Soy Protein Ameliorates Metabolic Abnormalities in Liver and Adipose Tissue of Rats Fed a High Fat Diet¹⁻³

Ivan Torre-Villalvazo,⁴,⁵ Armando R. Tovar,⁴ Victoria E. Ramos-Barragán,⁴ Marco Antonio Curbón-Cervantes,⁶ and Nimbe Torres⁴,*

¹Departamento de Fisiología de la Nutrición, Instituto Nacional de Ciencias Médicas y Nutrición Salvador Zubirán, México, DF, 14000 and ²Doctorado en Ciencias Biomédicas, Facultad de Medicina and ⁴Departamento de Biología, Facultad de Química, Universidad Nacional Autónoma de México, México, DF, 04510

Abstract

Chronic consumption of high-fat or -carbohydrate diets is associated with the development of obesity; however, it is not well established whether dietary protein plays a role in the development of abnormalities of lipid metabolism that occur during obesity. To determine the effect of different types of protein during diet-induced obesity on hepatic and adipocyte lipid metabolism, rats were fed casein (CAS) or soy (SOY) protein diets with 5% fat or high-fat diets with 25% fat (HF-CAS and HF-SOY) for 180 d. Rats fed soy diets had lower hepatic sterol regulatory element binding protein-1 (SREBP-1) expression and higher SREBP-2 expression than those fed casein diets, leading to less hepatic lipid deposition. On the other hand, long-term HF-SOY consumption prevented hyperleptinemia in comparison with rats fed HF-CAS. Rats fed soy protein diet showed higher adipocyte perilipin mRNA expression and smaller adipocyte area than those fed casein diets, which was associated with a lower body fat content. Furthermore, the lipid droplet area in brown adipose tissue was significantly lower in rats fed soy diets than in those fed casein diets and it was associated with higher uncoupling protein-1 (UCP-1) expression. As a result, rats fed the soy diets gained less weight than those fed the casein diets, in part due to an increase in the thermogenic capacity mediated by UCP-1. These results suggest that the type of protein consumed and the presence of fat in the diet modulate lipid metabolism in adipose tissue and liver. J. Nutr. 138: 462–468, 2008.

Introduction

Imbalance between energy intake and energy expenditure lead to metabolic disorders associated to obesity, which has increased rapidly around the world. The primary alteration in obesity is the dysfunction of the adipose tissue that results in an excessive release of FFA to other tissues. Accumulation of lipids that are not oxidized in nonadipose tissues induces cellular damage, a process named lipotoxicity (1).

To prevent the accumulation of lipids in nonadipose tissues, leptin, a hormone secreted by the adipose tissue, regulates food intake at the level of hypothalamus and stimulates fatty acid (FA) oxidation in peripheral tissues to avoid lipid deposition (2). However, chronic consumption of high-energy diets increases adipose tissue mass and leptin secretion. The establishment of obesity results in hyperleptinemia that induces leptin resistance (2–5), resulting in lipotoxicity. During the development of leptin resistance, there is an increased release of FFA from adipose tissue to other tissues, mainly the liver. As a result, hepatic esterification of FFA to triacylglycerols (TG) leads to the formation of fatty liver that is accelerated by an increased lipogenesis as a consequence of hyperinsulinemia and decreased FA oxidation.

Prevention of hyperinsulinemia may ameliorate metabolic abnormalities that occur in the liver as a consequence of obesity. There is evidence that the type of protein in the diet could play an important role in the secretion of insulin by the pancreas (6) and in the regulation of hepatic lipogenesis mediated by sterol regulatory element binding protein-1 (SREBP-1) (7). Nonetheless, it has not been established whether the type of protein has an effect on liver and adipose tissue during chronic intake of high fat diet. Thus, the aim of this work was to assess the biochemical and molecular adaptations that take place in the liver and adipose tissue in rats fed soy protein in a high-fat diet over a long term.

Materials and Methods

Animals and experimental protocol. Male Sprague-Dawley rats (4 wk old) were obtained from Harlan Mexico and maintained in the...
animal care facility at the National Institute of Medical Sciences and Nutrition S.Z. All rats were housed on a 12-h-light/dark cycle and were divided in 4 groups (8 rats per group). Two groups were fed a control diet containing 5% fat (11% of total energy) with casein (CAS) or soy protein (SOY) as the protein source and 2 groups were fed a high-fat diet containing 25% fat (45% of total energy) with casein (HF-CAS) or soy protein (HF-SOY) as the protein source. The protein concentration of the diets was 30% and it was adjusted on basis the protein content of the isolated protein (casein 90.6%, soy protein 86%). Rats were fed for 180 d and had free access to the diets and water. The composition of the experimental diets is presented in Table 1.

