



ELSEVIER

Nutrition Research 24 (2004) 603–611

**NUTRITION
RESEARCH**

www.elsevier.com/locate/nutres

Serum iron analysis of adults receiving three different iron compounds

Luciana Ferreira da Silva^a, José E. Dutra-de-Oliveira^{b,*},
Júlio Sérgio Marchini^b

^a*Nutritional Sciences, Pharmacy School, University of the State of São Paulo, Araraquara, Brazil and University of the State of Bahia, Faculty of Technology and Science, Salvador, Brazil*

^b*Department of Internal Medicine, Division of Nutrology, Medical School of Ribeirão Preto, University of São Paulo, Bandeirantes Avenue 3900, 14049-900 Ribeirão Preto, Brazil*

Received 25 July 2003; accepted 7 October 2003

Abstract

Iron deficiency is one of the most prevalent nutritional problems in the world. Iron compounds are used for the prevention and treatment of this deficiency. A double-blind randomized crossover study with 12 volunteers was carried out to compare the effect of three iron compounds, ferrous glycine chelate (GLY), ferric EDTA (EDTA), and ferrous sulfate (FS) on serum iron, and to examine serial serum iron levels. The products were offered as tablets, and blood samples were drawn hourly for 4 hours. FS produced higher serum iron levels than EDTA or GLY ($P < 0.05$). Data for GLY and EDTA were similar. The areas under the curve (AUC) for serum iron for the different compounds gave $AUC_{FS} > AUC_{GLY} = AUC_{EDTA}$ ($P < 0.05$). Iron from FS had a significantly greater bioavailability than for the other two compounds. © 2004 Elsevier Inc. All rights reserved.

Keywords: Iron; Ferrous sulfate; Sodium iron ethylenediaminetetraacetic acid; Iron-glycine chelate; Serum iron; Anemia prevention

1. Introduction

Iron deficiency and iron deficiency anemia has been and still is the most prevalent nutritional problem of developing countries. Despite all the available knowledge concerning

* Corresponding author. Fax: (5516) 633-6695.

E-mail address: jeddoliv@fmrp.usp.br (J.E. Dutra-de-Oliveira).

this problem, little progress towards its prevention has been made [1]. More than 2000 million persons still have iron deficiency or ferropenic anemia. It is highly prevalent in all developing countries and has a low prevalence in industrialized countries. The main factor leading to high frequencies of iron deficiency is a low dietary intake of bioavailable iron and the prevalence of absorption inhibitors in most cereal/legume staple food diets, which are consumed daily in developing areas of the world [2].

Suggestions and solutions to deal with the prevention and treatment of this iron deficiency are mainly based on changing diets and food habits, pharmaceutical iron supplementation, and food fortification. Improving diet and changing foods is a difficult task and a long-range goal. It is known that iron from animal sources is the best, but these are not available to low-level socioeconomic groups. Iron supplementation is more appropriate for use in iron anemia prevention and treatment programs [3]. Food fortification is accepted as the best approach to prevent iron deficiency. Several iron compounds have been used with this preventive objective.

One of them, ferrous sulfate, is well known and has been used worldwide in several anemia studies. Iron bioavailability and side effects from its therapeutic use have stimulated studies on other iron compounds. Iron EDTA and iron glycine are some of them. They also produce good results in the prevention and treatment of iron deficiency.

Sodium iron EDTA has been used since the seventies. Its iron content has been shown to have a good bioavailability, and it is not altered by inhibitors in the Brazilian diets. However, it was found to be less effective than ferrous ascorbate or hemoglobin [4]. In India, fortification of curry powder with NaFeEDTA decreased Fe-deficient anemia from 22% to 5% during a 2-year population study [5]. Recently, in 2001, studies on iron absorption from wild and mutant maize fortified with ferrous sulfate or iron EDTA in nonanemic women, using radiolabeled iron (extrinsic tag), showed iron EDTA to be more efficiently absorbed from food than was ferrous sulfate [6].

Iron bis-glycine chelate has been used as an effective compound to prevent and to treat ferropenic anemia during the last 10–15 years. Its effectiveness in the treatment of iron deficient anemia is said to be higher than that of ferrous sulfate [7]. Ferrous bis-glycine, offered to children 6–14 years old children in Riyadh, Saudi Arabia, decreased the prevalence of anemia from 25–23% to 5–9.6% in boys and girls [8]. Iron glycine studies in 9-month-old infants using stable isotope technology (^{57}Fe or ^{58}Fe) showed its iron bioavailability to be similar to that of ferrous sulfate [9].

