Consell Superior d'Investigacions Científiques
INSTITUT D'INVESTIGACIONS QUÍMIQUES I AMBIENTALS
DE BARCELONA "JOSEP PASCUAL VILA" (IIQAB)
Jordi Girona, 18-26 - 08034 Barcelona (Espanya)
Tel. (34) 93 400 61 00 - Tel. (34) 93 400 61.....
Telefax (34) 93 204 59 04 - E-mail:
Internet: http://www.cid.csic.es

IRON ABSORPTION AFTER ORAL ADMINISTRATION OF DIFFERENT DOSSAGE FORMS.

Efficiency of LIPOFER DISPERSIBLE iron absorption in rats

In order to study the efficiency of LIPOFER DISPERSIBLE on iron absorption, laboratory trials were carried out on rats.

Four groups of rats weighing 230-250 gr were stored in separate cages. Experiments started at least three days after arrival.

Three different iron forms, suspended in a 1% carboxymethyl cellulose, were administered orally directly in the esophagus:

LIPOFER DISPERSIBLE (Code:99 K)
ferrous sulfate
ferric pyrophosphate
control (Carboxymethyl Cellulose)

The iron content of the salts administered was equivalent in all cases (10 mg/kg of animal weight).

At different times rats were anesthetized and blood extracted through heart puncture (three animals per time).

Blood samples were centrifuged and the concentration of iron in the supernatants (blood serum) quantified through atomic absorption.

RESULTS

Results obtained are represented in Fig. 1, as iron concentration (µg/dL) versus Time (hours).

The pharmacokinetic profile of Lipofer 99K compared to ferric pyrophosphate shows higher values at any time.

Besides, comparing the pharmacokinetic profile of Lipofer 99K to that of ferrous sulfate one can appreciate that the iron concentration in blood is always higher with Lipofer 99K and there is also a sustained release.
Lipofer Dispersible 99K allows a sustained release of iron during the whole trial.

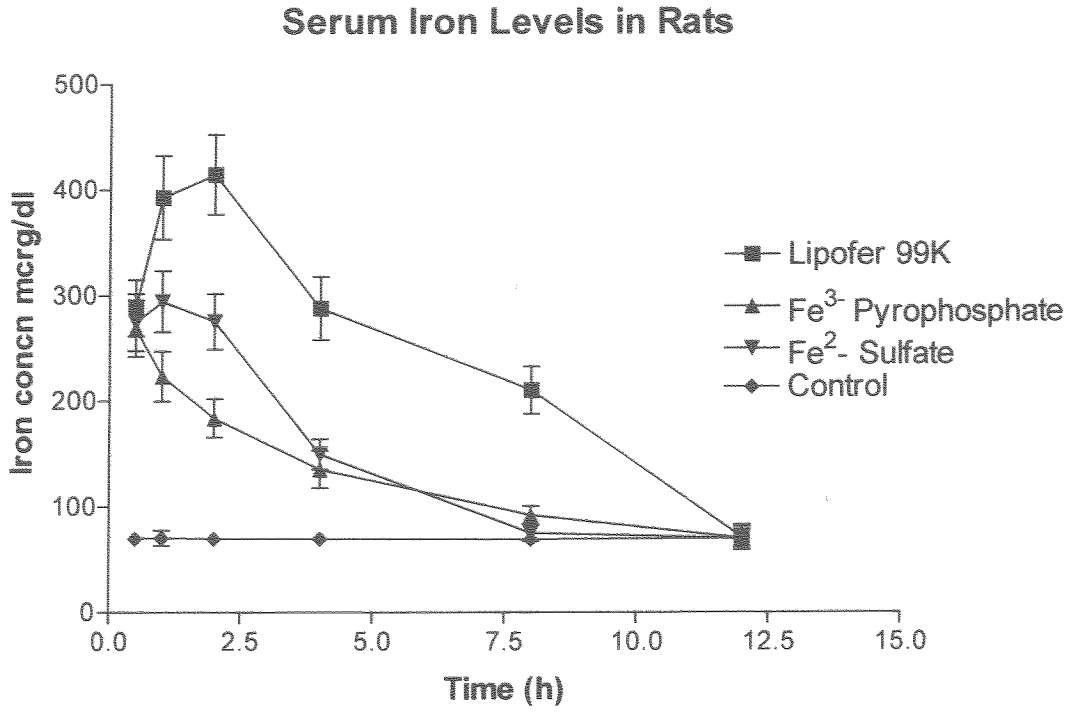


Figure 1.-Iron concentration measured at different times.

The corresponding area under the curve values calculated from media values applying the trapezoidal procedure give the following:

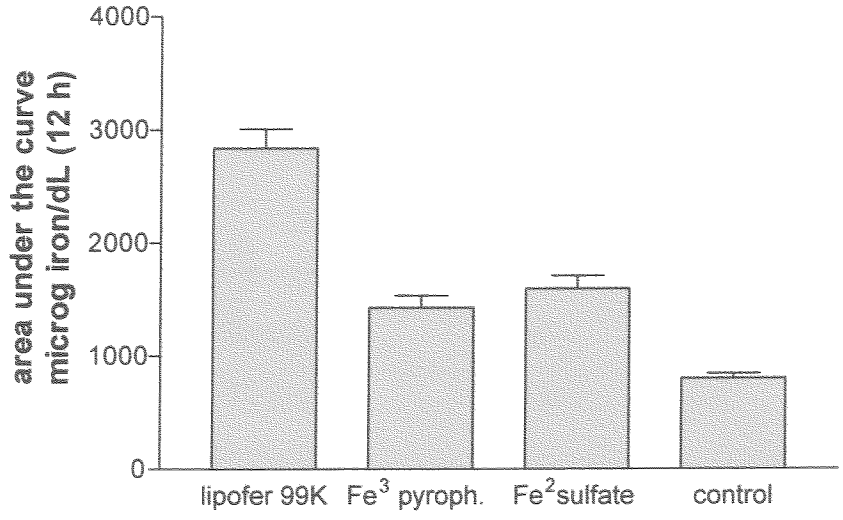
LIPOFER DISPERSIBLE (99K): 2826 µg/dl

Ferrous sulfate: 1550 µg/dl

Ferric pyrophosphate: 1380 µg/dl

Control: 806 µg/dl

These results are represented in Histogram 1



Histogram 1.- Area under the curve values for the different iron dosages.

CONCLUSIONS

Total iron absorption is higher when this element is administered in form of Lipofer 99K. Comparing the values under the curve obtained for free and microencapsulated (Lipofer 99K) ferric pyrophosphate, versus basal values, one can say that the increase in iron concentration in blood promoted by the microencapsulated form is 3.5 times higher than that of free salt. Besides, compared to ferrous sulfate this increase is 2.7 times higher. These differences are significant.

Dra. Francesca Reig Isart
Research Professor
Institute for Biological and Environmental Chemistry. CSIC.

January 1999

British Journal of Nutrition (2010), page 1 of 10
doi:10.1017/S0007114510005490

Efficacy of a microencapsulated iron pyrophosphate-fortified fruit juice: a randomised, double-blind, placebo-controlled study in Spanish iron-deficient women

Ruth Blanco-Rojo[1], Ana M. Pérez-Granados[1], Laura Toxqui[1], Carmen González-Vizcayno[2], Marco A. Delgado[3] and M. Pilar Vaquero[1]*

[1]*Department of Metabolism and Nutrition, Institute of Food Science and Technology and Nutrition (ICTAN), Spanish National Research Council (CSIC), C/José Antonio Novais 10, 28040 Madrid, Spain*
[2]*Clinical Analysis Laboratory, Madrid Salud, Madrid, Spain*
[3]*Grupo Leche Pascual, Aranda de Duero, Burgos, Spain*

(Received 12 July 2010 – Revised 29 November 2010 – Accepted 30 November 2010)

Abstract

Fe-deficiency anaemia is a worldwide health problem. We studied the influence of consuming an Fe-fortified fruit juice on Fe status in menstruating women. A randomised, double-blind, placebo-controlled study of 16 weeks of duration was performed. Subjects were randomised into two groups: such as P group (*n* 58) or F group (*n* 64), consumed, as a supplement to their usual diet, 500 ml/d of a placebo fruit juice or an Fe-fortified fruit juice, respectively. The Fe-fortified fruit juice, containing microencapsulated iron pyrophosphate, provided 18 mg of Fe (100 % of the RDA). At baseline and monthly, dietary intake, body weight and Fe parameters were determined: total erythrocytes, haematocrit, mean corpuscular volume (MCV), red blood cell distribution width (RDW), Hb, serum Fe, serum ferritin, serum transferrin, transferrin saturation, soluble transferrin receptor (sTfR-2) and zinc protoporphyrin (ZnPP). The fruit juice consumption involved increased intake of carbohydrates and vitamin C, and increased BMI within normal limits. Ferritin was higher in the F group after week 4 ($P<0{\cdot}05$) and became 80 % higher than in the P group after week 16 ($P<0{\cdot}001$), and transferrin decreased in the F group compared with the P group after week 4 ($P<0{\cdot}001$). RDW was higher at weeks 4 and 8 in the F group compared with the P group ($P<0{\cdot}05$). Transferrin saturation increased after week 8, and haematocrit, MCV and Hb increased after week 12, in the F group compared with the P group. Serum Fe did not change. sTfR and ZnPP decreased in the F group at week 16 ($P<0{\cdot}05$). Iron pyrophosphate-fortified fruit juice improves Fe status and may be used to prevent Fe-deficiency anaemia.

Key words: Fortification: Ferric pyrophosphate: Iron-deficiency anaemia: Iron status: Women

Nutritional Fe deficiency has been identified as one of the ten leading factors for disease, disability and death in the world today. An estimated two billion people are affected, and the population at risk includes women of child-bearing age and children. It is the only highly frequent nutritional deficiency in developing and developed countries[1].

