

Determination of iron absorption from intrinsically labeled microencapsulated ferrous fumarate (sprinkles) in infants with different iron and hematologic status by using a dual-stable-isotope method¹⁻⁴

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ABSTRACT

Background: The use of microencapsulated ferrous fumarate sprinkles is a new approach for home fortification. Iron and hematologic status may affect the absorption of iron from sprinkles.

Objective: The objective was to measure the absorption (corrected erythrocyte incorporation of ⁵⁷Fe) of 2 different doses of iron from sprinkles added to a maize-based complementary food provided to infants with different iron and hematologic status.

Design: Infants aged 6–18 mo were randomly assigned to receive either 30 (*n* = 45) or 45 (*n* = 45) mg elemental Fe as ⁵⁷Fe-labeled sprinkles added to a maize-based porridge on 3 consecutive days. A ⁵⁸Fe tracer (0.2 mg as ferrous citrate) was also infused intravenously (*n* = 46). Blood was drawn at baseline and 14 d later to determine erythrocyte incorporation of ⁵⁷Fe and ⁵⁸Fe by using inductively coupled plasma mass spectrometry. On the basis of hemoglobin and soluble transferrin receptor concentrations, subjects were classified as having iron deficiency anemia (IDA), iron deficiency (ID), or sufficient iron status.

Results: There was no significant effect of dose on iron absorption (*P* > 0.05). Geometric mean iron absorption was 8.25% (range: 2.9–17.8%) in infants with IDA (*n* = 32), 4.48% (range: 1.1–10.6%) in infants with ID (*n* = 20), and 4.65% (range: 1.5–12.3%) in iron-sufficient infants (*n* = 20). Geometric mean iron absorption was significantly higher in infants with IDA than in infants with ID or iron-sufficient infants (*P* = 0.0004); however, there were no significant differences between infants with ID and iron-sufficient infants.

Conclusion: During infancy, iron absorption from sprinkles in a maize-based porridge meets and surpasses requirements for absorbed iron and is up-regulated in infants with IDA. *Am J Clin Nutr* 2004;80:1436–44.

KEY WORDS Iron, iron absorption, erythrocyte incorporation of iron, stable isotopes, microencapsulated iron, ferrous fumarate, sprinkles, human infants, iron deficiency anemia, Ghana

INTRODUCTION

Iron deficiency is the most prevalent nutritional deficiency in the world and affects up to two-thirds of children in most developing countries (1). Typical homemade complementary foods used in developing countries are poor sources of bioavailable iron and thus are inadequate to meet infants' high iron requirements for rapid growth and blood volume expansion after 6 mo

of age (2, 3). It follows that there is an urgent need to find effective strategies for treating and preventing iron deficiency anemia (IDA) in infants.

Our research group recently developed a novel, practical home-fortification approach that provides microencapsulated ferrous fumarate, ascorbic acid, and vitamin A in powder form (sprinkles). The fortificant is packaged in single-dose sachets that can be added once daily to any complementary food immediately before serving. The iron is encapsulated with a soya-based hydrogenated lipid to prevent interaction with the food and thereby avoids any changes in color, taste, or texture. In trials conducted in Ghana, we showed that sprinkles were as efficacious as ferrous sulfate drops in treating anemia in infants aged 6–18 mo of age (4) and were well tolerated and better accepted than the drops (5). We believe that home fortification has the potential to decrease the burden of IDA in infants throughout the developing world. Thus, our goal is to make sprinkles available for widespread, international distribution (6). However, with large-scale, countrywide programs in the developing world, it is not feasible to differentiate iron status between infants. Thus, a sufficient amount of iron should be absorbed from sprinkles to both treat and prevent iron deficiency and associated anemia.

The extent to which microencapsulated ferrous fumarate is absorbed by infants after being added to a complementary food

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is unknown. Moreover, it has been suggested that iron absorption may vary depending on iron and hematologic status because of an internal regulation mechanism that monitors iron stores and erythropoiesis requirements, which results in up-regulation of iron absorption during states of iron deficiency (7). The present study was undertaken to test the hypotheses that the percentage of iron absorbed from sprinkles would increase with increasing iron needs and decrease with increasing iron dosage in infants aged 6–18 mo. Our objective was to determine with the use of an intrinsically labeled dual-stable-isotope method the absorption (corrected erythrocyte incorporation of ^{57}Fe) of 2 different doses of iron from sprinkles added to a maize-based complementary food provided to infants with different iron and hematologic status.

SUBJECTS AND METHODS

Study area, subjects, and recruitment

The study was conducted from February to May 2002 in the field study area for the Kintampo Health Research Centre located in the Kintampo district of Ghana. The study protocol was approved by the Research Ethics Committees at the Hospital for Sick Children (Toronto) and the Ministry of Health of Ghana through the Health Research Unit (Accra, Ghana). Verbal consent to conduct the study in the Kintampo district was obtained from the District Assembly of Elected Representatives. Written informed consent was obtained individually from the mothers of the infants before the start of the study.

Infants aged 6–18 mo were screened on the basis of the following inclusion criteria: ingestion of at least one complementary food in addition to breast milk, being free from major illness such as symptomatic malaria, being afebrile, and having a hemoglobin concentration ≥ 70.0 g/L. Infants found to be febrile or severely anemic (hemoglobin concentration < 70.0 g/L) were treated at no personal expense.

