Iron overload alters glucose homeostasis, causes liver steatosis, and increases serum triacylglycerols in rats

Maísa Silva, Marcelo E. Silva, Heberth de Paula, Cláudia Martins Carneiro, Maria Lucia Pedrosa

Abstract

The objective of this study was to investigate the effect of iron overload with a hyperlipidemic diet on the histologic feature of hepatic tissue, the lipid and glycemic serum profiles, and the markers of oxidative damage and stress in a rat model. Twenty-four male Fischer rats, purchased from Experimental Nutrition Laboratory, Federal University of Ouro Preto, were assigned to 4 equal groups, 2 were fed a standard cholesterol-free diet (group C or control and CI or control with iron) containing 8.0% soybean oil and 2 were fed a hyperlipidemic diet (group H or hyperlipidemic and HI or hyperlipidemic with iron) containing 1.0% cholesterol and 25.0% soybean oil. A total of 50 mg of iron was administered to rats in groups CI and HI in 5 equal doses (1 every 3 weeks for a 16-week period) by intraperitoneal injections of 0.1 mL of iron dextran solution (100 g Fe²⁺/L; Sigma, St Louis, Mo). The other rats in groups C and H were treated in a similar manner but with sterile saline (0.1 mL). Irrespective of the diet, iron excess enhanced serum triacylglycerols (P < .05) and reduced serum glucose and glycated hemoglobin levels (P < .05) but did not affect serum cholesterol concentration. Histologic analysis showed steatosis in groups H and to a lesser extent in HI. No significant differences (P > .05) were observed in paraoxonase activities or in serum levels of free or total sulfhydryl radicals, malondialdehyde, or total antioxidants. The findings suggest that iron excess in the rat probably modifies lipid metabolism and, as a consequence, alters glucose homeostasis and increases the level of serum triacylglycerols but not of cholesterol.

Keywords: Rat; Iron overload; Hyperlipemic diet; Hepatic steatosis; Serum glucose and lipids

Abbreviations:
ALT, alanine aminotransferase; ANOVA, analysis of variance; AOAC, Association of Official Analytical Chemists; HI, hyperlipemic iron group; MDA, malondialdehyde; PON, paraoxonase; SH, sulfhydryl; TIBC, total iron-binding capacity; TS, transferrin saturation; UIBC, unsaturable iron-binding capacity.

1. Introduction

There is considerable evidence indicating that a reduction in antioxidant defense capacity, and subsequent damage to macromolecules (ie, proteins and lipids), is related to the development of disease and ageing [1-3]. Endogenous mechanisms of antioxidant defense, involving both enzymatic and nonenzymatic processes, play important roles in transforming oxygen-reactive species into inert molecules [4]. More important, a number of dietary components can also modify the redox status by increasing or decreasing the antioxidant capacity in the body. In this context, high intracellular iron concentrations are associated with an increase in free radicals that can cause oxidative damage and trigger pathologic processes [4].

Iron metabolism is regulated by a sophisticated and complex mechanism, the purpose of which is to maintain a sufficient supply of the element to fulfill the demands of the...
cell while ensuring that the potentially toxic levels do not accumulate. However, iron excess can occur under certain circumstances, especially in individuals with diseases such as hereditary hemochromatosis, the most common inherited disorder among European descendents and a widespread disease in sub-Saharan populations [5]. Dietary iron supplementation can also lead to iron excess in otherwise healthy subjects. For instance in Sweden, where the diet has been supplemented with iron for some 50 years, 5% of men exhibited augmented levels of the mineral, whereas in 2% of the subjects, the levels were similar to those found in the initial stages of hemochromatosis [6,7]. Moreover, among elderly subjects, the intake of highly bioavailable forms of iron (ie, supplemental iron, red meat, and fruit) may promote high iron stores [8].

Iron excess has been linked to risks of development of certain chronic disorders such as diabetes [9], glucose intolerance [10], and cardiovascular diseases [11-13]. Iron excess induces cellular injury and functional abnormalities in hepatocytes by the process of lipid peroxidation [14].