These iron studies in anemic patients used several parameters. Hematological indicators, such as hemoglobin, total iron binding capacity, mean corpuscular volume, percent transferrin saturation, serum iron concentration, red cell distribution width, as well as ferritin, transferrin, and transferrin receptors have been used. Recently, isotope techniques have been introduced to measure the absorption of iron and advances in molecular biology studies concerning iron regulatory proteins and metabolism, which will certainly provide new insights into human iron metabolism and homeostasis.

Fairweather-Tait [10] pointed out that nuclear and even nonnuclear methods could be used as markers for studies on bioequivalence assessment of iron supplements. They can be of importance for preventive or therapeutic trials. Serum iron concentration, a traditional iron status parameter, is one of these measures. Iron serum level alone was previously reported

to be of limited value because of its daily variation [11]. It is also known that fasting serum iron values from the same individual, collected at the same time, have smaller variations [12]. An oral iron absorption test based on several serum samples was introduced in the 1960s [13]. The rise of serum iron levels after oral administration of iron supplements is dependent on iron absorption speed and on the rate of subsequent clearance to tissues [14].

Serial iron levels have been used for qualitative measurement of bioavailability [10,14]. These tests are shown to be of value to evaluate aspects of iron absorption and more specifically to test and compare different iron compounds. The maximum increase of serum iron after a test dose and serial serum iron levels measured 4–6 hours after ingestion is significantly correlated with the absorption of ^{59}Fe , measured by whole-body counting [10,15]. This could be used as a semiquantitative measurement of iron absorption [10,15]. Another important aspect of studies on serum iron concentration is that it allows simultaneous comparison of different iron compounds in nonanemic individuals. These are the individuals who, at a community level, should be protected against iron deficiency. Most reports compare therapeutic effects of iron compounds in anemic patients. It would be difficult to compare the prophylactic effects of iron compounds in anemic individuals.

The overall objective of our study was to compare serum iron levels after the intake of three different types of iron compounds (ferrous sulfate, EDTA iron, and glycine iron chelate) for possible use in anemia prevention programs.

2. Methods and materials

2.1. Subjects

A double-blind, crossover, randomized trial was carried out with 12 adult male volunteers aged 22–45 years, recruited from our University Hospital professional staff. Their health was checked by staff physicians and no abnormalities were found. These individuals maintained their daily activities and did not take vitamin and mineral supplements or laxatives, nor did they have diarrhea, pancreatic, hepatic, intestinal or liver disease. None of the subjects had received or donated blood during the previous 6 months and they did not report daily intake of iron-fortified foods. Their daily iron dietary intake was calculated, from a 24-hour dietary recall, to be 10–15 mg. The research protocol was reviewed and approved by the Ethics Committee of the University Hospital, Medical School of Ribeirão Preto. Written informed consent to participate in this study was obtained from each subject.

2.2. Study design

Each volunteer received one of the three iron supplements during three different study periods. A 1-week washout interval was used between studies to minimize intra- and interindividual variability. On all first days of each test period, fasting blood was collected in the morning, always starting at the same time, from each volunteer and after an 8-hour overnight fast for a biochemical checkup that included serum iron concentration. Next, and at random, each subject received two tablets with a total of 120 mg of elemental iron from

one of the three iron compounds, with 200 mL deionized water, under the supervision of a nurse. After the tablet intake, blood samples were collected for serum iron determination, every hour for 4 hours, under continuous fasting. Signs and symptoms such as gastric discomfort, eructation, vomiting or diarrhea, or flatulence were monitored in each participant, immediately and during the 24 hours after the tablet intake. No food or water was supplied to the patients during the 4-hour test.

2.3. Products

The iron compounds used in our study were as follows: ferrous sulfate heptahydrate from Laboratory Synth (Sulfato de Ferro.7H₂O Comercial, Diadema, SP, Brazil), sodium iron EDTA obtained from the laboratory of Dr. Paul Lohmann (Emmerthal, Germany), and iron-glycine aminochelete, Ferrochel, Albion Laboratories, Inc. (Clearfield, UT). For better compliance and reliability, the compounds were administered in two similar tablets prepared at the hospital pharmacy. Each contained the same amount (60 mg) of elemental iron. The tablets were manipulated using the same amount of cornstarch, a starch derivative and K30 polyvinyl pyrrolidone. The order in which the three compounds were administered to each volunteer was chosen by drawing lots.

