Ferrous Sulfate Is More Bioavailable among Preschoolers than Other Forms of Iron in a Milk-Based Weaning Food Distributed by PROGRESA, a National Program in Mexico[1,2]

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ABSTRACT After 1 y of distributing a milk-based fortified weaning food provided by the Mexican social program PROGRESA, positive effects on physical growth, prevalence of anemia, and several vitamin deficiencies were observed. There was no effect on iron status, which we hypothesized was related to the poor bioavailability of the reduced iron used as a fortificant in PROGRESA. The objective of this study was to compare the iron bioavailability from different iron sources added as fortificants to the weaning food. Children ($n = 54$) aged 2–4 y were randomly assigned to receive 44 g of the weaning food fortified with ferrous sulfate, ferrous fumarate, or reduced iron + Na_2EDTA. Iron absorption was measured using an established double-tracer isotopic methodology. Iron absorption from ferrous sulfate (7.9 ± 9.8%) was greater than from either ferrous fumarate (2.43 ± 2.3%) or reduced iron + Na_2EDTA (1.4 ± 1.3%) ($P < 0.01$). The absorption of log-^{58}Fe sulfate given with the iron source correlated with serum ferritin (s-ferritin) concentration ($n = 13$, $r = 0.63$, $P = 0.01$) and log-^{57}Fe absorption (reference dose) ($n = 14$, $r = -0.52$, $P = 0.02$). Absorption from ferrous fumarate and reduced iron + Na2EDTA did not correlate with s-ferritin or absorption of ^{57}Fe. The recommended daily portion of the fortified complementary food provides an average of 0.256, 0.096, 0.046 mmol (1.44, 0.54, and 0.26 mg) of absorbed iron, if fortified with sulfate, fumarate and reduced iron + Na_2EDTA, respectively. Ferrous sulfate was more bioavailable than either ferrous fumarate or reduced iron + Na_2EDTA when added to the milk-based fortified food and more readily met the average daily iron requirements for children 2–3 y of age. J. Nutr. 135: 64–69, 2005.

KEY WORDS: • *iron absorption* • *stable isotopes* • *fortification*

Anemia and iron deficiency are still public health problems globally. The most vulnerable group is young children whose prenatal iron stores are sufficient to maintain an adequate iron status for only the first 4–6 mo of life (1,2). After that period, the increased iron requirements for growth and a limited supply from breast milk (3) frequently result in iron deficiency with or without anemia, especially in many developing countries (4,5). Therefore, during this critical period, the primary sources of dietary iron are complementary foods with highly bioavailable iron or iron-fortified milk formulas (6). The high prevalence of iron deficiency anemia is of great relevance for public health in developing countries because of its negative effects on the survival (7) and future health of young children (8–11).

In 1999, 27% of Mexican children <5 y old were anemic. Iron deficiency (the percentage of transferrin saturation <16%) was present in 64% of anemic children and 48% of nonanemic children. Children from 12 to 23 mo of age were the most commonly affected, with a prevalence of anemia of 50% and of iron deficiency of 67% (12,13). Therefore, efforts are underway to improve the iron status of the population of this age. One of the interventions aimed at improving the iron status of small children that has been implemented by the Mexican Government is an integrated educational, health, and nutritional program. This program, PROGRESA (currently named "Oportunidades") serves >4 million impoverished families, at an annual cost of ~$2 billion (14). As a part of the nutrition intervention, the program provides a milk-based complementary food fortified with iron, zinc, folic acid, and vitamins A, C, E, and B-12 to children between 6 mo and 2 y of age (**Table 1**). An impact evaluation carried out in 1820 children 12 to 30 mo of age after 1 y of intervention, showed a positive effect on linear growth of 1 cm gain, and a 30% increase in the mean retinol and folic acid concentrations in intervened relative to nonintervened children (control group) (15). The prevalence of anemia in intervened children dropped from 46 to 40%, but there was no significant effect on