Body weight and food intake were measured daily. Eight rats per group were killed at the end of the experiment (d 180) after food deprivation for 12 h. We determined body composition and collected blood and tissue samples from liver, epididymal fat pads, brain, and interscapular brown adipose tissue (BAT). Tissues were rapidly excised, frozen in liquid nitrogen, and stored at −70°C. Institutional guidelines for animal care and use were followed. The animal protocol was approved by the Institutional Animal Care and Research Advisory Committee of the National Institute of Medical Sciences and Nutrition S.Z. in Mexico City.

**Serum measurements.** Serum glucose, cholesterol, and TG concentrations in food-deprived rats were measured by colorimetric kits (DiaSys Diagnostic Systems). Serum FFA concentration was measured with a FFA Half Micro test (Roche Applied Science). Serum insulin, leptin, and glucagon were analyzed by RIA kits (Linco Research Immunoassay).

**Lipoprotein separation.** Lipoproteins were isolated by sequential potassium bromide density gradient ultracentrifugation as previously described (8).

**Lipid content of liver.** Total lipids were extracted from liver according to Folch (9) and cholesterol and TG content were measured as above. To evaluate lipid droplets in hepatocytes, frozen liver sections were stained with oil red O and counterstained with hematoxylin.

**Histological analysis of liver and adipose tissue.** A total of 500 mg of liver and adipose tissue from epididymal fat pads and interscapular BAT were fixed in 10% phosphate buffered formalin. Liver sections (5 μm) were stained with periodic acid Schiff and Masson-trichrome. Epididymal adipose tissue and BAT sections were stained with hematoxylin and eosin.

**Liver glycogen concentration.** Glycogen was extracted from frozen samples (100 mg) according to Osterberger (10) modified by Good et al. (11) and glucose was assayed with a colorimetric kit (DiaSys Diagnostic Systems).

**Body composition.** Rats were eviscerated and the skin was removed. The carcass was ground with 3 volumes of distilled water, dried in a convection oven, and further ground to obtain a fine powder. Bromatologic analysis was carried out to determine total body nitrogen and fat content (12).

**Real-time quantitative PCR.** Total RNA from liver, BAT, hypothalamus, and epididymal fat pad was extracted according to Chomczynski and Sacchi (13). Samples of 300 ng of total RNA from 8 rats were run in triplicate and subjected to RT and PCR amplification using One-Step Master mix (Applied Biosystems). TaqMan fluorogenic probes and oligonucleotide primers were obtained from Applied Biosystems. The relative amounts of all mRNAs were calculated by using the Comparative CT method (User Bulletin no. 2, PE Applied Biosystems). 18S ribosomal RNA was used as the invariant control for liver analysis and β-actin for BAT, adipose tissue, and hypothalamus.

**Western blot.** Frozen liver samples from each rat were homogenized in ice-cold RadioImmunoprecipitation assay buffer containing a protease inhibitor cocktail (Roche Applied Science), separated in a 7.5% SDS-PAGE, transferred into polyvinylidene difluoride membranes (Hybond-P, GE Healthcare). Membranes were blocked with 0.4% nonfat dry milk and incubated overnight with anti-SREBP-1 (sc-367), or anti-SREBP-2 (sc-81510, Santa Cruz Biotechnology) antibodies. β-Actin was used as control.

**Immunohistochemistry.** Brain tissue sections (5 μm thick) were processed as described (14). The sections were incubated overnight with the primary antibody anti-leptin receptor Ob-R (sc-8991, Santa Cruz) at a 1:250 dilution (0.8 mg/L) and were incubated for 2 h at room temperature with biotin-conjugated goat anti-rabbit IgG (SC-2018, Santa Cruz) at a 1:100 dilution and developed according to the manufacturer instructions. Experiments were performed in duplicate.