Dietary strategies for combating Fe deficiency include Fe supplementation, dietary modification and diversification, and food fortification[2]. Our research group has recently observed in young women that the current RDA of 18 mg Fe/d[3,4] was not easily reached, even though the volunteers consumed five portions of red meat and two portions of poultry per week[5]. Supplementation with doses of Fe $\geq$100 mg/d is efficacious to increase Fe status, but its major limitation is low compliance due to gastrointestinal discomfort[2]. Finally, fortification is widely considered to be the most practical and cost-effective prevention programme[6]. However, Fe is the most challenging micronutrient to add to foods, because the Fe compounds that have the best bioavailability tend to be those that interact most strongly with food constituents producing undesirable organoleptic changes[7]. Among Fe fortificants, ferric pyrophosphate allows appropriate food processing, and it is easily and effectively absorbed while producing negligible colour and palatability changes[8–10].

It is well known that the food matrix strongly affects Fe bioavailability[11]. Therefore, in addition to the Fe salt,

Abbreviations: AA, ascorbic acid; F, fortified group; P, placebo group; RDW, red blood cell distribution width; STfR, soluble transferrin receptor; ZnPP, zinc protoporphyrin.

* **Corresponding author:** M. P. Vaquero, fax +34 915943627, email mpvaquero@ictan.csic.es

the effectiveness of consuming Fe fortificants is highly dependent on the type of food used.

Ascorbic acid (AA) is the most important enhancer of Fe absorption, both for its ability to improve Fe absorption in the lumen and for overcoming the negative effect of inhibitors[12–14]. However, there are doubts concerning the applicability of single-meal results to the practical diet.

Cook & Reddy[15] studied Fe absorption from three diets varying in AA and concluded that the effect of vitamin C on Fe absorption from a complete diet was far less pronounced than that from single meals. A better Fe status is reached when AA is consumed with meals containing substantial amounts of added Fe[10,13].

Nevertheless, studies that used foods with a naturally high content of AA are scarce[16]. Fruit juices can contain high quantities of vitamin C, low pH and no Fe absorption inhibitors, and should be considered as target products to fortify with Fe.

An orange juice fortified with iron sulphate (2 mg Fe/100 ml) given to Brazilian preschool children during 4 months (mean Fe intake 5·7 mg/d) increased Hb levels and decreased the percentage of anaemic children from 60 to 20 %[16]. Other studies have been performed using multiple-micronutrient-fortified powdered beverages containing iron bis-glycinate. These beverages reduced the overall prevalence of anaemia in children, adolescent girls and pregnant women of developing countries[17–21].

The present study therefore aims to investigate the influence of the consumption, as a part of the usual diet, of an Fe-fortified fruit juice on Fe metabolism in young Spanish Fe-deficient women.

This trial was registered at clinicaltrials.gov as NCT01135576.

Subjects and methods

The present study was designed and carried out following the statement guidelines of the Consolidated Standards of Reporting Trials[22].

Subjects

Volunteers were recruited by different announcements in press, university campus and web pages of Madrid. The study was also verbally promoted at public events.

The principal variable for the calculation of sample size was ferritin, with a mean value for the deficient population of 11 (SD 5) ng/ml. It was calculated that a minimum of sixty-three subjects with low Fe stores would be required in each group to demonstrate a difference of 2·5 units in serum ferritin between two treatments at 80 % power and confidence level at 95 %.

Women aged 18–35 years, non-smoker, non-pregnant, non-breast-feeding, with low Fe stores, defined as serum ferritin <40 ng/ml and Hb ≥ 110 g/l, were included in the present study. The cut-off value for serum ferritin was selected because a normal or elevated ferritin value does not exclude the presence of Fe deficiency, and cut-off ranges between 25 and 50 ng/ml are usually considered in studies dealing with predisposition to anaemia[5,23]. Subjects were excluded from the study if they had amenorrhoea, menopause or any known health problems likely to influence Fe status including Fe metabolism-related diseases (Fe deficiency anaemia, thalassaemia and haemochromatosis), chronic gastric diseases (inflammatory bowel disease, Crohn's disease, gastric ulcers, celiac disease and haemorrhagic diseases), renal disease or allergy to any of the components of the assay juices. Other exclusion criteria were blood donors or to have regularly consumed Fe or AA supplements within the 4 months before participating in the intervention.

A group of 259 women contacted the research group in order to participate in the study, but only 163 underwent screening. Out of the 163 women, thirty-three were excluded (twenty-eight did not meet the inclusion criteria and five refused to participate). Finally, a total of 130 women agreed to participate in the nutritional intervention. They were randomised into two groups: fortified (F) and placebo (P). All volunteers of the F group completed the assay while eight participants of the P group abandoned the intervention (Fig. 1).

Participating women were instructed not to deviate from their regular habits and to maintain their normal diet and exercise level during the 4 months.

The present study was conducted according to the guidelines laid down in the Declaration of Helsinki, and all procedures involving human subjects/patients were approved by the Clinical Research Ethics Committee of Hospital Puerta de Hierro, Madrid. Written informed consent was obtained from all subjects.

Study design

The study consisted of a randomised double-blind study controlled by placebo of 16 weeks of duration.

The volunteers recruited were randomly allocated into two groups, using the RAND function in Excel (Microsoft Office 2003). One group consumed, as a supplement to their usual diet, 500 ml/d of the Fe-fortified fruit juice (F group, *n* 64), whereas the other consumed 500 ml/d of the placebo fruit juice (P group, *n* 66). Fortified and placebo juices were manufactured in 500 ml cartons and in two different tastes (orange and peach apple) to achieve compliance. The fortified juice supplied 18 mg Fe/500 ml carton, in the form of microencapsulated iron pyrophosphate coated with lecithin, equivalent to 100 % of the RDA/d[3,4]. All juices were fortified with vitamin C. Nutritional composition of the juices was facilitated by the manufacturer (Grupo Leche Pascual, Burgos, Spain). Orange juices provided (per 100 ml) 188 kJ, 0·6 g of protein, 10·5 g of carbohydrate and 19 mg of vitamin C; the Fe-fortified orange juice provided 3·6 mg of Fe, whereas

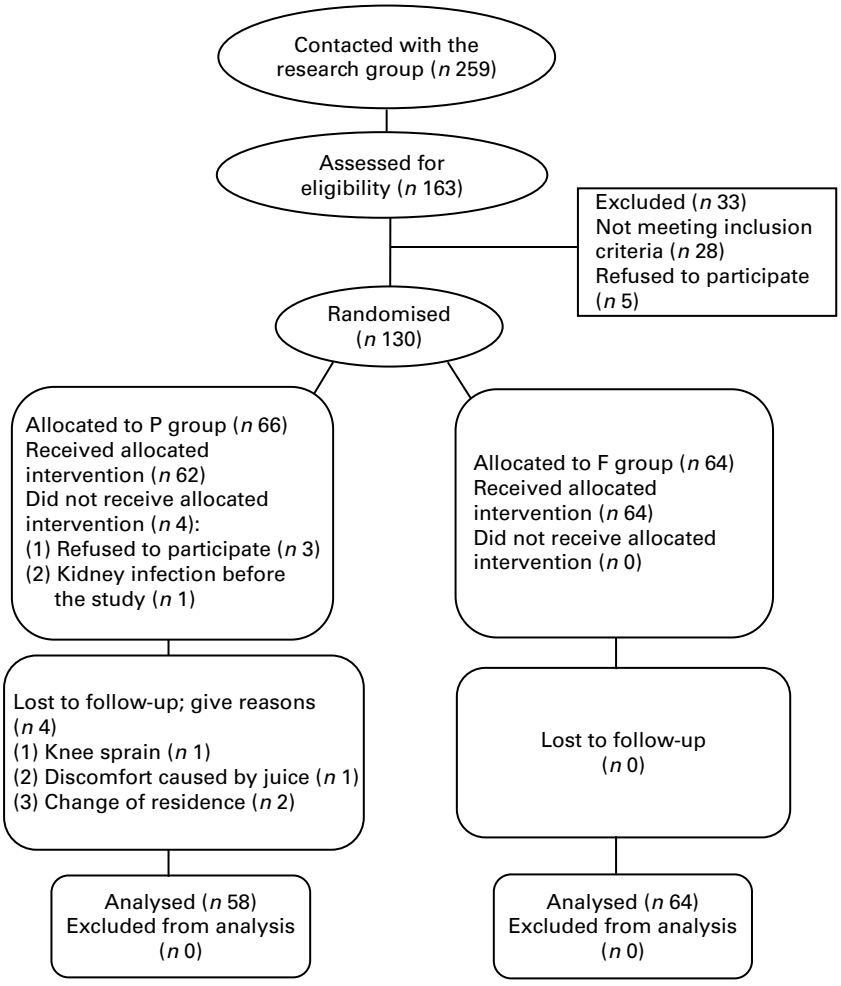


Fig. 1. Diagram of the Consolidated Standards of Reporting Trials.

the placebo juice had 0·084 mg. Peach apple juices provided (per 100 ml) 201 kJ, 0·6 g of protein, 11·3 g of carbohydrate and 19 mg of vitamin C; the Fe-fortified peach apple juice provided 3·6 mg of Fe, whereas the placebo juice had 0·136 mg.

Participants were instructed to alternate between juice flavours (orange juice one day, peach apple juice the next day). The 500 ml carton had to be drunk all at once separately from meals (by at least 2 h) and shaken before consumption. Volunteers who could not drink the juice one day were instructed to consume two juice cartons the following day.

