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 Nutritional Ferropenic Anemia

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The digestive utilization of Fe and its nutritive interaction with Ca, P, and Mg were studied in rats with nutritional ferropenic anemia. The diet contained 80% ferric citrate and 20% heme iron (80/20 diet). The weight gain, digestive utilization of Fe, and regeneration efficiency of hemoglobin and seric Fe were higher in iron-deficient rats (ID) fed the 80/20 diet than in iron-deficient rats fed the 50/50 diet (Campos et al., 1996). The phospho-calcic metabolism, which is adversely affected in ferropenic anemia, returned to normal values when iron was added to the diet. The digestive utilization of Mg, which fell with the 50/50 diet (Campos et al., 1996), returned to normal values when the ferropenic anemia was reversed with the 80/20 diet. In a state of iron deficiency, certain parameters related to the glucose and lipid metabolism are affected; the glucose and triglycerides values return to a normal range with the 80/20 diet.

Keywords: *Iron; calcium; phosphorus; magnesium; rat; heme; anemia*

INTRODUCTION

Anemia comprises one of the most serious health problems today, affecting all ages. According to the WHO, it is at present the second most significant problem of Public Health, only surpassed by energy−protein deficiency.

The nutritional importance of iron is universally recognized; it is essential for crucial metabolic functions such as the transport of O_2 in the bloodstream and for the redox processes of the respiratory system (Linder, 1988).

Although the main feature of the metabolism of iron is its continual reutilization, levels of Fe are also influenced by physiological and dietary factors (Beutler, 1988). The main factors contributing to the absorption of nonheme iron are proteins derived from animal tissues such as meat, poultry, and fish (Bjorn-Rassumsen and Hallberg, 1979) and ascorbic acid (Hoffman et al., 1991).

Among the inhibitors of absorption are phytates (Siegenberg et al., 1991), polyphenols (Siegenberg et al., 1991), and minerals such as zinc (Crofton et al., 1989), manganese (O'Dell, 1989), copper (Fritz et al., 1977), and calcium (Hallberg et al., 1991).

One of the strategies used to overcome inhibitory factors and to palliate the interference of iron absorption with that of other minerals in humans (Bothwell and MacPhail, 1992; Beard and Dawson, 1997) is the use of bovine lyophilized hemoglobin. The iron in the hemoglobin is absorbed to a high degree, as the porphyrin ring is not released before being captured by the mucous cells; thus, it is not affected by inhibitory dietary factors that might reduce the absorption of nonheme iron (Bothwell et al., 1979).

According to studies carried out on normal rats and those suffering nutritional ferropenic anemia, the addition of a subproduct of bovine blood to cereal milk formula improves the metabolic utilization of iron in both groups of animals (Pallarés et al., 1996b). Moreover, in iron-deficient rats when half of the supply of ferric citrate was replaced by heme iron, and despite the fact that digestive utilization of iron was good, the metabolism of calcium, phosphorus, and magnesium, in general, was affected (Campos et al., 1996). The above considerations led us to design another study with the same bovine blood subproduct, in an 80/20 proportion of nonheme and heme iron, attempting to minimize the interference with the metabolism of calcium, phosphorus, and magnesium.

MATERIALS AND METHODS

Experimental Design. For this study, we used two diets containing 100% ferric citrate or ferric citrate and bovine blood in an 80/20 proportion. These sources of iron provided a total iron content of 35 mg/kg of diet.

The diet was evaluated for an experimental period of 10 days using control and iron-deficient rats. We studied bioavailability and the therapeutic effect of iron by using nonheme and heme iron in an 80/20 proportion. A further object of study was the interaction with the metabolism of other minerals such as calcium, phosphorus, and magnesium.