**Statistical analysis.** Values are expressed as means ± SEM. Data that were not normally distributed were log transformed prior to the ANOVA. Data were tested using 2-way ANOVA with type of protein and fat content as independent variables. When the interaction was significant, Fisher’s protected least significant difference test was performed. Differences were considered significant at P < 0.05. Simple linear regression analysis (R value) was used to examine the relation between SREBP-1 mRNA abundance and the insulin:glucagon ratio, serum FFA, and adipocyte area, and serum glucagon and SREBP-2 mRNA abundance. The histological analysis was conducted in 4 rats from each group. Slides from adipose tissue were divided in 4 fields and the area of the lipid droplets from 10 cells per field of the 4 rats from each group was used for statistical analysis (Statview statistical analysis program V.4.5, Abacus Concepts).

**Results**

**Weight gain and body fat.** Weight gain in the 4 groups was similar during the first 50 d of the study (data not shown), indicating that both proteins met protein requirement of rats in the growing phase. At the end of the experiment, rats fed the HF diets gained more body weight than rats fed control diets; however, rats fed soy protein diets gained less weight than those fed casein diets (P < 0.05) (Table 2). Body weight gain differed despite the similar cumulative energy intake throughout the

---

**TABLE 1** Composition of experimental diets

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>SOY</th>
<th>CAS</th>
<th>HF-SOY</th>
<th>HF-CAS</th>
</tr>
</thead>
<tbody>
<tr>
<td>30% Soy protein (88% purity)</td>
<td>348.8</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>30% Casein (90.6% purity)</td>
<td></td>
<td>331.1</td>
<td>331.1</td>
<td></td>
</tr>
<tr>
<td>Cornstarch</td>
<td>269.7</td>
<td>278.6</td>
<td>169.7</td>
<td>178.6</td>
</tr>
<tr>
<td>Dextrin</td>
<td>269.7</td>
<td>278.6</td>
<td>169.7</td>
<td>178.6</td>
</tr>
<tr>
<td>Corn oil</td>
<td>50.0</td>
<td>50.0</td>
<td>50.0</td>
<td>50.0</td>
</tr>
<tr>
<td>Lard</td>
<td></td>
<td>200.0</td>
<td>200.0</td>
<td></td>
</tr>
<tr>
<td>Mineral mixture</td>
<td>50.0</td>
<td>50.0</td>
<td>50.0</td>
<td></td>
</tr>
<tr>
<td>Vitamin mixture</td>
<td>10.0</td>
<td>10.0</td>
<td>10.0</td>
<td></td>
</tr>
<tr>
<td>Choline citrate</td>
<td>1.7</td>
<td>1.7</td>
<td>1.7</td>
<td>1.7</td>
</tr>
</tbody>
</table>

1 Supro 710, Solae, México. Isoflavone analysis (mg/g protein): genistein 1.38, daidzein 0.71, glycitin 0.19. Soy protein amino acid concentration (g/100 g protein): Ala 4.1, Arg 7.5, Asp 11.9, Cys 1.3, Glu 21.5, Gly 4.2, His 2.6, Ile 4.9, Leu 8.1, Lys 6.3, Met 1.3, Phe 5.4, Pro 5.5, Ser 5.2, Thr 3.7, Trp 1.5, Tyr 4.0, and Val 4.5.
2 “Vitamin-free” casein, Harlan Teklad research diets. Casein amino acid concentration (g/100 g protein): Ala 2.8, Arg 3.4, Asp 6.3, Cys 0.3, Glu 20.5, Gly 1.6, His 2.5, Ile 4.7, Leu 8.2, Lys 7.2, Met 1.9, Phe 4.4, Pro 9.5, Ser 5.0, Thr 3.8, Trp 1.6, Tyr 4.7, and Val 6.0.
3 FA content (%): SFA 16.01, monounsaturated 28.4, Polyunsaturated 55.51.
4 FA content (%): SFA 31.5, monounsaturated 53.4, Polyunsaturated 15.1.
5 Rogers-Harper, Harlan Teklad research diets (33).
6 AIN-93-VX, Harlan Teklad research diets.
Interestingly, SREBP-1 mRNA levels correlated with the precursor and mature SREBP-1 protein located at the liver. Changes in SREBP-1 mRNA were associated with the amount of fat in the diet (Fig. 1A) and LDL-cholesterol (Fig. 1B) concentration than in those fed the HF-CAS diet. The pattern of SREBP-2 expression was similar to the pattern of serum glucagon concentration (Table 2).