Dietary control and compliance

Each subject's dietary intake was evaluated at baseline and monthly to control any possible changes in energy and nutrient intakes. They completed a 72 h detailed dietary intake report, previously validated and proved valuable to assess nutrient intake[24,25], specifying the types of food consumed and serving weights. Daily food, energy intake, nutrient intake and energy provided by macronutrients were calculated by an computer application using the Spanish Food Composition Database[26]. The compliance of the study was assessed monthly by questionnaires and personal interview when volunteers underwent blood sampling.

Anthropometric, blood pressure and physical activity determinations

Once a month, anthropometric measures were taken using standardised procedures. Body weight was measured with a calibrated Seca scale (to a precision of 100 g), and height was measured at baseline with a stadiometer incorporated into the scale. Duplicate waist circumference was taken to the nearest 0·1 cm using an inelastic, flexible tape measure. BMI was calculated as weight/height squared (kg/m^2). To avoid inter-examiner variability, one trained member of the research team did all anthropometric determinations. International manual procedures were used[27].

Physical activity was assessed by a validated questionnaire after weeks 4 and 12. This questionnaire was completed during face-to-face interviews conducted by a trained dietitian. Women were asked about their occupation, sleeping hours and additional activities at work

and during the rest of the day. The physical activity questionnaire included representative values expressed as multiples of resting energy expenditure. Average daily exercise was calculated taking into account the intensity level and time spent on each activity. Activities were divided into five categories and expressed as an activity factor (resting = 1, very light = 1·5, light = 2·5, moderate = 5 and heavy = 7)[28].

In addition, every 4 weeks, women completed a questionnaire about physical discomfort or health problems, medication use and changes in their normal routine.

Blood sampling and biochemical assays

Volunteers attended the laboratory facilities at baseline, 4, 8, 12 and 16 weeks. Blood samples were collected by venepuncture after a 12 h fasting period. Serum and plasma were obtained after centrifugation at 1000 ***g*** for 15 min and stored at −80°C.

Total erythrocytes, haematocrit, mean corpuscular volume, red blood cell distribution width (RDW) and Hb were determined following standard laboratory techniques using the Symex NE 9100 automated haematology analyser (Symex, Kobe, Japan). Serum Fe, serum ferritin and serum transferrin were determined by the Modular Analytics Serum Work Area analyser (Roche, Basel, Switzerland). Total iron-binding capacity (TIBC) and transferrin saturation were calculated as follows:

$$\text{TIBC}\,(\mu\text{mol/l}) = 25{\cdot}1 \times \text{transferrin}\,(\text{g/l}),$$

$$\text{Transferrin saturation} = \text{serum Fe}\,(\mu\text{mol/l})/\text{TIBC} \times 100.$$

Serum soluble transferrin receptor (sTfR) concentration was determined using an ELISA technique (sTfR Human ELISA; Biovendor, Heidelberg, Germany) and erythrocyte zinc protoporphyrin (ZnPP) by haematofluorophotometry (haematofluorophotometer AVIV 206; Izasa, Barcelona, Spain). Values of ZnPP μg/μg Hb were converted to μmol/mol haem of erythrocyte protoporphyrin by using a factor of 25·76 according to the AVIV conversion tables.

All determinations were subjected to the ISO 9001-2000 requirements, except for the transferrin receptor; the intra-assay CV of this determination was 3·5 % and the inter-assay CV was 4·3 %.

Statistical analysis

Data are presented as means and standard deviations. A normal distribution of variables was determined by the Kolmogorov–Smirnov test. Serum ferritin values were log-transformed for statistical testing. A two-way repeated-measures ANOVA was applied. Because a significant group × time interaction was found for the main variables (ferritin, transferrin and Hb, $P \leq 0{\cdot}001$), the repeated-measures ANOVA and the Bonferroni *post hoc* test were used to study the time effect within groups. Comparisons were also made between the F group and the P group using ANOVA. A P value of $< 0{\cdot}05$ was considered significant. Data were analysed using the SPSS statistical package for Windows (version 17.0).

Results

A total of 122 volunteers completed the study (Fig. 1). Ages of the volunteers were 24·5 (SD 5·1) and 24·2 (SD 4·6) years for the P and F groups, respectively. Energy and nutrient intakes at baseline and week 16 are shown in Table 1.

No significant differences between groups were found in the baseline characteristics of subjects (Tables 2 and 3). Compliance rate was confirmed to be very high (approximately 100 %).

Although energy intake did not show significant differences between baseline and week 16 in both groups, an increase between week 4 and baseline was observed to be significant in the F group (9767 *v.* 8807 kJ/d for week 4 and baseline, respectively, $P < 0{\cdot}05$). A significant decrease in the energy percentage from proteins and an increase in that from carbohydrates were observed during the study in both groups, and a decrease in the energy percentage from lipids was observed only in the F group. The differences between the groups were not significant (Table 1).

At baseline, Fe intake was slightly lower in the F group compared with the P group, and due to the Fe-fortified juice consumption, it was about twice that of the P group for the duration of the intervention. In contrast, Fe intake of the P group decreased with time, and it was significantly lower at week 16 compared with baseline. Vitamin C

Table 1. Energy and macronutrient intakes of iron-deficient women consuming placebo and iron-fortified fruit juices during 16 weeks

(Mean values and standard deviations)

	Baseline		Week 16		
Groups	Mean	SD	Mean	SD	Time effect (P)†
Energy (kJ)					
Placebo	9452	2179	9826	2493	NS
Fortified	8807	2108	9285	2292	NS
Protein (% energy)					
Placebo	15·2	3·9	13·0	2·4	<0·001
Fortified	14·6	2·4	13·6	2·3	0·004
Carbohydrate (% energy)					
Placebo	41·2	7·3	45·0	7·3	0·001
Fortified	41·2	6·2	44·6	6·6	<0·001
Lipid (% energy)					
Placebo	39·9	7·8	37·3	6·3	NS
Fortified	39·7	6·5	36·4	6·8	0·001
Fe (mg)					
Placebo	15·1	4·7	12·9	4·7	0·001
Fortified	13·7*	5·9	30·4***	7·5	<0·001
Vitamin C (mg)					
Placebo	145·9	60·1	199·8	64·9	<0·001
Fortified	118·3	59·3	190·2	66·0	<0·001

Mean values were significantly different from the placebo group at each point (measured using one-sided tests): * $P < 0{\cdot}05$, *** $P < 0{\cdot}001$.

† Time-point differences were analysed by repeated-measures ANOVA.

Table 2. Anthropometric values of iron-deficient women consuming placebo and iron-fortified fruit juices during 16 weeks

(Mean values and standard deviations)

	Baseline		Week 16		
Groups	Mean	SD	Mean	SD	Time effect (*P*)*
Weight (kg)					
Placebo	57·5	6·4	58·5	6·6	<0·0001
Fortified	59·6	7·6	60·5	7·8	<0·0001
BMI (kg/m^2)					
Placebo	21·6	2·2	22·0	2·2	<0·0001
Fortified	21·8	2·3	22·1	2·4	<0·0001
Waist circumference (cm)					
Placebo	68·7	4·7	68·8	4·6	NS
Fortified	69·6	5·4	69·8	5·4	NS

Q4 Mean values were not significant between the placebo and fortified groups at each point.

* Time-point differences were analysed by repeated-measures ANOVA.

intake increased in both groups from baseline, due to juice composition, without significant differences between the P and F groups (Table 1).

The intervention induced increases in body weight and BMI, within normal limits (Table 2). Waist circumference did not change during the intervention. Physical activity was unchanged during the study and did not show differences between the groups; it was classified between very light and light (activity factor, 1·68 *v.* 1·68 in the P and F groups).

Table 3 shows the results of haematological and biochemical markers. Increases in the values of total erythrocytes, haematocrit, mean corpuscular volume, RDW, Hb, serum Fe, serum ferritin and transferrin saturation show recovery from Fe deficiency, while increases in serum transferrin, sTfR-2 and ZnPP denote deterioration, as detailed below.

Total erythrocytes did not show significant differences between the groups. However, the F group showed significantly higher values at week 12 compared with week 4. Similarly, haematocrit values were significantly higher at week 12 compared with week 4 in this group, and higher haematocrit levels were shown at weeks 12 and 16 in the F group than in the P group ($P=0·01$). Mean corpuscular volume decreased slightly at week 12 in the P group, while it tended to increase in the F group; thus, differences between the groups were significant at week 12 ($P=0·03$). RDW was higher in the F group than in the P group at weeks 4 and 8 ($P<0·01$ and 0·05, respectively).

Hb concentrations did not vary in the P group during the assay (Table 3), but they increased in the F group and were significantly higher at week 8 compared with baseline, and at week 12 compared with the P group ($P < 0·05$). ZnPP increased in the P group and decreased in the F group during the assay, and the differences between the groups were significant at weeks 8 and 16 ($P < 0·05$).

No changes in serum Fe concentrations were observed due to either group or time (Table 3). Ferritin concentrations, the principal variable of the present study, significantly increased from week 4 in the F group, and the values became about 80 % higher compared with the P group at the end of the assay. In contrast, no changes were observed in the P group. Serum transferrin fluctuated above 3000 mg/l in the P group, while in the F group, it markedly decreased from week 4 to the end of the assay, and the differences between the groups were significant ($P<0·01$). Accordingly, transferrin saturation was significantly higher from week 8 in the F group with respect to the P group ($P<0·05$).

sTfR concentrations significantly decreased in the F group compared with baseline and the P group (significantly at week 16).

Discussion

The present study clearly shows that it is feasible to increase Fe status in an at-risk population by daily consumption of a microencapsulated iron pyrophosphate-fortified fruit juice and that the effects are detected in a short period of time (4 weeks). This consumption was compatible with the usual diet, and the extra daily 18 mg of Fe provided in each 500 ml juice carton was 100 % of the RDA[3,4]. This quantity of Fe is within the range of supplemental minerals added in European commercial foods (20 % of the RDA/100 ml).