Study design and protocol

Eligible subjects were stratified by age (6–12 mo and 13–18 mo) and hemoglobin status (hemoglobin concentration of 70–100 or ≥ 100 g/L) and then randomly assigned to receive either 30 or 45 mg elemental Fe as microencapsulated ferrous fumarate labeled with ^{57}Fe plus ascorbic acid and vitamin A on 3 consecutive days. Randomization was done by using sealed opaque envelopes containing color-coded group designations that were randomly selected by the mothers of the infants; all research staff and participants were blinded to the group assignments for the duration of the study and data analysis.

Classification with respect to iron and hematologic status was carried out post hoc because it was not possible to obtain immediate measures of iron status at the time of the blood sampling. Hemoglobin and soluble transferrin receptor (sTfR) concentrations were used to classify subjects into the following 3 subgroups: 1) subjects with IDA (hemoglobin concentration < 100 g/L and sTfR concentration > 8.5 $\mu\text{g}/\text{mL}$), 2) iron-deficient (ID) subjects (hemoglobin concentration ≥ 100 g/L and sTfR concentration > 8.5 $\mu\text{g}/\text{mL}$); and 3) nonanemic and iron-sufficient subjects (hemoglobin concentration ≥ 100 g/L and sTfR concentration ≤ 8.5 $\mu\text{g}/\text{mL}$).

The World Health Organization cutoff for anemia for children aged 6–59 mo is a hemoglobin concentration < 110 g/L; however, the World Health Organization has indicated that this cutoff

should be adjusted downward by 10 g/L in people of African extraction irrespective of age (8). In addition, the functional consequences of IDA become apparent at hemoglobin concentrations < 100 g/L (9). Thus, we used a hemoglobin concentration of < 100 g/L as the cutoff for anemia in the present study. The cutoff for iron deficiency was defined as an sTfR concentration > 8.5 $\mu\text{g}/\text{mL}$ (10, 11). Because ferritin is an acute phase reactant and is not a reliable reflection of iron stores in subjects with frequent infections (12, 13), it was not used to define iron and hematologic status in this study population, in whom malaria and other infections are common.

The total doses of elemental iron to be tested (30 and 45 mg) were based on estimates of iron absorption ranging between 2% and 8%, which is the range for nonheme iron sources (14). Because sprinkles are directly added to foods that are usually high in phytate and because the iron is encapsulated in a lipid coating, we predicted that the iron from sprinkles would be processed by the body in much the same way as nonheme iron. Thus, the total amount of available iron in the 30-mg dose would be between 0.6 and 3 mg, and the total amount of available iron in the 45-mg dose would be between 0.9 and 3.6 mg.

Eligible infants were studied at the Kintampo Health Research Centre on the mornings of days 1, 2, 3, and 17. On the morning of the first study day (day 1), each subject's hemoglobin concentration was determined, and a heparinized blood sample (2 mL) was withdrawn from an antecubital vein by using an intravenous cannula. The blood sample was stored for measurements of baseline iron isotopic composition of erythrocytes, iron status, and infection indexes (malaria and inflammation). Immediately after the blood sample was taken, the infusion set was flushed with saline to prevent clotting, and a 20-mL [^{58}Fe]ferrous citrate (0.2 mg elemental Fe) intravenous infusion was administered through a 1.2- μm filter over 10 min with careful monitoring of vital signs. At the end of the intravenous administration, the infusion set was flushed with saline to ensure that the entire intravenous ^{58}Fe tracer was infused. All blood draws and intravenous infusions were performed by a medical doctor and a nurse using sterile, pyrogen-free equipment and aseptic techniques.

No food or fluid other than water and breast milk was allowed for 4 h before the test meal administration. The test meal used throughout the study was made from locally available foods and was prepared at the research center before the arrival of the subjects and their mothers in the morning. The complementary food used consisted of 80% maize, 10% groundnuts, and 10% beans (15). The ingredients of the meal were roasted separately, mixed, and milled, and water and sugar were subsequently added to the mixture, which was cooked by bringing to a boil and allowed to cool before serving. To ensure that the entire ^{57}Fe -labeled sprinkles dose was fully ingested, it was mixed thoroughly with a small volume of the cooled meal (1–2 tablespoons) immediately before being fed to the infant in a dish, and the entire contents of the dish were fed to the infant. Subsequently, the dish and spoon used were rinsed several times with purified water, which was then consumed by the subject from a spoon or from the dish directly. Immediately thereafter, the infants were allowed to continue to breastfeed, and additional food was fed ad libitum.

All the infants were supervised closely by the field staff during consumption of the test meals and for an additional 2 h, during which no food or fluid other than water and breast milk was allowed. Any regurgitation that occurred during the meal administration was noted, and children who regurgitated were fed a

second isotope dose immediately. On days 2 and 3, the subjects received identical test meals under the same conditions. The infants and their mothers were discharged to their homes immediately after the test meal administration, and the mothers were asked to report any regurgitation that occurred during the following hour.

Fourteen days later (day 17), each subject's hemoglobin concentration was determined, and a final blood sample was collected from a finger prick (500 μ L) into a heparinized Microvette tube (Sarstedt Inc, Montreal) by using aseptic techniques to determine iron isotopic composition of erythrocytes and malaria status. Afterward, anthropometric measurements, including weight and length, were completed as previously described (4). After exclusion, withdrawal, or completion of the trial, all infants screened as being anemic (hemoglobin concentration < 100 g/L) were given a 2-mo supply of sprinkles to treat the anemia (4).

