

Bioavailability of iron from micro-encapsulated iron sprinkle supplement

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Abstract

To improve the iron status of infants an effort was made to increase the iron content of complementary foods by adding 12.5 mg of elemental iron to the meal in the form of micro-encapsulated ferrous fumarate coated with a lipid. The contents of the packet were sprinkled directly on to infant foods. Relative absorption of iron from this supplement was determined in a prospective randomized study with 39 infants (mean age 33.6 ± 5.2 weeks) with initial hemoglobin values greater than 100 g/L. They were fed two complementary foods (rice-based and wheat-based) in which the supplement labeled with stable isotopes of iron ^{57}Fe and ^{58}Fe was incorporated. The erythrocyte iron incorporation was measured in the blood by inductively coupled plasma mass spectrophotometry. The incorporation of iron was significantly higher 11.9% $p < .001$ and 13.3% $p < .001$ and no difference was observed with the type of cereal in complementary foods. The use of ferrous fumarate sprinkles has proved to be efficacious in increasing the available iron intake of the infants.

Key words: iron absorption, supplement, stable isotopes, ^{57}Fe , ^{58}Fe , infants

Introduction

Iron-deficiency anemia is the most common of all the nutritional deficiencies affecting millions of infants and

children in the developing world. It is prevalent in all the age groups of the Sri Lankan population in which 45% of preschool children are anemic [1]. Although a variety of infant foods that include pre-cooked cereals, cereal-fruit products, etc. are currently fortified with iron most infants and toddlers in the developing world have no access to these foods due to their prohibitive cost. Although iron supplementation is believed to be the most effective method of alleviating iron-deficiency anemia where the prevalence is high, it has not been shown to be an effective public health strategy. However, there are some potential problems with implementing an effective strategy of wide-scale iron supplement distribution to infants and young children. Iron supplements for infants and young children are presently given as a solution which has significant disadvantages as compared to tablets or pills including dispensing directions, shorter shelf-life, higher likelihood of dosage errors, possible staining of teeth, and a strong and unpleasant taste. As such, in an alternative delivery system for providing iron to infants and toddlers, a novel packaging method and a source of iron have been developed where iron is made available as micro-encapsulated ferrous fumarate in a packet as a single daily dose of 12 mg/per day.

The sprinkle-sized particles of ferrous fumarate are coated with a mono or diglyceride (hydrogenated soy lipid) and this thin coating protects the iron from the food (and food from the iron) and also masks the taste of the iron. The contents of the packet are sprinkled on the food that is served to the child. The iron will not react with the food altering its appearance or taste because it is encapsulated. The coating will dissolve in the stomach, releasing the iron salt, to be absorbed along with iron contained in the foods that constituted the meal. The availability of the added iron for absorption will be affected by inhibitors and enhancers of iron absorption that might be present in a meal fed to infants and toddlers. Knowledge of the bioavailability of iron supplements is therefore necessary for a sound approach to establishing strategies for meeting the needs of the absorbed iron in them. Of the

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several approaches that have been used to estimate the bioavailability of iron sources, the double stable isotope technique was selected to determine the absorption of iron from the erythrocyte iron incorporation.

Materials and methods

This was a randomized prospective study to determine the bioavailability of micro-encapsulated and non-encapsulated ferrous fumarate after mixing them with two different complementary foods.

Population

The study population consisted of 39 full-term healthy infants and toddlers between 7 and 12 months of age, who were of average birthweight and who attended the maternal and child health clinic at Godakanda, Galle. They had hemoglobin concentrations 100g/L or more and had already been introduced to complementary foods, that were similar to the test meals (porridge of rice or wheat), and clinically stable at the time of the initiation of the study. Consent was obtained from the parents to include their infants in the study after they were informed about the aims and procedures involved. The protocol was approved by the Ethical Committee of the Faculty of Medicine, University of Ruhuna, Sri Lanka.

The sample size was calculated to be large enough to detect a difference between the bioavailability of non-encapsulated versus encapsulated ferrous fumarate. As there had been no prior studies to estimate the variance in the outcome measure, a convenience sample of 40 infants were enrolled in the study. With an estimated dropout rate of 33% it was hoped to complete the study in about 30 subjects.

