Bioavailability of Microencapsulated Ferrous Sulfate in Powdered Milk Produced From Fortified Fluid Milk: A Prophylactic Study in Rats

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OBJECTIVE: We investigated the iron bioavailability of microencapsulated ferrous sulfate (SFE-171) in a diet based on powdered milk by using the prophylactic method in rats.

METHODS: The SFE-171 was added into fluid milk and industrially processed into powdered milk, which was then mixed in our laboratory with a normalized diet (17.2 ± 2.1 mg Fe/kg). A reference standard diet using ferrous sulfate as iron-fortifying source (19.8 ± 2.9 mg Fe/kg) and a control diet without added iron (4.6 ± 0.8 mg Fe/kg) were prepared in the laboratory in a similar way. These diets were administered to different groups of weaning rats for 28 d as the only solid nourishment. The iron bioavailability of the different sources was calculated as the relation between the mass of iron incorporated into hemoglobin during the treatment and the total iron intake per animal.

RESULTS: The iron bioavailability values of SFE-171 and ferrous sulfate in the fortified diets were $41.6 \pm 6.6\%$ and $42.6 \pm 4.2\%$, respectively; these results were significantly higher ($P < 0.01$) than the iron bioavailability of the control diet ($28.8 \pm 8.1\%$).

CONCLUSION: These results showed that iron-fortified powdered milk can be produced from fluid milk fortified with SFE-171. The bioavailability of SFE-171 in this rat model was not altered by the manufacturing process. *Nutrition* 2002;18:279–281. ©Elsevier Science Inc. 2002

KEY WORDS: iron, microencapsulation, food fortification, iron bioavailability, prophylactic method

INTRODUCTION

Iron fortification of foods is an effective approach to reduce the incidence of iron deficiency.[1,2] Different foods have been used as vehicles for iron-fortification purposes[3] such as milk and other dairy products, which are attractive vehicles because they are consumed widely and have high nutritional properties. Fluid cow's milk is consumed largely by children, a high-risk group with regard to iron deficiency.[4] In a study conducted in Argentina, for example, the administration of fluid whole cow's milk fortified with ferrous sulfate microencapsulated with phospholipids (SFE-171) for 4 mo corrected iron deficiency in a group of anemic children.[5]

Other dairy products such as powdered milk (PM), yogurt, and soft cheeses have been used in iron-fortification procedures. In particular, PM has been distributed in national nutritional programs because of its high nutritional value, long shelf life, reduced storing space, and low shipment costs. For these reasons, the importance of PM as a vehicle for iron-fortification purposes is widely supported, for example, by the successful trials performed in humans in Chile and Brazil.[6,7] However, when iron-soluble salts such as ferrous sulfate are added to PM at effective fortifications levels, the flavor and odor of the PM may be unpalatable unless it is packed in a protective environment. These problems are associated with iron oxidative properties that affect other nutrients[8] or

to the intrinsic metallic flavor of this element. Unfortunately, the use of less reactive iron sources is associated with low iron bioavailability[3] and is ineffective for the purpose of fortification.

For several years, SFE-171 has been used in Argentina and other countries as an iron-fortifying agent in milk and other dairy products, including PM. The SFE-171 is added to fluid milk, subjected to conventional dehydration processes (sprayed into a hot air flow at 205°C), and ground into fortified PM. The commercial product is satisfactorily consumed by the population, and it remains stable and palatable even after several months of storage.

SFE-171 was shown to have high iron bioavailability (BioFe) in fluid milk,[9] even after a heating period of 30 min at 100°C.[10] This finding indicated that the short thermal process used in the production of PM would not affect BioFe. Nevertheless, we investigated whether the intrinsic dehydration process of the nutritional matrix could affect the BioFe of SFE-171.

We used the prophylactic method[11] in rats. The BioFe of SFE-171 in a PM-based diet was compared with that of ferrous sulfate (reference standard) and measured in terms of relative biological value (RBV). In addition, the liver iron content (LIC) of each rat was determined at the end of the treatment to check the influence of each source on liver iron stores.

MATERIALS AND METHODS

The protocols of the prophylactic method[11] were adapted for this study. Twenty-seven female, inbred, Sprague–Dawley rats weaned at age 25 d were individually weighed (W_i, initial weight) and their initial hemoglobin concentrations (HbC_i) were determined by the

TABLE I.