2.4. Biochemical parameters

Blood samples for whole blood hemoglobin concentration and biochemical analysis were collected on the first test day. Hemoglobin was measured by the cyanomethemoglobin method. Serum iron concentration was determined by a colorimetric assay using Ferrozine (Roche Laboratories, Bern, Switzerland) [16]. Fasting serum iron concentration values were obtained in all participants and then hourly for 4 hours after the intake of each of the test compounds.

2.5. Statistical analysis

An analysis was performed of the maximum serum iron levels and the area under the curve (trapezoid method) [17]. Since the samples were paired, a nonparametric analysis of variance, equivalence, and the Friedman test and Dunn multiple comparisons test were used [18]. The level of significance was set at $P = 0.05$.

3. Results

The mean age of the 12 subjects was 33 ± 9 years; their mean body mass index was 25 ± 5 kg/m², and their mean hemoglobin was 15.2 ± 1.5 g/dL. The minimum hemoglobin level was 14 g/dL. Two of the volunteers presented minor side effects after receiving sodium iron EDTA; these consisted of general discomfort, slight ill-defined epigastric pain, nausea without vomiting, and one episode of soft stools in one of the two individuals. All symptoms

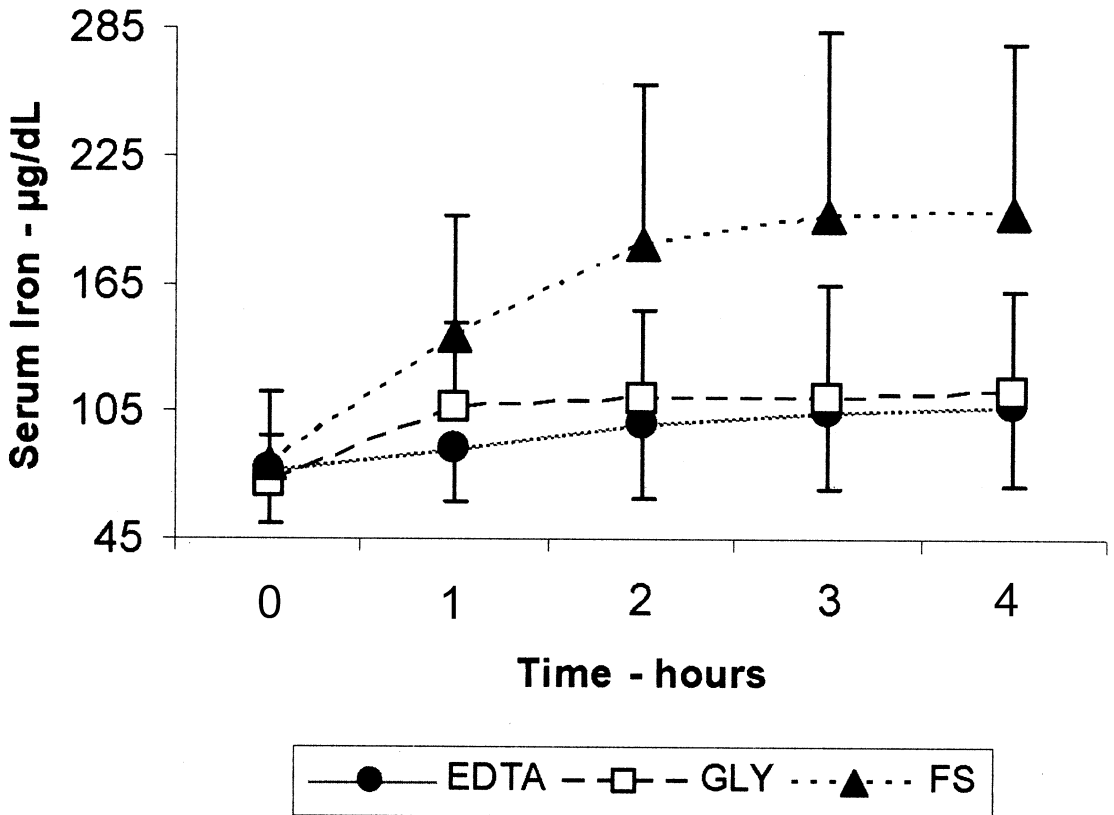


Fig. 1. Time 0, basal fasting serum iron concentrations; time 1, one hour iron levels after the intake of 120 mg elemental iron from each one of the 3 compounds; time 2, two hours levels after the intake; time 3, three hours levels after the intake and time 4, levels four hours after the intake of the three compounds. Statistical analysis; time 0 iron levels similar for the 3 compounds, time 1: FS > GLY > EDTA; times 2, 3, and 4: FS > GLY = EDTA.

disappeared within the first 8 hours after the product intake. No side effects were found for ferrous sulfate or chelate compounds intake.