Experimental design

The study subjects were selected during field visits by an investigator, and after obtaining written consent they were invited to the well-baby clinic on three days, two weeks apart, for the feeding trials. At each visit their body weight was measured and, a capillary blood sample was obtained before the test meal was given. A 10 μ l sample for hemoglobin was drawn onto a microcuvette, followed by another sample of about 250 μ l which was drawn into a heparinized plastic microtainer collection tube. The blood was transferred to the laboratory for subsequent ferritin and stable isotope analysis. Hemoglobin was determined immediately using the Heemocue photometer (AB Leo Diagnostics, Sweden).

Each infant was fed a test meal (rice-based) on the

first visit with a packet of sprinkles added and, the mothers were instructed to return in 14 days. At the second visit the infants were fed the second test meal (wheat-based) with an iron packet added after measuring their body weight and obtaining a blood sample. The mothers were asked to bring their infants back in 14 days and the third blood sample was drawn and their weights were recorded.

The normal food intake of the infants was not controlled except that test meals were given four hours after the last meal to that ensure their stomach was empty enough for proper utilization of iron and, the mothers were asked not to give any other iron supplement during the day.

Test meals

The complementary foods tested were primarily composed of brown country rice (low extraction) or semolina (high extraction) 17.5g, lentils 5g, carrots 12.5g, and butter 2.5g. The ingredients were cooked in double-distilled water until soft. Butter and salt were added after mashing the mixture to a thick porridge. Each serving was weighted and a packet of iron sprinkles (ferrous fumarate, encapsulated or non-encapsulated) either unlabeled or labeled with ^{57}Fe or ^{58}Fe added and thoroughly mixed just before serving. The test meals were fed by the mother under the supervision of the investigators, and the weight of waste and leftover food was measured to obtain the infants' actual intake. To make sure that the infants consumed the given amount of sprinkles completely, they were first fed a small proportion of the test meal mixed with the sprinkles followed by the rest of the meal.

Iron supplies

Each packet contained 12.5mg of elemental iron, consisting of three forms of iron ^{58}Fe , ^{57}Fe , and unlabeled ferrous fumarate. The proportion of each labeled iron was 1.5mg of ^{58}Fe , 5.0 mg of ^{57}Fe , and 5.5mg of (remainder) unlabeled ferrous fumarate.

The enriched ^{57}Fe and ^{58}Fe were purchased as ferric oxide from Oak Ridge National Laboratory, USA and, converted to labeled ferrous fumarate. Micro-encapsulation was done only on ^{58}Fe fumarate by coating with hydrogenated soybean lipid. ^{57}Fe ferrous fumarate was not encapsulated and was added directly to the packet.

Ferrous fumarate microcapsules were prepared by small-scale rotational suspension separation encapsulation. Briefly, ^{58}Fe ferrous fumarate was dispersed in molten hydrogenated soybean lipid (Durkee Industrial Foods Corp.) at 60°C. The resulting mixture was then poured slowly into a container of silicone oil (DF

55–2000, Silchem Inc.) at an equivalent temperature. The mixture was stirred with an impeller at 72 rpm and then rapidly cooled with chilled silicone oil to a temperature of 35°C. The resulting particles were separated from the silicone oil matrix by centrifugation at 2000X g and rapidly washed (5 x) with chilled heptane.

Analysis of blood samples

From the aliquots of blood (about 250 µl) that were drawn on each of three visits, 100 ml was separated and digested using nitric acid and heated until the digest was clear. The digests were stored in sterile screw capped containers at room temperature, and taken to the Division of Paediatrics and Nutritional Sciences of the Hospital for Sick Kids in Toronto, for determination of the erythrocyte incorporation of stable isotopes. The isotope in the erythrocytes was determined using the method of Zlotkin et al.[2]. The percentage of iron that is absorbed is incorporated into newly formed erythrocytes and, the incorporation was measured from the iron intensities of the erythrocytes [3] in the digested blood samples (3 samples per infant) using an inductively coupled plasma mass spectrometer (ICP-MS Elan Model 6000, SCIEX, Inc. Thornhill, Ontario Canada) operated in the isotope ratio mode (appendix 1). The remaining blood from each sample was used for the ferritin assay by well-established IRMA method [4] using Coat-A-Count Ferritin IRMA kits, DPC, Los Angeles, Calif., USA.