Prior to the study, iron deficiency was provoked in the animals by feeding them for 40 days with a low-

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Table 1. Composition of the Normal Diet

component	g/kg of dry weight
protein	
casein	200
DL-methionine/g	3
fiber (micronized cellulose)	50
fat (olive oil)	50
mineral suplement[a]	35
vitamin suplement	10
choline chloride	2
equal parts of saccharose and wheat starch to 1 kg	

[a] The mineral supplement contained the following (g/kg): calcium phosphate dibasic ($CaHPO_4$), 500.0; sodium chloride (NaCl), 74.0; potassium citrate, monohydrate ($K_3C_6H_5O_7 \cdot H_2O$), 220.0; potassium sulfate (K_2SO_4), 52.0; magnesium oxide (MgO), 24.0; manganous carbonate (43−48% Mn), 3.5; ferric citrate (16−17% Fe), 6.0; zinc carbonate (70% ZnO), 1.6; cupric carbonate (53−55% Cu), 0.3; potassium iodate (KIO_4), 0.01; sodium selenite ($Na_2SeO_3 \cdot 5H_2O$), 0.01; chromium potassium sulfate [$CrK(SO_4)_2 \cdot 12H_2O$],0.55; and finely powdered sucrose to make up to 1000 g (American Institute of Nutrition, 1977).

iron diet. The control animals were fed for 40 days with a diet including a normal iron content (ferric citrate), sufficient to satisfy the nutritional requirements of this species (AIN, 1993).

During the experimental period, we measured the food ingested, body weight, concentration of Ca, P, Mg, and Fe in the diet, hemoglobin regeneration efficiency, and levels of seric iron, calcium, and phosphorus. Also analyzed were PTH and alkaline phosphatase; GOT, GPT, CPK, LDH and, amylase; glucose, triglycerides, and cholesterol; and creatinin, uric acid, urea, and total protein levels. Finally, we measured the concentration of Fe, Ca, P, and Mg in the liver, femur, and sternum.

Diets. Table 1 summarizes the composition of a diet with a normal iron content (100% ferric citrate diet). The low-iron diet was obtained by omitting Fe from the mineral supplement. The 80/20 diet contained the same quantity of iron (35 mg/kg of diet) but was comprised of 80% ferric citrate and 20% heme iron.

Animals. The 48 experimental animals used were recently weaned white male rats of the Wistar Albina breed, with an approximate initial weight of 45−60 g, obtained from the Laboratory Animal Service of the University of Granada. The rats were divided into 4 groups of 12 animals. From the first day of experimentation, all the animals were housed in individual metabolic cages to facilitate the separate collection of feces and urine. The cages were situated in a well-ventilated room where the temperature was thermostatically controlled at a constant 21 °C with 12-h light/dark periods.

Experiment C: 100% Ferric Citrate. After being fed for 40 days with the diet established by AIN in 1977, the animals were given the same diet for an experimental period of 10 days.

Experiment D: 100% Ferric Citrate. After being fed for 40 days with a very low iron diet, the animals were given a diet containing iron provided as 100% ferric citrate for an experimental period of 10 days.

Experiment C: 80% Ferric Citrate + 20% Heme Iron. After being fed for 40 days with the diet established by AIN in 1977, the animals were given a diet containing iron provided as 80% ferric citrate and 20% heme iron for an experimental period of 10 days.

Experiment D: 80% Ferric Citrate + 20% Heme Iron. After being fed for 40 days with a very low iron diet, the animals were given a diet containing iron provided as 80% ferric citrate and 20% heme iron for an experimental period of 10 days.

The following Thomas-Mitchell biological technique (1923) was used in the experiments: for 40 days, the control group animals (C) were fed with a normal iron content diet and the iron-deficient group (D) given a very low iron content diet. This was followed by 3 days to allow the animals to adapt to the diet, and then a 7-day experimental period during which feces were collected. At the beginning and end of each experiment (days 43 and 50), body weight and food intake were recorded and the feces collected. The quantity of food ingested by each rat was determined from the quantity provided, the quantity refused and that spilt by each animal. During the study, all the animals were given double distilled water "ad libitum". During days 40 and 50, blood samples were taken from the caudal vein after a night's fast (12 h). The blood was immediately placed in tubes containing EDTA and used for hematological analysis. On day 50, the animals were anesthetized with sodium pentobarbital (5 mg/100 g of body weight) and totally bled by cannulation of the abdominal aorta. The total volume of blood was centrifuged to separate the serum, which was frozen at −30 °C for subsequent biochemical analysis. The liver, one femur, and the sternum were collected and frozen for posterior determination of their mineral content.

Biological Indices. The apparent digestibility coefficient was calculated using the following formula:

$$\text{percentage ADC} = \left(\frac{\text{absorbed}}{\text{intake}}\right) \times 100$$

where nutrient absorption = intake − fecal excretion.

