BIOAVAILABILITY OF Fe, Ca, P AND Mg IN Fe-DEFICIENT RATS TREATED WITH DIFFERENT SOURCES OF DIETARY IRON

M.S. Campos1,2*, Ph. D., I. Pallarés1, Ph. D., A. Moratalla1, Ph. D., I. López-Aliaga1, Ph. D., A.E. Gómez-Ayala1, Ph. D., S. Hartiti1, Ph. D., M.J.M. Alférez1, Ph. D., M. Barrionuevo1,2, Ph. D. and F. Lisbona1,2, Ph. D.

1 Departamento de Fisiología,
2 Instituto de Nutrición y Tecnología de Alimentos,
Universidad de Granada, E-18071 Granada, Spain

ABSTRACT

Iron deficiency (DO) markedly decreases the concentration of iron and calcium in the liver, femur and sternum. We investigated the effect of the source of dietary iron [ferric citrate alone (citrate diet), or combined with bovine blood at a proportion of 1:1 (citrate+heme diet), and ferric protein (protein-Fe diet)] on the nutritive utilization of Fe, Ca, P and Mg in anemic and healthy rats. All diets contained the same amount of Fe (40 mg/kg diet). In Fe-deficient rats (DT) fed citrate, citrate+heme or protein-Fe diet the apparent digestibility coefficient (ADC) of Fe were 39%, 37% and 94% greater than in the respective control groups (C); hemoglobin regeneration efficiencies (HRE) were respectively 47%, 51%, and 172% greater than in the control groups. The ADC of Ca was not affected by Fe deficiency, whereas the ADC of Mg decreased significantly, and that of P increased under our experimental conditions. In Fe-deficient rats fed Fe for 10 days (DT), the concentrations of hemoglobin approached normal values. However, serum Fe remained low, indicating that Fe reserves were still depleted. Hepatic and femoral Fe concentrations were also lower in all DT groups, whereas Fe concentrations in the sternum increased significantly, suggesting an increase in erythropoiesis. After supply of dietary Fe for 10 days in iron-deficient groups (DT), Ca and P concentrations increased in sternum, a site assumed to have higher requirements for these minerals. In the liver these values tended to normalize, whereas in the femur Ca and P stores remained depleted. Mg concentrations after the replenishment period generally approached normality in all three organs.


INTRODUCTION

The most common nutritional problem in both developing and industrialized countries is iron deficiency (1), which is usually attributable to inadequate iron intake, blood loss, or inadequate iron

* Corresponding author: Margarita Sánchez Campos, Departamento de Fisiología, Facultad de Farmacia, Campus de Cartuja, Universidad de Granada, E-18071, Spain.
absorption. Bioavailability is less affected by the iron content of a diet than the form of the iron and the composition of remainder of the diet. The present study investigated the bioavailability of two forms of non-heme Fe (ferric citrate and ferric protein), and of a mixture of non-heme + heme Fe (ferric citrate+bovine blood). Bioavailability in control and Fe-deficient animals was determined as the apparent digestibility coefficient (ADC) and hemoglobin regeneration efficiency (HRE), both of which are accurate estimations of total Fe utilization.

Iron bioavailability may be compromised by mineral interactions such as that between Ca and Fe, which is well known to limit Fe absorption (2-4) in healthy individuals. However, little is known about Fe-Ca, Fe-Mg or Fe-P interactions in states of Fe deficiency.

We investigated the effects of Fe deficiency and the dietary source of iron on the nutritive utilization of Fe, Ca, P and Mg, an approach has been little investigated thus far. Many forms of dietary Fe have been described, together with their effects on Fe absorption (5-10). Our choice of ferric citrate as the reference Fe supplement was based on the guidelines of the American Institute of Nutrition (1977) for mineral supplementation (11). With regard to the two remaining diets used here, a mixture of non-heme/heme Fe is frequently recommended to enhance Fe status in healthy individuals; and ferric protein (Fe-(OH),PO,-apoferitine) may represent a good approximation to the physiological factors (transferrin, ferritin, lactoferrin, etc) known to be potent enhancers of iron absorption in states of Fe deficiency (12).

To compare the effect of dietary Fe sources on the distribution of Fe, Ca, P and Mg in Fe-deficient and control rats, we determined the concentrations of these minerals in the liver, femur and sternum, organs thought to be the most directly involved in homeostasis of these metals.