The study fruit juice was fortified with micronised encapsulated iron pyrophosphate coated with lecithin. This form of Fe is dispersible in aqueous solution and has been demonstrated to be highly bioavailable. Its bioavailability is superior to that of non-micronised iron pyrophosphate, which has higher particle size, and to that of non-encapsulated iron pyrophosphate[9–11,29]. Roe *et al.*[30] enriched micronised iron pyrophosphate and ferrous sulphate with different Fe stable isotopes, and included each Fe form in apple juices to conduct an absorption experiment using the technique of the incorporation of Fe isotopes to erythrocytes. They concluded that the bioavailability of micronised iron pyrophosphate was higher relative to ferrous sulphate, indicating that it could be a useful fortifier for liquid food products.

Several studies using fortified food that supplied amounts of Fe similar to the present study have been reported, generally showing lower efficacy. The consumption of a wheat biscuit enriched with 10 mg of Fe (as encapsulated sulphate) during 22 weeks increased iron ferritin but not Hb levels in young women[31]. A recent study in female soldiers who received 56 mg of Fe/d in the form of food bars compared with placebo during 9 weeks has shown no changes in serum ferritin, transferrin saturation, %RDW and sTfR[32]. The difference between these two studies and ours could be explained by the presence of phytates and the absence of AA in their Fe-fortified products, while the fruit juices of the present study contained no phytate and were fortified with AA. When AA is present, as in one study giving 16 mg of Fe-fortified

Table 3. Haematological and biochemical markers of iron-deficient women consuming placebo and iron-fortified fruit juices during 16 weeks (Mean values and standard deviations)

	Baseline		Week 4		Week 8		Week 12		Week 16		
Groups	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Time effect (*P*)
Total erythrocytes ($\times 10^{-12}$/l)											
Placebo	4·50	0·35	4·49	0·33	4·48	0·29	4·51	0·34	4·45	0·30	NS
Fortified	4·51^{a,b}	0·30	4·49^{a}	0·32	4·53^{a,b}	0·32	4·56^{b}	0·31	4·53^{a,b}	0·30	0·046
Haematocrit (%)											
Placebo	39·3	2·9	39·2	2·8	38·9	2·5	38·9	2·6	38·7	2·6	NS
Fortified	39·0^{a}	2·8	39·4^{a}	2·5	39·4^{a}	2·7	40·1^{b**}	2·6	39·9^{a,b**}	2·4	0·006
Mean corpuscular volume (fl)											
Placebo	87·4^{a}	4·8	87·3^{a}	4·3	87·0^{a,b}	4·3	86·5^{b}	4·1	87·0^{a,b}	4·2	0·011
Fortified	86·6^{a}	5·1	87·9^{b,c}	3·9	87·1^{a,b}	4·2	88·1^{c*}	3·6	88·1^{c}	4·0	0·001
Red blood cell distribution width (%)											
Placebo	12·7^{a}	0·8	12·8^{a}	0·9	12·9^{a}	0·9	12·9^{a}	0·9	12·8^{a}	0·8	NS
Fortified	13·0^{a}	1·2	13·7^{b**}	2·3	13·5^{b*}	1·9	13·0^{a}	1·2	12·7^{a}	0·7	0·003
Hb (g/l)											
Placebo	133	9	133	9	132	8	132	8	132	8	NS
Fortified	132^{a}	9	133^{a}	8	135^{b}	9	136^{b*}	9	136^{b**}	8	<0·0001
Zinc protoporphyrin (μmol/mol haem)											
Placebo	–		79·3^{a,b}	26·9	79·9^{a,b}	29·4	76·1^{a}	32·5	86·1^{b}	33·7	0·049
Fortified	–		76·5^{a}	28·6	69·7^{b,c*}	26·1	67·1^{b}	24·1	74·6^{a,c*}	19·3	<0·0001
Serum Fe (mmol/l)											
Placebo	14·0	7·1	16·4	10·0	14·4	7·8	15·0	6·9	14·2	6·5	NS
Fortified	15·5	6·5	14·7	7·2	15·2	6·7	15·5	5·8	16·0	8·3	NS
Serum ferritin (ng/ml)											
Placebo	26·9	17·9	25·0	17·4	24·8	17·7	23·1	16·2	22·8	14·9	NS
Fortified	25·4^{a}	16·5	30·7^{b*}	13·6	34·1^{b,c**}	14·8	37·3^{c,d***}	16·5	40·7^{d***}	17·7	<0·0001
Serum transferrin (mg/l)											
Placebo	3213^{a}	676	3114^{b}	628	3208^{a,b}	618	3104^{a,b}	638	3232^{a,b}	718	0·001
Fortified	3102^{a}	519	2799^{b**}	449	2875^{b,c***}	486	2806^{b**}	438	2929^{c**}	509	<0·0001
Transferrin saturation (%)											
Placebo	19·2	7·9	19·6	10·3	17·8	9·7	19·1	9·0	17·8	11·7	NS
Fortified	19·7	10·7	23·7	15·3	22·2*	10·8	23·4*	9·9	23·0*	11·3	NS
Soluble transferrin receptor (mg/l)											
Placebo	1·41	0·37	–		1·40	0·35	–		1·45	0·53	NS
Fortified	1·48^{a}	0·60	–		1·33^{b}	0·46	–		1·28^{b*}	0·43	0·001

Mean values within the same row with unlike superscript letters were significantly different between the placebo and fortified groups at each point, measured using one-sided tests (repeated-measures ANOVA followed by the Bonferroni test): * $P<0·05$, ** $P\leq 0·01$, *** $P\leq 0·001$.

breakfast cereal with kiwifruit, increases in ferritin and Hb were observed[33], in agreement with the present study.

In the present study, the food matrix used was an acidic drink that contained AA with a molar AA:Fe ratio of 1·7:1. Several reports have discussed the importance of this ratio; however, there is no agreement on the optimal ratio to facilitate Fe absorption. A linear relationship between molar AA:Fe ratio and Fe absorption has been suggested[34], but Cook & Reddy[15] did not observe differences in Fe absorption between diets with AA:Fe ratios of 1·2:1, 2·4:1 and 4·5:1. This was attributed to the fact that Fe absorption was measured from a complete diet and not from individual meals. However, their study was criticised by Hunt[35] because subjects were instructed to select or avoid foods according to their AA content, which resulted in highly variable estimates of reported AA intakes. Shah *et al.*[36] compared Fe absorption in children consuming meals that were accompanied by either apple or orange juice, to which 5 mg of aqueous ferrous sulphate enriched with a stable isotope was added. They found that Fe absorption was similar to the orange and apple juices, even though the orange juice had much higher vitamin C content.

The effect of the AA:Fe ratio on Fe bioavailability depends on inhibitors present in the food[34], and several authors have suggested a ratio of 2:1 for low-phytate content foods[34,37], which is equivalent to the ratio of the juices assayed in the present study.

It is also important to consider that volunteers in the present study drank the 500 ml of juice separately at least 2 h after the meals; thus, the provided Fe could not interact with inhibitors present in the diet. Moreover, the low Fe status of these women at the beginning of the assay constituted a factor to favour Fe absorption[13]. Therefore, the Fe supplement given in the form of fruit juices was highly bioavailable and was efficacious to improve Fe status in Fe-deficient young women.

The short-term response observed in the present study was unexpected, and the effect was clearly shown in the principal variable, ferritin, a marker of Fe stores. To our knowledge, this is the first study that demonstrates a significant increase in this parameter after the consumption of an Fe-fortified functional beverage during 1 month. Therefore, it was found that in a very short period of time, and using a relatively low additional intake of Fe,

the biomarkers of Fe status improved in non-anaemic Fe-deficient women.

Moreover, it was observed that markers such as Hb or haematocrit, which were within normal ranges, increased after 3 months. The observed increase in RDW in the enriched group could also indicate a recovery from Fe deficiency. The RDW levels of the F group became higher in the first months, indicating greater variation in width, and returned to baseline levels at the end of the assay, which corresponds to the average lifespan of erythrocytes[38]. These results are consistent with the observed increase in mean corpuscular volume and haematocrit after the consumption of the Fe-enriched fruit juice.

Other indices, sTfR-2 and ZnPP, which are not widely available as standardised clinical determinations, also indicate an improvement of Fe status. Both reflect marrow Fe status for erythropoiesis and recovery from Fe deficiency[39].

The present study was performed in a European population at risk of Fe-deficiency anaemia (i.e. young menstruating women with low Fe stores), and it is important to indicate that intake of the juice portions was compatible with the usual diet, and that the amount of Fe ingested daily via the enriched juice was one-fifth of the usual therapeutic dose (100 mg) of Fe. Under these experimental conditions, ferritin levels increased by 80 %. It should be pointed out that once the assay started, none of the volunteers receiving the Fe-fortified juice dropped out and that they did not complain about digestive discomfort. Therefore, these results are outstanding and suggest that Fe-fortified juices could be used as part of the dietary treatment of anaemic patients in order to correct anaemia more effectively.

The slight increments of energy intake and body weight indicate high compliance, as all juices contained 10 g/100 ml of carbohydrates (non-added sugars). However, mean body-weight gain was only 1 kg, and BMI remained within normal limits during the whole intervention. This is a controversial issue because high consumption of fruit juices should be limited in order to prevent obesity, especially in children and young people. However, present results show that the percentage of energy from protein and fat is reduced by the inclusion of the daily juices in the diet, and the energy profile tended to be more balanced. In this respect, dietary guidelines recommend 45–60 % of energy from carbohydrates, and concerning sugar intake, even added sugar, the European Food Safety Authority has recently indicated that there were insufficient data to set an upper limit[40]. Moreover, Western populations show a low consumption of fruits and vegetables[41,42], and the beneficial effects of these fruit juices on other aspects of health should be explored. Nevertheless, sugar content could be reduced in future products that might be used as alternatives in subjects predisposed to anaemia and also obesity or diabetes.