Hemoglobin regeneration efficiency (HRE) was calculated as follows (Mahoney et al., 1974):

$$\text{hemoglobin − Fe (mg)} = \text{body wt (g)} \times \frac{\text{mL of blood}}{\text{g of body wt}} \times \frac{\text{g of hemoglobin}}{\text{mL of blood}} \times \frac{\text{mg of Fe}}{\text{g of hemoglobin}}$$

where

$$\frac{\text{mL of blood}}{\text{g of body wt}} \rightarrow \text{(assumed 0.067 mL)}$$

$$\frac{\text{mg of Fe}}{\text{g of hemoglobin}} \rightarrow \text{(assumed 3.35 mg)}$$

$$\text{percentage HRE} = \{[\text{mg of hemoglobin − Fe (final) − mg of hemoglobin − Fe (initial)}]/\text{mg of Fe consumed}\} \times 100$$

Analytical Methods. Water content in the diet, feces, liver, femur, and sternum was determined by drying the material at 105 ± 2 °C until the weight remained constant. A suitable quantity of the resulting material was baked at 450 °C, and the residue was extracted with 5 N HCl and brought up to an appropriate volume with double distilled water for Fe and P analyses or with lanthanum chloride solution (10 g/L) for Ca and Mg analyses to avoid possible interference by P.

A Perkin-Elmer 1100B atomic absorption spectrophotometer was used to determine the Fe, Ca, and Mg content. A Perkin-Elmer UV/vis spectrometer Lambda 16 was used to determine the P.

Table 2. Body Weight and Food Intake in Control and Iron-Deficient Rats Fed Diets with Different Sources of Fe (Mean Values with Standard Errors)

group	n	body wt (g) at day 43 (initial wt)	body wt (g) at day 50 (final wt)	wt change (g/rat/day)	food intake (g/rat/day)
C-100% citrate	12	275 ± 5	304 ± 5	4.1 ± 0.2[a]	19.1 ± 0.4
D-100% citrate	12	262 ± 6	282 ± 7	2.9 ± 0.2[b]	18.9 ± 0.6
C-80% citrate + 20% heme	12	301 ± 7	326 ± 6	3.8 ± 0.1	20.8 ± 0.5
D-80% citrate + 20% heme	12	267 ± 7	296 ± 8	3.8 ± 0.1	20.7 ± 0.4

[a] Significant difference between C-100% citrate and D-100% citrate. [b] Significant difference between D-100% citrate and D-80% citrate + 20% heme.

Table 3. Digestive Utilization of Fe, Ca, P, and Mg in Control and Iron-Deficient Rats Fed Diets with Different Sources of Fe (Mean Values with Standard Errors)

group	n	absorbed Fe (μg/rat/day)	ADC Fe%	absorbed Ca (mg/rat/day)	ADC Ca %	absorbed P (mg/rat/day)	ADC P %	absorbed Mg (mg/rat/day)	ADC Mg %
C-100%citrate	12	110 ± 12[a,b]	11 ± 2[b]	43 ± 3	45 ± 2	58 ± 3	60 ± 2[b]	3 ± 0.1	53 ± 3
D-100%citrate	12	163 ± 16[c]	18 ± 1[c]	46 ± 5	44 ± 3	64 ± 2	63 ± 3[c]	2 ± 0.3	36 ± 2[c]
C-80% citr. + 20% heme	12	141 ± 05[d]	19 ± 1	53 ± 2	50 ± 2	79 ± 2	77 ± 1	3 ± 1	51 ± 3
D-80% citr. + 20% heme	12	216 ± 08	30 ± 1	52 ± 2	48 ± 1	77 ± 1	75 ± 1	3 ± 1	50 ± 4

[a] Significant difference between C-100% citrate and D-100% citrate. [b] Significant difference between C-100% citrate and C-80% citrate + 20% heme. [c] Significant difference between D-100% citrate and D-80% citrate + 20% heme. [d] Significant difference between C-80% citrate + 20% heme and D-80% citrate + 20% heme.