Analysis of complementary foods (test meals)

Analysis of iron and calcium were made by atomic absorption spectrometry (Model 975; Varian, Techtron, Australia). Aliquots of the complementary foods were dried at 100°C and ashed for 24 hours at 600°C. The ash was dissolved in nitric acid to determine the iron concentration and measured by using a standard addition technique. For calcium, the ash was diluted with 1.5 mol hydrochloric acid per liter containing 0.5% lanthanum chloride. Duram wheat flour (reference material 8436, National Institute Standard Technology, Gaithersberg, Md., USA) was used as the reference. The phytate and tannin contents of the foods were determined using an Anion exchange method [5] and, modified FAS method [6], respectively.

Statistical analysis

The principle outcomes were the bioavailability of the encapsulated versus the non-encapsulated iron, and the effect of the type of diet on the bioavailability of the

two forms of iron. These outcomes were analyzed using paired t-tests since each infant received both forms of iron in the same dosage and both diets.

Results

All the infants in the study were within the second six months of life at the time of study (table 1). This age is a period for the development of iron depletion and for the onset of iron-deficiency anemia. The subjects were non-anemic, with a mean hemoglobin of 114.14 ± 0.76 g/L and without evidence of iron depletion (mean ferritin 67.38 ± 33.99 µg/L). The mean birthweight was 2.9kg with a mean body weight of 7.2kg at the time of study.

The mean weights for the test meals consumed were 73.7 ± 41.5 g for the rice-based and 80.9 ± 40.7 g for the wheat-based meal (table 2). Table 3 shows the mean isotopic ratio (MIR) of the stable isotopes of iron naturally occurring and, the baseline values for MIR in the study subjects. It is apparent that prior to study the subjects had the same isotopic ratio ($^{57}\text{Fe}/^{54}\text{Fe}$ and

TABLE 1. Characteristics of the infants included in the study

	N	Mean \pm SD
Birthweight (kg)	39	2.988 ± 0.36
Baseline		
Weight (kg)	39	7.202 ± 0.80
Age (weeks)	39	33.71 ± 5.82
Hemoglobin (g/L)	39	114.14 ± 0.76
Ferritin (µg/L)	21	67.381 ± 33.99
End		
Weight (kg)	39	7.891 ± 0.29
Hemoglobin (g/L)	39	11.08 ± 0.56
Ferritin (µg/L)	25	66.734 ± 30.74

TABLE 2. Intake of the test meals and the contents of iron, calcium and inhibitory factors in a meal

	Rice-based Meal	Wheat-based Meal
Iron (mg)	1.3 ± 0.8	1.3 ± 0.6
Calcium (mg)	20.4 ± 10.8	54.2 ± 26.8
Phytate (mg)	42.5 ± 2.4	18.4 ± 2.1
Tannins (mg)	0.0	0.0
Intake (g)	73.7 ± 41.5	80.9 ± 40.7

TABLE 3. Isotopic ratio report

Natural isotopic composition	$^{57}\text{Fe}/^{54}\text{Fe} = 0.379$
(Mean isotopic ratio, MIR)	$^{58}\text{Fe}/^{54}\text{Fe} = 0.0482$
Mean isotopic composition	$^{-57}\text{Fe}/^{54}\text{Fe} = 0.376 \pm 0.002$
(MIR) of the samples at baseline	$^{58}\text{Fe}/^{54}\text{Fe} = 0.046 \pm 0.001$

$^{58}\text{Fe}/^{54}\text{Fe}$) and, the MIR is quite close to the natural occurrence.

The effects of encapsulation and of the meal composition on erythrocyte incorporation are shown in table 4. It appears that the percentage of iron absorbed is directly related to the intake of the iron incorporated into the test meals. The higher the intake, the higher the absorption observed (11.9% and 13.3% absorption from the 5.07 mg of ^{57}Fe and, 1.8% and 2.2% absorption from 1.57 mg of ^{58}Fe). The iron content was the same in both types of meals, but the inhibitory factors (phytate and calcium) varies (table 2). However, for each form of iron (encapsulated or non-encapsulated) there was no effect of the type of cereal on the percentage incorporated. Absorption from non-encapsulated iron was significantly higher in both rice- and wheat-based meals.