**MATERIALS AND METHODS**

**Animals**

A total of 84 male rats were randomly divided into seven groups of 12 animals each. The animals were 4-week-old (recently weaned) male Wistar albino rats with an initial body weight of 50-65 g, reared in the University of Granada Laboratory Animal Service. The animals were housed from day 0 of the experiment in individual metabolic cages designed for the separate collection of feces and urine; the cages were located in a well-ventilated, thermostatically controlled room (21°C) with a 12 h light-12 h dark period.

**Diets**

Table 1 summarizes the composition of the diet 0 (without Fe supplement). The citrate, citrate+heme and protein-Fe diets were obtained by adding to this formula 40 mg Fe/kg diet as ferric citrate, equal amounts of ferric citrate and bovine blood and Fe-protein.

The amounts, by analysis, of Fe, Ca, P and Mg in the experimental diets were (mg/kg diet):

Diet 0 (without Fe supplement) Fe 6, Ca 5411, P 4534 and Mg 506; citrate diet, Fe 45, Ca 4910, P 4200 and Mg 514; citrate+heme diet, Fe 45, Ca 5157, P 5128 and Mg 491; and protein-Fe diet, Fe 47, Ca 4714, P 4987 and Mg 501.
Fe, Ca, P AND Mg IN Fe-DEFICIENT RATS

TABLE 1
Composition of the experimental diets.
Diet 0 (without Fe-supplement)

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>g/kg dry weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>Casein</td>
<td>200</td>
</tr>
<tr>
<td>DL-Methionine</td>
<td>3</td>
</tr>
<tr>
<td>Wheat starch</td>
<td>150</td>
</tr>
<tr>
<td>Sucrose</td>
<td>500</td>
</tr>
<tr>
<td>Micronized cellulose</td>
<td>50</td>
</tr>
<tr>
<td>Olive oil</td>
<td>50</td>
</tr>
<tr>
<td>Mineral supplement*</td>
<td>35</td>
</tr>
<tr>
<td>Vitamin supplement+</td>
<td>10</td>
</tr>
<tr>
<td>Choline bitartrate</td>
<td>2</td>
</tr>
</tbody>
</table>

**TOTAL** 1000

* The mineral supplement contained (g/kg): CaHPO₄ 500.00, NaCl 74.00, C₆H₆K₂O₂H₂O 220.00, K₂SO₄ 52.00, MgO 24.00, MnCO₃H₂O (430-480 mg Mn/g) 3.50, ZnCO₃ (700 mg ZnO/g) 1.60, CuCO₃Cu(OH)₂H₂O (530-550 mg Cu/g) 0.30, KIO₃ 0.01, Na₂SeO₃·5H₂O 0.01, KCr(SO₄)₂·12H₂O 0.55, and finely powdered sucrose to make up 1000 g.

+The vitamin supplement contained (g/kg): thiamine hydrochloride 0.6, riboflavin 0.6, pyridoxine hydrochloride 0.7, nicotinic acid (nicotinamide equivalent) 3.0, calcium pantothenate 1.6, folic acid 0.2, biotin 0.02, cyanocobalamin 0.001, retinol 0.12, cholecalciferol 0.0025, α-tocopherol 4.6, menadione 0.005 and finely powdered sucrose to make up 1000 g.

Experimental design

Experiment 1: we investigated the effect of Fe deficiency on the concentration of Fe, Ca, P and Mg in the liver, femur and sternum of rats made Fe-deficient by feeding for 40 days with a diet that lacked the Fe supplement (diet 0, D0 group). At the end of that experiment tail blood was collected after 12 h food deprivation into tubes containing EDTA; pentobarbital sodium (5 mg/100 g body weight) was then injected intraperitoneally; the animals were completely exsanguinated via an abdominal aorta cannula. Blood samples were centrifuged to separate serum, which was frozen at -30°C until analysis. The liver was removed and frozen at -80°C in nitrogen liquid, then stored at -40°C. The femur and sternum were also removed and stored at -40°C for mineral analyses.

