breast-fed initially for periods of 2 wk to 2 mo. At enrollment, infants were given low-iron (1.8 mg/L) formula (Similac®, Ross Products Division). Beginning 2 wk after isotope administration, there were no restrictions regarding feeding or dietary supplements. Formula-fed infants were fed iron-fortified formula (12 mg Fe/L, label claim) for at least several months.

Tracer (isotope) administration. The tracer, isotopically enriched ^{58}Fe, was obtained in elemental form from Cambridge Isotope Laboratory (^{58}Fe abundance 91–94 atom %). A precisely weighed quantity of isotopically enriched ^{58}Fe was converted to ferrous sulfate and made up to volume as previously described (5). Each infant was admitted to the Lora N. Thomas Pediatric Metabolic Unit within 4 d of age 168 d and given 2 doses of ^{58}Fe, each dose 3 h after a feeding and 1 h before the next feeding. Each dose consisted of ~0.9 mg of iron and 0.8 mg of ^{58}Fe-enriched ferrous sulfate in 5 mL of a 5% glucose solution containing 10 mg of ascorbic acid. Each dose was delivered from a syringe directly into the back of the oral cavity to decrease the likelihood of regurgitation. The syringe was rinsed twice with a 5% glucose solution and the rinses were administered to the infant. Infants were observed in the metabolic unit for 1 h after the 2nd dose to detect regurgitation if it occurred. No regurgitation occurred.

Blood collection, processing, and analysis. Capillary blood was collected by heel stick, using a disposable spring-loaded device (Tenderfoot, International Technidyne); ~1 mL of blood was collected into a heparin-treated tube. Hemoglobin concentration (Hb)[4] was measured immediately on whole blood using a Coulter AcT diff Hematology Analyzer (Coulter). The blood was then centrifuged at 2000 × g for 15 min to obtain plasma for determination of ferritin concentration (PF; by RIA using the Quantimune kit, Bio-Rad Laboratories). Plasma C-reactive protein (CRP) was quantified by a two-site ELISA developed in our laboratory, and following the general enzyme immunoassay protocol described by Flowers et al. (6). The capture antibody was sheep anti-human CRP (ICN Cappel Research), the primary detection antibody was rabbit anti-human CRP (EMD Biosciences Calbiochem), and the secondary detection antibody was alkaline phosphatase–conjugated goat anti-rabbit IgG (Sigma-Aldrich). Packed erythrocytes were saved for isotope ratio determination.

Isotope analyses. Isotope ratios ($IR_{58/57}$) were determined in erythrocytes as previously described (7). Isotope ratios were determined on a PlasmaQuad 3 inductively coupled plasma MS (ICPMS; VG Elemental). The PlasmaQuad 3 ICPMS instrument was modified for measurement of iron isotope ratios as follows: *1*) use of the PlasmaScreen option accompanied by a very low plasma radio frequency power setting; *2*) optimization of argon flow rates for minimization of background intensities at m/z = 58 and 57 with simultaneous maximization of analyte intensity for cobalt at m/z = 59; *3*) minimization of the nickel contribution to m/z = 58 by using copper rather than the customary nickel sampler and skimmer cones; *4*) use of the Cetac U-6000AT ultrasonic nebulizer for sample introduction; *5*) replacement of the original equipment discrete dynode electron multiplier detector and its vacuum chamber by a continuous dynode detector and housing that possesses very low electrical impedance, very high linearity of response, and minimal gain fatigue during exposure to high intensities. The ICPMS instrument is sequestered in a clean environment at constant temperature and humidity. In addition, the sample preparation procedures that are used minimize the contributions of Cr, CaOH, and Ni to m/z = 54, 57 and 58, respectively, by selective preconcentration of iron using ultrapure solvents and a chelating reagent highly specific for iron (III). Dilute hydrochloric acid, rather than the customary dilute nitric acid, is used for sample introduction to the ICPMS instrument to minimize formation of ArN^+ in the plasma and its contribution to background at m/z = 54. Parameters for operation of the PlasmaQuad 3 ICPMS instrument in the laboratory of Dr. Serfass resulted in a worst-case precision of measurement for $IR_{58/54}$ of 0.5% relative SD (rsd). The "internal precision of measurement," the mean value achieved by 10 sequential measurements of the same sample, was 0.33rsd.