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TABLE 1

Nutrient composition of the fortified milk-based weaning food

Nutrient	Dry matter[1]		Dry matter[2,3]
	g	*MJ/kg*	*g/kg*
Ingredient			
Whole milk powder		549	
Maltodextrins		372	
Sugar		57	
Flavor		0.3–18.2	
Minerals and vitamins premix		~3.2	
Proximate composition			
Energy, *MJ*		19.60	
Protein	144		137 ± 6
Fat	163		176 ± 15
Carbohydrate		671	
Iron	0.23		0.215 ± 0.015
Zinc	0.22		0.232 ± 0.020
Retinol		0.009	
α-Tocopherol		0.000136	
Vitamin C	1.136		1.374 ± 0.034
Vitamin B-12, *μg*		0.00015	
Riboflavin, *μg*		0.000018	
Folate		0.0011	

[1] Calculated on the basis of data from the nutritional facts label of the container.
[2] Values are means ± SD of 10 samples measured in our laboratory.
[3] Unless indicated otherwise.

their iron status. It is likely that the reduction in the prevalence of anemia was caused principally by the improvement in folate and vitamin A status.

The lack of effect on iron status was probably due to the low bioavailability of the reduced iron used as a fortificant in the complementary food (16). The response prompted by this limited success in reducing the prevalence of iron deficiency anemia was to explore alternate, more bioavailable, iron sources to improve the efficacy of such an intervention. Some successful public programs have added ferrous sulfate to milk (17). Ferrous fumarate, added as a sprinkle to weaning foods, is also well absorbed (18). However, the absorption of hydrogen-reduced iron proved to be very low when used as a fortifier in a rice-based meal (19) or added to a breakfast of cornflakes, semiskimmed milk, and tea (20). Although the literature is not entirely clear, disodium EDTA (Na_2EDTA) may enhance iron absorption in humans. The addition of Na_2EDTA may enhance iron absorption from ferrous sulfate (21), but not ferrous fumarate (21,22). Iron absorption is also greater from meals fortified with reduced iron and $Na_2FeEDTA$ than from reduced iron alone (20), addition of Na_2EDTA did not enhance iron absorption (20). This last study (20), however, was carried out in nonanemic women, consuming a Western breakfast (cornflakes), using fecal balances as the outcome variable. It is unclear whether results would be similar in a more iron-deficient population or in children, i.e., the populations that might benefit most from improved iron bioavailability. Thus, the efficacy of combining Na_2EDTA and reduced iron as food fortificants is not yet settled. The objective of the present study was to compare iron bioavailability from ferrous sulfate, ferrous fumarate, and reduced iron + Na_2EDTA, added as fortificants to the milk-based weaning food, distributed by a national program in Mexico. The aim of such a comparison was to provide feedback to decision makers to improve the efficacy of the intervention.

SUBJECTS AND METHODS

Subjects and study design. The study was conducted in Cuautlancingo, Puebla, a low-income community, 120 km east of Mexico City. Children were recruited after being screened for hemoglobin concentration. They were enrolled in the study if they were 2.0–3.0 y old, had hemoglobin concentration >80.0 g/L, had no respiratory infections, diarrhea, or fever, and were not chronically taking any medications or micronutrient supplements. The potential risks, benefits, and procedures of the study were explained in detail to each subject's parents or guardians and written informed consent was obtained from them. The study protocol was approved by the Ethical Committee of the National Institute of Public Health in Mexico and the Institutional Review Board for Human Subject Research for the Baylor College of Medicine and Affiliated Hospitals in Houston.

Children were randomly assigned, stratified by gender, to consume the milk-based weaning food fortified with ferrous sulfate, ferrous fumarate, or reduced iron with Na_2EDTA. To adapt to the supplement, children received a daily serving of the weaning food for 15 d before the stable isotope study. Due to the high prevalence of infection by helminthes, all children received 400 mg albendazole as a single oral dose on d 5 of this period.