Experiment 2: the influence of the source of dietary Fe (ferric citrate, ferric citrate+heme and ferric protein) on the digestive utilization of Fe, Ca, P and Mg, and on HRE, was studied in Fe-deficient rats (groups DT-citrate, DT-citrate+heme, and DT-protein-Fe) and in normal control rats (groups C-citrate, C-citrate+heme, and C-protein-Fe). Food intake, change in body weight, and intake and fecal excretion of Fe, Ca, P and Mg were determined, as were concentrations of Fe, Ca, P and Mg in the liver, femur and sternum, and serum Fe concentration. All control rats (C) were fed the semisynthetic citrate diet (with ferric citrate as the source of dietary Fe) for 40 days, while Fe deficient rats were fed diet 0 (without Fe supplement) during the same period. After the first 40 days, and during the experimental period, the citrate diet, the citrate+heme diet, or the protein-Fe diet was supplied (iron deficient groups treated, DT). The experiment was programmed in accordance with Thomas and Mitchell’s biological technique (13). A period of three days was allowed for adaptation to the diet, followed by a seven-day experimental period when feces were collected on alternate days. Food intake (the total amount consumed daily by each rat) was determined by weighing the amounts of diet given, refused and spilled, and body weight was
recorded at the beginning and at the end of the principal period, that is, on days 43 and 50 of that experiment. On days 43 and 50 tail blood was collected and on day 50 the animals were completely exanguinated. The serum and liver, femur and sternum were stored until analysis.

Food and double-distilled water were available ad libitum to all animals.

Biological indices

Percentage apparent digestibility coefficient (ADC) was calculated with the formula:

$$\text{percentage ADC} = \frac{\text{absorbed intake}}{\text{intake}} \times 100,$$

where nutrient absorption = intake - fecal excretion.

Hemoglobin regeneration efficiency was calculated as follows (8):

$$\text{Hemoglobin-Fe (mg)} = \text{body weight (g)} \times \frac{\text{ml blood}}{\text{g body weight}} \times \text{(assumed to be 0.067 ml)} \times$$

$$\frac{\text{g hemoglobin}}{\text{ml blood}} \times \frac{\text{mg Fe}}{\text{g hemoglobin}} \times (\text{assumed to be 3.35 mg})$$

$$\text{Percentage HRE} = \frac{\text{mg hemoglobin-Fe(final)} - \text{mg hemoglobin-Fe(initial)}}{\text{mg Fe consumed}} \times 100$$

Analytical methods

The water content of the diet and the feces was determined by drying at 105±2°C until no further weight change was observed. Samples were ashed by calcination of 1-2 g samples at 450°C; the resulting residue was extracted with 5 mol/l-HCI, and made up to an appropriate volume with double-distilled water for Fe and P analysis, or with lanthanum chloride solution (5 g/l) for Ca and Mg analysis to avoid possible interference with P. Atomic absorption spectrophotometry (Perkin-Elmer 1100B) was used to determine Fe, Ca and Mg; P was determined by visible light spectrophotometry with the technique of Fiske and Subbarow (1925) (14). Nitrogen was determined by Kjeldahl’s method, using a protein conversion factor of 6.25. Fat content was analyzed by the Weibull-Berntrop gravimetric method (15). Serum concentrations of Fe were found by colorimetry (16). The hemoglobin concentration was obtained with a Symex CC-130 automatic cell counter.

Statistical treatment

Values for each variable are given as means with their standard errors. Comparisons were made between groups (C, D and Fe deficient) and diets (citrate, citrate+heme and protein-Fe) by analysis of variance with the oneway procedure of the SPSS/PC software package. Means were compared with Duncan’s test. A p value of less than 0.05 was considered significant.
RESULTS

Body weight

At the end of the experimental period, changes in body weight caused by Fe deficiency were smaller in groups DT animals than in controls (C). Throughout this period, food intake in all experimental groups remained within normal values for the rat (17), despite the slight increase in DT animals fed the protein-Fe diet in comparison with those fed citrate or the citrate+heme diet (Table 2).