Data analysis

Estimation of body iron compartments. Total body iron (Fe_{tot}) was conceptualized as consisting of 3 compartments, circulating iron (Fe_{circ}), noncirculating active iron (Fe_{nca}), and storage iron (Fe_{stor}). Thus, $Fe_{tot} = Fe_{circ} + Fe_{nca} + Fe_{stor}$, where all components are in mg (1 mg Fe = 17.9 μmol), and Fe_{circ} represents iron in circulating erythrocytes and ignores the small amounts of iron present in circulating iron-containing enzymes and the iron in circulating transport proteins (e.g., transferrin, ferritin). Because circulating non-Hb iron is such a small percentage of circulating iron, we contend that little error is introduced by ignoring it. It was calculated as Fe_{circ} = BV × Hb × 3.47, where BV is blood volume in L, assumed to be 0.65 L/kg body weight, Hb is hemoglobin concentration in g/L, and 3.47 is the concentration of iron (mg/g) in hemoglobin. Fe_{nca} consists of iron in myoglobin and in iron-containing enzymes. Fe_{nca} was assumed by analogy with normal adult men using representative values as suggested by Dallmann et al. (8), which resulted in a value of 455 mg. For a 75 kg adult, Fe_{nca} is therefore 6 mg/kg.

Storage iron (Fe_{stor}) consists of iron in ferritin and hemosiderin. In the absence of inflammation, PF is an index of Fe_{stor} (9–11). Storage iron was thus calculated from PF on the assumption that, after correction for body size, PF bears the same relation to storage iron in infants (excluding the early months of life) and toddlers as in adults. Although this assumption is untested, we showed in most of our studies of infants (12–16) and in a study of preadolescent children (17) that erythrocyte incorporation of administered ^{58}Fe is inversely correlated with PF. Thus, we estimated Fe_{stor} on the basis of analogy with adults using the equation describing the relation between PF and storage iron that Hallberg et al. (18) derived from the data of Skikne et al. (19). This equation predicts the absence of storage iron when PF is ≤15.9 μg/L.

For purposes of estimating Fe_{stor}, it was necessary to obtain PF values that were reflective of storage iron. Because ferritin is an acute-phase reactant, PF may not be reflective of iron nutritional status during inflammation or infection. We had planned to use CRP as an indication of the presence of an acute-phase reaction and to ignore values for PF obtained concurrently with elevated CRP. However, in analyzing a large amount of data from another study, it was evident that in some infants, PF was not elevated in the presence of elevated CRP and in others, PF was elevated when CRP was normal. We presume that these circumstances reflect either laboratory errors in one of the measurements or a lack of concurrence between the timing of increase and subsequent decrease of the 2 acute-phase reactants (CRP and PF). We chose to identify spuriously elevated PF without considering CRP. We established the rule that a PF value was classified as spurious if it was 50% higher than the mean of the preceding and the immediately following values. For the purpose of calculating Fe_{stor}, such spurious values were replaced by "adjusted" values (PF_{adj}), calculated as the mean of the adjacent values. In the

[4] Abbreviations used: ^{58}A, mass abundance of ^{58}Fe in excess of natural abundance (unless otherwise stated as molar abundance $^{57}A^{tr}$, $^{58}A^{tr}$, $^{57}A^{N}$); CRP, C-reactive protein; Fe_{circ}, mass Fe in circulation; Fe_{gain}, mass gain in Fe; $^{58}Fe_{inc}$, prompt (within 4 wk) erythrocyte incorporation of ^{58}Fe; Fe_{loss}, mass loss of Fe; Fe_{nca}, mass noncirculating active Fe; Fe_{stor}, mass storage Fe; Fe_{tot}, mass total body iron; $^{58}Fe_{tot}$, mass ^{58}Fe in excess of natural abundance; Hb, hemoglobin; ICPMS, inductively coupled plasma MS; $IR_{58/57}$, molar isotope ratio $^{58}Fe/^{57}Fe$; PF, plasma ferritin; rsd, relative SD.

case of a spurious value at age 26 mo, the 2 preceding values were used to obtain a corrected value.

