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Bioavailability of Stabilised Ferrous Gluconate with Glycine in Fresh Cheese Matrix: a Novel Iron Compound for Food Fortification

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Abstract Iron fortification of foods continues to be one of the preferred ways of improving the iron status of the population. Dairy product is a common product in the diet; therefore, it is a plausible vehicle for iron fortification. This study aims to investigate the bioavailability of ferrous gluconate stabilised with glycine (FGSG) in a fresh cheese fortified with zinc. The iron bioavailability of fresh cheese fortified with either FGSG and with or without zinc and FGSG in aqueous solution and a water solution of ferrous ascorbate (reference dose) was studied using double radio iron (^{55}Fe and ^{59}Fe) erythrocyte incorporation in 15 male subjects. All subjects presented with normal values for iron status parameters. The geometric mean of iron bioavailability for the water solution of FGSG was 38.2 %, adjusted to 40 % from reference doses (N.S.). Iron bioavailability in fresh cheese fortified with Ca and Zn was 15.4 % and was 23.1 % without Zn, adjusted to 40 % from reference doses (N.S.). The results of the present study show that the novel iron compound ferrous gluconate stabilised with glycine in a fresh cheese matrix is a good source of iron and can be used in iron fortification programmes.

Keywords Iron bioavailability · Iron absorption · Ferrous gluconate

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Introduction

The World Health Organization considers iron deficiency the number one nutritional disorder in the world. In children, iron deficiency causes development delays and behavioural disturbances and in pregnant women it increases the risk for preterm delivery and delivering low birth weight newborns [1, 2].

Iron supplementation is believed to be the most effective method of alleviating iron deficiency anaemia where the prevalence is high. However, therapeutic doses of iron supplements may cause gastrointestinal side effects, such as nausea, vomiting, constipation, diarrhoea, dark coloured stools, and abdominal distress [1, 2].

Iron fortification of foods continues to be one of the preferred ways of improving the iron status of the population. However, there are a variety of technical considerations of importance when iron will be used as the fortificant: (a) the food vehicle must be consumed in the habitual form for the target population, (b) the iron absorption of the fortified food must be regulated by the iron status of the subject, (c) the type of iron fortificant used, (d) the level of fortification, (e) incorporation of other microminerals like zinc and copper must be related to the quantity of iron and (f) the health status and environmental factors that affect iron metabolism.

Dairy products are common in the diet; therefore, they are a plausible vehicle for iron fortification. Nevertheless,

some researchers have raised concerns about the interactions between iron, zinc and calcium. When iron and zinc supplements are given together in a water solution and without food, greater doses of iron may decrease zinc absorption. However, the effect of supplemental iron on zinc absorption does not appear to be significant when supplements are consumed with food. Likewise, there is evidence that calcium from supplements and dairy foods may inhibit iron absorption, but it has been difficult to distinguish between the effects of calcium on iron absorption and the effects of other inhibitory factors, such as phytate, that are also present in the food [2–5].

The aim of our study was to measure the iron bioavailability of ferrous gluconate stabilised with glycine (FGSG) when used as the iron source in an iron–zinc–calcium-fortified fresh cheese. For comparative purposes, we also studied the iron bioavailability of FGSG in an iron–calcium-fortified fresh cheese and in a water solution.

Subjects and Methods

Subjects

This was an open, paired-design study. Fifteen apparently healthy males aged 21 to 45 years were selected to participate. All subjects presented a body mass index below 30 kg/m^2, serum ferritin >12 μg/L and Hb concentration >130 g/L. They had not consumed nutritional supplements containing iron or zinc in the prior 6 months. This study was conducted according to the guidelines laid down in the Declaration of Helsinki, and all procedures involving human subjects were approved by the Ethics Committee of the Institute of Nutrition and Food Technology (INTA), University of Chile #2007-3. Written informed consent was obtained from all subjects/patients.

Study Design

Iron isotopes (^{59}Fe and ^{55}Fe) were used as tracers (NEN, Life Science Products, Boston, MA, USA) for the intrinsic labelling of the test meals. The nutritional matrix of studied foods belongs to the family of fresh cheese fermented by *Lactobacillus lactis* subsp. *cremoris* and *L. lactis* subsp. *lactis*, which is specifically manufactured by the industry Danone SA Argentina and prepared before administration. This cheese is composed of 6.2 % protein, 3.5 % fat, 15.3 % total sugars, 4.5 % fruit (pasteurised strawberries) and 70.5 % humidity. The amount of Fe, Zn and Ca in the cheese before the addition of the minerals was 1.1±0.1, 5.2±0.6 and 956±5 mg/100 g, respectively. The fresh cheese was fortified with iron as FGSG (2.1 mg) (Lipotech SA, Buenos Aires, Argentina), zinc as zinc gluconate stabilised (2.1 mg) (Lipotech SA, Buenos Aires, Argentina) and calcium (240 mg) as calcium citrate (Tate & Lyle, London, UK) and calcium lactate (Purac, Gorinchem, The Netherlands) per 90 g of product. A second fresh cheese was prepared with the same minerals but without zinc. The meal portions used in the study were provided with a temperature indicator to ensure that transport and storage conditions were compliant. The mineral content for iron, zinc and calcium, as declared in the composition of the test meals, was measured by atomic absorption spectrometry (Perkin-Elmer, Model 2280, The Perkin-Elmer Corporation, Norwalk, CT, USA).