Stable-isotope labels and dosing

Isotopically enriched ^{57}Fe and ^{58}Fe in elemental iron form were purchased from Trace Sciences International (Richmond Hill, Canada). The abundances of the different iron isotopes, as measured by the manufacturer with the use of inductively coupled plasma mass spectrometry, were as follows (in percent by weight): enriched ^{57}Fe (^{57}Fe , 95.85%; ^{58}Fe , 3.19%; ^{56}Fe , 0.95%; ^{54}Fe , 0.01%); enriched ^{58}Fe (^{58}Fe , 84.60%; ^{57}Fe , 7.34%; ^{56}Fe , 8.01%; ^{54}Fe , 0.05%).

The isotopes were converted to [^{57}Fe]ferrous fumarate and [^{58}Fe]ferrous citrate by one of the major commercial suppliers of iron-fortification compounds, Dr Paul Lohmann GmbH KG (Emmerthal, Germany). For the preparation of [^{57}Fe]ferrous fumarate, ^{57}Fe was dissolved in sulfuric acid and combined with an aqueous solution of sodium fumarate. The iron fumarate precipitate was washed with water until no residual sulfate could be detected, and then the precipitate was dried and ground in an agate mortar to a fine powder (16). For the preparation of [^{58}Fe]ferrous citrate, the iron citrate was prepared by reaction of ^{58}Fe and an aqueous solution of citric acid. The iron citrate precipitate was washed with ethanol, dried, and ground as described above.

The [^{57}Fe]ferrous fumarate was microencapsulated specifically for the study by Particle Dynamics Inc (St Louis) by using the same encapsulation process and specifications that are used for their commercial product Descote Ferrous Fumarate 60% Ultra. Particle Dynamics Inc's proprietary process encapsulates ferrous fumarate in a lipid-based matrix (soya-based hydrogenated lipid). This process masks the taste of ferrous fumarate while maintaining its dissolution characteristics. The lipid encapsulation represents 40% of the product by weight. The minimum amount of ferrous fumarate that could be commercially encapsulated was 100 g. Because of the cost of the isotope, we were able to produce only one batch of encapsulated ferrous fumarate, which was made up of a homogeneous mixture of [^{57}Fe]ferrous fumarate and standard food-grade ferrous fumarate. Particle Dynamics Inc was supplied with sufficient [^{58}Fe]ferrous fumarate to attain a ratio of [^{57}Fe]microencapsulated ferrous fumarate to standard food-grade microencapsulated ferrous fumarate approximately equal to 1:4.4. Thus, from this batch, we planned to produce 2 ^{57}Fe -labeled iron doses, which would contain a total of either 30 or 45 mg elemental Fe with the following composition: 30-mg dose (5.5 mg elemental Fe as

[^{57}Fe]microencapsulated ferrous fumarate and 24.5 mg elemental Fe as standard food-grade microencapsulated ferrous fumarate); 45-mg dose (8.3 mg elemental Fe as [^{57}Fe]microencapsulated ferrous fumarate and 36.7 mg elemental Fe as standard food-grade microencapsulated ferrous fumarate).

The minimal dose of ^{57}Fe to be administered over 3 consecutive days (16.5 mg) was calculated on the basis of estimates of the quantity of total circulating iron in the subjects, the expected range of iron absorption, and the attainable reproducibility of the iron isotopic analysis. More specifically, this dose was based on a minimal detection limit for iron absorption of 1% measured with an external precision < 2% (17, 18). Total doses of ^{57}Fe used in the present study were higher than those commonly used in pediatric iron absorption studies, which usually range from 5 to 15 mg (19). The decision to use the larger dose was based on the study by Fomon et al (20), which concluded that infants incorporate far less than 80% of absorbed iron into erythrocytes.

Iron doses were individually weighed into color-coded, opaque Eppendorf tubes (Sarstedt Inc) into which 50 mg ascorbic acid and 300 μ g retinol equivalents retinol acetate were added by experienced laboratory personnel at the Hospital for Sick Children (Toronto). Ascorbic acid and vitamin A were added to each dose because sprinkles are routinely formulated to contain these vitamins, which may enhance the absorption of iron from food (21–24).

The ^{58}Fe -labeled intravenous infusion was manufactured by the pharmacy at the Hospital for Sick Children (Toronto) with the use of sterile, pyrogen-free equipment and aseptic techniques. The [^{58}Fe]ferrous citrate powder was dissolved in saline to reach a final concentration of 0.01 mg ^{58}Fe /mL as previously used in premature infants (25). Subsequently, the final solution was filtered through a 0.22- μ m filter and stored in individual sterile injection vials (20 mL), which were purged with nitrogen, sealed, and kept refrigerated until use. The final solution was tested for sterility, pyrogenicity, and stability before use as previously described (18).

The intrinsically labeled microencapsulated ferrous fumarate used in our study was compared with its commercial equivalent by measuring the mean particle size of a random sample and the relative contribution of the lipid encapsulate (by weight). The results were virtually identical. The isotopic compositions of the final ^{57}Fe -labeled oral doses and ^{58}Fe -labeled intravenous infusion were validated by using inductively coupled plasma mass spectrometry (Activation Laboratories, Ancaster, Canada).

Blood samples

Whole blood samples collected at baseline and on day 17 were used to make thick blood smears. The remaining blood was frozen at -40°C for analysis of iron isotopic composition of erythrocytes. At baseline, plasma was separated by centrifugation ($12\,000 \times g$, 10 min, room temperature), divided into aliquots, and frozen for analysis of ferritin, sTfR, and C-reactive protein (CRP) concentrations.