Fiske and Subbarow's technique (1925) was used to measure the phosphorus content. Hemoglobin concentration was determined with a Symex CC-130 automatic cell counter. The seric concentrations of Fe, Ca, and P were measured by colorimetry (Trinder, 1956; Sarkar and Chauhan, 1967; Drewes, 1972). The total bilirrubin was determined by a color test, DPD method (Boehringer Mannhein GmbH Diagnostica). Creatinin was determined by the creatinin combination test, Jaffe method without deproteinization, Color Test (Boehringer Mannhein GmbH Diagnostica). Uric acid was determined by the uric acid combination test, PAP method, Enzymatic Color Test (Boehringer Mannhein GmbH Diagnostica). Urea was determined by an enzymatic UV test (Boehringer Mannhein GmbH Diagnostica). Total protein levels were determined by a combination test, using the Biuret method, color test (Boehringer Mannhein GmbH Diagnostica). Glucose was determined by the Gluco-quant combination test, hexocinase method, UV test (Boehringer Mannhein GmbH Diagnostica). Cholesterol was determined by the CHOD-PAP method, enzymatic test, peridochrom cholesterol (Boehringer Mannhein GmbH Diagnostica). Triglycerides were determined by a colorimetric enzymatic test, triglycerides GPO-PAP (Boehringer Mannhein GmbH Diagnostica). GOT/ASAT was determined by an ASAT/ALT/GPT monotest, according to IFCC, UV test (Boehringer Mannhein GmbH Diagnostica). GPT/ASAT was determined by an ALAT/ALT/GPT monotest, according to IFCC, UV test (Boehringer Mannhein GmbH Diagnostica). CPK was determined by an activated CK-NAC monotest, optimized standard method, UV test (Boehringer Mannhein GmbH Diagnostica). Dehydrogenated lactate was determined by an optimized LDH monotest, optimized standard method, UV test (Boehringer Mannhein GmbH Diagnostica). PTH was determined by a radioimmune assay, C-terminal/average, and parathyroid hormone molecule (measured on a PACKARD counter, Nichols Institute). Alkaline phosphatase was determined by an optimized alkaline phosphatase monotest, optimized standard method, color test (Boehringer Mannhein GmbH Diagnostica). Cortisol was determined by a radioimmune assay, measured on a PACKARD counter (Euro-Diagnostica BV).

Quality Control. Given the importance of obtaining an accurate determination of the different parameters studied, a quality control test of these determinations was carried out. This included the analysis of a set of primary standards and problem samples. There were two types of primary standards: those related to each determination and lyophilized control serum. Results showed that neither the standard deviation of the means between the primary standards nor that related to the problem samples was significant in any case throughout the experimental period.

Statistical Treatment. All values are expressed as the mean ± the standard error. The experimental results and analysis were statistically compared by a variance test, using one way SPSS/PC software. The means were compared with the Duncan test, with values of $p < 0.05$ being considered significant.

RESULTS

Chemical Analysis. The content of the low-iron diet was the following (mg/kg of diet): Fe, 4.43; Ca, 4769; P, 5139; Mg, 514. The mineral content of the iron-normal diet was the following (mg/kg of diet): Fe, 40.61 (as ferric citrate); Ca, 5020; P, 5430; Mg, 524. The mineral content of the 80/20 diet was the following (mg/kg of diet): Fe, 40.02 (80% ferric citrate + 20% heme iron); Ca, 5121; P, 4945; Mg, 534.

Biological Analysis. In the rats fed with the 100% ferric citrate diet the change in body weight was smaller in iron-deficient rats than in the control animals. When the diet containing 80% ferric citrate + 20% heme iron was administered, weight gain was the same for both the control and the iron-deficient groups: it was greater in iron-deficient rats fed the 80/20 diet than in iron-deficient rats fed the 100% ferric citrate diet (Table 2).

The control rats receiving the 80/20 diet presented an increase in the ADC of iron and of HRE compared to the control rats fed with 100% ferric citrate (Tables 3 and 4). Levels of seric iron in the control rats were within the normal limits established in the bibliography (Table 4).

In the iron-deficient rats fed with the 80/20 diet, the digestive utilization of iron, the HRE, and seric iron

Table 4. Hemoglobin (Hb) Values and Serum Values of Iron and Hemoglobin Regeneration Efficiency (HRE) in Control and Iron-Deficient Rats Fed Diets with Different Sources of Fe (Mean Values with Standard Errors)

		Hb (g/L)			serum Fe
	n	initial	final	HRE (%)	(μg/L)
C-100% citrate	12	145 ± 2	154 ± 2	25.9 ± 2.1[a]	1180 ± 70[b]
D-100% citrate	12	73 ± 4	106 ± 3	40.1 ± 1.9[c]	300 ± 38[c]
C-80% citrate + 20% heme	12	138 ± 2	150 ± 3	37.2 ± 3.5	1190 ± 160
D-80% citrate + 20% heme	12	84 ± 3	128 ± 4	77.5 ± 6.4	920 ± 90

[a] Significant difference between C-100% citrate and C-80% citrate + 20% heme. [b] Significant difference between C-100% citrate and D-100% citrate. [c] Significant difference between D-100% citrate and D-80% citrate + 20% heme.