Mean daily gain in total body iron (Fe_{gain}) during the period 13–26 mo was calculated as the difference in Fe_{tot} at the beginning and end of the period divided by the number of days in the period.

Isotope data. The first step was calculation of tracer abundance (^{58}A) from measured isotope ratios. Tracer abundance was defined as concentration of tracer isotope in excess of natural abundance isotope. Thus, calculation of tracer abundance included correction for natural abundance of ^{58}Fe. Abundance at time t ($^{58}A^t$) was expressed in mass units (mg/g) and was calculated as

$$^{58}A^t = \frac{^{58}A^{tr} \cdot {}^{57}A^N \cdot (IR^t_{58/57} - IR^N_{58/57}) \cdot 57.933 \cdot 1000}{[{}^{57}A^{tr} \cdot (IR^{tr}_{58/57} - IR^t_{58/57}) + {}^{57}A^N \cdot (IR^t_{58/57} - IR^N_{58/57})] \cdot 55.845}$$

where $^{58}A^{tr}$ is the abundance (molar) of ^{58}Fe (tracer) in the enriched ^{58}Fe preparation, $^{57}A^N$ is the abundance (molar) of ^{57}Fe in natural iron (0.021191), $IR^t_{58/57}$ is the measured ^{58}Fe /^{57}Fe isotope ratio (molar) in the blood sample at time t, $IR^N_{58/57}$ is the natural ^{58}Fe / ^{57}Fe isotope ratio (molar) (0.133032), $^{57}A^{tr}$ is the ^{57}Fe abundance (molar) in the enriched ^{58}Fe preparation, $IR^{tr}_{58/57}$ is the ^{58}Fe /^{57}Fe isotope ratio (molar) of the enriched ^{58}Fe preparation, 57.933 is the molar mass of ^{58}Fe, and 55.845 is the molar mass of natural Fe.

The amount of tracer at time t ($^{58}Fe^t_{tot}$) was calculated as

$$^{58}Fe^t_{tot} = Fe^t_{tot} \cdot {}^{58}A^t$$

where Fe^t_{tot} is Fe_{tot} at time t.

Loss and absorption of iron between 13 and 26 mo of age

Principle. After a tracer has equilibrated with the tracee pool, a decrease (dilution) of tracer concentration (abundance) can occur only through addition of tracee. The decrease in tracer abundance is therefore proportional to the addition of tracee (by iron absorption). Conversely, a decrease in the amount of tracer (= abundance × pool size) can occur only if the tracer is removed, which can occur only in association with tracee. The decrease in the amount of tracer is therefore proportional to the amount of tracee removed (iron loss).

Calculations. The linear regression of $\log^{58}A^t$ against time was calculated for each subject for the period 13–26 mo. The slope (k_{abs}) of the regression reflects the fractional absorption of iron, expressed as fraction of total body iron per unit of time. The mean quantity of iron absorbed each day (mg/d) between 13 and 26 mo (Fe_{abs}) was calculated as

$$Fe_{abs} = k_{abs} \cdot \overline{Fe}_{tot}$$

where $\overline{Fe}_{tot}$ is mean total body iron between 13 and 26 mo, calculated as amount at the midpoint (19.5 mo) of the linear regression of Fe_{tot} against time.

The linear regression of $\log {}^{58}Fe^t_{tot}$ against time was calculated for each subject for the period 13 to 26 mo. The slope (k_{loss}) of the regression reflects the fractional loss of iron, expressed as fraction of total body iron per unit of time. The mean amount of iron lost each day (mg/d) between 13 and 26 mo (Fe_{loss}) was calculated as

$$Fe_{loss} = k_{loss} \cdot \overline{Fe}_{tot}$$

Initial erythrocyte incorporation. Erythrocyte incorporation of tracer ($^{58}Fe_{inc}$), expressed as a percentage of the dose, was determined from tracer abundance determined 28 d after isotope administration using calculations described previously (7).

Statistical analysis. Data were analyzed using SAS version 6.12 (SAS Institute). Changes in Fe status were compared by paired t tests, and differences were considered significant with $\alpha = 0.05$. Slopes representing fractional change in $\log^{58}A$ and $\log^{58}Fe_{tot}$ were determined using least-squares regression. Slopes of the regression of isotope abundance on age were assessed for gender effects and homogeneity using mixed-model ANOVA (PROC MIXED). Values presented are means ± SD.