Liver plays a central role in iron metabolism and in the maintenance of glucose and lipid homeostasis. Thus, hepatic injury triggered by iron excess may increase the concentration of secondary serum metabolites, such as cholesterol, triacylglycerols, and glucose, which are considered risk factors for cardiovascular diseases. The association between iron and a lipid-rich diet may exacerbate this situation because lipids also cause oxidative stress and steatohepatitis [15]. Various experimental models involving iron excess have been applied to assess the risk factors of cardiovascular diseases and alterations in liver histologic conditions [16-19].

In the present study, nontoxic doses of iron have been used to assess the effects of the metal for a long period and in association with a hyperlipidic diet. To investigate the interaction between hyperlipidemic diet and the effects of iron overload, we have targeted serum markers of antioxidant defenses and oxidative stress as well as glucose and lipid levels in rats receiving iron treatment and cholesterol-free or lipid-rich diets. Furthermore, we evaluated the effect of these treatments on the markers of hepatic function and histologic conditions.

2. Methods and materials

This study was approved by the Ethical Committee on Research of the Universidade Federal de Ouro Preto, Ouro Preto-MG, Brazil. All experimental procedures were approved and performed in accordance with the principles defined by the Brazilian School of Animal Experimentation [20].

2.1. Animals and experimental design

Male Fischer rats (n = 24), weighing approximately 100 g, were maintained in metabolic cages at 24°C under a constant relative humidity of 55% and a 12-hour light/dark cycle. All rats were provided food and water ad libitum. The rats were divided into 4 groups as follows: the control group (C) was fed a standard cholesterol-free diet containing 8.0% soybean oil; the control iron group (CI) was fed the same standard diet and given iron dextran by intraperitoneal injections; the hyperlipemic group (H) was fed a diet containing 1.0% cholesterol and 25.0% soybean oil in accordance with Turbino-Ribeiro et al [18]; and the hyperlipemic iron group (HI) was fed the same high-cholesterol diet and given iron dextran injections.

A total of 50 mg of iron was administered to rats in groups CI and HI in 5 equal doses (1 every 3 weeks for a 16-week period) by injection of iron dextran solution (100 g Fe²⁺/L; Sigma, St Louis, Mo). Rats in groups C and H were similarly injected with 0.1 mL of sterile saline during the same period. At the end of the treatment period, rats were subjected to overnight food deprivation and euthanized by an intraperitoneal injection of pentobarbital (Sigma) at a dose of 60 mg/kg body weight. For the determination of the levels of serum components, blood samples were collected from the brachial plexus in 5-mL test tubes (containing EDTA for the determination of glucose) and centrifuged at 12 800 g for 15 minutes. The livers were removed, weighed, and portions frozen for subsequent determination of iron and fat content; the remaining liver samples, together with the aorta (thoracic and abdominal), were stored in 3.7% buffered formaldehyde for histologic analysis.

2.2. Dietary composition

The control diet contained the following (g/kg): cornstarch (686; Unilever Bestfoods, Mogi-Guaçu, São Paulo, Brazil), casein (150; Isofar, Duque de Caxias, Rio de Janeiro, Brazil), soybean oil (80; Sadia, São Paulo, São Paulo, Brazil), salt mixture (50), cellulose (20; Merck, Darmstadt, Germany), vitamin mixture (10), and choline (4; Labsynth, Diadema, São Paulo, Brazil), with a total energy of 4064 kJ/kg. The hyperlipemic diet contained the following (g/kg: cornstarch (311), cellulose (200), soybean oil (250), casein (125), salt mixture (50), cholesterol (10), vitamin mixture (10), and choline (4), with a total energy of 4084 kJ/kg [21]. The salt mixture contained the following (g/kg): NaCl (139.3), KI (0.79), MgSO₄·7H₂O (57.3), CaCO₃ (381.4), MnSO₄·H₂O (4.01), FeSO₄·7H₂O (27.0), ZnSO₄·7H₂O (0.548), CuSO₄·5H₂O (0.477), CoCl₂·6H₂O (0.023), and KH₂PO₄ (389.0) [21]. The vitamin mixture contained (g/kg unless otherwise stated) the following: retinyl acetate (2 000 000 IU), cholecalciferol (200 000 IU), p-aminobenzoic acid (10.00), L-inositol (10.00), niacin (4.00), calcium pantothenate (4.00), riboflavin (0.80), thiamine hydrochloride (0.50), pyridoxine hydrochloride (0.50), folic acid (0.20), biotin (0.04), vitamin B₁₂ (0.003), sucrose (qsp 1000), choline (200.0), and dl α-tocopherol (10 000 IU) [21].