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>Day 43 (Initial weight)</th>
<th>Day 50 (Final weight)</th>
<th>Weight change (g/rat per d)</th>
<th>Food intake (g dry weight/rat per d)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Experiment 1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DO</td>
<td>263.4 ± 9.9</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Experiment 2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C-citrate</td>
<td>276.3 ± 5.5</td>
<td>307.2 ± 5.3</td>
<td>4.4 ± 0.2*</td>
<td>19.0 ± 0.5</td>
</tr>
<tr>
<td>DT-citrate</td>
<td>260.5 ± 7.8</td>
<td>279.3 ± 8.1</td>
<td>2.7 ± 0.1</td>
<td>20.8 ± 0.6</td>
</tr>
<tr>
<td>C-citrate + heme</td>
<td>255.1 ± 5.2</td>
<td>303.7 ± 6.2</td>
<td>6.9 ± 0.3</td>
<td>23.4 ± 0.7</td>
</tr>
<tr>
<td>DT-citrate + heme</td>
<td>264.8 ± 5.3</td>
<td>285.2 ± 5.2</td>
<td>2.9 ± 0.2</td>
<td>21.7 ± 0.6</td>
</tr>
<tr>
<td>C-protein-Fe</td>
<td>251.8 ± 6.9</td>
<td>291.7 ± 7.9</td>
<td>5.7 ± 0.3</td>
<td>20.2 ± 0.8</td>
</tr>
<tr>
<td>DT-protein-Fe</td>
<td>259.5 ± 6.0</td>
<td>285.7 ± 7.7</td>
<td>3.7 ± 0.5</td>
<td>24.3 ± 0.8*</td>
</tr>
</tbody>
</table>

Mean values ± standard errors, n = 12.

DO: iron deficient rats non treated with Fe; C-citrate: control rats treated for 10 days by citrate diet.

DT-citrate: iron deficient rats treated for 10 days by citrate diet; C-citrate + heme: control rats treated for 10 days by citrate + heme diet; DT-citrate + heme: iron deficient rats treated for 10 days by citrate + heme diet; C-protein-Fe: control rats treated for 10 days by protein-Fe diet; DT-protein-Fe: iron deficient rats treated for 10 days by protein-Fe diet.

* Mean values were significantly different from those in DT-citrate group.

† Mean values were significantly different from those in DT-citrate + heme group.

‡ Mean values were significantly different from those in DT-protein-Fe group.

For details of diets and procedures, see Table 1 and pp 2.

Apparent digestibility coefficient of Fe, Ca, P and Mg

The ADC of Fe was lower in C animals fed the citrate + heme or protein-Fe diet than in Fe-deficient animals given the same diet. The highest ADC of Fe was found in rats (C and DT) that consumed the protein-Fe diet in comparison with those fed the citrate diet. A mixture of ferric citrate and bovine blood significantly increased Fe ADC in DT animals compared with those fed citrate alone (Table 3).

The ADC of Ca remained within normal values for the rat (18), despite the slight increase in C and DT animals fed the protein-Fe diet in comparison with those fed the citrate + heme diet.
and C animals fed the protein-Fe diet in comparison with those fed the citrate diet. Fe deficiency slightly decreased Ca ADC in rats fed citrate+heme (Table 3).

The ADC of P increased significantly in DT rats fed the citrate+heme or protein-Fe diet in comparison with their respective controls. P ADC was higher in DT animals fed protein-Fe than in those fed the citrate+heme or citrate diet. P ADC was also higher in C rats fed protein Fe diet than those fed citrate+heme (Table 3).

Fe deficiency led to a marked decrease in the ADC of Mg in animals fed the citrate or citrate+heme diet or the protein-Fe diet. In C rats fed the protein-Fe diet, Mg ADC was significantly greater than in animals fed the citrate or the citrate+heme diet. Similarly, in DT animals fed protein-Fe, the ADC of Mg was greater than in animals given either of the other diets (Table 3).

TABLE 3
Digestive utilization of Fe, Ca, P and Mg in control and iron-deficient rats fed on different diets.