Subjects arrived at the community health center in the morning of d 16 of the study. Parents had been asked to not give them any breakfast before coming to the center. They received 1 serving of one of 3 complementary foods fortified with ferrous sulfate, ferrous fumarate, or reduced iron with Na_2EDTA, labeled with 1.5 mg of ^{58}Fe in the chemical form matched to the fortifier type they had been receiving at home previously. The total iron content of the test meal, including the tracer, was 1.79 mmol (10 mg). All of the children consumed the entire labeled meal as served. The test meals were administered in plastic, trace element–free containers by one member of the research team. The following day, again after not receiving a breakfast, children were administered an oral reference dose of 0.716 mmol (4.0 mg) of $^{57}FeSO_4$ with 2.83 mmol (50 mg) of ascorbic acid that had been diluted in 70 mL of nonvitamin-enriched grape juice. Only water was allowed for the 2 h after isotope administration. Fourteen days later (d 31 of the study), a 4-mL venous blood sample was obtained to determine the ferritin and C-reactive protein (CRP) concentrations in plasma and the isotopic enrichment of the RBC. Weight and height were measured according to standard methods (23).

Characteristics of the complementary food. The complementary food was prepared for this study by the same manufacturer that supplies the governmental program PROGRESA (Liconsa), following the formulation shown in Table 1. In short, the main ingredients included powdered milk (54.9%), maltodextrins (37.2%), sugar (5.7%), flavoring substances, and iron, zinc, retinol, riboflavin, vitamin B-12, α-tocopherol, vitamin C, and folate. In the manufacturing plant, the ingredients were mixed in a mechanical type "V" mixer until homogenized in the following sequence: milk powder, maltodextrins (from hydrolyzed cornstarch), flavoring substances, and vitamins and minerals premixed with an aliquot of hydrolyzed cornstarch, and finally sugar. Complementary foods were prepared by end users for consumption by adding ~25 mL of water (2 tablespoons) to 44 g of powder, giving it a purée consistency. Each serving provided 1.79 mmol (10 mg) of iron and 2.11 mmol (37.4 mg) of vitamin C.

Labeling of the test meals. ^{57}Fe and ^{58}Fe (>90% enriched (Trace Sciences International) were purchased and used to prepare the iron tracers. ^{58}Fe labeled ferrous fumarate was synthesized by the manufacturer by a standard method (24) and provided as a dry powder. Powdered, reduced ^{58}Fe was provided by the manufacturer at a particle size of 325 mesh (same particle size of the reduced iron used as fortificant for the complementary food). No further analyses were performed to assess compatibility with the commercial compound. To make ferrous sulfate, reduced iron isotopes, in ingots, were dissolved with H_2SO_4 to obtain $^{57}Fe\ SO_4$ and $^{58}Fe\ SO_4$ solutions (25). The isotopic enrichment of solutions was verified by magnetic sector thermal ionization MS (MAT 261, Finnigan Thermoquest). Glass vials were filled with isotope solutions and stored at 4°C.

Labeling of the milk-based weaning food. The reduced ^{58}Fe powder was mixed with Na_2EDTA in a 1:1 molar ratio, added to a small portion of weaning food powder, and thoroughly mixed. The

isotope container was rinsed several times to ensure the transference of the entire isotope dose. Then the weaning food powder was reconstituted with water, mixed again, and divided into 18 equal doses using a precision scale. $^{58}Fe\ SO_4$ and fumarate already in aqueous solution were extracted from the vial as individual doses with plastic syringes and added to small portions of previously reconstituted weaning food of equal weight. The unlabeled weaning food (~398 g/iron compound) was weighed on a calibrated scale with a precision of ±0.1 g and divided into 18 equal test meals (~22.1 g/test meal).

