Effect of Thioacetamide and Dexamethasone on Serum Lipids in Rats Fed on High-Fat Sunflower or Olive Oil Diets

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Summary We have previously reported that high-fat diets develop hepatic steatosis and, depending on the fat quality, affect serum lipid levels differently (J Nutr Sci Vitaminol, 1997, 43, 155–160). The aim of this work is to study the influence of high-fat diets (14% sunflower or olive oils) on serum lipids in a model of hepatic acute damage induced by thioacetamide, and their influence when dexamethasone is administered before thioacetamide injection. Serum lipids and hepatic collagen have been evaluated using biochemical methods, and the steatotic process by histological staining. The results showed that hepatic steatosis and fibrosis are developed either by high-fat diets or thioacetamide injection. Pretreatment with dexamethasone did not decrease the hepatic collagen content. Thioacetamide injection alone or pretreatment with dexamethasone produced increases in serum triglycerides (TG), total cholesterol (TC) and LDL-C in both high-fat diet groups, and a HDL-C increase in the olive-oil group, even though the atherogenic indices (HDL/TC and HDL/TG) were different depending on the enriched diet. The administration of high-fat diets to study the influence of the fat quality on health and disease should be interpreted carefully due to the ability of the diets themselves to cause hepatic damage.

Key Words serum lipids, steatosis, fibrosis, glucocorticoids

The Mediterranean diet is supposed to be rich in olive oil, vegetables, salads, fresh fruit and fish, and has been related to a low incidence of cardiovascular pathologies (1). In addition, anti-atherogenic and anti-inflammatory properties have been attributed to this type of diet, in particular to olive oil (2, 3). Olive oil may also play a protector role in decreasing the degree of cirrhosis consequent on hepatic damage (4, 5). Thus, the type of dietary fat may be of general importance for patients with chronic liver disease, whose medical management continues to present difficult problems (6).
Recent studies have suggested that the health advantages of olive oil over other dietary oils or fats are based on its high content of mono-unsaturated fatty acid (7). Moreover, it is well-known that the type of fat in the diet affects serum lipids and lipoprotein levels, as well as liver morphology and biochemical parameters (8), and the influence of dietary fat composition on serum lipids and hepatic fibrosis is receiving much attention (9, 10). Thus, we have shown that different dietary fats affect serum lipid levels in hepatic steatosis (8).

The clinical use of glucocorticoids in treating a variety of pathologies characterized by collagen formation is also controversial in many situations (11), although some studies have shown an inhibitory effect of dexamethasone on connective tissue biosynthesis (12). In addition, it has been demonstrated that dexamethasone may influence the serum lipid profile (13).

In the present work, we have studied the short-term effects of diets high in fats of different saturation grades on serum lipid and lipoprotein levels after inducing acute hepatic damage with thioacetamide. The possibility of treating acute liver damage with diets of different fat composition, alone or together with dexamethasone, has been evaluated taking into account that high-fat diets showed a steatotic effect by themselves (8).

**Experimental**

High-fat diets (fat content 14%), semi-purified and balanced, were prepared as previously described (8). The standard (control) diet was a commercially available rat feed (fat content 4–5%). All of the animals had free access to the diet and water.

The animal experiments were carried out according to E.U. guidelines on the use of animals for biomedical research (86/609/EU). Sixty-three male, weaned Wistar rats (3 wk old), independently caged, were divided into three groups of 21 rats each, and fed ad libitum for a month on standard, high-fat olive-oil or high-fat sunflower-oil diets, respectively; all animals were fasted 12 h before being sacrificed, and they had free access to water only. As expected, body and liver weights, as well as the food intake, underwent a decrease after thioacetamide or dexamethasone plus thioacetamide treatments mainly in the high-fat diet groups (data not shown).

Seven animals from each diet group were used as controls, and another seven from each diet group were injected intraperitoneally with thioacetamide (Sigma, 500 mg/kg) 4 d before being sacrificed. The remaining seven rats from each diet group were injected intraperitoneally with dexamethasone (Sigma, 3 mg/kg) 45 min before injecting thioacetamide as described above.

For sample collection, all rats were anesthetized with Ketolar (Parke Davis, 150 mg/kg intraperitoneally) and injected with heparin via the dorsal vein of the penis (500 IU/kg). A laparatomy was performed and blood was collected from the mesenteric vein. Serum lipid measurements and statistical calculations were carried out as described elsewhere (8).
After collecting blood, the livers were immediately perfused through the portal vein with 20–30 mL 0.001 M phosphate-buffered saline (PBS) gassed with 95% O₂/5% CO₂, followed by 100–120 mL 4% paraformaldehyde in 0.1 M phosphate buffer. The livers were then dissected out, cut into small blocks and immersed in the same fixative for 3 h at 4°C, followed by immersion in 30% sucrose in 0.1 M phosphate buffer overnight at 4°C. Free-floating sections (40-μm-thick) were obtained for collagen quantitation by the colorimetric method described elsewhere (14). Histological examination for fatty infiltration was performed on 20-μm-thick sections stained with 1% OsO₄ 0.1 M phosphate buffer for 1 h at 4°C and mounted in PBS: glycerol (1:1).