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>Absorbed Fe (%)</th>
<th>ADC Fe (µg/rat/d)</th>
<th>Absorbed Ca (%)</th>
<th>ADC Ca (mg/rat/d)</th>
<th>Absorbed P (%)</th>
<th>ADC P (mg/rat/d)</th>
<th>Absorbed Mg (%)</th>
<th>ADC Mg (mg/rat/d)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Experiment 2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C-citrate</td>
<td>106 ± 22</td>
<td>12.1 ± 2.4</td>
<td>41 ± 3</td>
<td>44.2 ± 2.4</td>
<td>55 ± 2</td>
<td>62.0 ± 1.7</td>
<td>4.4 ± 0.2</td>
<td>56.4 ± 2.2</td>
</tr>
<tr>
<td>DT-citrate</td>
<td>156 ± 14</td>
<td>16.8 ± 1.4</td>
<td>45 ± 3</td>
<td>43.8 ± 1.8</td>
<td>61 ± 2</td>
<td>64.0 ± 1.6</td>
<td>2.8 ± 0.1</td>
<td>33.7 ± 1.7</td>
</tr>
<tr>
<td>C-citrate + heme</td>
<td>169 ± 12</td>
<td>15.8 ± 0.8</td>
<td>55 ± 2</td>
<td>46.0 ± 0.8</td>
<td>71 ± 4</td>
<td>58.5 ± 1.7</td>
<td>5.6 ± 0.3</td>
<td>60.1 ± 1.4</td>
</tr>
<tr>
<td>DT-citrate + heme</td>
<td>212 ± 08</td>
<td>21.6 ± 0.4*</td>
<td>45 ± 2</td>
<td>40.3 ± 1.2</td>
<td>77 ± 3</td>
<td>68.4 ± 0.5*</td>
<td>2.6 ± 0.1</td>
<td>30.1 ± 1.1*</td>
</tr>
<tr>
<td>C-protein-Fe</td>
<td>373 ± 29</td>
<td>39.5 ± 2.4*</td>
<td>50 ± 3</td>
<td>52.6 ± 2.4*</td>
<td>66 ± 3</td>
<td>66.0 ± 1.8*</td>
<td>6.3 ± 0.4</td>
<td>66.8 ± 2.4*</td>
</tr>
<tr>
<td>DT-protein-Fe</td>
<td>873 ± 31</td>
<td>76.8 ± 1.5*</td>
<td>53 ± 3</td>
<td>46.5 ± 2.3*</td>
<td>60 ± 3</td>
<td>74.3 ± 1.1*</td>
<td>6.5 ± 0.3</td>
<td>57.8 ± 1.7*</td>
</tr>
</tbody>
</table>

Mean values ± standard errors, n = 12.
* Mean values were significantly different from those in C-citrate group.
† Mean values were significantly different from those in DT-citrate group.
‡ Mean values were significantly different from those in C-citrate+heme group.
¶ Mean values were significantly different from those in C-protein-Fe group.
Mean values were significantly different from those in DT-citrate+heme group.
Abbreviations: see note to Table 2.

Hematological data

Percent HRE was significantly higher in DT animals than in each of their respective control groups. Hemoglobin regeneration was more efficient in the control group fed citrate+heme than in protein-Fe or citrate-fed rats. In Fe-deficient rats fed the citrate+heme diet, HRE was higher than in animals fed citrate (Table 4).

Serum concentrations of Fe were markedly lower in all Fe-deficient animals than in controls; among DT animals, serum Fe was significantly higher in those fed the citrate+heme or protein-Fe diet in comparison with the citrate diet. There were no significant differences in serum concentrations of Ca, P or Mg, all values remaining within normal limits for the rat (19).
### TABLE 4

Hemoglobin (Hb) values, serum values of Fe and hemoglobin regeneration efficiency (HRE) in control and iron-deficient rats fed different diets.

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>Initial Hb (g/l)</th>
<th>Final Hb (g/l)</th>
<th>HRE (%)</th>
<th>Serum Fe (μmol/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Experiment 1</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>D0</td>
<td>66 ± 3</td>
<td></td>
<td></td>
<td>8.2 ± 0.5</td>
</tr>
<tr>
<td><strong>Experiment 2</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C-citrate</td>
<td>147 ± 2</td>
<td>155 ± 2</td>
<td>26.7 ± 2.4</td>
<td>21.4 ± 1.0</td>
</tr>
<tr>
<td>DT-citrate</td>
<td>72 ± 3</td>
<td>106 ± 3</td>
<td>39.3 ± 1.8*</td>
<td>5.0 ± 0.6*</td>
</tr>
<tr>
<td>C-citrate + heme</td>
<td>140 ± 1</td>
<td>166 ± 2</td>
<td>44.3 ± 2.2*</td>
<td>23.0 ± 2.6</td>
</tr>
<tr>
<td>DT-citrate + heme</td>
<td>69 ± 3</td>
<td>135 ± 2</td>
<td>66.8 ± 2.5†</td>
<td>11.3 ± 1.3†</td>
</tr>
<tr>
<td>C-protein-Fe</td>
<td>136 ± 2</td>
<td>141 ± 2</td>
<td>22.9 ± 2.5‡</td>
<td>23.4 ± 2.6</td>
</tr>
<tr>
<td>DT-protein-Fe</td>
<td>66 ± 3</td>
<td>136 ± 2</td>
<td>62.4 ± 1.5†</td>
<td>9.6 ± 0.8†</td>
</tr>
</tbody>
</table>

Mean values ± standard errors, n = 12.