A physiological adaptation appears to occur, since the increase in body weight was observed at the beginning of the assay (data not shown); thus, it is possible that energy from foods other than the juice decreased to compensate for the energy provided by the juices. It should be pointed out that none of the Fe-deficient women participating in this intervention were obese, and that their physical activity was unchanged during the study.

Fe-fortified juice consumption should be recommended to individuals with predisposition to Fe-deficiency anaemia but not to those at risk of excessive Fe intake who do not need to increase their Fe supply, such as patients with Fe overload[43]. Therefore, consumption of an Fe-fortified fruit juice may be considered as a supplement to prevent Fe-deficient anaemia in population risk groups, such as women of child-bearing age, pregnant women or children. These groups have a high acceptance of fruit juices, and the concept of functional foods also has high acceptance in developed countries. The present study can have repercussions on public health as prevention of one of the most widespread diseases will have important economic impact, decreasing the need to use public health services and pharmaceutical Fe supplements. The cost benefits of the consumption of this Fe-fortified food compared with those of the pharmaceutical therapy and health care services should be studied. In fact, in the present study, consumption of the Fe-fortified juice led to a recovery from depleted Fe stores (ferritin <12 ng/ml), with only one woman remaining depleted.

Further studies should be focused on the effectiveness of this Fe-fortified beverage in subjects according to their genetic background, in the line of previous studies[43,44]. Consumer aspects such as the optimal amount of fruit juice to be drunk, according to concentration of the Fe salt to be included, cost, palatable aspects and acceptability should also be investigated. Finally, the effect of such Fe-enriched juices on subjects with Fe-deficiency anaemia, obesity, diabetes or CVD and their corresponding nutrigenomic aspects should also be studied.

Acknowledgements

The present study was supported by Grupo Leche Pascual and Projects Ref. AGL2006 09519/ALI and Ref. AGL2009 11437. R. B.-R. was supported by a JAE-predoc grant from European Social Fund and CSIC, Spain, and L. T. was supported by a predoc grant from CONACyT, Mexico. The authors are grateful to I. Wright for revising the English text and to the volunteers who participated in the study. The authors' responsibilities were as follows: R. B.-R., A. M. P.-G. and L. T. contributed to the study design, data collection, analysis and manuscript preparation; R. B.-R. and A. M. P.-G. implemented the recruitment and follow-up of study participants; C. G.-V. contributed to the analytical determinations; M. A. D. manufactured the beverages; and M. P. V. was the principal investigator of the study, contributed to the study design and manuscript preparation. M. A. D. works for Grupo Leche Pascual. R. B.-R., A. M. P.-G., L. T., C. G.-V. and M. P. V. had no conflict of interest.

References

1. World Health Organization (2008) Worldwide prevalence of anaemia 1993–2005. In *WHO Global Database on Anaemia* [EM de Benoist, I Egli and M Cogswell, editors]. Geneva: World Health Organization.
2. Lynch SR (2005) The impact of iron fortification on nutritional anaemia. *Best Pract Res Clin Haematol* **18**, 333–346.
3. Moreiras O, Carbajal A, Cabrera L, *et al.* (2009) Ingestas recomendadas de energía y nutrientes para la población española (Recommended intakes of energy and nutrients for the Spanish population). In *Tablas de composición de alimentos (Food Composition Tables)*, 13th ed., pp. 227–230. Madrid: Pirámide, Grupo Anaya, SA.
4. Institute of Medicine and Food and Nutrition Board (2001) *Dietary Reference Intakes for Vitamin A, Vitamin K, Arsenic, Boron, Chromium, Copper, Iodine, Iron, Manganese, Molybdenum, Nickel, Silicon, Vanadium, and Zinc*. Washington, DC: National Academic Press.
5. Navas-Carretero S, Pérez-Granados AM, Schoppen S, *et al.* (2009) Iron status biomarkers in iron deficient women consuming oily fish versus red meat diet. *J Physiol Biochem* **65**, 165–174.
6. Hurrell RF (2002) Fortification: overcoming technical and practical barriers. *J Nutr* **132**, 806S–812S.
7. WHO/FAO (2004) *Guidelines on Food Fortification with Micronutrients* [L Allen, B de Benoist, O Dary and Richard Hurrell, editors]. Geneva: World Health Organization.
8. Navas-Carretero S, Sarria B, Pérez-Granados AM, *et al.* (2007) A comparative study of iron bioavailability from cocoa supplemented with ferric pyrophosphate or ferrous fumarate in rats. *Ann Nutr Metab* **51**, 204–207.
9. Navas-Carretero S, Pérez-Granados AM, Sarriá B, *et al.* (2007) Iron bioavailability from pate enriched with encapsulated ferric pyrophosphate or ferrous gluconate in rats. *Food Sci Tech Int* **13**, 159–163.
10. Fidler MC, Walczyk T, Davidsson L, *et al.* (2004) A micronised, dispersible ferric pyrophosphate with high relative bioavailability in man. *Br J Nutr* **91**, 107–112.
11. Moretti D, Zimmermann MB, Wegmuller R, *et al.* (2006) Iron status and food matrix strongly affect the relative bioavailability of ferric pyrophosphate in humans. *Am J Clin Nutr* **83**, 632–638.
12. Reddy MB, Hurrell RF & Cook JD (2000) Estimation of nonheme-iron bioavailability from meal composition. *Am J Clin Nutr* **71**, 937–943.
13. Hurrell R & Egli I (2010) Iron bioavailability and dietary reference values. *Am J Clin Nutr* **91**, 1461S–1467S.
14. Conrad ME & Schade SG (1968) Ascorbic acid chelates in iron absorption: a role for hydrochloric acid and bile. *Gastroenterology* **55**, 35–45.
15. Cook JD & Reddy MB (2001) Effect of ascorbic acid intake on nonheme-iron absorption from a complete diet. *Am J Clin Nutr* **73**, 93–98.
16. Nogueira de Almeida C, Crott G, Ricco R, *et al.* (2002) Control of iron-deficiency anaemia in Brazilian preschool children using iron-fortified orange juice. *Nutr Res* **23**, 27–33.
17. Ash DM, Tatala SR, Frongillo EA Jr, *et al.* (2003) Randomized efficacy trial of a micronutrient-fortified beverage in primary school children in Tanzania. *Am J Clin Nutr* **77**, 891–898.
18. Abrams SA, Mushi A, Hilmers DC, *et al.* (2003) A multinutrient-fortified beverage enhances the nutritional status of children in Botswana. *J Nutr* **133**, 1834–1840.
19. Makola D, Ash DM, Tatala SR, *et al.* (2003) A micronutrient-fortified beverage prevents iron deficiency, reduces anemia and improves the hemoglobin concentration of pregnant Tanzanian women. *J Nutr* **133**, 1339–1346.
20. Solon FS, Sarol JN Jr, Bernardo AB, *et al.* (2003) Effect of a multiple-micronutrient-fortified fruit powder beverage on the nutrition status, physical fitness, and cognitive performance of schoolchildren in the Philippines. *Food Nutr Bull* **24**, S129–S140.
21. Hyder SM, Haseen F, Khan M, *et al.* (2007) A multiple-micronutrient-fortified beverage affects hemoglobin, iron, and vitamin A status and growth in adolescent girls in rural Bangladesh. *J Nutr* **137**, 2147–2153.
22. Moher D, Schulz KF & Altman DG (2001) The CONSORT statement: revised recommendations for improving the quality of reports of parallel-group randomized trials. *Ann Intern Med* **134**, 657–662.
23. Koulaouzidis A, Cottier R, Bhat S, *et al.* (2009) A ferritin level >50 microg/L is frequently consistent with iron deficiency. *Eur J Intern Med* **20**, 168–170.
24. de Groot CP, van Staveren WA, Dirren H, *et al.* (1996) Summary and conclusions of the report on the second data collection period and longitudinal analyses of the SENECA Study. *Eur J Clin Nutr* **50**, Suppl. 2, S123–S124.
25. Navas-Carretero S, Pérez-Granados AM, Schoppen S, *et al.* (2009) An oily fish diet increases insulin sensitivity compared to a red meat diet in young iron-deficient women. *Br J Nutr* **102**, 546–553.
26. DIAL, Alce Ingenieria, Spain. http://www.alceingenieria.net/nutricion.htm (accessed December 2009).
27. National Health and Nutrition Examination Survey (NHANES) (2007) Anthropometry procedures manual. http//www.cdc.gov/nchs/data/nhanes/ (accesed September 2008).
28. National Research Council (1989) *Recommended Dietary Allowances*, 10th ed. Washington, DC: National Academic Press.
29. Navas-Carretero S, Pérez-Granados AM, Sarria B, *et al.* (2009) Iron absorption from meat pate fortified with ferric pyrophosphate in iron-deficient women. *Nutrition* **25**, 20–24.
30. Roe MA, Collings R, Hoogewerff J, *et al.* (2009) Relative bioavailability of micronized, dispersible ferric pyrophosphate added to an apple juice drink. *Eur J Nutr* **48**, 115–119.
31. Zimmermann MB, Winichagoon P, Gowachirapant S, *et al.* (2005) Comparison of the efficacy of wheat-based snacks fortified with ferrous sulfate, electrolytic iron, or hydrogen-reduced elemental iron: randomized, double-blind, controlled trial in Thai women. *Am J Clin Nutr* **82**, 1276–1282.
32. Karl JP, Lieberman HR, Cable SJ, *et al.* (2010) Randomized, double-blind, placebo-controlled trial of an iron-fortified food product in female soldiers during military training: relations between iron status, serum hepcidin, and inflammation. *Am J Clin Nutr* **92**, 93–100.
33. Beck K, Conlon CA, Kruger R, *et al.* (2010) Gold kiwifruit consumed with an iron-fortified breakfast cereal meal improves iron status in women with low iron stores: a 16-week randomised controlled trial. *Br J Nutr* (Epublication ahead of print version).
34. Lynch SR & Stoltzfus RJ (2003) Iron and ascorbic acid: proposed fortification levels and recommended iron compounds. *J Nutr* **133**, 2978S–2984S.
35. Hunt JR (2001) How important is dietary iron bioavailability? *Am J Clin Nutr* **73**, 3–4.
36. Shah M, Griffin IJ, Lifschitz CH, *et al.* (2003) Effect of orange and apple juices on iron absorption in children. *Arch Pediatr Adolesc Med* **157**, 1232–1236.
37. Hurrell RF, Lynch S, Bothwell T, *et al.* (2004) Enhancing the absorption of fortification iron. A SUSTAIN Task Force report. *Int J Vitam Nutr Res* **74**, 387–401.
38. Dugdale AE (2006) Predicting iron and folate deficiency anaemias from standard blood testing: the mechanism and implications for clinical medicine and public health in developing countries. *Theor Biol Med Model* **3**, 34.