2.3. Assay methods

Serum iron concentration and total iron-binding capacity (TIBC) were determined in nonhemolyzed serum samples by spectrophotometric analysis using Labtest kits no. 38 and 41.
(Labtest, Lagoa Santa, MG, Brazil), respectively, with a standard iron solution (89.5 μmol/L). Transferrin saturation (TS) was estimated from the ratio of serum iron/TIBC. Serum triacylglycerols and cholesterol were measured enzymatically using Labtest kits no. 59-4/50 and 60-2/100, respectively, with glycero1 or cholesterol standards as appropriate. After precipitation of low-density lipoprotein and very low-density lipoprotein with phosphotungstic acid/MgCl₂, high-density lipoprotein (HDL-C) was determined in the supernatant (Labtest kit no. 13). Glucose, hemoglobin, alanine aminotransferase (ALT), and aspartate aminotransferase (AST) levels were measured with Labtest kits no. 84, 43, 53, and 52, respectively. To determine enzymatic activity, a calibration curve (supplied by the manufacturer) was used to correlate the obtained readings with the standardized values (U/L). The percentage of glycated hemoglobin was assayed using Labtest kit no. 17.

The total antioxidant status was determined with a Calbiochem kit no. 615700 (Calbiochem, San Diego, Calif). Lipid peroxidation was estimated spectrophotometrically (Calbiochem kit no. 437634) via assay of 4-hydroxynonenals (HAE) and malondialdehyde (MDA) with 1,1,3,3-tetramethoxypropane in Tris-hydrochloride as the external standard. The concentrations of free and total sulfhydryl (SH) radicals were determined using the Ellman test according to the method of Sedlak et al [22].

Paraoxonase (PON) activity toward phenyl acetate (arylesterase activity, AE) was determined by measuring the initial rate of substrate hydrolysis (monitored at 270 nm for 3 minutes) in an assay mixture (3 mL) containing 2 mmol/L of substrate, 2 mmol/L of CaCl₂, and 10 μL of plasma in 50 mmol/L of Tris-HCl (pH 8.0). The background rate associated with the nonenzymatic hydrolysis of substrate, determined in a blank sample prepared as described above but without plasma, was subtracted, and the enzymatic activity was calculated assuming the molar extinction coefficient of phenyl acetate to be 1310 mol/L·cm. The results were expressed in U/mL, where 1 U of arylesterase hydrolyzes 1 μmol of phenyl acetate per minute [23].

2.4. Iron and lipid levels in the liver

Liver samples were digested at 100°C in HNO₃, the excess acid was evaporated, and the iron quantified by colorimetric analysis using the Association of Official Analytical Chemists (AOAC) o-phenanthroline assay protocol [21] with a standard iron solution (89.5 μmol/L). The percentage of total lipids was determined after the AOAC procedure [21] in which a powdered liver sample, obtained by grinding 1 g of tissue with 12 g of clean sand in a porcelain mortar and pestle, was dried at 105°C for 2 hours, placed in the cellulose cartridge of a Soxhlet apparatus, and the lipids were extracted with petroleum ether for 6 hours.