RESULTS

Of the 35 infants administered ^{58}Fe at 168 d of age, 5 subjects did not complete the study as planned because the parents found it difficult to keep the scheduled appointments. Three of these subjects were withdrawn before 13 mo of age, but Subjects 7814 and 8106 were not withdrawn until after the 23-mo visit and their data are included. Unless specified otherwise, data for the last-mentioned 2 subjects are included with the 26-mo data for all other subjects. In 1 subject who had the lowest initial erythrocyte incorporation of ^{58}Fe (1.18%), tracer abundance was below the detection limit after 17 mo of age, and data for that subject are not included. Thus, results presented here concern 31 subjects, 15 boys and 16 girls, who completed the study to at least 23 mo of age. Between 13 and 26 mo of age, 6 blood samples were available from 17 subjects, 5 samples from 11 subjects, and 4 samples from 3 subjects. Six subjects had PF (unadjusted) <10 μg/L at one or more time points between 13 and 26 mo of age and thus were iron deficient by the conventional classification.

Body iron compartments. Hemoglobin concentration changed little throughout the period of observation **(Table 1)**. Final Hb values (data not shown) at age 23 or 26 mo were <110 g/L in only 2 of the 31 subjects. As was anticipated, PF_{adj} decreased from age 168 d (5.6 mo) to age 13 mo ($P < 0.001$), and there was a further decrease from age 13 to 26 mo ($P = 0.027$). Iron stores (Fe_{stor}), whether expressed in milligrams or as a percentage of Fe_{tot}, also decreased with increasing age (Table 1). Iron stores were depleted (defined as $Fe_{stor} = 0$, corresponding to PF ≤ 15.9 μg/L) in 9 subjects at 13 mo of age and in 12 subjects at 26 mo. Six subjects were depleted at both ages. The 12 subjects with depleted iron stores at 26 mo included 4 of the subjects who had PF < 10 μg/L at some time between 13 and 26 mo. At age 168 d, Fe_{circ} accounted for 74.6% of Fe_{tot}, Fe_{nca} for 16.0% and Fe_{stor} for 9.4%. By age 26 mo, Fe_{circ} had increased to 79.6% and Fe_{stor} had decreased to 3.1% of Fe_{tot}.

Loss and absorption of Fe between 13 and 26 mo. After isotope administration at 168 d of age, log tracer abundance (^{58}A) increased in erythrocytes as the absorbed tracer entered the circulation **(Fig. 1**; representative subject #7818). There was then a decrease in abundance as shown before (16) that reached a nadir at ~11 mo of age. After a slight rise in

TABLE 1

Body weight, hemoglobin, plasma ferritin, and estimated body iron compartments of study subjects[1]

	Age		
	168 d	13 mo	26 mo
Weight, *g*	7600 ± 690	10338 ± 905	13097 ± 1091
Hb, *g/L*	124.1 ± 7.8	121.4 ± 7.6	123.1 ± 7.8
PF_{adj}, *μg/L*	44.6 ± 26.6[a]	25.6 ± 11.8[b]	21.4 ± 9.9[c]
Fe_{tot}, *mg*	286.2 ± 33.3	364.2 ± 37.6	457.0 ± 58.8
mg/kg	37.6 ± 2.1	35.2 ± 2.1	34.9 ± 2.3
Fe_{circ}, *mg*	212.7 ± 23.0	284.1 ± 31.4	363.8 ± 41.6
%	74.6 ± 4.8	78.0 ± 3.9	79.6 ± 3.5
Fe_{nca}, *mg*	45.6 ± 4.1	62.0 ± 5.4	78.6 ± 6.5
%	16.0 ± 0.9	17.1 ± 1.1	17.3 ± 1.1
Fe_{stor}, *mg*	27.9 ± 17.6	18.1 ± 16.6	14.9 ± 17.8
%	9.4 ± 5.3	4.9 ± 4.4	3.1 ± 3.9

[1] Values are means ± SD, $n = 31$ subjects (168 d and 13 mo) or 29 (26 mo). Means in a row with superscripts without a common letter differ, $P < 0.05$.