Mean basal fasting serum iron levels of the 12 subjects on the test days before intake of sodium iron EDTA, iron-glycine amino chelate, or ferrous sulfate iron supplements were, respectively, 77 ± 24 , 72 ± 22 , and 80 ± 34 $\mu\text{g/dL}$ ($P > 0.05$). The mean relative increases in serum iron 1 hour after administration of EDTA, GLY, and FS were, respectively, 14%, 49%, and 75%; after 2 hours they were 30%, 54%, and 130%; after 3 hours they were 37%, 54%, and 146%; and after 4 hours: 42%, 58% and 148% (Fig. 1). At time 1 (1 hour after intake of the iron compound), a significant difference was observed in serum iron values only for ferrous sulfate and sodium iron EDTA. The mean serum iron was higher for ferrous sulfate than for EDTA. No significant differences in serum iron were observed when sodium iron EDTA was compared to iron-glycine amino chelate at times 2, 3, and 4. Mean serum iron was significantly higher for ferrous sulfate compared to EDTA and the amino chelate at times

Table 1

Maximum serum iron concentration (Cmax) after intake of 120 mg of elemental iron from sodium iron EDTA (EDTA), iron-glycine aminochelate (GLY), and heptahydrate ferrous sulfate (FS)

Volunteer	EDTA Cmax ($\mu\text{g/dL}$)*	GLY Cmax ($\mu\text{g/dL}$)*	FS Cmax ($\mu\text{g/dL}$)*
1	83	114	106
2	110	207	279
3	124	89	133
4	136	200	299
5	167	121	294
6	163	142	148
7	180	148	316
8	67	99	106
9	95	105	219
10	81	108	245
11	76	70	150
12	76	52	144
Mean*	113	121	203
SD	40	47	80
Median	103	111	185

* Statistical analysis: $P < 0.05$, with FS > EDTA = GLY.

Cmax = maximum serum iron concentration.

2, 3, and 4. The maximum serum iron value reached with the sulfate was the only parameter that was more than double the mean fasting values after the test dose. The time to reach the maximum iron level was similar for the three compounds.

Both maximum serum iron concentration and delta area under the curve were significantly ($P < 0.05$) higher for ferrous sulfate compared to sodium iron EDTA and iron-glycine aminochelate (Tables 1 and 2). No difference was observed between the latter two compounds. There was no significant correlation between overall baseline iron values and the area under the curve (Spearman coefficient = 0.184, $P > 0.05$), but there was a significant correlation between the overall maximum iron concentration after iron intake and baseline iron values (Spearman coefficient = 0.581, $P < 0.05$). Also, the iron sulfate baseline group values were significantly correlated with the corresponding maximum iron concentration (Spearman coefficient = 0.907, $P < 0.05$). The iron EDTA baseline group values were also significantly correlated with the corresponding maximum iron concentration (Spearman coefficient = 0.820, $P < 0.05$). On the other hand, there was no significant correlation between GLY group baseline values and the corresponding maximum values (Spearman coefficient = 0.482, $P > 0.05$).

4. Discussion

Complex iron compounds, such as iron EDTA and iron chelates are being used as an alternative to ferrous sulfate to prevent and to treat iron deficiency and iron anemia. Serial serum iron level studies were performed in adult volunteers. After the intake of the same oral dose of elemental iron from ferrous sulfate, ferric EDTA, and ferrous glycine chelate, serum

Table 2

Area under the curve for volunteers after intake of 120 mg of elemental iron from sodium iron EDTA (EDTA), iron-glycine amino chelate (GLY), and heptahydrate ferrous sulfate (FS)

Volunteer	EDTA Area ($\mu\text{g/dL/h}$)	GLY Area ($\mu\text{g/dL/h}$)	FS Area ($\mu\text{g/dL/h}$)
1	-2	58	165
2	147	480	588
3	71	131	269
4	67	285	340
5	147	122	617
6	93	69	215
7	163	206	504
8	26	101	104
9	80	73	406
10	45	105	404
11	63	17	254
12	42	-39	245
Mean*	78	134	342
SD	51	137	164
Median	69	103	304

* Statistical analysis: $P < 0.05$, with FS > EDTA = GLY.