Hemoglobin concentrations were measured with the use of a portable HEMOCUE B-Hemoglobin photometer (Hemocue Inc, Angelholm, Sweden) by trained technicians using standardized techniques (26). Thick blood smears were prepared on glass slides, stained with Giemsa buffer solution, and examined under oil immersion with a light microscope for the presence of malaria parasites by a trained microscopist at the Michener Institute

(Toronto). Ferritin and sTfR concentrations were assayed in duplicate with the use of commercial enzyme-linked immunosorbent assay kits (Ramco Laboratories, Houston). Similarly, CRP was analyzed in duplicate by using a commercial enzyme-linked immunosorbent assay kit (Alpha Diagnostic International, San Antonio, TX); concentrations $> 8 \mu\text{g/mL}$ were considered elevated as suggested by the manufacturer. Commercially available external reference standards (BioRad Laboratories, Anaheim, CA; Ramco Laboratories) were assayed in duplicate along with all samples analyzed for ferritin, sTfR, and CRP.

Analysis of isotopic composition of blood samples

Iron was separated from 100- μL aliquots of whole blood and dissolved in 1% (by vol) HCl:H₂O to bring the analyzed iron concentration to 2 $\mu\text{g/mL}$ (27, 28). The $^{58}\text{Fe}:^{54}\text{Fe}$ and $^{57}\text{Fe}:^{54}\text{Fe}$ ratios of the dilute HCl solutions were determined by using inductively coupled plasma mass spectrometry (PlasmaQuad3 with PlasmaScreen option; Thermo Elemental, Franklin, MA). The sampler and skimmer cones were solid copper and platinum tipped, respectively. Each sixth sample was preceded and followed by blank acid extract and by certified iron isotopic reference material (IRMM-014; European Commission Joint Research Centre Reference Laboratory for Isotopic Measurements, Geel, Belgium). Intensities at masses 53, 54, 57, 58, and 60 were measured, and 10 successive replicates per sample were obtained. Intensities for masses 54, 57, and 58 for blank acid extract were subtracted from the corresponding intensities for aliquots of samples and isotopic reference material. Intensities for masses 54 and 58 were further compensated for contributions from ^{54}Cr and ^{58}Ni on the basis of the measured intensities for masses 53 and 60 and of the known natural abundance ratios of ^{54}Cr to ^{53}Cr and ^{58}Ni to ^{60}Ni . Sample ratios were compensated for mass bias on the basis of the calculated and the certified ratios for the aliquots of isotopic reference material. The mean relative precisions (percentages relative to SDs) obtained for $^{57}\text{Fe}:^{54}\text{Fe}$ and $^{58}\text{Fe}:^{54}\text{Fe}$ measurements were 0.3% and 0.5%, respectively.

Calculation of iron absorption

Calculations were adapted from Fomon et al (27) and Zlotkin et al (18). The atom isotopic ratios of administered isotopes (^{57}Fe and ^{58}Fe) were determined relative to ^{54}Fe in the blood sample, and the quantity of administered isotopes incorporated into the erythrocytes (Fe_{inc}) 14 d after administration of the last test meal (day 17) was determined as described by Fomon et al (27). Blood volume was assumed to be 65 mL/kg body weight on the basis of studies involving infants ranging from 6 to 15 mo of age (16, 29, 30).

The ^{57}Fe label contained a small amount of ^{58}Fe and vice versa. Therefore, the following equation was developed to correct for cross-contamination of ^{58}Fe in the ^{57}Fe label on the basis of the same assumptions made by Kastenmayer et al (31):

$$^{58}\text{Fe}_{\text{inc/corrected}} = ^{58}\text{Fe}_{\text{inc}} - [^{57}\text{Fe}_{\text{inc}} \times (3.19/95.85)] \quad (1)$$

where 3.19 and 95.85 (weight percentages) are the amounts of ^{58}Fe and ^{57}Fe , respectively, in the ^{57}Fe label. The quantity of administered ^{58}Fe or ^{57}Fe incorporated into erythrocytes, $\% \text{Fe}_{\text{inc}}$, was expressed as a percentage of the administered dose of ^{58}Fe or ^{57}Fe as described by Zlotkin et al (18).

Statistical analyses

Sample-size calculations were performed by using our previous data on iron absorption from microencapsulated ferrous fumarate (32). We estimated that 17 infants per group would be sufficient to detect a 5% difference in iron absorption with a 5% SD on the basis of a type I error set at 0.05 and a 0.8 probability of detecting a true difference between the 30- and 45-mg doses. Assuming a 30% dropout rate, we planned to include 22 eligible subjects per subgroup.

All iron absorption calculations were made by using EXCEL (Microsoft Office 1997; Microsoft Corporation, Seattle), and data were analyzed with the use of SAS, version 8.0 (SAS Institute Inc, Cary, NC). Hemoglobin concentrations, anthropometric z scores (growth reference values from the Centers for Disease Control and Prevention, 2000), and data from erythrocyte incorporation of iron from the ^{57}Fe - and ^{58}Fe -labeled oral and intravenous administrations, respectively, were normally distributed and therefore are presented as means \pm SDs. All other data were skewed and thus were transformed to natural logarithms before statistical analyses to compensate for nonnormal distributions and are presented as geometric means and ranges. Results were retransformed to the antilogarithms to recover the original units after statistical analyses.