Laboratory determinations. Samples of the meals were digested with nitric acid and their iron content determined by atomic absorption spectrometry (Analyst 300, Perkin Elmer). Hemoglobin concentrations were determined from capillary blood samples using a portable photometer (HemoCue). Blood samples were drawn into trace metal–free, evacuated tubes with EDTA (Vacutainer, Beckton Dickinson). Plasma was separated after centrifugation at 1400 × *g* for 15 min and preserved in liquid nitrogen until ferritin and CRP were determined. The quantitative measurement of ferritin was determined by sandwich immunoassay (ELISA, Opus Behring Laboratories) using commercial kits (Dade Behring). CRP in serum was determined by an immunonephelometry system using a commercial kit (Dade-Behring). The cutoff point used to detect abnormal values was >3 g/L, as suggested by the manufacturer (26).

Determinations of isotope ratios. RBC were washed with saline and then frozen until analysis. Iron absorption was calculated from the enrichment in the RBC of the iron isotopes as described previously (27). Briefly, 0.3–0.5 mL RBC were digested with 10 mL of 15 mol/L nitric acid on a hot plate overnight. The digest was allowed to cool and redissolved in 0.6 mL of 6 mol/L ultrapure hydrochloric acid before being separated by anion exchange. A column (8-cm long × 0.4-cm diameter) was loaded with anion exchange resin (AG 1-X8; Bio-Rad Laboratories) and prewashed with 4 and 1 mL of 6 mol/L ultrapure hydrochloric acid. The sample was then loaded onto the column, followed by 6 mL of 6 mol/L ultrapure hydrochloric acid and 0.5 mL of 0.5 mol/L ultrapure hydrochloric acid. The sample was eluted from the column with 1 mL of 0.5 mol/L ultrapure hydrochloric acid into a teflon vial, dried on a hot plate, and dissolved in 40 μL of 3% ultrapure hydrochloric acid. Then 10 μL of the sample was loaded onto rhenium filaments with 2 μL of 0.7 mol/L phosphoric acid and 6 μL silica gel; the iron isotope ratio was measured with a thermal ionization magnetic sector MS (MAT 261; Finnigan). Replicate blocks of 10 scans each were performed until the required degree of precision (<0.2%) was achieved. The results were expressed as the ratio of ^{58}Fe to iron-56. The ratio of the 2 nonadministered isotopes (iron-56 and iron-54) was used to correct for temperature-specific differences in fractionation (27). Trace element–free reagents and disposables were used throughout.

Calculations. Incorporation into erythrocytes of the administered ^{57}Fe and ^{58}Fe (Fe_{inc}) was calculated from enriched (enr) and baseline (base) isotope ratios as follows:

$$^{57}Fe_{inc} = \left(\frac{^{57}Fe/^{56}Fe - {}^{57}Fe/^{56}Fe_{base}}{^{57}Fe7/^{56}Fe_{base}} \right) \times {}^{57}Fe_{cir} \times NA$$

where NA is the natural abundance of the isotope. Fe_{inc} is the total circulating iron estimated assuming a total blood volume of 65 mL/kg, the measured Hb concentration, and the iron concentration in Hb (3.47 mg/g). The same calculation was done for ^{58}Fe. Correction of cross-contamination of stable isotopes was made using tracer/tracee methodology. Results are expressed as the percentage of administered dose of each isotope incorporated into erythrocytes.

Sample size calculations were based on a 5% variability of iron absorption and expected differences among fortificants of 2.8%, as observed in previous studies (28,29). Considering a power of 80% and a 5% level of significance, a total sample size of 54 children (18 per group) including attrition was determined.

Statistical analysis. Iron absorption for each iron compound was measured from the incorporation of ^{58}Fe into RBC at d 14 as described previously; a figure of 90% of absorbed iron incorporated into RBC 14 d after dosing is typically applied at this age (30).

The absolute differential iron absorption among the iron sources tested was assessed by ANOVA and analysis of covariance, corrected by ferritin and the absorption of the reference dose as covariates. Then, to account for iron-deficient populations, absolute iron absorption was adjusted to 40% of the absorption of the reference dose. The resulting equation. separately regressing iron absorption from each iron compound tested on the iron absorption from the reference dose, was used for this 40% adjustment. Student's *t* test and a post-hoc Tukey-Kramer test were used for comparisons of iron absorption values among iron source groups. Differences were considered significant at $P < 0.05$. For comparisons among discrete variables, we used χ^2 tests. Correlations among iron absorption from the tested meal, the reference dose, and serum ferritin concentrations were assessed by Pearson's correlation. Serum ferritin and iron absorption values were not normally distributed; thus, they were normalized by log transformation before statistical analyses. Data are presented as geometric means, SD, and proportions. Data analyses were performed with Stata statistical software (V. 7.0).