Area = area under the curve.

levels were measured and analyzed to compare results. These compounds are water soluble and all are known to have high iron bioavailability.

Human studies have been carried out with these different iron compounds for prevention, and especially for treatment, of iron deficiency anemia [19,20]. In general, the compounds are tested against iron sulfate, and tested in different individuals, and not tested simultaneously. Studies with iron EDTA and iron chelates in anemic patients, when compared with ferrous sulfate, have yielded similar results, and sometimes have given even better responses than with the sulfate reference standard. It has also been found that other complex iron compounds do not have their absorption impaired by food inhibitors, as sometime happens with this inorganic salt [21,22].

Various aspects of iron absorption and metabolism have been previously explored in experimental and human studies, but they are not always simultaneously tested. Our earlier studies in a prophylactic anemia assay in rats, testing several iron compounds, including EDTA and chelate iron, showed iron complexes to have a similar biodisponibility and liver iron content when compared to ferrous sulfate. These values confirmed previously published data but with better controls, as they were assessed simultaneously with the same methodology and compared to individuals receiving ferrous sulfate and a control group receiving no iron [23].

Our experimental design on humans, using the same person receiving the same amount of elemental iron in successive and at random crossover periods, was used to minimize inter- and intraindividual variations in serum iron levels, giving more credibility to the results. Although there are some questions concerning the real meaning of serum iron levels, they represent the absorption, transport, and clearance of iron from the blood stream. At any moment, the amount of iron found in the serum is considered a function of iron entering and

leaving the blood stream. Iron absorption is a complex mechanism affected by food iron, its bioavailability, the gastric and small intestinal environment, and intestinal cell functions. Once in the blood stream, iron circulates, reaches various organs and tissues, is stored in many cells, and is excreted in very small amounts.

Serum iron, iron load, and tolerance tests have been used for many years. They were first used to measure iron absorption and more recently have been recommended to compare pharmaceutical products. It has been noted that this method could be used for the bioequivalence assessment of iron supplements [10].

The experimental design of our investigation was to use a simple and practical parameter and to compare in the same person the effect of the same amount of elemental iron on hourly levels of serum iron. Serum iron concentration decreases with iron anemia and low values are also found in infections and some other diseases. It is also known that the levels depend not only on absorbed iron but also on blood clearance rates [13,24]. Isolated serum iron values are not always considered a reliable parameter, although measurements made at the same time and after an overnight fast show little variation. The analysis of our 36 fasting values measured on different days, gave similar values with low standard deviations.

We found that ferrous sulfate produced significantly higher levels and a greater maximum iron serum increase when compared to iron EDTA and iron glycine chelate. This would mean higher iron absorption and/or higher qualitative bioavailability and/or increased iron supply to the tissues from iron sulfate than for the other iron complexes. Ferrous sulfate under our experimental research conditions and in healthy subjects gave higher values than the other compounds.

The overall results on serial serum levels from ferrous sulfate, iron EDTA and iron chelate in a double blind, crossover design allow us to conclude that iron EDTA and iron chelate resulted lower serum levels than did ferrous sulfate in healthy adults. They also had a lower bioavailability. Higher blood levels and maximum iron peak during a shorter time, and a decrease afterwards, mean a faster iron clearance rate to the body tissues. These findings support ferrous sulfate as a reference standard to compare different iron compounds and certainly support its utilization for the prevention and treatment of iron deficiency.

It is also important to point that our data showed higher C_{max} serum iron values for ferrous sulfate than for glycine or EDTA irons. If we take into consideration the findings of Ekenved et al [15] of a high correlation of C_{max} with the amount of iron absorbed measured when more precise whole-body counter method was used, the conclusion is that iron from ferrous sulfate is better absorbed than from other salt compounds.

We emphasize that our study was carried out with non-anemic subjects for preventive effects of iron fortification and not to treat iron anemia. Iron sulfate is available everywhere and cheaper than the other compounds, a practical advantage for developing countries.

Acknowledgments

We thank and appreciate the support of Luiz Sakamoto Maçao for his assistance in the preparation of the iron tablets at the University Pharmacy.