Stepwise backwards selection was used to determine the best fitting model examining the relation of percentage and total erythrocyte incorporation of iron and of percentage and total iron absorption (natural logarithm) with the following baseline variables: malaria, inflammation (CRP), age (natural logarithm), and sex. General linear modeling (GLM) was used to evaluate the main effect, iron-status subgroup (subjects with IDA: hemoglobin concentration $< 100 \text{ g/L}$ and sTfR concentration $> 8.5 \mu\text{g/mL}$; ID subjects: hemoglobin concentration $\geq 100 \text{ g/L}$ and sTfR concentration $> 8.5 \mu\text{g/mL}$; iron-sufficient subjects: hemoglobin concentration $\geq 100 \text{ g/L}$ and sTfR concentration $< 8.5 \mu\text{g/mL}$), on erythrocyte incorporation and absorption of iron. Baseline hematologic indexes, infant characteristics, the incorporation of iron from the ^{58}Fe -labeled intravenous infusion, and iron absorption values from the oral dose were compared between iron status subgroups by using GLM with Tukey's correction. Because ferritin is an acute phase reactant and is elevated with infection or inflammation (as reflected by a CRP concentration $> 8 \mu\text{g/mL}$), ferritin values from subjects with elevated CRP (22 of 72 subjects) were excluded from statistical analyses (13). Statistically significant differences were indicated by $P < 0.05$.

RESULTS

Infant characteristics

Ninety-six infants were screened (**Figure 1**). Five infants were ineligible because they were severely anemic, and one infant was ineligible because of fever. Therefore, 90 eligible infants were enrolled in the 2 study groups, with equal numbers in each group. Forty infants in the 30-mg/d group and 38 infants in the 45-mg/d group completed the study. The rates of withdrawal and exclusion were similar between the 2 groups; in total, 5 infants withdrew from the study, and 7 were excluded during the trial because of fever, diarrhea, or insufficient blood collection. Eleven regurgitations were noted throughout the entire study, all on day 1. In one of these cases, the infant regurgitated 2 h after the meal was consumed; because gastric emptying time in infants is $\approx 2 \text{ h}$, we

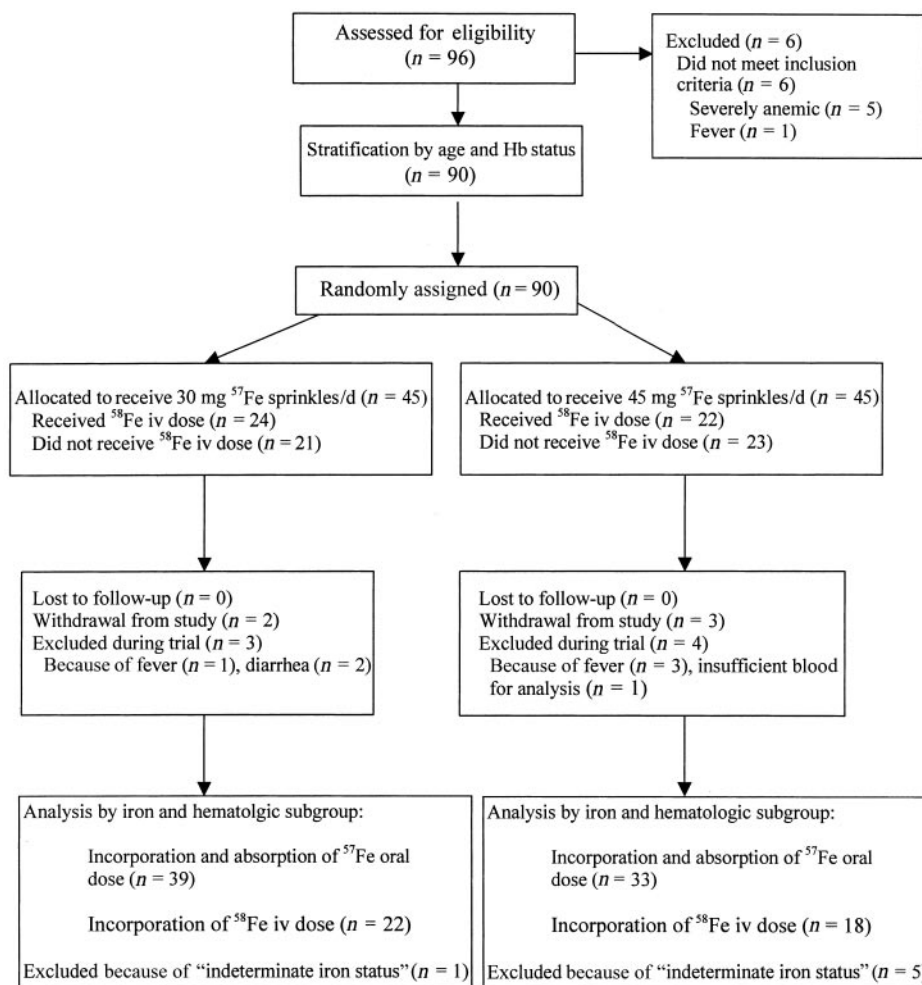


FIGURE 1. Trial profile. Hb, hemoglobin; iv, intravenous.

assumed that the isotope dose had already been absorbed (33). In the other 10 cases, the infant regurgitated while eating the meal and was fed an additional isotope dose right away. There were no reports of regurgitation occurring outside of the center after the meal administration on days 2 and 3.

The characteristics and baseline hematologic indexes of the infants who completed the study are shown in **Table 1** by iron-status subgroup. Age, sex distribution, and anthropometric *z* scores (weight-for-age, height-for-age, and weight-for-height) did not differ significantly between the infants in the 3 iron-status subgroups. Despite evidence suggesting the presence of iron deficiency in many of the subjects, most plasma ferritin concentrations were within the range expected for healthy subjects. In addition, evidence of an acute phase response, as reflected by elevated CRP concentrations, among $\approx 30\%$ of the subjects supported our rationale for classifying subjects' iron status on the basis of hemoglobin and sTfR concentrations. At baseline, 10 of 77 subjects had positive malaria smears, and at the end, 15 of 76 tested positive. There were no significant differences in the prevalence of positive malaria smears between the 3 iron-status subgroups at baseline or at the end. Six subjects in total were classified as having "indeterminate iron status" and were excluded from the analysis. We assumed that these subjects were anemic due to causes other than iron deficiency. As planned, there were

significant differences in hemoglobin, ferritin, and sTfR concentrations between the subgroups at baseline.