**Hepatic glycogen and the insulin:glucagon ratio.** Rats fed the HF diets had significantly higher serum insulin concentrations than those fed the control diets (Table 2). Furthermore, hepatic glycogen content was higher in rats fed CAS and HF-CAS diets than in those fed SOY and HF-SOY diets, and rats fed HF diets showed higher glycogen content than rats fed control diets. There was no significant protein × fat interaction in hepatic glycogen concentration (Table 3). Hepatic glycogen content also differed in the histological analysis (Supplemental Fig. 1). Serum glucagon concentrations were greater in the SOY group than in the HF-SOY and CAS groups, which did not differ, and were greater in these groups than in the HF-CAS group (Table 2). The insulino-glucagon ratio was higher in animals fed CAS and HF-CAS diets than those fed the SOY and HF-SOY diets, respectively. In addition, rats fed HF diets had a higher ratio than those fed control diets (Table 2).

**Hepatic SREBP-1 and SREBP-2 mRNA concentration.** SREBP-1 is a transcription factor that is positively regulated by insulin and negatively regulated by glucagon (15). SREBP-1 mRNA content was 1.5-fold greater in the liver of rats fed HF-CAS than in those fed the CAS or HF-SOY diet (Fig. 1C). Changes in SREBP-1 mRNA were associated with the amount of the precursor and mature SREBP-1 protein located at the endoplasmic reticulum and nucleus, respectively (Fig. 1D). Interestingly, SREBP-1 mRNA levels correlated with the insulin:glucagon ratio (r = 0.85; P = 0.004). These results clearly established that the consumption of a specific type of dietary protein modulates the insulin:glucagon ratio, which in turn controls hepatic lipogenesis mediated by SREBP-1. On the other hand, SREBP-2 mRNA (Fig. 1E) and protein expression (Fig. 1F) increased more in rats fed SOY diets than rats fed CAS diets. The pattern of SREBP-2 expression was similar to the pattern of serum glucagon concentration (Table 2).

**Serum TG and cholesterol.** Consumption of soy protein significantly reduced the serum TG concentration (Table 3). Serum cholesterol was greater in rats fed CAS than in those fed SOY and was greater in those fed the HF diets compared with their controls (Table 3). Reduction in serum lipids in rats fed SOY and HF-SOY diets was associated with a lower VLDL-TG concentration (Fig. 1A) and LDL-cholesterol concentration (Fig. 1B) than in those fed CAS and HF-CAS diets.

**Serum leptin concentration.** Rats fed HF-CAS developed hyperleptinemia, whereas those fed HF-SOY maintained serum leptin concentration within the physiological levels (≤ 14 pg/L) (16). Leptin concentration did not differ significantly between the control groups and HF-SOY (Table 2). Serum leptin concentration depended on the type of protein consumed and the amount of fat in the diet (P < 0.05), indicating that soy protein intake prevented hyperleptinemia induced by the consumption of a high-fat diet.

**Histological analysis of the liver.** Oil red O stain showed numerous red-stained macrovesicular lipid droplets in livers of rats fed HF-CAS (Supplemental Fig. 1) associated with high

<table>
<thead>
<tr>
<th>Variable</th>
<th>CAS</th>
<th>SOY</th>
<th>HF-CAS</th>
<th>HF-SOY</th>
<th>Protein</th>
<th>Fat</th>
<th>Protein × fat</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight gain, g/180 d</td>
<td>380 ± 7b</td>
<td>330 ± 9b</td>
<td>481 ± 7b</td>
<td>390 ± 10b</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>0.01</td>
</tr>
<tr>
<td>Cumulative energy intake, MJ/180 d</td>
<td>22.9 ± 0.4</td>
<td>22.6 ± 0.4</td>
<td>23.0 ± 0.3</td>
<td>23.1 ± 0.6</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Body fat, mg/g carcass</td>
<td>79.5 ± 2.2a</td>
<td>85.1 ± 12.9b</td>
<td>129 ± 6.4b</td>
<td>94.7 ± 7.4b</td>
<td>0.0076</td>
<td>0.0002</td>
<td>0.02</td>
</tr>
<tr>
<td>Body protein, mg/g carcass</td>
<td>139 ± 11</td>
<td>131 ± 13</td>
<td>138 ± 10</td>
<td>132 ± 1</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Serum insulin, pmol/l</td>
<td>184 ± 25</td>
<td>205 ± 21</td>
<td>370 ± 19</td>
<td>345 ± 22</td>
<td>NS</td>
<td>&lt;0.0001</td>
<td>NS</td>
</tr>
<tr>
<td>Serum glucagon, ng/l</td>
<td>84.5 ± 9b</td>
<td>183 ± 8b</td>
<td>55.5 ± 1b</td>
<td>94.4 ± 28b</td>
<td>0.0001</td>
<td>0.0025</td>
<td>0.05</td>
</tr>
<tr>
<td>Insulin/glucagon</td>
<td>10.9 ± 1.2</td>
<td>6.50 ± 0.8</td>
<td>32.0 ± 4.4</td>
<td>20.1 ± 4.8</td>
<td>0.039</td>
<td>&lt;0.0001</td>
<td>NS</td>
</tr>
<tr>
<td>Serum leptin, pg/l</td>
<td>4.1 ± 4.6a</td>
<td>3.0 ± 0.5b</td>
<td>16.5 ± 1.8a</td>
<td>6.4 ± 1.9b</td>
<td>0.0012</td>
<td>&lt;0.0001</td>
<td>0.008</td>
</tr>
</tbody>
</table>