2.5. Histologic evaluation

Slides containing sections of formalin-fixed, paraffin-embedded liver tissue were coded (to avoid observer bias), stained with hematoxylin and eosin for routine histologic examination, with Masson trichrome for collagen and with Perls’ Prussian blue for iron, and examined under the light microscopy. Steatosis, hydropic degeneration, inflammation, iron deposits, and collagen neoformation were evaluated under a ×40 objective in 20 random fields. Fragments of the aorta (thoracic and abdominal) were stained with hematoxylin and eosin, Masson trichrome, Weigert, and periodic acid-Schiff stains to identify alterations characteristic of atherosclerosis.

2.6. Statistical analysis

Statistical analyses were performed with the aid of Minitab 13 statistical software (Minitab, State College, Pa). All data were expressed as means ± SD. Treatment differences were determined using 2-way analysis of variance (ANOVA). Classification factors were iron and diet as well as their interactions. When the interactions were significant, means of the 4 treatment groups were compared using the Tukey multiple range test. Differences were considered significant at P < .05.

### Table 1

<table>
<thead>
<tr>
<th>Variables</th>
<th>C</th>
<th>CI</th>
<th>H</th>
<th>HI</th>
<th>ANOVA (P)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum iron (μmol/L)</td>
<td>14.54 ± 4.63b</td>
<td>25.45 ± 5.18a</td>
<td>21.30 ± 4.25a</td>
<td>20.87 ± 1.3a,b</td>
<td>NS</td>
</tr>
<tr>
<td>UIBC (μmol/L)</td>
<td>36.41 ± 7.39</td>
<td>38.00 ± 13.77</td>
<td>43.13 ± 9.76</td>
<td>50.20 ± 6.68</td>
<td>&lt;.05</td>
</tr>
<tr>
<td>TIBC (μmol/L)</td>
<td>50.96 ± 8.69</td>
<td>63.58 ± 11.39</td>
<td>64.44 ± 11.02</td>
<td>71.07 ± 6.74</td>
<td>&lt;.05</td>
</tr>
<tr>
<td>TS (%)</td>
<td>28.42 ± 8.09b</td>
<td>40.34 ± 7.16a</td>
<td>33.38 ± 6.40a,b</td>
<td>29.58 ± 3.27b</td>
<td>NS</td>
</tr>
<tr>
<td>Liver iron (μg/100 mg)</td>
<td>7.71 ± 3.96</td>
<td>53.36 ± 33.47</td>
<td>8.01 ± 7.12</td>
<td>47.89 ± 27.58</td>
<td>NS</td>
</tr>
</tbody>
</table>

Groups of animals were fed on a cholesterol-free control (C and CI) or a hyperlipemic (H and HI) diet and received 5 injections (0.1 mL each) of iron dextran solution (CI and HI) or sterile saline (C and H) for a 16-week period. Values are means ± SD; n = 6. Data were tested by 2-way ANOVA; NS indicates that mean values/interactions were not significant; when interactions were significant (P < .05), Tukey post hoc tests were performed to determine the specific differences between mean values. Within each row, mean values bearing different superscript letters (a and b) are significantly different according to Tukey test at P < .05.
3. Results

3.1. Iron status and histologic aspects of the liver

Serum iron concentration was 75% higher in animals receiving the cholesterol-free control diet together with iron dextran treatment (group CI) compared with those of the untreated control group C (Table 1). In contrast, no significant differences in iron concentration could be detected between the hyperlipemic groups H and HI, and the serum iron levels in these groups were not different from that observed in the control group CI. As was expected, rats fed on a control diet and treated with iron dextran (group CI) presented higher TS, but this effect was not observed in iron-treated animals fed on a hyperlipemic diet. It seems, therefore, that the hyperlipemic