39. Labbe RF & Dewanji A (2004) Iron assessment tests: transferrin receptor *vis-à-vis* zinc protoporphyrin. *Clin Biochem* **37**, 165–174.
40. European Food Safety Authority (2010) Scientific opinion on dietary reference values for carbohydrates and dietary fibre (EFSA) panel on dietetic products, nutrition and allergies. *EFSA J* **8**, 1462.
41. Casagrande SS, Wang Y, Anderson C, *et al.* (2007) Have Americans increased their fruit and vegetable intake? The trends between 1988 and 2002. *Am J Prev Med* **32**, 257–263.
42. Lloyd-Jones DM, Hong Y, Labarthe D, *et al.* (2010) American Heart Association Strategic Planning Task Force and Statistics Committee. Defining and setting national goals for cardiovascular health promotion and disease reduction: the American Heart Association's strategic Impact Goal through 2020 and beyond. *Circulation* **121**, 586–613.
43. Toxqui L, De Piero A, Courtois V, *et al.* (2010) Iron deficiency and overload. Implications in oxidative stress and cardiovascular health. *Nutr Hosp* **25**, 350–365.
44. Sarriá B, López-Parra AM, Navas-Carretero S, *et al.* (2007) Hepcidin, transferrin (exon 7) and hemochromatosis genotyping suggests that haplotype block analysis is the best strategy for predicting iron deficiency phenotype in women. *Nutr Res* **27**, 672–678.

ELSEVIER

Nutrition 25 (2009) 20–24

NUTRITION

www.elsevier.com/locate/nut

Applied nutritional investigation

Iron absorption from meat pate fortified with ferric pyrophosphate in iron-deficient women

Santiago Navas-Carretero, Ph.D., Ana M. Pérez-Granados, Ph.D., Beatriz Sarriá, Ph.D., and M. Pilar Vaquero, Ph.D.*

Department of Metabolism and Nutrition, Instituto del Frío, Food Science and Technology and Nutrition Institute (ICTAN), Spanish Council for Scientific Research, Madrid, Spain

Manuscript received December 19, 2007; accepted July 5, 2008.

Abstract

Objective: Preventing iron deficiency has been a main target of the World Health Organization since 1992. Difficulties to reach dietary recommended iron intakes and to enhance iron absorption should be overcome. We compared in iron-deficient women the bioavailability of iron of three meat pate products enriched with ferrous sulfate, ferric pyrophosphate encapsulated in liposomes, or ferric pyrophosphate encapsulated in liposomes plus a hemoglobin-based meat pigment.

Methods: Seventeen women with low iron stores (ferritin <30 μg/L) took part in a three-way, randomized, crossover, double-blind postprandial intervention. Test meals consisted of 80 g of the three different enriched meat pate products, which were spread on two slices of white bread. The pate composition was 13.5 g of protein/100 g, 30 g of fat/100 g (49% monounsaturated fatty acids, 35% saturated fatty acids, 16% polyunsaturated fatty acids), 1 g of carbohydrates/100 g, and 19 mg of total iron (including 15 mg of iron from the test fortificants). Blood samples were taken at baseline and each hour for 6 h after eating the meal and serum iron was determined.

Results: Serum iron concentration evolution during the postprandial study was similar with the three meals, and maximum concentrations were obtained between hours 2 and 4. The effect of type of fortificant was not significant.

Conclusion: Consumption of meat pate fortified with ferric pyrophosphate encapsulated in liposomes can be part of a dietary strategy for preventing iron deficiency in humans. The addition of larger amounts of a meat pigment rich in heme iron should be further studied.

Keywords: Iron absorption; Iron-deficient women; Iron-fortified food; Ferric pyrophosphate; Postprandial intervention

Introduction

Iron deficiency anemia is one of the most important nutritional deficiencies, and its prevention has been a main target for the World Health Organization since 1992 [1]. Iron deficiency anemia is considered "a public health condition of epidemic proportions," with children and women being at-risk populations. In developing countries 52% of women are estimated to have iron deficiency and 22% in developed countries [2,3]. Iron deficiency affects more people than any other condition in the world [2].

Because dietary recommended iron intake is difficult to achieve from food alone, strategies for preventing iron deficiency have focused on food fortification with iron salts, mainly cereal products such as rice, flours, and bread [4–7], although an effective iron fortification of food remains a challenge. Ideal iron fortificants should permit supplementing high doses of iron in food without changing their physical, chemical, or sensory properties [8], allow appropriate food processing, and from a consumer's point of view be easily and effectively absorbed. Recently, the most studied

This study was supported by La Piara SA and the Spanish Ministry of Education and Science. La Piara SA provided the iron-fortified pate test meals. S. Navas-Carretero was granted by a Comunidad de Madrid–European Social Fund FPI fellowship.

* Corresponding author. Tel.: +34-91-549-0038; fax: +34-91-549-3627.

E-mail address: mpvaquero@if.csic.es (M. P. Vaquero).

0899-9007/09/$ – see front matter
doi:10.1016/j.nut.2008.07.002

iron fortificants have been ferrous bis-glycinate [5,9], ferrous fumarate [4], elemental iron [10,11], ferrous lactate [12], and ferric pyrophosphate [8,13–16]. Ferric pyrophosphate produces neither color nor palatability changes.

The use of meat-based products as vehicles for iron fortification is an unexplored pathway in iron deficiency prevention strategies. An important advantage is the natural content of heme iron and the presence of the "meat factor," a well-known enhancer of iron absorption [17–19].

Fortificants made from hemoglobin have also been studied, because they contain almost exclusively heme iron, which is well absorbed and improves the rate of iron absorption in rats and humans from the common iron pool in the gut [20,21]. Blood pigments containing heme iron are widely used in the meat industry, but their potential enhancing effect of iron absorption has not been explored yet.

Meat pate is a ready-to-eat spreadable product consumed by different population groups. It is an excellent source of highly bioavailable iron due to its content in heme iron, animal protein [22], and saturated fat [23]; therefore, it may be an adequate matrix for iron fortification. In a recent study in rats our group compared the relative iron absorption from two forms of iron, ferrous gluconate and ferric pyrophosphate encapsulated in liposomes (Lipofer, Lipofoods, Gavá, Barcelona, Spain.), when used as fortificants in meat pate, and observed that iron bioavailability was similar from both sources [15].

The present work compared the iron bioavailability in young iron-deficient women of meat pate products enriched with ferrous sulfate, ferric pyrophosphate encapsulated in liposomes, or ferric pyrophosphate encapsulated in liposomes plus a hemoglobin-based meat pigment.

Materials and methods

Study design

This study was approved by the clinical research ethics committee of the Hospital Clinico San Carlos and the ethics committee of the Spanish Council for Scientific Research (Madrid, Spain).

The study was a three-way, randomized, crossover, double-blind postprandial intervention.

Volunteer recruitment was carried out through advertisements in the Complutense University campus and advertising in Web pages of nutritional interest.

Twenty healthy, non-smoking, non-pregnant, non-anemic (hemoglobin >110 g/L), menstruating women, 21–25 y of age, with low iron stores (serum ferritin <30 μg/L), who had had iron deficiency anemia or had a family history of anemia showed their interest in participating in the intervention trial. None had taken iron supplements in the 12 mo before the start of the study, were blood donors, or taking any medication that could influence their iron metabolism. Seventeen women took part in the study after giving written informed consent.

The volunteers visited the laboratory facilities three times at 1-wk intervals. On each occasion the subjects fasted overnight for ≥12 h. After a cannula (ABOCATH 20G, Abbott Laboratories, Abbott Park, IL, USA) was inserted into a vein for blood sampling, baseline samples were obtained and the volunteers received the pate with the sliced bread. Blood samples were collected at 1-h intervals for 6 h after the end of the meal. On their first study day, volunteers were randomly assigned to an individual sequence of eating, starting with the P1, P2, or P3 pate (see following section), until every woman had completed the postprandial study with all three meals.

Test meals

A total of 90 pate cans, 80 g each, were provided by La Piara SA (Spain). They were distributed in three groups of 30 cans: P1, pate fortified with ferrous sulfate; P2, pate fortified with ferric pyrophosphate encapsulated in liposomes (Lipofer); and P3, pate fortified with Lipofer and an added pigment (Aprored). Total iron contents per can were 20.19 ± 1.08, 18.42 ± 1.47, and 18.95 ± 0.93 mg, respectively (values are means of 10 measurements). Because the pate enriched in ferrous sulfate (P1) had a slightly higher iron content, 6.5 g of P1 pate was discarded just before eating to provide the volunteers an equivalent iron content in each meal. The cans were kept at room temperature in a dry place until use.