1 Values are means ± SEM, n = 8. Means in a row with superscripts without a common letter differ, P < 0.05.

---

**TABLE 3** Serum glucose, serum and hepatic lipids, and hepatic glycogen in rats fed soy protein or casein with or without high fat in the diet for 180 d

<table>
<thead>
<tr>
<th>Variable</th>
<th>CAS</th>
<th>SOY</th>
<th>HF-CAS</th>
<th>HF-SOY</th>
<th>Protein</th>
<th>Fat</th>
<th>Protein × fat</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum glucose, mmol/L</td>
<td>5.8 ± 0.1</td>
<td>5.5 ± 0.1</td>
<td>7.8 ± 0.4</td>
<td>5.7 ± 0.2</td>
<td>NS</td>
<td>0.049</td>
<td>NS</td>
</tr>
<tr>
<td>Serum cholesterol, mmol/L</td>
<td>3.8 ± 0.1</td>
<td>2.1 ± 0.1</td>
<td>4.2 ± 0.1</td>
<td>2.5 ± 0.2</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>NS</td>
</tr>
<tr>
<td>Serum TG, mmol/L</td>
<td>1.2 ± 0.9a</td>
<td>0.53 ± 0.0b</td>
<td>1.9 ± 0.1a</td>
<td>0.58 ± 0.1c</td>
<td>&lt;0.0001</td>
<td>0.0001</td>
<td>0.0005</td>
</tr>
<tr>
<td>Serum FFA, mmol/L</td>
<td>1.1 ± 0.1</td>
<td>1.0 ± 0.0</td>
<td>1.8 ± 0.1</td>
<td>1.3 ± 0.0</td>
<td>0.0036</td>
<td>&lt;0.0001</td>
<td>NS</td>
</tr>
<tr>
<td>Liver cholesterol, mmol/g</td>
<td>0.01 ± 0.0d</td>
<td>0.01 ± 0.0d</td>
<td>0.04 ± 0.0d</td>
<td>0.02 ± 0.0d</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>0.0001</td>
</tr>
<tr>
<td>Liver TG, mmol/g</td>
<td>0.02 ± 0.0d</td>
<td>0.03 ± 0.0d</td>
<td>0.06 ± 0.0d</td>
<td>0.02 ± 0.0d</td>
<td>0.0002</td>
<td>&lt;0.0001</td>
<td>0.019</td>
</tr>
<tr>
<td>Liver glycogen, mg/g</td>
<td>6.8 ± 0.9</td>
<td>3.4 ± 0.5</td>
<td>19.8 ± 0.9</td>
<td>10.7 ± 0.7</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>NS</td>
</tr>
</tbody>
</table>

1 Values are means ± SEM, n = 8. Means in a row with superscripts without a common letter differ, P < 0.05.

---

464 Torre-Villalvazo et al.
hepatic cholesterol and TG concentrations (Table 3) in livers of the control group, whereas it did not occur in livers of rats fed HF-SOY. There was no evidence of inflammation in livers of HF-SOY rats. Masson trichrome stain revealed collagen deposition (blue) in livers of the rats HF-CAS, whereas there was no evidence of fibrosis in rats fed the HF-SOY diet or in the control groups (Supplemental Fig. 1). Hepatic fat accumulation, collagen deposition, and glycogen content in HF-CAS rats was associated with high circulating leptin levels (Table 2).