DIETARY IRON CONTENT AND TOTAL IRON INTAKE, INITIAL WEIGHT, INITIAL HEMOGLOBIN CONCENTRATION, AND INITIAL
HEMOGLOBIN IRON OF THE ANIMALS IN THE STUDY*

Group	n Animals	DIC (mg/kg)	ToFeIn (mg) animal†	W_i (g)	HbC_i (g/dL)	$HbFe_i$ (mg)
Control	9	4.6 ± 0.8‡	1.6 ± 0.4‡	52 ± 2	11.7 ± 0.7	1.4 ± 0.1
SFE-171	9	17.2 ± 2.1	6.3 ± 1.3	55 ± 7	11.0 ± 1.1	1.4 ± 0.3
Standard reference	9	19.8 ± 2.9	6.1 ± 1.3	53 ± 3	11.2 ± 0.5	1.4 ± 0.4

* Results are presented as mean ± standard deviation.
† ToFeIn/animal was calculated as the product of the DIC multiplied by the amount of food consumed by each animal during the experiment.
‡ Significantly different from other groups ($P < 0.01$).
DIC, dietary iron content; HbC_i, initial hemoglobin concentration; $HbFe_i$, initial hemoglobin iron, SFE-171, microencapsulated ferrous sulfate; ToFeIn, total iron intake; W_i, initial weight.

cyanomethahemoglobin method.[12] The animals were segregated into three similar groups comprising nine rats according to their W_i and HbC_i values and then housed in stainless steel cages in a temperature- and light-controlled environment.

Three experimental diets were prepared in our laboratory and given to the animals during the treatment.

SFE-171 Diet

This diet was based on a sample of whole PM (La Serenísima, Buenos Aires, Argentina) industrially fortified with 110 mg Fe/kg as SFE-171 (Lipotech, Buenos Aires, Argentina). The fortifying agent was added to the fluid milk that was then processed into the fortified PM.

Standard Reference Diet

This diet was based on a sample of whole PM (La Serenísima) fortified at our laboratory with 110 mg Fe/kg as $FeSO_4 \cdot 7H_2O$ (Fluka, Buchs, Switzerland).

Control Diet

This diet was based on a sample of whole PM (La Serenísima) without iron additions.

To meet the nutritional requirements of the rats, other food components were added to each diet. The final compositions of all diets were adjusted to the specifications of the AIN-93 diet for rodents.[13] As a consequence, the final iron concentration in the fortified diets was reduced to 17 to 20 mg Fe/kg.

These diets were available ad libitum to the different groups of rats (which henceforth are referred to by the particular diet). No other solid nourishment was given to the animals and the amount of consumed food was registered daily. The iron concentration of each diet was determined by the Ferrozine technique as modified for foods.[14] All rats had free access to deionized water (Ametek, Plymouth, MA, USA).

The treatment continued for 28 d. After that period, each animal was weighed again (W_f). The rats were then treated with 1500 IU of heparin per kilogram of body, anesthetized with diethyl ether, and killed; between 3 and 4 mL of blood per animal was collected. The hemoglobin concentration in the collected blood of each rat (HbC_f) was measured the same way as HbC_i. Whole livers were removed from all rats, washed with deionized water, weighed, and stored at −20°C. The LICs were determined with the Ferrozine technique.[14]

Six parameters were calculated as described elsewhere:[15] dietary iron concentration, total iron intake, initial hemoglobin iron, final hemoglobin iron, %BioFe, and RBV. The LIC was calculated as the product of the iron concentration in each liver multiplied by the organ mass.

Statistical analysis of the results was done with one-way analysis of variance followed by Scheffé's test.[16] $P < 0.01$ was considered statiscally significant.

RESULTS

The dietary iron concentrations of the diets and the total iron intake per animal are shown in Table I. For both parameters, significantly lower ($P < 0.01$) values were obtained for the control group than for the other groups. Table I also shows that non-significant differences were found in the W_i, HbC_i, and initial hemoglobin iron values between groups.

Table II shows that the values of W_f, HbC_f, and final hemoglobin iron in the control group were significantly lower ($P < 0.01$) than those in the SFE-171 and standard reference groups.