Anemia was defined as a hemoglobin concentration <110 g/L (corrected by altitude) and iron deficiency as a serum ferritin <26.9 pmol/L (12 μg/L).

RESULTS

The groups did not differ in age, gender distribution, weight, height, hemoglobin, or serum ferritin concentrations (**Table 2**). Serum ferritin ranged from 5.84 to 187.6 pmol/L (2.6 to 86.2 μg/L); 11% of the subjects in the sample were anemic and 20% were iron deficient.

The overall geometric mean of the absorbed reference dose was 18.7%, assuming that 90% of iron is incorporated into erythrocytes. Absorption of the reference dose (^{57}Fe) did not differ among groups (ANOVA, $P = 0.215$). Due to logistic misfortunes in the field, the final sample size for each group was slightly different than originally planned, as discussed below. The geometric mean of iron incorporated into erythrocytes from ferrous sulfate was 7.9% ± 9.8 ($n = 17$). It was higher than ferrous fumarate (2.4% ± 2.3, $n = 19$) and reduced iron with Na_2EDTA (1.4% ±1.3, $n = 17$) (ANOVA, $P < 0.0001$; Tukey-Kramer test, $P < 0.01$). Iron absorption from ferrous fumarate was higher than that from reduced iron + Na_2EDTA (Tukey-Kramer test t, $P < 0.01$) (**Table 3**). The incorporation of iron into erythrocytes corrected by 40% of the absorption of reference dose was 14.4% for ferrous sulfate. 5.4% for ferrous fumarate, and 2.6% for reduced iron + Na_2EDTA

Inflammation indicated by a high CRP confounds iron status; thus, it is common practice to remove such cases from analysis. After removing from the analyses the cases with elevated CRP (>3 mg/L, $n = 11$), there was a negative correlation among log serum ferritin and the log ^{58}Fe absorption from the test meal fortified with ferrous sulfate ($n = 13$, $r = -0.63$, $P = 0.01$). Log s-ferritin and log ^{58}Fe absorption from ferrous fumarate ($n = 12$, $r = -0.52$, $P = 0.06$), and reduced iron + Na_2EDTA ($n = 13$, $r = -0.44$, $P = 0.12$) tended to correlate, though, not significantly. However, log ^{57}Fe absorption correlated with log-ferritin ($n = 14$, $r = -0.56$, $P = 0.02$), and log ^{58}Fe absorption ($n = 14$, $r = -0.52$, $P = 0.02$) in the sulfate group, but not in the fumarate or reduced iron groups.

DISCUSSION

Iron absorption from the complementary food fortified with ferrous sulfate was greater (7.9 ± 9.8%) than ferrous fumarate (2.43 ± 2.3%) and reduced iron + Na_2EDTA (1.4 ± 1.3%) ($P < 0.01$). Our data demonstrate that hydrogen-reduced iron is poorly absorbed from the milk-based weaning food tested. This result confirms other observations in which iron powders were tested both in vivo and in vitro (16). Even when

TABLE 2

Characteristics of the 53 children treated with the labeled weaning food, fortified with 3 sources of iron