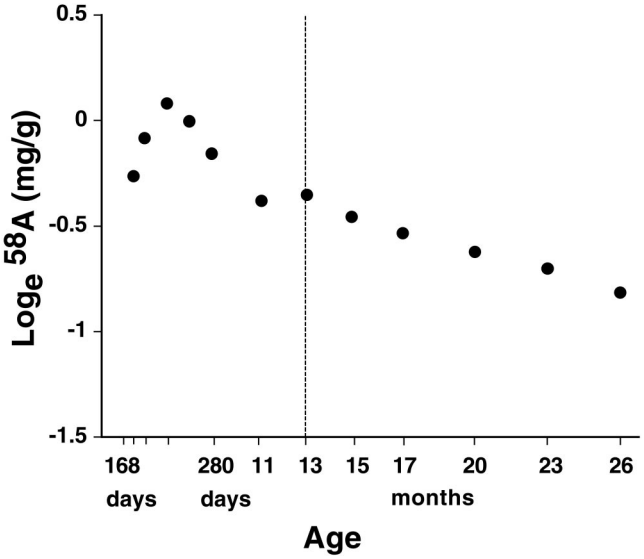

FIGURE 1 Time course of enrichment of circulating iron ($\log^{58}A^t$) plotted against age in a representative subject (#7818).

abundance, a steady linear decline began. The beginning of the linear decline of $\log^{58}A$ represents the time point at which the tracer is thought to have equilibrated with Fe_{tot}. The linear decline in log tracer abundance was described previously by Green et al. (3). Inspection of the plots of the other subjects showed that the individual $\log^{58}A$ began to decline by 13 mo of age; in a few subjects, the decline began at 11 mo. We concluded that the administered isotope had equilibrated with Fe_{tot} by 13 mo of age. When data were grouped by gender (**Table 2**), there were no gender-related differences; therefore, all data were combined in the statistical analyses.

The regression of $\log^{58}Fe^t_{tot}$ on age from 13 to 26 mo for the individual subjects varied widely (**Fig. 2**). The slope of the regression (k_{loss}) represents fractional loss of iron. Correlation coefficients of individual subjects ranged from 0.445 to 0.995. The wide differences in slopes are evidence of the large differences in fractional iron loss. The vertical spread of the intercepts, as indicated by the tracer abundance at 13 mo, is due largely to differences in initial absorption of the tracer, although differences in Fe_{tot} also influenced the intercepts. Mean fractional loss of iron from 13 to 26 mo was 22.7 ± 10.5%/y of Fe_{tot}, and Fe_{loss} was 0.25 ± 0.12 mg/d. Interindividual differences in iron loss were large, with values ranging

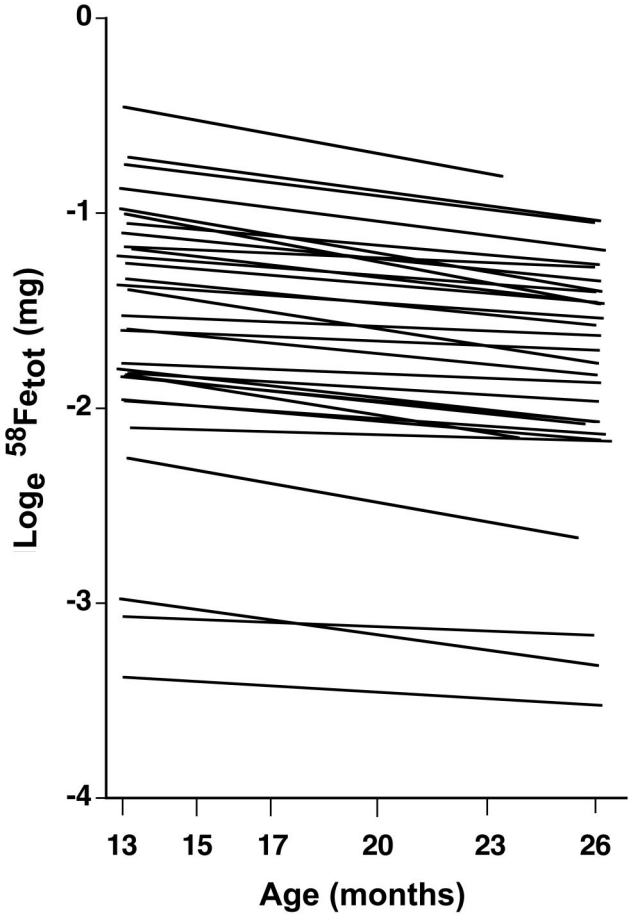

FIGURE 2 Fitted values for regressions of $\log^{58}Fe^t_{tot}$ on age of individual infants.

from 5.7 to 42.3%/y (0.07–0.55 mg/d). The mixed-model ANOVA rejected the null hypothesis that iron loss was equal in all subjects.