Aprored is a chromatically stable meat pigment, completely soluble in water, with no taste, that contains heme iron (66% of proteins present are hemoglobin) [24] and is employed in meat-based products to enhance the natural color of meat. It was used in P3 in the proportion of 2.5 g of Aprored/kg.

Macronutrient pate composition per kilogram was 10 g of carbohydrates, 135 g of protein, and 302 g of fat (49% monounsaturated fatty acids, mainly oleic acid; 35% saturated fatty acids, mainly palmitic acid; and 16% polyunsaturated fatty acids).

On each study day, volunteers ate two slices of white bread (25 g) with the pate. They were not allowed to eat or drink anything more except tap water during the 6 h of the study.

Analytical methods

Hematologic baseline parameters were determined according to standard laboratory techniques and using the Symex NE 9100 automated hematology (Symex, Kobe, Japan) and the Modular Analytics Serum Work Area (Roche, Basel, Switzerland) analyzers. Measurements of hemoglobin concentration, serum ferritin, hematocrit, serum transferrin, and serum iron were carried out and total iron-binding capacity (micromoles per liter; 25.1 × serum transferrin [grams per liter]) and transferrin saturation (serum iron/total iron-binding capacity × 100) were calculated.

The postprandial blood samples were collected into tubes containing ethylene-diaminetetra-acetic acid and centri-

fuged at 1000 × *g* for 30 min, and serum was obtained and kept frozen in aliquots of 1 mL at −80°C.

Pate samples were dry-ashed in a muffle furnace at 500°C. Ashes were dissolved in an acid solution ($HCl/0_3$/H_2O: 1/2/1; Suprapur, Merck, Darmstadt, Germany). Iron was determined by atomic absorption spectrophotometry (model 1100B, Perkin-Elmer, Norwalk, CT, USA). A stock standard solution of iron (1 g/L) was prepared from Tritrisol (Merck; $FeCl_3$ in 15% HCl, 1.000 g; SD 0.002 g). Calibration solutions were prepared from the stock standard solutions by serial dilution with demineralized water (MilliQ Plus, Millipore Iberica, Madrid, Spain.) A blank solution was also used.

Postprandial serum iron concentrations were determined according to the protocol developed by Martinek [25]. This colorimetric technique allows determining serum iron in lipemic serum samples, such as those obtained after consuming the meat pate, by using Nitroso-R-salt as chromogenic agent without deproteinization.

Pig lyophilized kidney (certified reference material CRM 186; Community Bureau of Reference, Brussels, Belgium) was used as an external control, and yielded a concentration of 295.24 ± 16.42 μg/g (certified value 299 ± 10 μg/g), with an interassay variation coefficient of 5.56%.

Statistical analysis

The sample size was calculated to be 16 subjects given a power of 80% to detect a difference of 5% in serum iron, with a standard deviation of 5% at an α-value of 0.05 (95% confidence).

To examine the influence of the three pate meals, the influence of time and the possible interaction of meal type by time, data were analyzed by two-way analysis of variance with repeated measures followed by Bonferroni's post hoc test. SPSS 15.0 for Windows (SPSS, Inc., Chicago, IL, USA) was used. $P < 0.05$ was considered statistically significant.

Results

Hematologic values at the beginning of the study corresponding to the 17 volunteers are listed in Table 1.

No significant differences were observed in serum iron concentration during the 6-h postprandial study due to the type of pate consumed and there was no interaction between the type of meal and time (Fig. 1). The time influence was significant ($P = 0.005$). Serum iron concentration increased steadily in hours 1 and 2 after eating the meals ($P = 0.001$, compared with basal values and between hours 1 and 2), and the maximal concentrations were reached between hours 2 and 4 (no significant differences between values). A slight decrease was observed in hours 5 and 6 (no significant differences across hours 4, 5, and 6), without returning to baseline values.

Table 1
Hematologic characteristics of participants ($n = 17$)

Parameter	Mean	SD
Hemoglobin (g/dL)	13.03	0.97
Hematocrit (%)	40.21	2.55
Ferritin (ng/mL)	18.03	12.26
Serum iron (μmol/L)	14.53	7.39
Serum transferrin (mg/dL)	307.87	46.10
Transferrin saturation (%)	25.16	13.47

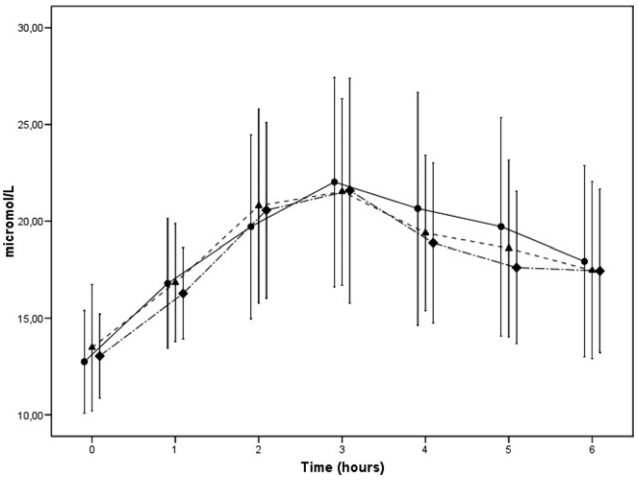


Fig. 1. Serum iron increase during the postprandial study after eating the enriched pate meals. Values are mean ± SEM of 17 volunteers. The effect of time was significant ($P = 0.005$; further data in RESULTS). There was no effect of type of food. There was no interaction of food with time. Circles, pate enriched with ferrous sulfate (P1); squares, pate enriched with ferric pyrophosphate (P2); triangles, pate enrich with ferric pyrophosphate and Aprored (P3).

Discussion

The use of serum iron increases is a feasible and reliable method to determine iron absorption that has been recently validated [26–28].

Our data of serum iron concentration at time 0 (baseline) are in accordance with reported values [26,29–32]. The evolution of serum iron concentration is in agreement with previous studies [31,32]. Ekenved et al. [31] reported a maximum serum iron increase of 12 μmol/L 3 to 4 h after consuming a labeled meal containing 25 mg of ^{59}Fe. Navarro and Wood [32] reported similar results in serum iron evolution and increase using a multivitamin and mineral supplement containing 60 mg of ascorbic acid and 18 mg of iron. As expected, after 6 h serum iron concentration did not return to baseline values [26,27,31,32].

Some investigators have reported the existence of a significant within-day serum iron variation, observing increases between 12% and 30% with respect to baseline values in starved subjects during 6 h [26,29,31,33]. However, other studies have shown that the diurnal variation is not consistent [30,32].

Hoppe et al. [26–28] adjusted the serum iron increase for diurnal variation by subtracting a standarized area under the curve from the serum iron increases observed in their subjects. Alternatively, Navarro and Wood [32] compared the evolution of different serum parameters when eating a meal with or without the mineral and vitamin supplement and showed that serum iron did not vary when the subjects consumed the meal without the supplement.

The present study was designed to be a randomized crossover trial, so such a correction was not considered, because we aimed to compare the response of each subject to different iron-fortified meals, and subtracting a constant area under the curve obtained from a mean population would not have modified the results obtained.

We are conscious that the method we have employed in this study has some limitations, the most important being the low sensitivity to changes in serum iron concentration, due mainly to the different within-subject and between-day responses of serum iron. Nevertheless, the measurement of serum iron concentration is a good way to discriminate iron salts that may present different bioavailabilities depending on the food matrix, as in our case, and its advantage is the feasibility and low cost of the colorimetric determination. This method is useful to screen for candidate fortification salts and to study new food matrices in a fast and effective way. To confirm the results obtained in this study, a new assay using stable isotopes of iron or a long-term nutritional intervention is needed. An assay with stable isotopes imply the production of iron salts containing different isotopes, e.g., ^{54}Fe, ^{57}Fe, and ^{58}Fe, and a long-term study must reproduce real conditions of consumption and the measurements should include classic parameters of iron metabolism and specific new biomarkers.

Ferric pyrophosphate has been widely studied in recent years, showing very different values of bioavailability [8,13–16]. Because this salt is poorly soluble in dilute acid and in gastric juice, efforts were focused on enhancing its solubility by its encapsulation [8]. Wegmüller et al. [14] suggested a 40/60 capsule/substrate ratio as optimal for maximal iron bioavailability. In our case the capsule/ferric pyrophosphate ratio was 30/70, and previous studies in rats have confirmed its effectiveness [15]. The particle size of ferric pyrophosphate also influences iron bioavailability [14]. Particle size reduction, from 2.5 to 0.5 μm, improved the relative bioavailability of pyrophosphate. Lipofer has a size of 7 μm and yielded iron bioavailability results similar to ferrous sulfate in the present study, which suggests that this salt is a potential candidate for fortification of meat-based products.

The food matrix can strongly affect the bioavailability of ferric pyrophosphate. Moretti et al. [34] stated that the relative bioavailability of ferric pyrophosphate varied from 62% when it was included in wheat-milk infant cereal to 15–25% in a rice meal. In previous rat studies carried out by our group, cocoa enriched with Lipofer presented low iron absorption and low utilization of the iron absorbed, probably due to the interaction and formation of chelates between ferric pyrophosphate and polyphenols [16]. In contrast, pate enriched with Lipofer presented a good iron bioavailability in rats [15], which is consistent with the present study in humans.