Fig. 1. Histologic analysis of liver sections derived from rats fed a control diet and untreated (A, E, and I) or treated with iron dextran (B, F, and J) and from rats fed a hyperlipemic diet and untreated (C, G, and K) or treated with iron dextran (D, H, and L). Sections were submitted to hematoxylin and eosin (A-D), Masson (stain) trichrome (E-H), or Perls’ Prussian blue (I-L) stains and are shown at ×600 magnification. Section B shows iron deposition (black arrows); C shows intense steatosis and inflammation; D shows discreet steatosis and inflammation; F shows the absence of collagen neoformation; G and H show collagen neoformation; J and L show iron deposition (blue); and K shows intense steatosis and the absence of iron deposition.
different superscript letters (a, b, and c) are significantly different according to Tukey test at significant (\(P < .05\)). Tukey post hoc tests were performed to determine the specific differences between mean values. Within each row, mean values bearing different superscript letters (a, b, and c) are significantly different according to Tukey test at \(P < .05\).

Table 2

Levels of cholesterol, HDL-C, triacylglycerols, and PON in the serum and the level of lipid in the liver of rats

<table>
<thead>
<tr>
<th>Variables</th>
<th>C</th>
<th>CI</th>
<th>H</th>
<th>HI</th>
<th>ANOVA ((P))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cholesterol (mmol/L)</td>
<td>1.78 ± 0.22</td>
<td>1.75 ± 0.21</td>
<td>3.84 ± 1.03</td>
<td>3.97 ± 0.63</td>
<td>&lt;.05 NS NS</td>
</tr>
<tr>
<td>HDL-C (mmol/L)</td>
<td>0.73 ± 0.31</td>
<td>0.76 ± 0.26</td>
<td>0.24 ± 0.07</td>
<td>0.25 ± 0.16</td>
<td>&lt;.05 NS NS</td>
</tr>
<tr>
<td>Triacylglycerols (mmol/L)</td>
<td>1.04 ± 0.25</td>
<td>2.29 ± 1.42</td>
<td>2.12 ± 0.71</td>
<td>2.64 ± 0.94</td>
<td>NS &lt;.05 NS</td>
</tr>
<tr>
<td>Paraoxonase (U/mL)</td>
<td>124.43 ± 11.49(^a)</td>
<td>117.68 ± 1.41(^b)</td>
<td>90.88 ± 8.89(^p)</td>
<td>114.63 ± 16.65(^a)</td>
<td>&lt;.05 NS &lt;.05</td>
</tr>
<tr>
<td>Liver lipids (%)</td>
<td>13.52 ± 3.33</td>
<td>16.98 ± 8.03</td>
<td>33.19 ± 8.77</td>
<td>38.88 ± 3.45</td>
<td>&lt;.05 NS NS</td>
</tr>
<tr>
<td>Liver weight (g)</td>
<td>6.51 ± 0.64(^a)</td>
<td>6.32 ± 0.39(^b)</td>
<td>8.25 ± 0.48(^b)</td>
<td>10.20 ± 1.56(^a)</td>
<td>&lt;.05 NS &lt;.05</td>
</tr>
</tbody>
</table>

Groups of animals were fed on a cholesterol-free control (C and CI) or a hyperlipemic (H and HI) diet and received 5 injections (0.1 mL each) of iron dextran solution (CI and HI) or sterile saline (C and H) for a 16-week period. Values are means ± SD; n = 6. Data were tested by 2-way ANOVA; when interactions were significant (\(P < .05\)).

Table 3

Aspartate aminotransferase and ALT activities and levels of glucose, hemoglobin, and glycated hemoglobin in serum of rats