The correlation coefficients for the regression of $\log^{58}A$ on age from 13 to 26 mo, whose slope represents fractional absorption of iron, ranged from 0.914 to 0.999 (excepting 1 subject in whom the coefficient was 0.692), indicating excellent agreement with the exponential model. The fractional absorption of iron was 44.0 ± 10.2%/y of Fe_{tot} (data not included in Table 2) and Fe_{abs} was 0.49 ± 0.13 mg/d. Inter-

TABLE 2

Plasma ferritin, initial erythrocyte incorporation of isotope, and loss and absorption of iron in toddlers between 13 and 26 mo of age[1]

				13–26 mo			
	n	168 d PF[2]	196 d $^{58}Fe_{inc}$[3]	Fe_{loss}		Fe_{abs}	Fe_{gain}
		μg/L	*% of dose*	*%/y*	*mg/d*		
Boys	15	56.1 ± 69.9	14.2 ± 7.2	20.7 ± 10.8	0.24 ± 0.12	0.49 ± 0.13	0.24 ± 0.07
Girls	16	50.9 ± 29.1	14.9 ± 9.6	24.6 ± 10.2	0.27 ± 0.12	0.50 ± 0.13	0.22 ± 0.09
Total	31	53.3 ± 51.1	14.6 ± 8.4	22.7 ± 10.5	0.25 ± 0.12	0.49 ± 0.13	0.23 ± 0.08

[1] Values are means ± SD.
[2] Plasma ferritin (unadjusted).
[3] Prompt erythrocyte incorporation of ^{58}Fe.

individual differences in Fe_{abs} were also substantial, but somewhat less than those of Fe_{loss}, with values ranging from 21.9 to 67.9%/y (0.24–0.79 mg/d). Fe_{abs} and Fe_{loss} (both in mg/d) were closely correlated ($r = 0.789$, $P < 0.001$, slope 0.824), as illustrated in **Figure 3**.

For a growing individual to maintain adequate iron status, iron absorption must not only replace losses but provide iron for growth. The gain in iron for growth (Fe_{gain}) was 0.23 ± 0.08 mg/d. Fe_{abs} was indeed nearly equal to the sum of Fe_{gain} plus Fe_{loss}. In individual subjects, the difference between Fe_{abs} and (Fe_{gain} + Fe_{loss}) ranged from −0.05 to + 0.05 mg/d. It bore no relation to the change in PF_{adj}. Neither the correlation of Fe_{gain} with Fe_{abs} ($r = 0.338$, $P = 0.063$) nor the correlation of Fe_{gain} with Fe_{loss} ($r = -0.277$, $P = 0.132$) was significant.

Initial erythrocyte incorporation of iron. Erythrocyte incorporation of tracer ($^{58}Fe_{inc}$) 28 d after administration was 14.6% of the dose with a range from 1.5 to 41.1%. Erythrocyte incorporation of tracer ($^{58}Fe_{inc}$) was inversely correlated at a borderline level of significance with (unadjusted) PF at 168 d of age ($r = -0.511$, $P = 0.051$). Erythrocyte incorporation of tracer was not significantly correlated with Fe_{loss} ($r = 0.354$, $P = 0.051$) nor with Fe_{abs} ($r = 0.160$, $P = 0.341$).

DISCUSSION

We administered ^{58}Fe, the least abundant stable isotope of iron, to infants at 168 d of age and obtained blood samples at regular intervals until the subjects were 26 mo old. The administered isotope had equilibrated with Fe_{tot} by 13 mo of age. Thus, from 13 to 26 mo of age, ^{58}Fe was a valid tracer for Fe_{tot}; during this age interval, we were able to determine endogenous loss of iron and absorption of iron by following the ^{58}Fe abundance in circulating erythrocytes. This is the first determination of iron loss and absorption in toddlers using isotopes.