References

- [1] World Health Organization. Nutrition for health and development. Progress and prospects on the eve of 21st century. Geneva: World Health Organization, 1999.
- [2] Charlton RW, Bothwell TH. Iron absorption. *Annu Rev Med* 1983;34:55–68.
- [3] Hurrell RF. Preventing iron deficiency through food fortification. *Nutr Rev* 1997;55:210–22.
- [4] Viteri FE, Garcia-Ibanez R, Torun B. Sodium iron NaFeEDTA as an iron fortification compound in Central America. Absorption studies. *Am J Clin Nutr* 1978;31:961–71.
- [5] Ballot DE, MacPhail AP, Bothwell TH, Gillooly M, Mayet FG. Fortification of curry powder with NaFe(111)EDTA in an iron-deficient population: report of a controlled iron-fortification trial. *Am J Clin Nutr* 1989;49:162–9.
- [6] Mendonza C, Viteri FE, Lonnerdal B, Raboy V, Young KA, Brown KH. Absorption of iron from unmodified maize and genetically altered, low-phytate maize fortified with ferrous sulfate or sodium iron EDTA. *Am J Clin Nutr* 2001;73:80–5.
- [7] Pineda O, Ashmead HD, Perez JM, Lemus CP. Effectiveness of iron amino acid chelate on the treatment of iron deficiency anemia in adolescents. *J Appl Nutr* 1994;46:2–13.
- [8] Osman AK, al-Othaimeen A. Experience with ferrous bis-glycine chelate as an iron fortificant in milk. *Int J Vitam Nutr Res* 2002;72:257–63.
- [9] Fox TE, Eagles J, Fairweather-Tait SJ. Bioavailability of iron glycine as a fortificant in infant foods. *Am J Clin Nutr* 1998;67:664–8.
- [10] Fairweather-Tait SJ. Iron. *J Nutr* 2001;131(Suppl 4):1383S–6S.
- [11] Borel MJ, Smith SM, Derr J, Beard H. Day-to-day variation in iron status indices in healthy men and women. *Am J Clin Nutr* 1999;54:729–35.
- [12] Dale JC, Burritt MF, Zinsmeister AR. Diurnal variation of serum iron, iron-binding capacity, transferrin saturation, and ferritin levels. *Am J Clin Pathol* 2002;117:802–8.
- [13] Hallberg L, Solvell L. A method for simultaneous determination of iron absorption, plasma volume, and plasma iron turnover in man. *Scand J Haematol* 1965;2:187–94.
- [14] Verloop MC, Meeuwissen JET, Blokhuis EWM. Comparison of the iron absorption test with the determination of the iron-binding capacity of serum in the diagnosis of iron deficiency. *Br J Haematol* 1958;4:70–81.
- [15] Ekenved G, Pharm B, Norrby A, Solvell L. Serum iron increase as a measure of iron absorption—studies on the correlation with total absorption. *Scand J Haematol* 1976;28:S31–S49.
- [16] Stookey LL. Ferrozine—a new spectrophotometric reagent for iron. *Anal Chem* 1970;42:779–81.
- [17] Matthews JN, Altman DG, Campbell MJ, Royston P. Analysis of serial measurements in medical research. *Br Med J* 1990;300:230–5.
- [18] Hollander M, Wolfe DA. Nonparametric statistical methods. New York: John Wiley & Sons, 1973.
- [19] Brise H, Hallberg L. Absorbability of different iron compounds. *Acta Med Scand* 1962;171:23–37S.
- [20] Nair MK, Raman L, Ramalakshmi BA, Sreeramulu D. Plasma iron tolerance of ferrous glycine sulphate and ferrous sulphate in women. *Food Nutr Bull* 1995;16:72–4.
- [21] International Nutritional Anemia Consultative Group (INACG). Iron EDTA for food fortification: a report of the INACG. INACG, Washington, DC: Nutrition Foundation, 1993.
- [22] Layrisse M, Martinez-Torres C. Fe(III)–EDTA complex as iron fortification. *Am J Clin Nutr* 1977;30:1166–74.
- [23] Dutra-de-Oliveira JE, Freitas ML, Ferreira JF, Gonçalves AL, Marchini JS. Iron from complex salts and its bioavailability to rats. *Int J Vitam Nutr Res* 1995;65:272–5.
- [24] Heinrich HC. Intestinal iron absorption in man. Methods of measurement, dose relationship, diagnostic and therapeutic applications. In: Hallberg L, Harwerth HG, Vannotti A, editors. Iron deficiency: pathogenesis, clinical aspects, therapy. London: Academic Press, 1970. p. 213–96.