Results

Hepatic steatosis was evaluated by histology in the different experimental groups (Fig. 1). Control rats fed on standard diet showed lipid accumulation in very few hepatocytes (Fig. 1A). However, thioacetamide-injected rats on standard diet showed periportal steatosis, with and without dexamethasone treatment (Fig. 1B, C). The rats fed on the high-fat olive-oil diet showed periportal steatosis in the non-treated, thioacetamide-injected, and dexamethasone plus thioacetamide conditions (Fig. 1D, E, F). On the other hand, the animals fed on the high-fat sunflower-oil diet showed only periportal lipid accumulation in the non-treated rats (Fig. 1G), but development of periportal and pericentral steatosis in the thioacetamide-injected rats (Fig. 1H), and necrotic pericentral areas were observed in those rats treated with dexamethasone prior to the administration of thioacetamide (Fig. 1I).

The results of collagen quantification (μg collagen/mg protein; mean ± SE) in the different experimental groups show a significant increase (p < 0.05) in hepatic collagen in the control rats fed the two high-fat diets (olive oil: 127 ± 3; sunflower oil: 120 ± 4) in relation to the control rats fed the standard diet (101 ± 6). As expected, and independently of the diet, rats with thioacetamide-induced hepatic damage showed greater amounts of collagen (standard: 159 ± 2; olive oil: 162 ± 2; sunflower oil: 162 ± 2) than the control rats (see above). Administration of dexamethasone did not reduce collagen formation in the livers of the rats fed the three different diets (standard: 171 ± 7; olive oil: 169 ± 7; sunflower oil: 187 ± 8).

Serum lipid and lipoprotein cholesterol values are shown in Table 1. In the control state, the serum values of triglycerides (TG) were slightly higher in the standard and sunflower-oil dietary groups than in the olive-oil group. In thioacetamide-injected animals, these values increased mainly in the standard and sunflower-oil dietary groups, even though the differences did not reach statistic significance. On the other hand, dexamethasone administration led to a significant increase in the serum TG values in both high-fat diet groups, the highest TG values being observed in the sunflower-oil group.

In the present experiment, serum total cholesterol (TC) values were higher in both high-fat diet groups than in the standard diet group, although this increase
was only statistically significant in the sunflower-oil group. After thioacetamid
injection, serum TC increased in all three dietary groups. Dexamethasone and
thioacetamide injection resulted in a significantly higher TC level in the olive-oil
group.
Table 1. Serum lipid levels (mean ± SE).

<table>
<thead>
<tr>
<th>Diet</th>
<th>TG  (mm)</th>
<th>TC  (mm)</th>
<th>LDL-C (mm)</th>
<th>HDL-C (mm)</th>
<th>HDL/TC</th>
<th>HDL/TG</th>
</tr>
</thead>
<tbody>
<tr>
<td>Standard</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>0.42 ± 0.04*</td>
<td>1.26 ± 0.02*</td>
<td>0.29 ± 0.02*</td>
<td>0.78 ± 0.01*</td>
<td>0.62 ± 0.01*</td>
<td>1.96 ± 0.17*</td>
</tr>
<tr>
<td>T</td>
<td>0.53 ± 0.09*</td>
<td>2.15 ± 0.13*</td>
<td>0.67 ± 0.12*</td>
<td>1.24 ± 0.20*</td>
<td>0.57 ± 0.06*</td>
<td>2.39 ± 0.33*</td>
</tr>
<tr>
<td>D + T</td>
<td>0.54 ± 0.06*</td>
<td>2.09 ± 0.12*</td>
<td>0.55 ± 0.05*</td>
<td>1.30 ± 0.10*</td>
<td>0.62 ± 0.02*</td>
<td>2.50 ± 0.25*</td>
</tr>
<tr>
<td>Olive oil</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>0.30 ± 0.07*</td>
<td>1.52 ± 0.08*</td>
<td>0.58 ± 0.03*</td>
<td>0.80 ± 0.04*</td>
<td>0.53 ± 0.01*</td>
<td>3.08 ± 0.48*</td>
</tr>
<tr>
<td>T</td>
<td>0.35 ± 0.06*</td>
<td>2.18 ± 0.08*</td>
<td>0.89 ± 0.19*</td>
<td>1.13 ± 0.21*</td>
<td>0.51 ± 0.08*</td>
<td>3.17 ± 0.57*</td>
</tr>
<tr>
<td>D + T</td>
<td>0.96 ± 0.27*</td>
<td>3.30 ± 0.20*</td>
<td>1.45 ± 0.20*</td>
<td>1.42 ± 0.31*</td>
<td>0.43 ± 0.08*</td>
<td>2.06 ± 0.70*</td>
</tr>
<tr>
<td>Sunflower oil</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>0.38 ± 0.04*</td>
<td>1.87 ± 0.10*</td>
<td>0.32 ± 0.05*</td>
<td>1.38 ± 0.07*</td>
<td>0.74 ± 0.01*</td>
<td>4.14 ± 0.89*</td>
</tr>
<tr>
<td>T</td>
<td>0.55 ± 0.08*</td>
<td>1.90 ± 0.03*</td>
<td>0.42 ± 0.04*</td>
<td>1.23 ± 0.06*</td>
<td>0.65 ± 0.03*</td>
<td>2.42 ± 0.39*</td>
</tr>
<tr>
<td>D + T</td>
<td>1.61 ± 0.22*</td>
<td>2.55 ± 0.35*</td>
<td>1.08 ± 0.22*</td>
<td>0.74 ± 0.19*</td>
<td>0.30 ± 0.06*</td>
<td>0.47 ± 0.11*</td>
</tr>
</tbody>
</table>