<table>
<thead>
<tr>
<th>Variables</th>
<th>C</th>
<th>CI</th>
<th>H</th>
<th>HI</th>
<th>ANOVA ((P))</th>
</tr>
</thead>
<tbody>
<tr>
<td>AST (U/L)</td>
<td>13.91 ± 7.67</td>
<td>40.55 ± 8.85</td>
<td>23.43 ± 15.01</td>
<td>30.47 ± 18.86</td>
<td>NS &lt;.05 NS</td>
</tr>
<tr>
<td>ALT (U/L)</td>
<td>22.49 ± 4.61</td>
<td>39.47 ± 5.04</td>
<td>36.14 ± 16.89</td>
<td>27.73 ± 5.29</td>
<td>NS NS NS</td>
</tr>
<tr>
<td>Glucose (mmol/L)</td>
<td>6.37 ± 0.64</td>
<td>5.57 ± 0.39</td>
<td>5.29 ± 0.99</td>
<td>4.78 ± 0.46</td>
<td>&lt;.05 &lt;.05 NS</td>
</tr>
<tr>
<td>Hemoglobin (μmol/L)</td>
<td>21.95 ± 1.30</td>
<td>23.14 ± 1.40</td>
<td>19.92 ± 1.09</td>
<td>20.60 ± 2.12</td>
<td>&lt;.05 NS NS</td>
</tr>
<tr>
<td>Glycated hemoglobin (%)</td>
<td>8.52 ± 3.28</td>
<td>6.36 ± 2.55</td>
<td>5.18 ± 2.21</td>
<td>2.25 ± 0.55</td>
<td>&lt;.05 &lt;.05 NS</td>
</tr>
</tbody>
</table>

Groups of animals were fed on a cholesterol-free control (C and CI) or a hyperlipemic (H and HI) diet and received 5 injections (0.1 mL each) of iron dextran solution (CI and HI) or sterile saline (C and H) for a 16-week period. Values are means ± SD; n = 6. Data were tested by 2-way ANOVA; when interactions were significant (\(P < .05\)).
hyperlipemic diet did not affect the activities of either of the aminotransferases.

3.4. Antioxidant status and MDA, HAE, and SH radical levels

Serum MDA and HAE concentrations were increased by the hyperlipemic diet but not by administration of iron dextran. Neither diet nor iron treatment affected serum total antioxidant status or the levels of free or total SH radicals (Table 4).

4. Discussion

The model used in the present study, which involved administration of iron dextran by injection of rats for a 16-week period, avoided any indirect mechanisms of up-regulation that could be caused by the disturbance in nutrient absorption associated with oral loading. The increase in hepatic iron in the experimental animals was well below the critical threshold (>22-fold increase) in which hepatocellular injury has been reported [24] and moderate in comparison with that observed in patients with symptomatic hemochromatosis (ie, 16-fold to 30-fold increase) [25].

The findings with respect to serum iron and TS suggest that a hyperlipidemic diet affects iron metabolism in rats. These results confirm those of Dabbagh et al [26] who found that the levels of serum iron in rats fed a hyperlipidemic diet were unaffected by treatment with iron pentacarbonyl. In addition, Du et al [27] observed that hepatic levels of metal-related genes, such as transferrin and ceruloplasmin, as well as those related to bile acid synthesis, were up-regulated in female Long-Evans Cinnamon rats that had been treated with a mixture of docosahexaenoic acid-rich soybean oil. In the present work, the intracellular iron stores were more evident in group HI rats as compared with those in group CI, suggesting that the hyperlipidemic diet affected iron homeostasis.

The present study demonstrated that the diet used was effective in inducing rats to become hyperlipemic. Interestingly, histopathologic analysis of thoracic and abdominal aorta showed no atherosclerosis in hyperlipemic rats confirming the findings of other authors [28].

The data for serum cholesterol levels and iron are in agreement with those obtained by Araujo et al [29], who also observed the absence of alteration in serum cholesterol in rabbits receiving iron dextran injections but contradicts previous results from our laboratory [18]. In our earlier study, although the same dose of iron was used, the period of application was shorter, and the distribution of iron among the various compartments of the liver might not have been the same as in the model used in the present work. Thus, the transit pool of free iron may have been different between the 2 studies and, consequently, cholesterol metabolism or circulating cholesterol uptake could have been affected in a different manner. It is noteworthy, however, that the effects of diet on serum cholesterol levels were observed to be the same in both of our studies.