In-vitro studies of release of iron from micro-encapsulated ferrous fumarate

In an attempt to further understand the potential release of iron after micro-encapsulation, two commercial sources of micro-encapsulated ferrous fumarate were used and differences in the release of iron were studied by an in vitro simulated "gastric digestion model" modified to mimic the gastric pH, temperature, fluid volume, and gastric emptying time of the infants between 6 to 12 months of age. The differences in the release of iron were striking between the two sources of encapsulated iron. Over a two-hour time span there was virtually no release of iron from one as compared to 70% release from the other. It is apparent, therefore, that the method of micro-encapsulation will have a significant effect on the release and bioavailability of iron and that one cannot necessarily assume equivalent bioavailability among the different forms of micro-encapsulated iron.

Discussion

The results of this study provide information on the absorption of iron from the two homemade comple-

mentary meals. Ferrous fumarate (non encapsulated) was reasonably well absorbed despite the phytate and calcium content of the complementary foods used in the study. There was no significant difference in absorption between the rice- and the wheat-based meals.

The encapsulated ferrous fumarate, however, was less well absorbed than the non-encapsulated ferrous fumarate. Theoretically, the micro-encapsulated iron will not react with the food it comes in contact with, yet the coating will readily dissolve in the low pH of the stomach. It is quite likely that the coating on the isotopically-labeled ferrous fumarate adversely affected the absorption of the iron in this study. The encapsulation of the isotopically-labeled ferrous fumarate was done in a university laboratory since a commercial form of isotopically-labeled iron was not available. The ratio of coating to iron was 10-fold higher with the laboratory prepared product, as compared to the commercially encapsulated non-isotopically-labeled iron. It is therefore most likely that because of the increased amount of coating, digestion of the coating was incomplete. Even with commercially micro-encapsulated iron, a significant difference in potential absorption and bioavailability would be expected depending on the process of micro-encapsulation. The results of the current study, therefore, cannot be directly extrapolated to estimate iron dosage for clinical use, since the release of iron appears to be highly dependent on the method of micro-encapsulation. However, the estimate of the absorption of isotopically-labeled ferrous fumarate will be useful in the determination of an appropriate dose of encapsulated iron to be included in a single dose packet.

One explanation of the high prevalence of iron-deficiency anemia in developing countries is the low iron content of typical complementary foods combined with the low bioavailability of the iron due to the relatively high fiber and phytate contents of the food [7]. In the industrialized world, the problem has been addressed by the fortification of commercial complementary foods with iron. Since the routine use of iron-fortified commercial complementary foods is not an option for most infants in the developing world, alternate strategies have to be employed. One strategy is to add iron in a soluble form to the home-prepared complementary

TABLE 4. The intake doses of labeled iron and percentage of erythrocyte incorporation of isotopes from micro-encapsulated ($n = 32$) versus non-encapsulated ($n = 39$) iron from rice-based and wheat-based meals

Iron isotope and intake doses	Percent absorption from meal					
	Rice-based		Wheat-based		Combined	
	% \pm SD	<i>p</i>	% \pm SD	<i>p</i>	% \pm SD	<i>p</i>
^{57}Fe non-encapsulated	5.07 \pm 0.11 mg	.001	13.3 \pm 9.6	.001	12.5 \pm 7.6	.001
^{58}Fe encapsulated	1.56 \pm 0.03 mg		2.2 \pm 2.5		2.0 \pm 2.0	

food, without changing its taste, color, or appearance. In the present study the form of soluble iron (ferrous fumarate) tested caused no organoleptic changes and, the thin coating also masked the taste of the iron. As such, the use of a single dose supplement added directly to complementary foods has much appeal and this is especially true in developing countries because of the high prevalence of micronutrient deficiencies.

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Appendix 1

Isotope ratio mode

Samples:	Digested whole blood (1:3 HNO_3) diluted with deionized water
Instrumentation:	PE Elan 6000
Analytical standard:	Iron high-purity standards (Cat. No. 100026-1)
Interference correction:	Isotopic- ^{54}Fe ^{40}Ar ^{14}N Isobaric- ^{58}Fe (^{58}Ni) ^{57}Fe ^{40}Ar ^{160}H ^{58}Fe ^{40}Ar 180
Mass-discrimination correction:	Used in all isotopic ratios