	Ferrous sulfate	Ferrous fumarate	Reduced iron + Na_2EDTA
n	17	19	17
Sex, *Male:Female*	9:8	10:9	8:9
Age,[1] *y*	2.8 ± 0.8	2.7 ± 0.5	2.8 ± 0.5
Weight,[1] *kg*	13.5 ± 1.7	12.9 ± 1.9	13.2 ± 1.6
Height, *cm*	89.9 ± 4.6	87.9 ± 5.0	90.1 ± 4.0
Hemoglobin, *g/L*	128 ± 15	129 ± 13	129 ± 14
s-Ferritin,[2,3] *pmol/L*	93.3 (5.8–193.6)	42.7 (7.2–78.6)	45.1 (5.8–85.4)
ng/mL	44.2 (2.6–86.2)	19.0 (3.5–35.0)	20.1 (2.6–38)
Iron deficiency,[4] %	29	42	23
CRP,[2,3] *g/L*	19.5 (0.19–38.8)	60.7 (0.19–121)	5.6 (0.19–10.9)
Prevalence of high CRP,[5] %	11.0	10.5	17.6

[1] Values are means ± SD.
[2] Values are medians (range).
[3] Conversion factor used: 1 ng/mL ferritin = 2.247 pmol/L.
[4] Iron deficiency: s-ferritin ≤ 26.9 pmol/L.
[5] High CRP: ≥30 g/L.

Na_2EDTA was combined with reduced iron to fortify the complementary food, the incorporation of iron into RBC (1.4%) was very low. There are some possible explanations for this negative result. It is possible, for example, that the time allowed for equilibration between Na_2EDTA and reduced ^{58}Fe, once water was added to the powdered mixture, was not long enough for EDTA chelation of iron.

Absolute iron absorption from ferrous sulfate was the greatest among the 3 iron sources tested (7.9%). It was similar to that observed in Jamaican children for ferrous sulfate added to chocolate-flavored milk (31), but slightly lower than the absorption observed in Chilean children when ferrous sulfate was added to powdered milk (10%) (17). Iron absorbed from ferrous fumarate was much lower (2.4%) than the percentage absorbed from iron sulfate. It was comparable, however, to the absorption of iron from ferrous fumarate in a chocolate drink (2.8%), in adults (32).

According to figures of iron absorption presented here, a recommended daily portion of the weaning food fortified with ferrous sulfate would allow a mean of absorbed iron per day equal to 0.141 mmol/d (0.79 mg/d) [range = 0.046–0.758 mmol/d (0.26–4.24 mg/d)]. Fortification with ferrous fumarate would provide 0.042 mmol/d (0.24 mg/d) [range = 0.010–0.195 mmol/d (0.06–1.09 mg/d)]. Finally, fortification with reduced iron + Na_2EDTA would provide 0.025 mmol/d (0.14 mg/d) [range = 0.001–0.096 mmol/d (0.01–0.54 mg/d)]. The daily requirement of absorbed iron proposed for children 0.5–5.5 y of age ranges from 0.096 to 0.125 mmol (0.54–0.7 mg) (33). On this basis, the mean absorbed iron from ferrous sulfate and ferrous fumarate when added to the fortified weaning food could meet 113–146% of the daily requirement for children 1–3 y of age. Absorption from reduced iron + Na_2EDTA (20–26% of the requirements) may be inadequate to produce a measurable effect on the iron status of children receiving the fortified weaning food tested in this study. The study population had a high incidence of iron deficiency; thus, they would require more than the "maintenance" level of iron to correct this. Indeed, the actual amount of iron absorbed by infants in the present study from one serving of PROGESSA fortified with reduced iron varied between 0.001 and 0.89 mmol/d (0.01 and 0.5 mg/d).

There was a negative correlation between the log ^{58}Fe absorption and log ferritin concentration from ferrous sulfate; in contrast this relation was not significant for ferrous fumarate and reduced iron + Na_2EDTA. Nevertheless, the log ^{57}Fe absorption correlated with log ferritin only in the sulfate group, but not in the other 2 groups. This differential lack of correlation might have several causes. First, in this population with a high risk of iron deficiency, ferritin concentrations tend to be low, and variability is therefore very limited. Thus, in the majority of cases, iron absorption is as high as possible, and there are an insufficient number of subjects with high iron

TABLE 3

Absorption of ^{58}Fe (added to weaning food) and ^{57}Fe (reference dose) from ferrous sulfate, ferrous fumarate, and reduced iron + Na_2EDTA by children in the study[1,2]