* Mean values were significantly different from those in C-citrate group.
† Mean values were significantly different from those in DT-citrate group.
‡ Mean values were significantly different from those in C-citrate + heme group.
§ Mean values were significantly different from those in C-protein-Fe group.

### TABLE 5

Iron concentration in several organs in control and iron-deficient rats fed different diets

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>Liver (μg/g dry weight)</th>
<th>Femur (μg/g dry weight)</th>
<th>Sternum (μg/g dry weight)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Experiment 1</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>D0</td>
<td>120 ± 8</td>
<td>45 ± 1</td>
<td>90 ± 5</td>
</tr>
<tr>
<td><strong>Experiment 2</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C-citrate</td>
<td>320 ± 22‡</td>
<td>64 ± 2*</td>
<td>96 ± 2</td>
</tr>
<tr>
<td>DT-citrate</td>
<td>130 ± 04†</td>
<td>39 ± 1†</td>
<td>116 ± 3†</td>
</tr>
<tr>
<td>C-citrate + heme</td>
<td>351 ± 13*</td>
<td>50 ± 2*</td>
<td>97 ± 3</td>
</tr>
<tr>
<td>DT-citrate + heme</td>
<td>192 ± 08‡</td>
<td>42 ± 2‡</td>
<td>119 ± 5‡</td>
</tr>
<tr>
<td>C-protein-Fe</td>
<td>269 ± 12†</td>
<td>72 ± 3‡</td>
<td>82 ± 3†</td>
</tr>
<tr>
<td>DT-protein-Fe</td>
<td>127 ± 17†</td>
<td>57 ± 2‡</td>
<td>87 ± 4‡</td>
</tr>
</tbody>
</table>

Mean values ± standard errors, n = 12.

* Mean values were significantly different from those in D0 group.
† Mean values were significantly different from those in C-citrate group.
‡ Mean values were significantly different from those in DT-citrate + heme group.
§ Mean values were significantly different from those in C-protein-Fe group.

Abbreviations: see note to Table 2.
TABLE 6

Ca, P and Mg concentrations in several organs in control and iron-deficient rats fed different diets

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>Liver (per g dry weight)</th>
<th>Femur (per g dry weight)</th>
<th>Sternum (per g dry weight)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ca (µg)</td>
<td>P (mg)</td>
<td>Mg (mg)</td>
</tr>
<tr>
<td>Experiment 1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>D0</td>
<td>60±4</td>
<td>10.6±0.2</td>
<td>0.82±0.01</td>
</tr>
<tr>
<td>Experiment 2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C-citrate</td>
<td>80±3*</td>
<td>7.7±0.2*</td>
<td>0.68±0.01*</td>
</tr>
<tr>
<td>DT-citrate</td>
<td>78±4</td>
<td>8.5±0.1†</td>
<td>0.61±0.01†</td>
</tr>
<tr>
<td>C-citrate+heme</td>
<td>75±3*</td>
<td>8.6±0.1⊥</td>
<td>0.70±0.01⊥</td>
</tr>
<tr>
<td>DT-citrate+heme</td>
<td>76±3†</td>
<td>8.9±0.1†</td>
<td>0.61±0.01†</td>
</tr>
<tr>
<td>C-protein-Fe</td>
<td>97±3‡</td>
<td>6.9±0.2‡</td>
<td>0.74±0.01‡</td>
</tr>
<tr>
<td>DT-protein-Fe</td>
<td>92±3†</td>
<td>8.2±0.2†</td>
<td>0.65±0.02†</td>
</tr>
<tr>
<td></td>
<td>119±4</td>
<td>43±3</td>
<td>2.4±0.1</td>
</tr>
</tbody>
</table>

Mean values ± standard errors, n = 12.
* Mean values were significantly different from those in D0 group.
† Mean values were significantly different from those in C-citrate group.
‡ Mean values were significantly different from those in C-citrate+heme group.
⊥ Mean values were significantly different from those in C-protein-Fe group.
‡‡ Mean values were significantly different from those in DT-citrate+heme group.
‡‡‡ Mean values were significantly different from those in DT-citrate group.
Abbreviations: see note to Table 2.
Fe, Ca, P and Mg in different organs

In the liver and femur, Fe and Ca concentrations were much lower in D0 group than in control rats, whereas P and Mg concentrations in the liver were significantly higher and femoral P concentrations were significantly lower (Tables 5 and 6).