The BioFe values of 41.6 ± 6.6 and 42.6 ± 4.2 were obtained for the SFE-171 and standard reference groups, respectively (Table III); those values were significantly higher ($P < 0.01$) than those for the control group (28.8 ± 8.1). The RBV of the SFE-171 group was 98%, which is similar to that of ferrous sulfate, arbitrarily fixed at 100%. Non-significant differences in LIC were found between the SFE-171 and standard reference groups but were significantly higher ($P < 0.01$) than that of the control group.

TABLE II.

FINAL WEIGHT, FINAL HEMOGLOBIN CONCENTRATION, AND
FINAL HEMOGLOBIN IRON OF THE ANIMALS IN THIS STUDY*

Group	W_f (g)	HbC_f (g/dL)	$HbFe_f$ (mg)
Control	155 ± 9†	5.2 ± 0.6†	1.8 ± 0.2†
SFE-171	185 ± 22	9.4 ± 0.7	3.9 ± 0.4
Standard reference	187 ± 13	9.5 ± 0.8	4.0 ± 0.3

* Results are presented as mean ± standard deviation.
† Significantly different from and between groups ($P < 0.01$).
HbC_f, final hemoglobin concentration; $HbFe_f$, final hemoglobin iron; SFE-171, microencapsulated ferrous sulfate; W_f, final weight.

TABLE III.

IRON BIOAVAILABILITY, RELATIVE BIOLOGICAL VALUES OF IRON SOURCES, AND LIVER IRON CONTENT OF THE ANIMALS IN THIS STUDY[*]

Group	BioFe (%)	RBV (%)	LIC (mg Fe)
Control	28.8 ± 8.1[†]	—	0.07 ± 0.01[†]
SFE-171	41.6 ± 6.6	98	0.29 ± 0.06
Standard reference	42.6 ± 4.2	100	0.25 ± 0.04

[*] Results are presented as mean $\pm$ standard deviation. The BioFe values were calculated as the percentage ratio between the hemoglobin iron variation during the treatment and the total iron intake. The RBV of SFE-171 was calculated as the percentage ratio between the BioFe value of this group and that of the standard reference group. The LIC values were calculated as the product of the liver iron concentration multiplied by the liver mass.

[†] Significantly different from the other groups ($P < 0.01$).

BioFe, iron bioavailability; LIC, liver iron content; RBV, relative biological value; SFE-171, microencapsulated ferrous sulfate.

DISCUSSION

The between-groups differences for W_i, HbC_i, and initial hemoglobin iron concentration were not significant, so the animals' iron status was satisfactorily homogeneous at the beginning of the study. Moreover, non-significant differences were found in the dietary iron concentration of the SFE-171 and standard reference diets, which also had identical compositions, except for the iron-fortification source. Thus, any difference in iron absorption between these two groups would depend on the intrinsic BioFe of the different sources. This premise was supported by the fact that no significant differences in total iron intake were found between these two groups.

No significant differences in W_i were found between the three groups. In contrast, W_f was significantly lower in the control group than in the other groups. The only difference between the compositions of the control diet and the other diets was iron concentration. Food consumption was similar for all groups. Therefore, there was a clear relation between the iron ingesta and the growing rate. In addition, the iron provided by SFE-171 and ferrous sulfate likely had similar positive effects on the animals' growth.

These three groups had similar hemoglobin concentration and iron hemoglobin content (Table I). At the end of the study, the values of these parameters remained similar for the SFE-171 and standard reference groups, although in both cases the values were significantly higher ($P < 0.01$) than those in the control group (Table II). Therefore, the iron provided by ferrous sulfate and SFE-171 was positively and equally incorporated into the hemoglobin of the animals consuming those diets.

The BioFe and RBV of SFE-171 and ferrous sulfate are given in Table III. The BioFe provided by both sources was similar. The LIC value of the SFE-171 group was slightly higher than, although not significantly different from, that of the standard reference group, showing that the iron provided by both sources was stored similarly in the rat livers.

The present BioFe and RBV results are very similar to those obtained in a previous analogous work[15] in which the AOAC basal diet[17] was fortified with the same iron source. In that study, the BioFe of SFE-171 was similar to that of ferrous sulfate. The present results showed that the production of iron-fortified PM from fluid milk fortified with SFE-171 is feasible; the procedure did not alter the BioFe in this rat model.

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(For an additional perspective, see Editorial Opinions.)