Erythrocyte incorporation and absorption of iron

Oral stable-isotope label (^{57}Fe)

After processing, the specific ratio of [^{57}Fe]microencapsulated ferrous fumarate to standard food-grade microencapsulated ferrous fumarate was $\approx 1:4$. Thus, the 2 ^{57}Fe -labeled iron doses had approximately the following composition: 30-mg dose (6 mg elemental Fe as [^{57}Fe]microencapsulated ferrous fumarate and 24 mg elemental Fe as standard food-grade microencapsulated ferrous fumarate); 45-mg dose (9 mg elemental Fe as [^{57}Fe]microencapsulated ferrous fumarate and 36 mg elemental Fe as standard food-grade microencapsulated ferrous fumarate). The [^{57}Fe]microencapsulated ferrous fumarate was structurally identical to the standard food-grade microencapsulated ferrous fumarate, Descote Ferrous Fumarate 60% Ultra (Particle Dynamics Inc).

Erythrocyte incorporation of intravenous iron (^{58}Fe)

The final concentration of the ^{58}Fe -labeled intravenous infusion (after filtration) was found to be 0.01135 mg $^{58}\text{Fe}/\text{mL}$. As

TABLE 1
Infant characteristics and baseline iron and hematologic indexes¹

	Iron deficiency anemia (n = 32)	Iron deficiency (n = 20)	Iron sufficiency (n = 20)	P
Infant characteristics				
Age (mo)	11.8 (6.2–18.6) ^{a,2}	14.4 (8.9–18.6) ^b	11.0 (6.6–18.6) ^a	0.03
Sex (%M)	63	40	65	0.2
Height-for-age (z score)	-0.5 ± 1.0 (-2.7 to 1.4) ^{a,3}	-0.7 ± 1.0 (-3.2 to 1.5) ^a	-0.9 ± 1.4 (-4.6 to 1.2) ^a	0.4
Weight-for-age (z score)	-1.2 ± 1.4 (-4.3 to 1.8) ^a	-1.6 ± 0.9 (-4.0 to -0.4) ^a	-1.8 ± 1.7 (-6.2 to 0.8) ^a	0.3
Weight-for-height (z score)	-0.7 ± 1.0 (-2.7 to 1.4) ^a	-1.8 ± 1.0 (-3.4 to 0.4) ^a	-1.1 ± 1.2 (-3.6 to 1.0) ^a	0.3
Baseline indexes				
Hemoglobin (g/L)	88.4 ± 7.9 (72–98) ^a	107.7 ± 7.3 (100–127) ^b	109.7 ± 7.2 (100–121) ^b	<0.0001
Anemia [n (%)] ⁴	32 (100) ^a	0 (0) ^b	0 (0) ^b	<0.0001
Ferritin (μg/L) ⁵	17.9 (4–166) ^a	38.7 (11–287) ^b	27.2 (7–157) ^b	0.01
Iron depletion [n (%)] ⁶	11 (34.4) ^a	1 (5.0) ^b	2 (10.0) ^b	0.01
sTfR (μg/mL)	16.5 (9–38) ^a	13.5 (9–44) ^a	7.1 (5–8.4) ^b	<0.0001
Iron deficiency [n (%)] ⁷	32 (100) ^a	20 (100) ^a	0 (0) ^b	<0.0001

¹ sTfR, soluble transferrin receptor. Differences between groups were determined by using general linear modeling with Tukey’s correction to determine individual group differences. Values in the same row with different superscript letters are significantly different, *P* < 0.05.

² Geometric \bar{x} ; range in parentheses (all such values). Analyses were done with log-transformed values because results were not normally distributed.

³ \bar{x} ± SD; range in parentheses (all such values).

⁴ Defined as a hemoglobin concentration < 100 g/L.

⁵ Because ferritin is an acute phase reactant protein, samples from subjects with elevated C-reactive protein concentrations were excluded. In total, 22 values were excluded from the ferritin analysis.

⁶ Depleted iron stores, defined as a ferritin concentration < 12 μg/L (34).

⁷ Tissue iron deficiency defined as an sTfR concentration > 8.5 μg/mL.

might have been expected in a study with infants as subjects, it was not always possible to obtain intravenous access because of the infants’ very small veins; 49% (44 out of 90) of our sample did not receive the ⁵⁸Fe-labeled intravenous infusion. Using stepwise backwards selection, which included age, sex, infection (CRP), malaria, the main effect of iron-status subgroup, and their interactions, age was the only variable found to be associated with the percentage erythrocyte incorporation of iron (*P* = 0.002, *r* = 0.4). The mean percentage erythrocyte incorporation of ⁵⁸Fe from the intravenous dose was found to be 76.6 ± 11.5% (range: 59.5–100%) in the IDA group (*n* = 15), 75.6 ± 15.2% (range: 48.8–100%) in the ID group (*n* = 13), and 72.7 ± 11.0% (range: 53.0–88.6%) in the iron-sufficient group (*n* = 8). No significant differences were detected between the groups after adjustment for age (*P* > 0.05); thus, the overall mean of

75% was used to calculate iron absorption in the infants who did not receive the ⁵⁸Fe-labeled intravenous infusion.