Data with the same letter as superscript showed statistical significance for at least p < 0.05.
Serum low-density lipoprotein-cholesterol (LDL-C) levels were highest in the animals fed the olive-oil diet, in controls as well as in the thioacetamide and dexamethasone-thioacetamide groups.

High-density lipoprotein-cholesterol levels (HDL-C) showed a significant increase in rats fed the sunflower-oil diet. After thioacetamide injection, the HDL-C values were similar in the three diet groups. On the other hand, serum HDL-C values decreased in the dexamethasone-treated animals on the sunflower-oil diet.

HDL/TG and HDL/TC indices were higher in the control state when the animals were fed the sunflower-oil diet. However, after thioacetamide injection, HDL/TG was highest in the olive-oil group, whereas the HDL/TC index was higher in the sunflower-oil group in the controls and after thioacetamide injection. The highest HDL/TC levels after the administration of dexamethasone and thioacetamide were observed in the standard diet group.

Discussion

Hepatic steatosis is a process characterized by lipid accumulation within hepatocytes, and can be produced by high-fat diets (8). In addition, steatosis can result from long-term alcohol intake, and may progress to chronic liver disease (15). Damage to the rat liver induced by thioacetamide also produces qualitative and quantitative changes in hepatic lipid droplets (16).

Both of the high-fat diets (olive or sunflower oil) produced an increase in hepatic accumulation of fat droplets, as previously observed (8). The acute injury produced by thioacetamide led to an increase in hepatic steatosis, especially in the animals fed the sunflower-oil diet; this may be related to a differential influence of polyunsaturated fatty acids on lipid metabolism in hepatic cirrhosis (17). Dexamethasone treatment did not alter hepatic steatosis in these animals.

Collagen quantification is a good index of liver damage (14). In comparison with sunflower oil and other polyunsaturated oils, olive oil has previously been found to play a protective role in the development of hepatic fibrosis in rats (4). A hypercholesterolemic diet can lead to collagen deposition and may produce a moderate to pronounced centrolobular liver fibrosis (18). On the other hand, cholesterol supplementation enhanced the degree of fibrosis but prevented necrosis and inflammation (19). Our results showed that high-fat diets can increase collagen deposition in the rat liver. In addition, acute injury with thioacetamide increased the accumulation of collagen in the liver both with standard and high-fat diets, although we did not detect differences between the diet types. Dexamethasone pretreatment did not decrease the amount of collagen detected in the liver, in agreement with results from other authors (20). Taking into account that high-fat diets produce a toxic effect by themselves, because they induce, as showed by our results, some degree of fibrosis and steatosis, the administration of high-fat diets in studies focused on the influence of lipid saturation grade on different biochemical (2, 21), immunological (2, 22), and morphological (8) parameters ought to be interpreted carefully. In addition, the fibrogenic effect of high-fat diets may be
related to Ito cell activation (\textsuperscript{10}).

The liver plays a central role in lipid metabolism, and it is not surprising that high-fat diets may affect serum lipid levels in different ways (\textsuperscript{8}). Hepatic injury leads to changes in serum lipids, lipoprotein, cholesterol, triglycerides, and some related enzymatic activities (\textsuperscript{23}). In addition, hepatic injury can be influenced by changes in polyunsaturated fatty acids (\textsuperscript{24}). In this study, we have observed an increase in serum HDL-C in thioacetamide-injected animals on standard or olive-oil diets. However, levels of LDL-C were only increased in the olive-oil group. Similar results were obtained when the animals were pretreated with dexamethasone. These data, independently of serum TC values, suggest a greater atherogenicity when animals are fed high-fat diets, especially when they are treated with dexamethasone and thioacetamide. We speculated that this important result may be a consequence of the hormonal imbalance produced under our experimental conditions, which is supported by the fact that high-fat diets develop insulin resistance (\textsuperscript{25}). In this sense, and according to previous works, dexamethasone-induced hyperlipidemia has been related to the increased synthesis and secretion of serum lipids and lipoproteins (\textsuperscript{26}) and to a decrease in the binding of LDL to hepatocytes (\textsuperscript{13}).

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