Studies with meat-based products as vehicles for iron fortification are scarce, although the potent enhancing power of the “meat factor” in iron absorption is well known [17–19,22]. This may be due to the high cost of meat products, which limits its use in strategies for preventing iron deficiency anemia in developing countries. However, almost 22% of women in developed countries have iron deficiency [2], and thus the use of this iron-fortified pate may be relevant in Western countries, where this spreadable ready-to-eat meat product is generally very well accepted by a wide range of the population, and depending on the food processing involved in its manufacture it can be stored, refrigerated or at room temperature, for long periods.

The fat content of meat pate also contributes to it being a good matrix for iron fortification. Kapsokefalou and Miller [23] studied the effects of different protein and fat sources on iron absorption in rats, stating that independently of the fat source (beef fat, milk fat, or partially hydrogenated vegetable fat), diets containing lean beef presented the highest iron absorption, with the highest in combination with beef fat. Highly unsaturated fat has been shown to alter iron metabolism in rats [35]. The proportions of protein and fat in the study meat pate (13.5% and 30%), in addition to the lipid profile of this fat (49% monounsaturated fatty acids, 35% saturated fatty acids, and 16% polyunsaturated fatty acids), make this food a suitable enhancer and vehicle for iron fortificants.

In this study, the inclusion of Aprored together with Lipofer, under the experimental conditions of the trial, did not show an increase in serum iron concentration, mainly due to the small quantity added (2.5 g of Aprored/kg of pate). Previous studies carried out with heme-derived compounds, used for fortifying infant weaning foods, have reported an increase of iron bioavailability without observing major changes in the organoleptic characteristics of these foods [20,21]. Lisbona et al. [36] suggested, in rats, a proportion of 20/80 heme/nonheme iron in diets as the most adequate for recovering from iron deficiency anemia. A combination of ferric pyrophosphate with a meat pigment derived from hemoglobin providing a similar ratio would be feasible and effective for pate fortification that should be explored, considering technologic, organoleptic, and health effects.

Conclusions

Consumption of pate fortified with ferric pyrophosphate encapsulated in liposomes can be part of a dietary strategy for preventing iron deficiency in humans. Long-term studies in which this pate is part of the usual diet are needed to confirm benefits in populations at risk of iron deficiency anemia, although the possible additional effects of adding a meat pigment rich in heme iron should be further studied.

Acknowledgments

The authors are grateful to the volunteers for their participation. They also thank Laura Barrios (Informatics and Statistics Service, Spanish Council for Scientific Research) for her statistical advice, and Monica Miquel (Nutrition Division, La Piara SA) for technical advice.

References

[1] World Health Organization. The prevalence of anaemia in women: a tabulation of available information. Document WHO/NUT/MCM/92.2. Geneva: World Health Organization; 1992.
[2] World Health Organization. Iron deficiency anaemia. Available at: http://www.who.int/nutrition/topics/ida/en/index.html. Last accessed, October 2007.
[3] World Health Organization. Iron deficiency anaemia: assessment, prevention and control. A guide for programme managers. Document WHO/NHD/01.3. Geneva: World Health Organization; 2001.
[4] Davidsson L, Kastenmayer P, Szajewska H, Hurrell RF, Barclay D. Iron bioavailability in infants from an infant cereal fortified with ferric pyrophosphate or ferrous fumarate. Am J Clin Nutr 2000;71:1597–602.
[5] Layrisse M, García-Casal MN, Solano L, Baron MA, Arguello F, Llovera D, et al. Iron bioavailability in humans from breakfast enriched with iron bis-glycine chelate, phytates and polyphenols. J Nutr 2000;130:2195–9.
[6] Hernández M, Sousa V, Moreno A, Villapando S, López-Alarcón M. Iron bioavailability and utilization in rats are lower from lime-treated corn flour than from wheat flour when they are fortified with different sources of iron. J Nutr 2003;133:154–9.
[7] Kloots W, Op den Kamp D, Abrahamse L. In vitro iron availability from iron-fortified whole-grain wheat flour. J Agric Food Chem 2004;52:8132–6.
[8] Hurrell RF. Fortification: overcoming technical and practical barriers. J Nutr 2002;132:806S–12.
[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] Roe MA, Fairweather-Tait SJ. High bioavailability of reduced iron added to UK flour. Lancet 1999;353:1938–9.
[11] Hoppe M, Hulthén L, Hallberg L. The relative bioavailability in humans of elemental iron powders for use in food fortification. Eur J Nutr 2006;45:37–44.
[12] Kapsokefalou M, Alexandropoulou I, Komaitis M, Politis I. In vitro evaluation of iron solubility and dialyzability of various iron fortificants and of iron-fortified milk products targeted for infants and toddlers. Int J Food Sci Nutr 2005;56:293–302.
[13] Fidler MC, Walczyk T, Davidsson L, Zeder C, Sakaguchi N, Juneja LR, Hurrell RF. A micronised, dispersable ferric pyrophosphate with high relative bioavailability in man. Br J Nutr 2004;91:107–12.
[14] Wegmüller R, Zimmermann MB, Moretti D, Arnold M, Langhans W, Hurrel RF. Particle size reduction and encapsulation affect the bioavailability of ferric pyrophosphate in rats. J Nutr 2004;134:3301–4.
[15] Navas-Carretero S, Pérez-Granados AM, Sarriá B, Schoppen S, Vaquero MP. Iron bioavailability from pate enriched with encapsulated ferric pyrophosphate or ferrous gluconate in rats. Food Sci Tech Int 2007;13:159–63.
[16] Navas-Carretero S, Sarriá B, Pérez-Granados AM, Schoppen S, Izquierdo-Pulido M, Vaquero MP. A comparative study of iron bioavailability from cocoa supplemented with ferric pyrophosphate or ferrous fumarate in rats. Ann Nutr Metab 2007;51:204–7.
[17] Engelmann MD, Davidsson L, Sandstrom B, Walczyk T, Hurrell RF, Michaelsen KF. The influence of meat on nonheme iron absorption in infants. Pediatr Res 1998;43:768–73.
[18] Baech SB, Hansen M, Bukhave K, Jensen M, Sorensen SS, Kristensen L, et al. Nonheme-iron absorption from a phytate-rich meal is increased by the addition of small amounts of pork meat. Am J Clin Nutr 2003;77:173–9.
[19] Navas-Carretero S, Pérez-Granados AM, Sarriá B, Carbajal A, Pedrosa MM, Roe MA, et al. Oily fish increases iron bioavailability of a phytate rich meal in young iron deficient women. J Am Coll Nutr 2007;27:96–101.
[20] Hertrampf E, Olivares M, Pizarro F, Walter T, Cavazzo M, Heresi G, et al. Haemoglobin fortified cereal: a source of available iron to breast-fed infants. Eur J Clin Nutr 1990;44:793–8.
[21] Walter T, Hertrampf E, Pizarro F, Olivares M, Llaguno S, Letelier A, et al. Effect of bovine-hemoglobin–fortified cookies on iron status of schoolchildren: a nationwide program in Chile. Am J Clin Nutr 1993;57:190–4.
[22] Hurrell RF, Reddy MB, Juillerat M, Cook JD. Meat protein fractions enhance nonheme iron absorption in humans. J Nutr 2006;136:2808–12.
[23] Kapsokefalou M, Miller DD. Lean beef and beef fat interact to enhance nonheme iron absorption in rats. J Nutr 1993;123:1429–34.
[24] Oliver MA, Polo J, Panella N, Arnau J, Contreras M, Moreras S, Ruiz J, Gil M. Effect of natural stabilised pork haem pigment on the colour, colour stability and texture of cooked hams from pale, soft and exudative meat. Food Sci Tech Int 2006;12:429–35.
[25] Martinek RG. Simplified determination of serum iron and binding capacity by nitroso-R-salt without deproteinization. Clin Chim Acta 1973;43:73–80.
[26] Hoppe M, Hulthén L, Hallberg L. Serum iron concentration as a tool to measure relative iron absorption from elemental iron powders in man. Scand J Clin Lab Invest 2003;63:489–96.
[27] Hoppe M, Hulthén L, Hallberg L. The validation of using serum iron increase to measure iron absorption in human subjects. Br J Nutr 2004;92:485–8.
[28] Hoppe M, Hulthén L. Validation of the clinical approach of using the induced serum iron increase after 1h as a measure of iron absorption. Clin Nutr 2006;25:163–5.
[29] Statland BE, Winkel P, Bokelund H. Factors contributing to intra-individual variation of serum constituents: 1. Within-day variation of serum constituents in healthy subjects. Clin Chem 1973;19:1374–9.
[30] 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.
[31] Ekenved G, Norrby A, Sölvell L. Serum iron increase as a measure of iron absorption – studies on the correlation with total absorption. Scand J Haematol 1976;28:31–49.
[32] Navarro M, Wood RJ. Plasma changes in micronutrients following multivitamin and mineral supplement in healthy adults. J Am Coll Nutr 2003;22:124–32.
[33] Statland BE, Winkel P, Bokelund H. Variation of serum iron concentration in young healthy men: within-day and day-to-day changes. Clin Biochem 1976;9:26–9.
[34] Moretti D, Zimmermann MB, Wegmüller R, Walczyk T, Zeder C, Hurrell RF. Iron status and food matrix strongly affect the relative bioavailability of ferric pyrophosphate in humans. Am J Clin Nutr 2006;83:632–8.
[35] Pérez-Granados AM, Vaquero MP, Navarro MP. Iron metabolism in rats consuming oil from fresh or fried sardines. Analyst 1995;120:899–903.
[36] Lisbona F, Reyes-Andrada MD, López-Aliaga I, Barrionuevo M, Alférez MJM, Campos MS. The importance of the proportion of heme/nonheme iron in the diet to minimize the interference with calcium, phosphorus and magnesium metabolism on recovery from nutricional ferropenic anemia. J Agric Food Chem 1999;47:2026–32.