Erythrocyte incorporation of oral iron (⁵⁷Fe)

Data on total and percentage erythrocyte incorporation of iron from the 2 doses of ⁵⁷Fe-labeled sprinkles are shown in **Table 2**. Ten infants regurgitated immediately during 1 of the 3 meals containing the labeled iron, which means that only a minimal amount, if any, of the isotope would have been absorbed. With the use of GLM procedures, the mean values for erythrocyte incorporation of iron from the iron-status subgroups of the infants who regurgitated were not significantly different from the mean values from the iron-status subgroups of those who did not regurgitate (*P* > 0.05). Therefore, all infants were included in the main analysis. After a stepwise selection procedure with the use

TABLE 2
Erythrocyte incorporation and absorption of iron from oral ingestion of [⁵⁷Fe]ferrous fumarate sprinkles in infants with iron deficiency anemia, iron deficiency, iron sufficiency¹

	Erythrocyte incorporation of iron		Iron absorption ²	
	Total	Percentage of dose	Total	Percentage of dose
	mg Fe	%	mg Fe	%
Iron deficiency anemia (n = 32)	2.5 ± 1.0 ^{a,3}	6.9 ± 2.8 ^a	3.03 (1.1–5.9) ^{a,4}	8.25 (2.9–17.8) ^a
Iron deficiency (n = 20)	1.5 ± 0.8 ^b	3.9 ± 2.1 ^b	1.64 (0.03–4.8) ^b	4.48 (1.1–10.6) ^b
Iron sufficiency (n = 20)	1.5 ± 0.9 ^b	4.1 ± 2.3 ^b	1.67 (0.5–4.7) ^b	4.65 (1.5–12.3) ^b

¹ There were no significant main effects of dose and no significant dose-by-iron status interaction. Differences between groups were determined by using general linear modeling with Tukey’s correction to determine individual group differences. Values in the same column with different superscript letters are significantly different, *P* < 0.05.

² Analyses were done with log-transformed values because results were not normally distributed.

³ \bar{x} ± SD (all such values).

⁴ Geometric \bar{x} ; range in parentheses (all such values).

of GLM, only the main effect (iron-status subgroup: $P = 0.0001$, $r = 0.4$) was found to be significantly associated with total and percentage erythrocyte incorporation when sex, age, malaria or inflammation (CRP), and their interactions with iron-status subgroup were considered. There was no significant association between total or percentage erythrocyte incorporation of iron and iron dose ($P = 0.9$). Total erythrocyte incorporation of iron and percentage erythrocyte incorporation of iron were significantly higher in the IDA group than in the ID or iron-sufficient groups ($P < 0.0001$).

Iron absorption (^{57}Fe)

The data on total and percentage iron absorption from the 2 doses of ^{57}Fe -labeled sprinkles are also presented in Table 2. Not surprisingly, the range of percentage iron absorption values was wide because of the large intersubject variation associated with this measure (35). In addition, the wide ranges may have been due to the lack of standardization of the serving size and the ingestion of breast milk before or after ingestion of the test meal in some cases. After a stepwise selection procedure with the use of GLM, only the main effect (iron-status subgroup: $P = 0.0005$, $r = 0.4$) was found to be significantly associated with total and percentage iron absorbed when sex, age, malaria or inflammation (CRP), and their interactions with iron-status group were considered. There was no significant association between total and percentage absorption and iron dose ($P = 0.2$). Total and percentage iron absorption were significantly higher in the IDA group than in the ID or iron-sufficient groups ($P < 0.001$). When the associations of percentage iron absorption with plasma ferritin and sTfR concentrations were examined independently of iron-status subgroup, an inverse association was found between percentage absorption and log ferritin ($P = 0.01$, $r = -0.32$), and a positive association was found between percentage absorption and sTfR concentration ($P = 0.007$, $r = 0.32$).

DISCUSSION

In this study, we determined iron absorption from microencapsulated ferrous fumarate in powder form after it was sprinkled onto a small volume of maize-based complementary food provided to infants. We showed that iron absorption in the infants with IDA was nearly twice that in the iron-deficient infants (8.3% compared with 4.5%) and that among nonanemic infants, iron absorption did not differ significantly between iron-deficient and iron-sufficient infants (4.5% compared with 4.7%). Somewhat to our surprise, increasing the dose of iron provided to the infants from 30 to 45 mg had no significant effect on erythrocyte incorporation or absorption of iron from sprinkles independently of iron and hematologic status. The difference between the 2 iron doses studied may not have been large enough to detect a difference.

To our knowledge, this is the first study of iron absorption to report the use of an intravenous stable isotope administered concurrently with an oral stable isotope in infants aged 6–18 mo with different iron and hematologic status. Other studies of children in which a single isotope dose of iron was administered orally have typically assumed that 90% of absorbed iron is promptly incorporated into erythrocytes (19, 36). Very little is known about iron metabolism during infancy, and the extent to which newly absorbed iron is incorporated into erythrocytes in infants has not been validated (20, 31, 37). The advantage, therefore, of using the

current dual-stable-isotope method is that it allowed for the direct measurement of iron absorption, albeit in only about half of the children who completed the study ($n = 36$). However, data from the children who received the intravenous isotope allowed us to estimate a mean value for the percentage of absorbed iron that was promptly incorporated into erythrocytes for those children who did not receive the intravenous dose. A limitation of taking this extrapolated mean value is the degree of uncertainty associated with the calculation of iron absorption. We believe, however, that the use of this mean value is as justifiable as using an arbitrary value of between 80% and 90%. The approach taken in the present study used observed data among the same population rather than using a value derived from an adult population.