Iron loss from 13 to 26 mo. We determined that iron loss as a fraction of Fe_{tot} was 22.7 ± 10.5%/y. In absolute terms, iron loss was 0.25 ± 0.12 mg/d. Moreover, iron loss had very high intersubject differences, with an almost 8-fold range of values (0.07–0.55 mg/d).[2] Because the mixed-model analysis excluded the possibility that the values were derived from a single population, the variability could not be attributed to random errors. If iron loss is indeed "inevitable," the observed variability suggests that iron loss is mediated by more than one mechanism involved, the results indicate that it is operating at different levels of efficiency, possibly the result of genetic variation. It is, of course, also possible that rather than being inevitable, iron loss is regulated or that it is influenced by other factors. One such factor might be the amount of iron absorbed. The high degree of correlation between and Fe_{loss} and Fe_{abs} (Fig. 3) is consistent with this possibility.

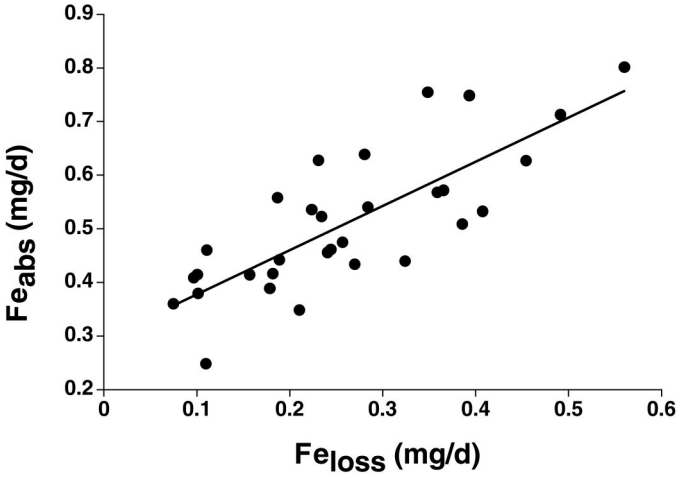

FIGURE 3 Regression of absorbed iron (Fe_{abs}, mg/d) on inevitable iron loss (Fe_{loss}, mg/d) ($r = 0.789$, $P < 0.001$, slope = 0.824).

Endogenous iron loss was determined in adults (3) and in adolescents (2) using methods comparable to that used in the present study, i.e., after equilibration of isotopic tracers with total body iron. With body weight slightly <12 kg for the interval 13 to 26 mo of age, mean iron loss by toddlers in the present study was 0.022 mg/(kg · d). Per unit of body weight, this was somewhat more than the loss by men and adolescents. Assuming that the mean weight of the men studied by Green et al. (3) was 70 kg, the loss of 1 mg/d was 0.014 mg/(kg · d). Adolescent boys weighing 56 kg (2) lost 0.70 mg/d, which is also 0.014 mg/(kg · d). Because loss of iron from the gastrointestinal tract makes the largest contribution to iron loss, the age-related difference in iron loss is probably explained by greater gastrointestinal loss per unit of body weight by toddlers than by older subjects.

There are no previous reports on endogenous iron loss by toddlers and only 1 report on iron loss by infants. Belsten et al. (20) administered ^{58}Fe intravenously to infants at 6, 9, and 12 mo of age and determined fecal excretion of the isotope 7–14 d after administration. Endogenous fecal iron was assumed to derive from an "exchangeable" iron pool, whose size was estimated by 2 different methods. At 12 mo of age, endogenous iron loss was 3 μg/(kg · d) by one method and 8 μg/(kg · d) by the other. However, because the isotope could not have equilibrated with total body iron and it is not known whether it had equilibrated with the exchangeable iron pool, the results are difficult to evaluate.