After the replenishment period (experiment 2), hepatic Fe concentration was still lower in all DT groups. In rats fed the citrate+heme diet, these values increased in C and DT animals compared with those fed protein-Fe (Table 5).

In the femur, DT animals fed citrate, citrate+heme or protein-Fe had lower concentrations of Fe than their respective controls. The highest concentrations were found in C and DT animals given protein-Fe as compared to rats fed the citrate+heme diet (Table 5). Nevertheless, Fe deficiency increased Fe concentrations in the sternum of rats fed citrate alone or mixed with heme; the lowest concentration was found in DT animals fed protein-Fe in comparison with those fed the two remaining diets, and in C animals fed protein-Fe versus those fed the citrate or citrate+heme diet (Table 5).

Hepatic Ca concentration was significantly greater in C animals fed the protein-Fe diet than in rats fed either of the other diets (Table 6). Ca concentrations decreased significantly in the femur of DT animals, whereas in the sternum, these concentrations increased under the same experimental conditions (Table 6).

Hepatic P concentration increased in DT animals fed the citrate or the protein-Fe diet. These values were higher in C animals fed the citrate+heme diet than in rats given citrate alone or protein-Fe. Similarly, DT animals fed citrate+heme had higher concentrations of P than DT animals fed the citrate or protein-Fe diet (Table 6).

Femoral P concentration decreased significantly in Fe-deficient animals fed the citrate or citrate+heme diet. The opposite was found in the sternum of these animals (Table 6).

Fe deficiency decreased Mg concentration in the liver of rats fed citrate alone or mixed with heme or the protein-Fe diet. As in Ca, protein-Fe increased the liver Mg concentration in C animals compared with those given the citrate or citrate+heme diet (Table 6).

Mg concentration was higher in the femur of C and DT animals fed the protein-Fe diet compared with those fed citrate+heme. In DT animals given the protein-Fe diet, Mg content in the sternum was greater than in DT animals fed the citrate or citrate+heme diet (Table 6).

DISCUSSION

Fe deficient rats (DT groups) showed smaller changes in body weight in comparison with their respective control groups, regardless of the diet consumed. This finding suggests that Fe deficiency reduces feeding efficiency (20,21).

The ADC of Fe was markedly higher in DT rats compared with their respective controls. This digestive efficiency was greater than what we found in an earlier study with a lower dietary supply of protein (12%) (10). In this connection, Gordon and Godber (7) noted that Fe absorption was affected by both the amount and the quality of the protein tested.

The best digestive efficiency of Fe was found in C and DT animals fed protein-Fe in comparison with their respective C and DT rats given citrate+heme or citrate alone. The increase in inorganic Fe absorption associated with iron-deficient states has been well documented (10,12,22), but the mechanism by which inorganic Fe is absorbed is preferably
passive. Subcellular fractionation studies have proposed several models to explain Fe absorption. According to Huebers et al. (12), apotransferrin is secreted from mucosal cells and is loaded with iron in the intestinal lumen, and the intact complex is taken into the cell. Within the cell, Fe is released and transferred to the blood stream, whereas iron-free transferrin returns to the brush border to be recycled. These studies support the role of mucosal transferrin as a shuttle protein for Fe absorption. Conrad et al. speculated that integrins might facilitate the uptake of Fe from intestinal lumen, and that the entire absorptive process is driven by a cascade of differences in the binding constants of proteins such that iron moves from luminal mucin to mucosal mobilferrin to plasma transferrin (23). The significant increase in Fe absorption in C and DT rats fed the diet containing ferric protein supports the importance of the binding of Fe to protein; according to Conrad's hypothesis, this would strengthen the first step in the iron absorption model (23,24). According to this model, the iron binding pathway (mucin-mobilferrin-interin pathway) is important in facilitating the mucosal uptake of Fe by intestinal cells which do not have transferrin receptors in their luminal surfaces (24). This model would go part of the way toward explaining the large ADC of Fe obtained in Fe-deficient rats fed protein-Fe (DT-protein-Fe group) during the replenishment period, as the carrier system that allows Fe to pass through the membrane into mucosal cell might have been affected by Fe-deficiency (8).