Data from the intravenous infusion of ^{58}Fe indicated that infants incorporated infused iron into erythrocytes to a similar extent, on a percentage basis, independently of their iron and hematologic status. Anemic infants incorporated $\approx 77\%$ (range: 59.5–100%) of infused iron, whereas nonanemic, iron-sufficient infants incorporated $\approx 73\%$ (range: 53.0–88.6%). Therefore, infants aged 6–18 mo with IDA behave differently from anemic adults, who have been shown to incorporate 100% of absorbed iron into erythrocytes (38). The mean value of 75% is also much higher than the values reported by Fomon et al (20), who found that geometric mean iron utilization of absorbed iron ranged from 16.8% to 35.6% in 18 infants aged between 20 and 215 d. The reason for the higher values reported in the present study may be related to differences in the study design and populations, because the infants in the present study were older.


Our study population was selected to investigate the effect of different iron and hematologic status on iron absorption from sprinkles. Iron deficiency can be identified best by using multiple indexes of iron status, including plasma ferritin, zinc protoporphyrin, and sTfR (12, 39). However, plasma ferritin and zinc protoporphyrin have been shown to be unreliable indicators of iron status in populations exposed to a high prevalence of infection (12, 13). Thus, in the present study, the combination of sTfR and hemoglobin was used as an indicator of iron deficiency and IDA (12, 40).

Although the mechanisms are still unclear, iron absorption is probably regulated by 2 different mechanisms, one known as the stores regulator and the other as the erythropoietic regulator (41, 42). However, whether these 2 biological controls of iron absorption are fully functional in infancy is unknown. Data from the present study show that the infants with IDA absorbed iron from sprinkles more efficiently than did the infants who were either iron deficient or iron sufficient but did not have anemia. These results suggest, as speculated by Domellof et al (37), that the erythropoietic regulator may be mature in infants after 6 mo of age and thus may up-regulate iron absorption for hemoglobin synthesis in states of anemia. There was no significant difference between nonanemic iron-deficient and iron-sufficient subjects, which suggests that tissue iron deficiency alone, as indicated by sTfR, may not affect iron absorption in this population.

The daily median requirement for absorbed iron in nonanemic infants after 6 mo of age is ≈ 0.7 mg/d (43). This requirement has been calculated to account for basal iron losses and for increases in hemoglobin mass and tissue and storage iron required for growth. It follows that infants with iron deficiency and IDA would need to absorb additional iron to further increase hemoglobin mass or replenish depleted iron stores. The doses of iron tested in the present study (30 and 45 mg) resulted in amounts of

absorbed iron that exceeded the daily requirement for this age group (Table 2). Thus, a lower dose of iron in sprinkles may be more appropriate for iron-intervention programs that would include iron-sufficient infants as well as iron-deficient and anemic infants. The current World Health Organization/UNICEF dose recommendation for program settings in which the prevalence of anemia in infants and young children is >40% is 12.5 mg Fe/d from 6–24 mo of age (44). If we assume equivalent rates of iron absorption, as were observed in the present study, with a 12.5-mg dose of iron, infants with IDA would absorb an average of 1.03 mg/d, whereas nonanemic infants would absorb 0.56 mg/d. Thus, sprinkles added to complementary foods at a 12.5-mg dose of iron would probably be adequate to ensure that the infant's diet (including breastmilk and intrinsic iron from complementary foods) would meet the daily absorbed iron requirement and provide sufficient iron to iron-deficient and anemic children to correct the anemia or replenish depleted iron stores.

Because iron deficiency and anemia in infancy have a detrimental effect on motor, cognitive, and social-emotional development, which may not be reversible (9), ensuring adequate iron intake and iron absorption in infants throughout the developing world is crucial. The present study provides evidence that administration of sprinkles in a complementary food given to infants aged 6–18 mo would result in iron absorption that would meet or exceed their iron needs. Wide intersubject variation was found in the present study. Results may have differed had the serving size been standardized and breast milk not been ingested before and after the administration of the test meal. For example, some of the variation in erythrocyte incorporation of iron from sprinkles could have been related to differences in the total amount of phytic acid ingested with the test meal. However, in rural Ghana, it was not possible to adhere to a strictly standardized protocol. The values reported here probably reflect the wide range of iron-absorption values that would be found in free-living persons, for whom sprinkles could be added to various serving sizes of complementary foods depending on the age, growth rate, and appetite of the child.

Although the results of this study can be generalized only to countries where maize-based complementary foods are the norm, the absorption characteristics of iron from sprinkles would probably be similar in rice- or wheat-based complementary foods with similar or lower amounts of phytic acid. Ongoing research with intrinsically labeled ferrous fumarate sprinkles is exploring the role of ascorbic acid and the effect of the addition of zinc to the sprinkles sachet on iron absorption. 

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MCT was involved in the design of the experiment, the collection and analysis of data, and the writing of the manuscript. CSS was involved in the design of the experiment and the writing of the manuscript and contributed significant advice. ALC was involved in the analysis of the data and the writing of the manuscript. KPA was involved in the collection of data. SN was the acting director of the Kintampo Health Research Centre after the death of Paul Arthur, co-principal investigator. SN provided significant consultation and supervision of fieldworkers. RES analyzed the isotopic composition of the blood samples and contributed significant advice. SHZ was involved in the design of the experiment, provided significant advice and consultation, and was involved in the writing of the manuscript. In addition, SHZ owns the intellectual property right to Supplefer Sprinkles. The HJ Heinz Company

Ltd is supporting the technical development of the sprinkles on a “cost-recovery” basis. None of the other authors had any conflicts of interest.

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