**Hypothalamic leptin receptor and BAT thermogenesis.** Soy protein increased gene expression of the leptin receptor in the hypothalamus of both the control and HF groups compared with rats fed CAS and HF-CAS (Fig. 2A; P = 0.009). These findings were confirmed by immunohistochemical analysis that showed increased leptin receptor content in the arcuate and paraventricular nuclei of the hypothalamus (Fig. 2B). These results imply that consumption of soy protein during obesity maintains leptin signaling capacity, allowing an accurate control

**FIGURE 1** Serum lipoprotein cholesterol (A) and TG (B), SREBP-1 mRNA abundance (C), protein content (D), SREBP-2 mRNA abundance (E), and protein content (F) in rats fed SOY, CAS, HF-SOY, and HF-CAS diets. Values are means ± SEM, n = 8. Means without a common letter differ, P < 0.05.

**FIGURE 2** Leptin receptor mRNA abundance in hypothalamus (A), immunohistochemistry of brain sections incubated with anti-leptin receptor (B), hematoxylin and eosin staining of interscapular BAT (C), brown adipocyte lipid droplets area (D), BAT mRNA relative abundance of PPARγ (E), and UCP-1 (F) in rats fed SOY, CAS, HF-SOY, and HF-CAS diets. Values are means ± SEM, n = 8. Means without a common letter differ, P < 0.05.
of energy metabolism. To elucidate whether the increment in hypothalamic leptin receptors in rats fed HF-SOY correlates with higher BAT activity as has been previously proposed (17), we conducted histological analysis and mRNA expression of PPARγ and uncoupling protein 1 (UCP-1) in BAT. Histological analysis of brown adipocytes (Fig. 2C) of rats fed the HF-CAS diet resulted in enlarged lipid droplets (Fig. 2D), suggesting an impaired lipid utilization. In contrast, BAT from rats fed HF-SOY resulted in smaller \( P = 0.001 \) adipocytes than those fed HF-CAS but higher than adipocytes from control groups. The enlarged adipocytes from HF-CAS rats are indicative of impaired lipid oxidation and thermogenesis. Gene expression analysis in BAT showed that PPARγ mRNA was higher in BAT of rats fed SOY and HF-SOY than rats fed CAS and HF-CAS (Fig. 2E). PPARγ directs brown adipocyte proliferation and metabolism by controlling the expression of BAT-specific genes such as the mitochondrial (UCP-1) (18). As expected, UCP1 mRNA content was also higher in rats fed SOY (Fig. 2F). These results indicate that rats fed soy protein with or without high fat showed enhanced thermogenic capacity reflected in body fat reduction compared with those fed casein despite equal energy intake (Table 2).

**Adipocyte size, serum FFA, and adipose PPARγ and perilipin mRNA concentration.** Adipose tissue of rats fed HF diets showed higher adipocyte area than those fed control diets (Fig. 3A). Nonetheless, rats fed SOY and HF-SOY diets showed significantly smaller adipocyte area than those fed CAS and HF-CAS diets (Fig. 3B). The number of adipocytes in the HF-SOY group was 44% higher than in those fed HF-CAS (data not shown). Dysfunctional adipocytes increase the release of FFA to circulation, enhancing their uptake by the liver (19), where FA uptake depends mainly on serum FFA. Serum FFA concentration was higher in rats fed CAS and HF-CAS diets than those fed SOY and HF-SOY diets and rats fed HF diets had increased serum FFA concentration compared with rats fed control diets (Table 3). Serum FFA was correlated with adipocyte area \( r = 0.97; P < 0.05 \); the lower the adipocyte area, the lower the serum FFA. On the other hand, adipose tissue of rats fed HF diets had a significantly lower PPARγ mRNA concentration than those fed control diets (Fig. 3C). Rats fed control diets showed higher perilipin mRNA expression than those fed HF diets (Fig. 3D), although those fed SOY and HF-SOY diets had higher perilipin expression than rats fed CAS and HF-CAS diets. These results suggest that decreased perilipin expression in adipocytes of rats fed casein diets increases the exposition of the neutral lipid core of the lipid droplets to the hormone-sensitive lipase, facilitating the hydrolysis of TG and releasing more FFA, mainly to the liver.