Table 5. Iron Concentration in Several Organs in Control and Iron-Deficient Rats Fed Diets with Different Sources of Fe (Mean Values with Standard Errors)

group	*n*	liver (μg/g of dry wt)	femur (μg/g of dry wt)	sternum (μg/g of dry wt)
C-100% citrate	12	322 ± 20[a]	62 ± 3[a]	98 ± 8
D-100% citrate	12	135 ± 10	41 ± 2	111 ± 9
C-80% citrate + 20% heme	12	312 ± 16[b]	54 ± 4	103 ± 16
D-80% citrate + 20% heme	12	152 ± 11	45 ± 4	112 ± 12

[a] Significant difference between C-100% citrate and D-100% citrate. [b] Significant difference between C-80% citrate + 20% heme and D-80% citrate + 20% heme.

levels were greater than in iron-deficient rats fed with 100% ferric citrate diet (Tables 3 and 4).

In control and iron-deficient rats fed with the 80/20 diet for 10 days, the ADC of calcium and phosphorus were within the values given in the bibliography for rats, although they were higher than those found for the 100% ferric citrate diet (Table 3).

It is noteworthy that, in the iron-deficient rats fed with the 80/20 diet, the absorption of Mg is practically equal to that of the control group and greater than that of the iron-deficient rats fed the 100% ferric citrate diet (Table 3).

In iron-deficient rats the concentration of iron in the liver was significantly decreased in comparison with the control group, for both diets. The concentrations of calcium, phosphorus, and magnesium in the liver, femur, and sternum were higher with the 80/20 diet than with the 100% ferric citrate diet, in both the control and the iron-deficient rats, except for the concentration of calcium in the sternum, which was higher for the iron-deficient rats fed the 100% ferric citrate diet (Tables 5 and 6).

In iron-deficient and control rats fed the two diets being tested, that is, 100% ferric citrate and the 80/20 diet, the levels of PTH were within the normal range of values (Table 7).

The values recorded for the various parameters related to hepatic and pancreatic metabolism were within the wide range of variability observed in this species, (Table 8).

By day 40 of iron deficiency, seric levels of triglycerides had risen, while those of cholesterol had fallen. When iron was administered, irrespective of the sources and proportion, these values returned to normal in the case of the triglycerides (Table 9).

All values related to protein metabolism, both in the control rats and in iron-deficient animals, were within the normal limits for this species (Table 10).

DISCUSSION

Nutritional ferropenic anemia reduces the efficiency of food intake for weight gain (Beard, 1987; Greger and Lyle, 1988; Pallarés et al., 1996a,b). When the iron-deficient group was fed the 80/20 diet, the weight of these animals rose to equal that of the respective control animals, thus demonstrating the positive effect on weight gain of the addition of heme iron in this proportion to the diet. This effect might be due to the improved utilization of Fe, which was 25% greater than observed by Campos et al. (1996) for the 50/50 diet.

In the control rats, the 80/20 proportion is adequate for efficient utilization of iron, which is in agreement with the conclusions of Gordon and Godber (1989), Pallarés et al. (1993), Layrisse and Martínez-Torres (1972), and Gómez-Ayala et al. (1997) in that the coexistence of nonheme and heme iron increases the absorption of this mineral. On comparison with the 50/50 proportion used by Campos et al. (1996), the 80/20 diet is seen to produce more positive results on the metabolism of iron in the control rats.