	^{58}Fe Ferrous sulfate	^{57}Fe reference dose	^{58}Fe ferrous fumarate	^{57}Fe reference dose	^{58}Fe reduced iron + Na_2EDTA	^{57}Fe reference dose
n	17		19		17	
				%		
Absorption	7.9 ± 9.8*	16.2 ± 9.7	2.43 ± 2.3**	16.1 ± 5.4	1.4 ± 1.3	24.6 ± 14.4

[1] Values are geometric means ± 1 SD. * Different from ferrous fumarate and reduced iron + Na_2EDTA, $P < 0.0001$; ** different from reduced iron + Na_2EDTA, $P < 0.001$.
[2] Assuming 90% iron incorporation into RBC.

status to represent the reduced rate of iron absorption seen at this end of the spectrum. Second, the absorption of the reference dose and plasma ferritin may not correct individual variations in iron absorption as was suggested by Davidsson et al. (34). Finally, ferritin might not be the best indicator of iron status in children in this age group. Margolis et al. (35) found that the best predictor for response to iron supplementation in children was hemoglobin compared with s-ferritin, transferrin saturation, and erythrocytes protoporphyrin. Hershko et al. (36) found that ferritin was not the most reliable index of iron status in Israeli children. In that study, the iron absorption from ferrous sulfate, in both the test meal and the reference dose, was the only one to have a clearly significant correlation with iron status. Further, iron absorption from ^{58}Fe and ^{57}Fe ferrous sulfate correlated well between them ($R^2 = 0.32$, $\beta = 0.62$, $P = 0.01$), but similar correlations between ^{58}Fe and ^{57}Fe from ferrous fumarate or reduced iron were not significant ($P = 0.2$). On the basis of these findings, we speculate that the full water solubility of ferrous sulfate allows iron absorption to maintain a clear association with the iron status of the subjects tested. On the contrary, the lower water solubility of ferrous fumarate and reduced iron require an acidic milieu to be absorbed. Reduced acid output in *Helicobacter pylori* infections is an important limiting factor of iron absorption, especially for ferrous fumarate, altering it irregularly independently of iron status (37). The prevalence of this infection is as high as 50% in Mexican children (38) as in many other developing countries.

In an attempt to ensure that the tracer compounds were absorbed to the same extent as the specific iron fortificants that they were meant to label, the tracers were provided in the same chemical form as the respective fortificants. However, the physical forms of the tracers varied slightly, in that the ferrous sulfate was added to the prepared meals as an aqueous solution, whereas the ferrous fumarate and reduced iron + Na_2EDTA were added as dry powders. Nevertheless, this difference in the physical forms of the tracers was unlikely to have confounded the results because ferrous sulfate is readily water soluble over a wide pH range. Thus, even if the ferrous sulfate had been added as a powder, it would have entered into solution very rapidly. By contrast, ferrous fumarate and reduced iron + Na_2EDTA are soluble only at pH < 2 (39). Therefore, it would not have been appropriate to add these tracers to the test meals as low-pH solutions, because it could have altered the meals natural condition. For this reason, the later tracers were added as dry powders, conserving the meals and tracers in their natural condition.

This study tested a single food; if its results are considered in making programmatic decisions, consideration must be given to the balance between iron absorption enhancers and inhibitors in the whole meal consumed with the supplement, and the prevalence of parasitic infections of the target population, which are known causes affecting iron status. The government authorities of Mexico decided to change the iron source to sulfate for use in the weaning food. Such a decision was based primarily on the data presented here.

We presented an example of the need to conduct bioavailability studies for planning effective interventions with iron-fortified foods. To be effective, food iron-fortification strategies must not only ensure the content of adequate amounts of iron in the food vehicle, a successful delivery system, its acceptance, and its consumption by the target population, but they also must guarantee that the form of iron added to the particular food vehicle of choice is absorbed in adequate amounts. If the latter condition is not in place, the intervention will be ineffective, regardless of how satisfactory the other conditions are.

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