On day 1, the subjects received 90 g of the fresh cheese fortified with FGSG, zinc and calcium. The FGSG was labelled with 111 kBq ^{55}Fe. On day 2, the subjects drank a ferrous ascorbate solution prepared with 3 mg of iron as ferrous sulphate ($FeSO_4 \cdot 7H_2O$, Merck, Darmstadt, Germany) and ascorbic acid (L-ascorbic acid, Sigma Chemical Co., St. Louis, MO) in a 1:2 molar proportion labelled with 37 kBq ^{59}Fe (reference dose). On day 14, a venous blood sample was obtained to measure the circulating isotopes and to determine the iron status of the subjects. Later the same day, the subjects received 90 g of the fresh cheese fortified with FGSG and calcium but without zinc. Again, the FGSG was labelled with 111 kBq ^{55}Fe. On day 15, the subjects received a powder of FGSG intrinsically labelled with 37 kBq ^{59}Fe dissolved in deionized water. Finally, on day 28, a final venous sample was obtained to measure the increase in red blood cell isotopes. All the test meals were consumed after an overnight fast, and no food or beverages other than water were allowed during the following 4 h. Because there are no data concerning the bioavailability in humans of iron in a matrix of fresh cheese fortified with zinc and calcium and because bioequivalence studies generally include a minimum of 12 subjects, 15 was considered a sufficient number of volunteers for the purposes of the current study. For purposes of comparison, all studies currently refer to 40 % absorption of the reference dose of ferrous ascorbate.

Laboratory Analysis

Venous blood samples obtained on days 14 and 28 were used to measure the circulating isotopes and to determine the iron status of the subjects. It was measured with Hb and mean cell volume (MCV) (CELL-DYN 1700, ABBOTT Diagnostics, Abbott Park, IL, USA), erythrocyte zinc protoporphyrin (Znpp) (Hematofluorometer Model 206D, AVIV Biomedical Inc., Lakewood, NJ, USA), serum iron, total iron binding capacity, transferrin saturation (Sat) [6] and serum ferritin (SF) [7].

In order to calculate the total isotopes ingested, four aliquots of compounds were used as standards. Blood isotopes determinations were performed in duplicate according

to the Eakins and Brown technique [8]. Samples were counted a sufficient time to obtain a counting error of <3 % in a liquid scintillation counter (TRICarb 2000, Canberra Packard, Downers Grove, Illinois, USA). The absorption percentages were calculated on the basis of blood volume estimated for height and weight [9], assuming an 80 % incorporation of the radioisotope into erythrocytes [10]. This method is reproducible in our laboratory with a CV of 5 %.

Statistical Methods

Because the percentages of iron absorption and SF have skewed distributions, these values were first transformed to their logarithms before calculating means and standard deviations (SD) and all statistical analyses were performed on log values. The results were retransformed using the antilogarithm to recover the original units and then expressed as geometric means and ±1 SD ranges. All statistical tests were considered significant at the 5 % level of significance. Student's *t* tests on repeated measures were used to characterise significant differences on iron absorption. All analyses were performed using SPSS (Chicago, IL, USA) and Excel (XP 2002; Microsoft, Seattle, WA, USA).

Results

All subjects presented a normal iron status: the mean ±SD of Hb was 158±7 g/L; MCV, 88±3 fL; Znpp, 0.85±0.18 μmol/L RBC; Sat, 31.2±8.6 % and SF, 65±23 μg/L. There were no exclusions or premature withdrawals during the study. Therefore, the intention-to-treat and per-protocol population were the same and we performed analyses among only one population.

Table 1 shows the iron bioavailability results. There was no statistically significant difference in iron bioavailability between the FGSG and ferrous ascorbate in water solution. The iron bioavailability corrected to 40 % absorption of the reference dose was 15.4 % in the fresh cheese fortified with Ca and Zn and 23.1 % without Zn ($p>0.2$).