When we compare iron-deficient rats with the control animals, the ADC of Fe is approximately 58% higher, which agrees with the findings of Campos et al. (1996), Gordon and Godber (1989), and Pallarés et al. (1993) that there is a greater utilization of iron in anemic rats. The digestive utilization of Fe is greater with the 80/20 diet than with the 50/50 diet used by Campos et al. (1996). The most likely explanation for this is, as previously suggested by Gordon and Godber (1989), that a state of anemia is accompanied by a depletion of the active mechanisms for the absorption of heme iron. Huebers et al. (1990), however, found no such depletion, but rather an increase in the absorption of the heme group by active mechanisms. Of the two hypotheses proposed, we are more inclined to accept that of Gordon and Godber (1989) as, given the lower proportion of heme iron, the greater absorption of iron found with the 80/20 diet was fundamentally due to a greater absorption of iron by passive mechanisms, or alternatively, to a greater digestive utilization of iron compounds such as ferric citrate (Zhang et al., 1989). This latter hypothesis is confirmed by the fact that the "ferric protein" diet, which comprises a manitol−ovoalbúmina−iron complex, as described by Campos et al. (1996), has a greater ADC than the 80/20 diet. It is obvious that the iron absorption pathways are not exclusive.

The beneficial effect of the 80/20 proportion is also reflected at the metabolic level, particularly concerning the hemoglobin regeneration efficiency parameter and that of the seric concentration of iron, even though the deposits of iron in the liver and femur are at low levels due to the short period of repletion. Iron content in the sternum increases in anemic rats, in agreement with the findings of Campos et al. (1996), Pallarés et al. (1993), and Milne et al. (1990).

In both the control and the iron-deficient rats, the 80/20 proportion improved the digestive utilization of Ca and P; nevertheless, with both of the diets being tested, these values were within the normal range (Pallarés et al., 1996b; Greger et al., 1987). In other words, the supply of the bovine blood subproduct improved the digestive utilization of iron, calcium, and phosphorus. Neither were any important alterations observed in the concentrations of Ca and P in the liver or sternum (Greger et al., 1987; Barrionuevo et al., 1989; Planells et al., 1995; Campos et al., 1996; Pallarés et al.,

Table 6. Ca, P, and Mg Concentrations in Several Organs in Control and Iron-Deficient Rats Fed Diets with Different Sources of Fe (Mean Values with Standard Errors)

		liver (per g of dry wt)			femur (per g of dry wt)			sternum (per g of dry wt)		
group	*n*	Ca (μg)	P(mg)	Mg (mg)	Ca (mg)	P(mg)	Mg (mg)	Ca (mg)	P(mg)	Mg (mg)
C-100% citrate	12	82 ± 3[a]	8.0 ± 0.3[a]	0.66 ± 0.03	228 ± 4	108 ± 2	3.7 ± 0.1	107 ± 3	46 ± 1[a]	2.3 ± 0.1
D-100% citrate	12	80 ± 4[b]	8.4 ± 0.1	0.62 ± 0.02	195 ± 3[b]	95 ± 2	3.9 ± 0.2	158 ± 5[b]	54 ± 3[b]	2.2 ± 0.2
C-80% citrate + 20% heme	12	99 ± 2	9.7 ± 0.5	0.71 ± 0.06	232 ± 5	106 ± 3	4.1 ± 0.3	108 ± 4	63 ± 2	2.1 ± 0.1
D-80% citrate + 20% heme	12	104 ± 4	8.9 ± 0.7	0.74 ± 0.04	225 ± 4	104 ± 6	4.2 ± 0.4	106 ± 6	63 ± 2	2.2 ± 0.2

[a] Significant difference between C-100% citrate and C-80% citrate + 20% heme. [b] Significant difference between D-100% citrate and D-80% citrate + 20% heme.

Table 7. Phosphocalcic Metabolism in Control and Iron-Deficient Rats (Mean Values with Standard Errors)

groups	*n*	PTH (pg/mL)	serum calcium (mg/dL)	serum phosphorus (mg/dL)	alkaline phosphatase (UI/L)
C-100% citrate	12	110 ± 4[a]	10.2 ± 0.2	8.0 ± 0.1	400 ± 33
D- 100% citrate	12	88 ± 7	10.2 ± 0.2	8.3 ± 0.3	350 ± 16
C-80% citrate + 20% heme	12	108 ± 3[b]	10.7 ± 0.7	6.9 ± 0.3	333 ± 15[b]
D-80% citrate + 20% heme	12	86 ± 3	10.4 ± 0.2	7.3 ± 0.2	415 ± 13

[a] Significant difference between C-100% citrate and D-100% citrate. [b] Significant difference between C-80% citrate + 20% heme and D-80% citrate + 20% heme.