The ADC of Fe in animals given a mixture of ferric citrate and heme was lower than we expected, and contradicted enhancement by blood reported by others (25). Our findings might be explained by Zhang's hypothesis: the function of the carrier system implicated in heme-Fe absorption may be reduced by several factors (eg, reduced Fe enzyme activity, reduced energy supply or low O2 supply) in severe Fe-deficiency (8). The intestine's capacity to take up heme is limited (26), but the critical factor in this limitation remains unclear. According to hypothesis proposed by Roberts et al. (25), some degree of adaptation of receptors to heme iron may occur in moderate Fe-deficiency. In severe Fe-deficiency such as that caused in the animals studied here, the insufficient O2 supply (a consequence of anemia) may have limited the efficiency of heme Fe receptors; an increase in their numbers may have been insufficient to enhance the Fe status of anemic rats. In this regard, Zhang et al. (8) confirmed a reduction in heme iron receptor activity under conditions of severe anemia.

The digestive utilization of Ca or P was not seriously affected by Fe deficiency, and the protein-Fe diet improved the ADC of both minerals in C and DT animals. However, the ADC of Mg was significantly lower in all DT animals, especially those given the citrate or citrate+heme diet. Nevertheless, in rats fed protein-Fe, the difference in Mg ADC between C and DT animals decreased. These findings support the hypothesis of Zhang et al. (8) that Fe diffusion by the enterocyte is significantly increased in anemic rats. Similarly, the amount of citrate anion in the intestinal lumen may increase; the anion would then bind to Mg to form less readily absorbed divalent compounds of this cation (10,27).

The greater digestive utilization of Fe by Fe-deficient rats treated with Fe (DT groups) was reflected in the HRE, which increased markedly to 39.3% with the citrate diet, to 66.8% after citrate+heme, and to 62.4% after protein-Fe. Percentage HRE in all groups of control rats was similar to that reported by other authors (8-10,28-30). The percent values of HRE we found after supplying different dietary sources of Fe were similar to those reported in earlier studies (7-10,29-33).

However, neither the better digestive utilization of Fe nor the near normal levels of hemoglobin were reflected by serum Fe concentrations. In all Fe-deficient groups, these values remained low after the replenishment period, regardless of the diet given. This finding indicates
that Fe reserves in these animals were not completely replenished (34). The lower concentration of Fe in the liver and femur in all Fe deficient animals supports this hypothesis. The slight increase in sternum Fe concentrations in anemic rats might be explained by the hypothesis proposed recently by Vreugdenhil et al. (35): under conditions of Fe deficiency, Fe ligands may facilitate the release of Fe from storage sites and its transport toward the sternum.

In DO group Ca concentrations decreased in the liver, femur and sternum. Because the Ca cation is absorbed preferentially through active transport (36,37), and because anemia is associated with impaired metabolic processes (38), Ca absorption may have been decreased, and deposits of this cation may therefore have been partially depleted.

Iron deficiency mobilized P and Mg deposits toward the liver. The increase in liver P concentrations may have been due to the increased catabolism of glucose in the Fe-deficient state (38). Hepatic Mg may have increased as a result of certain pathological situations associated with Fe deficiency (39), since nearly all enzymes involved in glucose metabolism are Mg-dependent.

Mg concentrations after recovery with an adequate Fe supply usually approached normality in all three organs analyzed here.

After the replenishment period, Ca and P concentrations increased in the sternum, a site assumed to have higher requirements for these minerals. This finding reflects the increased demand for minerals during hematopoiesis.

In the liver these values tended to normalize, whereas in the femur Ca and P stores remained depleted.

In summary: in anemia deposits of minerals were mobilized in different organs. Longer studies are necessary to elucidate whether replenishment of Fe deposits normalizes the stores of Ca and P in bone tissue. Our findings show clearly that anemia is a risk factor. If these findings can be extrapolated to humans (7), the iron deficiency may be of significance in populations susceptible to osteoporosis. Therefore, in anemic states, the metabolism of other minerals in addition to Fe should be controlled closely.

ACKNOWLEDGEMENTS

This study was partially supported by the CICYT of Spain through project no. ALI90-0616. We thank Ms Elisa Alcover for her expert secretarial assistance, Ms Rosa Jiménez for her competent technical assistance, and Karen Shashok for improving the English of the manuscript.

REFERENCES


Accepted for publication October 3, 1995.