Discussion

Previous work performed in animals has shown that FGSG used to fortify fresh cheese has a 95 % relative bioavailability compared to ferrous sulphate and does not show negative interaction effects with zinc [11, 12]. However, there have been no data available on the bioavailability for this kind of iron source in humans or in a food matrix fortified with zinc and calcium in either species.

Table 1 Iron bioavailability of fresh cheese fortified with iron as ferrous gluconate stabilised with glycine (FGSG) and reference dose

Subject	Iron absorption (% of dose)			
	Fresh cheese + FGSG, Ca and Zn (Day 1) ^{55}Fe	Ferrous ascorbate solution (Day 2) ^{59}Fe	Fresh cheese + FGSG and Ca (Day 14) ^{55}Fe	FGSG solution (Day 15) ^{59}Fe
1	7.7	15.5	8.5	27.0
2	27.7	63.3	27.8	44.7
3	4.8	2.6	10.8	19.7
4	4.0	13.1	6.3	6.9
5	1.8	1.6	2.4	7.8
6	4.5	17.4	3.1	4.0
7	16.3	20.3	22.1	27.0
8	4.8	28.1	7.9	7.1
9	15.0	32.0	18.2	26.3
10	1.0	9.7	2.4	6.0
11	4.9	10.5	4.5	12.2
12	5.0	13.6	11.8	15.0
13	3.8	14.4	16.9	6.7
14	4.7	22.8	7.9	22.8
15	10.1	34.5	11.0	31.8
G Mean	5.6	14.5	8.4	13.9
−1DE	2.4	5.7	3.9	6.6
+1DE	13.0	37.2	18.1	29.3
	15.4 a		23.1 ab	38.2 b

Values not sharing the same letter are statistically different ($p<0.05$)

Milk and dairy products have high nutritional value but low iron content. Dairy product is a common product in the diet; therefore, it is a plausible vehicle for iron fortification. Therefore, they are attractive food vehicles for iron fortification. Nevertheless, there exist concerns about possible antagonistic interactions with the iron absorption inhibitors that are present in this kind of food, such as casein, milk serum proteins, phosphates and calcium. Iron absorption may also be affected by a combination of other different factors, such as the type of ingested iron, the iron status of the individual and the presence of absorption activators or inhibitors coexisting with iron in the intestinal lumen [13–19]. For calcium in particular, there are some contradictions regarding the degree of iron absorption inhibition and the mechanism through which this effect may take place. Several research groups studied the effect of different calcium sources on iron absorption and demonstrated that the chemical form in which the calcium is found as well as the physiological iron status are factors that determine the inhibitory effect of calcium on iron absorption [17, 20–26]. In addition, concomitant fortification with other micronutrients such as zinc is of concern because antagonistic interactions between the two trace elements when they were consumed in a solution have been reported, although this interference was not present when they were consumed in a meal with a 1 to 1 molar ratio [27]. Therefore, because large quantities of dairy products are consumed by the general population, especially by children (a high-risk group with regard to iron deficiency), and because it has good palatability, a fresh cheese (petit-suisse) was selected as the main food vehicle for the iron source under study.

In this work performed in humans, the bioavailability of FGSG in a fresh fortified cheese was studied. We considered the 40 % iron absorption of ferrous ascorbate in a water solution as the reference standard because it is the absorption expected in subjects who are borderline iron-deficient [28]. According to this standard, the results showed that the bioavailability of iron from FGSG is similar (95 % of relative bioavailability) to that of ferrous ascorbate when both were administered in a water solution. When this iron source was used to fortify a complex food matrix like a fresh cheese, the bioavailability values varied depending on the presence of other minerals like calcium and zinc. In the fresh cheese fortified with calcium only, the iron bioavailability from FGSG was 23.1 %. When it was used in a food matrix fortified with calcium and zinc, the iron bioavailability was 15.5 %. Taking into account that previous studies in humans have shown that ferrous sulphate microencapsulated with the phospholipids (SFE171) in milk has a bioavailability of 9.2 % [29], and that the mean iron absorption from ferrous sulphate (the iron reference) in milk is about 4 % [5], the iron bioavailability of FGSG in the fresh cheese fortified with calcium and zinc is a promising result for the potential use of a petit-suisse cheese as an iron fortification source food suitable for consumption by children. Moreover, according to the WHO/FAO classification of nonheme iron absorption, the bioavailability of FGSG in this food matrix fortified with calcium and zinc can be considered high [30].

The results of the present study show that FGSG is as well absorbed as ferrous sulphate in aqueous solution. The high relative iron bioavailability of ferrous gluconate stabilised with glycine indicates the potential usefulness of this compound for food fortification.

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