Data presented in this study indicate that iron increased fat content, although lipid percentages in the H and HI rats were the same. Higher liver weights, similar lipid percentages, and a marked reduction in steatosis (as detected in the histologic evaluation) within the HI group probably indicate liver hyperplasia in these animals and a more efficient liver lipid metabolism. The observation that treatment with iron increased serum triacylglycerols agrees with the findings of Rodriguez et al [30].

Because an association between hepatic iron stores and insulin-resistance syndrome has been reported previously [5,6,31], parameters related to glycemic levels were determined in our experimental rat model. Interestingly, no relationship between the increase in iron stores and resistance to insulin was detected but rather iron treatment (associated or not with a hyperlipidic diet) was observed to promote a decrease in the levels of glucose and glycated hemoglobin. The resistance of rodents to iron-induced diabetes has recently been demonstrated in iron overload hepcidin-deficient mice [32].

The design of our study did not allow us to propose a mechanism for the observed alterations in serum glucose and glycated hemoglobin levels. Nevertheless, it is noteworthy that several authors have reported functional abnormalities in mitochondrial membranes caused by iron excess. Masini et al [33] observed that iron-induced oxidative stress gave rise to irreversible mitochondrial dysfunctions with resultant derangement of mitochondrial
energy-transducing capabilities arising from a reduction in the respiratory chain enzyme activities. Such irreversible oxidative anomalies caused a dramatic drop in tissue ATP levels. In addition, Ceccarelli et al [34] observed induction of lipid peroxidation and consequential abnormalities in mitochondria and calcium transport, after feeding rats with a diet supplemented with 2.5% of iron pentacarbonyl for 40 days. The present study revealed that, irrespective of the diet applied, iron treatment did not dramatically stress the antioxidant system, as indicated by the absence of alteration in the levels of free and total SH radicals and total antioxidant status in the serum. In contrast, Lykkesfeldt et al [35] have recently demonstrated an increase in the biochemical markers of oxidative stress and damage in response to increasing concentrations of iron in the liver. In our study, we observed that iron treatment produced an increase in AST activity, which is a marker for mitochondrial lesions. A reduction in mitochondrial capacity and impaired ATP production would lead to reduced levels of gluconeogenesis, and this could explain the observed reduction in serum glucose and glycated hemoglobin levels.

Paraoxonase is a serum enzyme that is closely associated with HDL and prevents and inhibits lipoprotein oxidation, a basic mechanism involved in the initiation and progression of atherosclerosis. Under certain conditions, oxidative stress is associated with the reduction of PON activity [36,37]. In the present study, arylesterase activity toward phenyl acetate was decreased in rats fed a hyperlipidemic diet, which is in agreement with previous reports [37,38]. Moreover, Nguyen and Sok [39] proposed that metal-catalyzed oxidation may be a primary cause for the decrease in HDL-associated PON1 activity observed under oxidative stress. Administration of iron might, therefore, give rise to a reduction in PON activity. This hypothesis is supported by Trudel et al [40] who reported that PON activity was decreased in rat and human liver microsomes in the presence of increased iron concentrations. However, these authors found that the administration of iron did not alter PON activity in rat serum, a result that was confirmed in the present study. It is likely that the iron dextran treatment used in our investigation did not cause the oxidative stress required to bring about an alteration in PON activity. This possibility is supported by the finding that MDA and HAE levels were altered by diet but not by administration of iron dextran.

In conclusion, the present study revealed that when hyperlipidemic rats are subjected to iron dextran therapy, there are consequential changes in glucose homeostasis and in the levels of triacylglycerols in the serum and in the liver. These aspects, together with distinct histologic features observed in H and HI rats, suggest an alteration of hepatic lipid metabolism and/or liver hyperplasia when iron is administered in association with a hyperlipemic diet. On the basis of the model used to promote excess iron in the present study, it would appear that rats do not exhibit the tendency toward abnormal glucose tolerance and diabetes frequently observed in humans with iron overload [5,6,31].

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