Most fecal iron derives from desquamated enterocytes, which contain iron predominantly in the form of ferritin. Ferritin is indeed present in feces, but it is not known what percentage of endogenous iron is accounted for by ferritin iron. Feces contain small amounts of heme, mostly in the form of hemoglobin, which accounts for some of the fecal iron. We demonstrated (21) that in the majority of infants, especially older infants, fecal iron loss in the form of hemoglobin amounts to ~2–4 μg/(kg · d). With cow's milk feeding, fecal blood loss can increase considerably. The greatest loss of hemoglobin iron induced by cow's milk that we observed was ~250 μg/(kg · d), although in most infants with unequivocal cow's milk–induced blood loss, fecal loss of hemoglobin iron was much less. Nevertheless, these observations make it appear possible that fecal blood loss could explain some of the variability of fecal iron loss by toddlers. Whether this is indeed the case cannot be verified because there are no reports of fecal blood loss by toddlers.

Iron absorption from 13 to 26 mo. Iron absorption as a fraction of Fe_{tot} was 44.0 ± 10.2%/y. In absolute terms, iron absorption (Fe_{abs}) was 0.49 ± 0.13 mg/d. As with iron loss, iron absorption had high intersubject variability, with a 3-fold range of values (0.24–0.79 mg/d).[2] The agreement between iron absorption and the sum of loss and gain of iron was good, suggesting that our estimates of loss and absorption were reasonable. Subjects with greater Fe_{loss} had greater Fe_{abs} (Fig. 3). One interpretation of this relation is that greater loss of iron somehow stimulates greater iron absorption. This is the more plausible explanation for the relation than the alternative explanation mentioned earlier. It is consistent with the well-established inverse relation between PF and iron absorption, which was also confirmed in the present study. Although

this relation is well established, nothing is known about the mechanism(s) involved.

Prompt erythrocyte incorporation of administered isotope. Incorporation of an administered isotope into erythrocytes has been used extensively as a surrogate of iron absorption even in infants, although we showed (16) that in infants, <80% of newly absorbed iron is promptly (within 4 wk) incorporated into erythrocytes. In the present study, incorporation 28 d after isotope administration was 14.6% of the dose.

Erythrocyte incorporation of administered isotope was borderline correlated with iron loss between 13 and 26 mo of age, an observation suggesting that indices of iron metabolism determined in infancy may be predictive of indices during y 2 of life. If this is confirmed in future studies, it would suggest that there are intersubject differences in iron metabolism among "normal" subjects, i.e., subjects without iron overload. It is, of course, well appreciated that mutations in a number of proteins involved in iron metabolism can lead to iron overload. Our observations suggest that there are identifiable differences in iron metabolism among subjects who remain in iron balance. Whether these differences have clinical consequences is not known; however, it is reasonable to speculate that differences in inevitable iron loss may affect an individual's risk of developing iron deficiency.

Adequacy of iron absorption. During y 1 of life, Fe_{stor} decreases as Fe_{circ} increases. Because blood volume continues to expand during y 2 of life, Fe_{circ} continues to increase, but little is known about changes in Fe_{stor}. In the cohort of toddlers in the present study, there was a slight but significant decrease in Fe_{stor} from age 13 to 26 mo. At 13 mo of age, 9 of 31 subjects were iron deficient ($Fe_{stor} = 0$) and by 23 or 26 mo of age, 12 of 31 subjects were iron deficient. Thus, it is evident that iron absorption during y 2 of life lagged behind the needs for growth and replacement of losses.

What we observed in our study may reflect findings in toddlers generally. Iron deficiency is acknowledged to be prevalent during y 2 of life (22) as observed in the present study. The estimated iron intake by toddlers of ~10 mg/d (23,24) appears to be insufficient to maintain iron stores. The quantity of iron consumed is, perhaps, less important than its rather low bioavailability from the toddler diet. The majority of toddlers consume some meat, principally chicken and turkey (25), but the amounts are unknown and it seems unlikely that heme iron makes a major contribution to total iron intake. The sources of the nonheme iron are not well identified, but a considerable portion of the dietary iron may come from iron-fortified "noninfant" cereals, which most toddlers consume at least once daily (25). The iron fortificant of these cereals is "reduced iron" (26). Reduced iron is a member of the family of elemental iron powders which, as used in food fortification, are thought to be of low bioavailability (27–33). Perhaps the most important factor affecting iron absorption by toddlers is their widespread consumption of cow's milk (24,34). Both calcium (35–37) and cow's milk proteins (38) are potent inhibitors of iron absorption.

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