Table 8. Hepatic and Pancreatic Metabolism in Control and Iron-Deficient Rats (Mean Values with Standard Errors)

groups	*n*	GOT/ASAT (UI/L)	GPT/ALAT (UI/L)	CPK (UI/L)	LDH (UI/L)	AMILASA (UI/L)
C-40 days	12			1347 ± 80	1302 ± 58	3633 ± 129
D-40 days	12			959 ± 74	1360 ± 31	5022 ± 113
C-100% citrate	12	138 ± 10	30 ± 1	812 ± 49	1590 ± 161	5420 ± 150
D-100% citrate	12	170 ± 23	29 ± 2	870 ± 95	1370 ± 142	4350 ± 121
C-80% citrate + 20% heme	12	126 ± 07	20 ± 1	883 ± 55	1184 ± 23	4995 ± 204
D-80% citrate + 20% heme	12	189 ± 14	31 ± 2	1228 ± 91	1170 ± 25	4762 ± 174

Table 9. Glucose and Lipid Metabolism in Control and Iron-Deficient Rats (Mean Values with Standard Errors)

groups	*n*	glucose (mg/dL)	cortisol (ng/mL)	cholesterol (mg/dL)	triglycerides (mg/dL)
C-40 days	12			78 ± 3[a]	65 ± 4[a]
D-40 days	12			64 ± 2	136 ± 14
C-100% citrate	12	124 ± 06	20 ± 1	80 ± 6	73 ± 6
D-100% citrate	12	111 ± 04	17 ± 2	72 ± 3	75 ± 5
C-80% citrate + 20% heme	12	127 ± 12	15 ± 1	72 ± 4	67 ± 4
D-80% citrate + 20% heme	12	106 ± 07	17 ± 2	64 ± 4	84 ± 8

[a] Significant difference between C-40 days and D-40 days.

Table 10. Protein Metabolism in Control and Iron-Deficient Rats Fed Diets with Different Sources of Iron (Mean Values with Standard Errors)

groups	*n*	creatinine (mg/dL)	uric acid (mg/dL)	urea (mg/dL)	total protein (g/dL)
C-40 days	12	0.59 ± 0.02[a]	1.4 ± 0.1	51 ± 2[a]	
D-40 days	12	0.51 ± 0.02	1.5 ± 0.1	38 ± 3	
C-100% citrate	12	0.50 ± 0.04	1.6 ± 0.3[b]	30 ± 2	6.1 ± 0.2
D- 100% citrate	12	0.48 ± 0.02	2.1 ± 0.3	27 ± 2	5.7 ± 0.1
C-80% citrate + 20% heme	12	0.60 ± 0.03	2.8 ± 0.1	35 ± 1	5.7 ± 0.1
D-80% citrate + 20% heme	12	0.52 ± 0.03	2.7 ± 0.1	31 ± 2	5.5 ± 0.1

[a] Significant difference between C-40 days and D-40 days. [b] Significant difference between C-100% citrate and C-80% citrate + 20% heme.

1996a,b). Campos et al. (1996) observed that the stocks of Ca and P in the femur in iron-deficient rats remained depleted even after the 10 days' repletion of iron. In the present study, however, with the 80/20 diet and the same repletion period, the content of Ca and P in the femur increased at the same rate as in the control animals. This might be explained by the increases in the digestive utilization of Ca and P, which were 20% and 10%, respectively, in contrast with the 50/50 diet.

The seric concentration of calcium and phosphorus did not vary in anemic rats with respect to the control animals fed with the 80/20 diet. A comparison of these seric levels with those of day 40 of iron deficiency, before repletion with iron, reveals that they were all within the normal limits for this species (Campos et al., 1998).

PTH levels in the iron-deficient and control rats for the 100% ferric citrate diet and the 80% citrate + 20% heme diet were within the normal range, as described in the bibliography (Planells et al., 1995), while in a severe state of iron deficiency, before supplying iron in the diet, the levels of this hormone had increased by approximately 40% (Campos et al., 1998). Therefore, the greater content of Ca and P in the femur in iron-deficient rats fed with the 80/20 diet must be attributed to the greater digestive utilization of these minerals with this diet and not to hormonal changes; as with all three diets, PTH levels were within normal limits.

In the control rats, the digestive utilization of Mg and its content in the liver, femur, and sternum when the 80/20 diet was supplied coincided with the results of many other authors (Aranda et al., 1987, 1989; López-Aliaga et al., 1991; Greger et al., 1989; Lerma et al., 1993; Pallarés et al., 1996a,b; Campos et al., 1996). However, in the iron-deficient rats given the 80/20 diet for 10 days, it is striking that the digestive utilization of Mg was practically the same as in the control animals, contrasting with the results of Campos et al. (1996) using the 50/50 diet.

This latter result might be due to the fact that, with this nonheme/heme iron proportion, the recovery of the metabolism of iron is better, as suggested by its greater digestive utilization (ADC) and the improved HRE. Consequently, the quantity of available oxygen and of ATP increases, and thus the membrane receptors for

the transport of heme iron and the receptors of the ferric citrate complex (Zhang et al., 1989; Conrad and Umbreit, 1993) must return to normal more quickly with the 80/20 diet. Moreover, the receptors of magnesium recover more efficiently, thus favoring absorption by active transport. Furthermore, the passive component of magnesium absorption is less influenced by the presence of free citrate in the lumen, wich would form magnesium citrate, a low solubility compound (López de Novales, 1974). This is because part of the citrate is absorbed as an iron complex, by active transport. This explanation is supported by the results obtained by Campos et al. (1996) with the ferric protein diet, wich showed that an improved recovery of iron absorption is accompanied by greater absorption of magnesium.

The content of magnesium in the liver, femur, and sternum in both the control rats and the iron-deficient animals was within the normal range described in the bibliography for this species (Lerma et al., 1993; Pallarés et al., 1996a,b; Greger et al., 1989).

The biochemical parameters related to the metabolism of glucose and lipids in control rats fed with the 80/20 diet were within the normal range described in the bibliography for this species (Llopis et al., 1989), as was the case with the 100% ferric citrate diet.

In rats with nutritional ferropenic anemia, significant changes in the metabolism of glucose were observed, with increased seric levels of glucose and cortisol (Henderson et al., 1986; Mattews and Battezzati, 1994; Rodríguez-Matas et al., 1998a,b), showing that anemia-induced stress leads to an increase in the seric levels of cortisol and thus a rise in glucemia. These data, obtained from iron-deficient rats, must be a consequence of the iron deficiency as the cited values return to normal when iron is added to the diet. With regard to the metabolism of lipids, iron deficiency provokes an increase in the levels of triglycerides and a reduction in cholesterol levels. This increase in the seric levels of triglycerides is in agreement with the findings of Sherman et al. (1978, 1979), who attributed it to an increase in the endogenous production of triglycerides, but is in contrast to the results of Rao et al. (1983). The increase in the levels of triglycerides could be explained by the increase in the levels of cortisol in serum, a consequence of the stress provoked by iron deficiency (Campos et al., 1998; Rodríguez-Matas et al., 1998a,b) which would produce a mobilization of triglycerides and an increase in their levels in plasma. The fall in the levels of cholesterol during a state of iron deficiency was also observed by Rao et al. (1983). On the other hand, Lewis and Iammarino (1971) found no change, while Sherman et al. (1978, 1979) observed an increase in cholesterol levels. Although iron deficiency produces significant changes in the metabolism of lipids, it is difficult to make generalizations, as observed by Rao et al. (1983), since many of the effects are influenced by factors such as age and strain of the experimental animal model and the dietary regime. When the diet contains iron in sufficient quantity, whether in 80/20 or as 100% ferric citrate, the triglycerides return to normal, while cholesterol levels remain low with the 80/20 diet.

In summary, the 80/20 diet constitutes a suitable supply of iron for recovery from nutritional ferropenic anemia, as shown by the adequate utilization of Fe. Particularly noteworthy is the improved utilization of Ca, P, and especially Mg, with no evident effects on the biochemical parameters related to glucose, lipid, or protein metabolism or on parameters related to hepatic and pancreatic functioning. Thus, the supply of 80% nonheme iron and just 20% heme iron reduces the interactions that would otherwise occur with calcium, phosphorus, and magnesium metabolism in recovery from nutritional ferropenic anemia. Moreover, according to Gordon and Godber (1989), these results obtained from rats, with care, could be extrapolated to humans.

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