**Discussion**

It is clearly established that long-term consumption of a high-fat diet accelerates the development of obesity (20). However, the type of some nutrients, such as dietary protein, can play an important role in the progression of obesity, as demonstrated in this study. Rats fed a HF-SOY diet for 180 d gained significantly less weight than those fed a HF-CAS diet and the difference was mainly due to a lower body fat content rather than changes in body protein content or cumulative energy intake. The difference in weight gain between these groups was not associated with the quality of the protein consumed, because rats fed with casein or soy protein grew at the same rate during the first 50 d of the study, when the protein requirement is higher than during the maintenance period (21). The possible reason why different types of proteins in a high-fat diet may contribute more or less to the development of obesity could be in part due to their specific amino acid patterns on the release of hormones such as insulin (6) and possibly glucagon (22).

During the development of obesity, which may take months or years, there are gradual changes in serum insulin and leptin concentration that, in a given time, will result in hyperinsulinemia or hyperleptinemia. Furthermore, in vivo and in vitro studies with pancreatic islets have shown that soy protein or its isoflavones stimulate insulin secretion in a lower extent than casein (6). At the end of this study, insulin concentration increased in rats fed high-fat diets, whereas serum leptin concentration increased depending on fat content and the type of protein consumed. These results indicate that the consumption of a specific type of dietary protein can modulate the insulin: glucagon ratio, which in turn can control hepatic lipogenesis mediated by SREBP-1. As a consequence, there was decreased accumulation of TG in the liver and reduced assembly and deposition in epididymal white adipose tissue.
release of VLDL-TG particles in rats consuming SOY and HF-SOY diets. It has been shown that insulin upregulates SREBP-1 activity and expression, whereas glucagon has the opposite effect (15,23). Our results indicated that the insulin:glucagon ratio could be modified by consumption of soy protein despite the high-fat content in the diet preventing lipid deposition in the liver and the development of hepatic steatosis. In addition, leptin resistance may contribute to the development of fatty liver and its progression to cirrhosis and fibrosis (24). We observed that rats fed HF-CAS had fatty liver and collagen deposition, associated with increased body weight, and hyperleptinemia that induces leptin resistance. Rats fed soy protein even in the presence of a high-fat diet had serum leptin concentrations in the normal range, resulting in low hepatic lipid accumulation due to the leptin action to stimulate FA oxidation (25).

In addition, the serum glucagon concentration correlated with SREBP-2 mRNA abundance ($r = 0.93; P < 0.05$), a transcription factor that regulates the expression of many genes involved in cholesterol synthesis and uptake in liver (26). When cellular sterol levels fall, SREBP-2 is transcriptionally active and activates cholesterogenic target genes to increase synthesis and uptake of cholesterol in the liver (27). Our results showed that soy protein consumption decreased liver cholesterol and increased SREBP-2. The possible mechanism by which glucagon increases SREBP-2 is not known; however, it could be mediated via cAMP-activating CREB, because SREBP-2 gene promoter shows 3 potential cAMP response elements (28).

The establishment of obesity is accompanied by concomitant alterations not only in the liver but also in adipose tissue, where adipocytes become dysfunctional (2). Functional adipocytes release several adipocytokines to protect nonadipose tissues from lipid overload (1). Long-term consumption of high-fat or high-carbohydrate diets induces adipocyte hypertrophy and eventually dysfunction, characterized by an excessive release of FFA to circulation (29). Our results showed that soy protein also may play a role in the adipocyte-decreasing serum FFA associated with small adipocytes.

The release of FA from adipocytes depends on the rate of TG lipolysis mediated by the hormone-sensitive lipase (30). The activity of this enzyme on the lipids stored in adipocytes is restricted by the abundance of perilipins surrounding the lipid droplet membrane (31). Interestingly, groups fed SOY and HF-SOY showed higher perilipin expression than those fed CAS and HF-CAS, limiting the release of FA.

At the end of the study, only rats fed the HF-CAS diet showed hyperleptinemia and it was associated with lower amounts and mRNA abundance of leptin receptor in the arcuate nucleus, indicating less sensitivity to the leptin action in the hypothalamus as a result of leptin resistance.

Because the hypothalamus controls BAT thermogenic activity (32), the decrease in leptin action in rats fed CAS and HF-CAS diets was associated with reduced PPARγ and UCP-1 mRNA in BAT. A major characteristic of brown adipocytes is the presence of multilocular lipid droplets enriched with mitochondria. However, rats fed HF-CAS resulted in numerous lipid-filled unilocular adipocytes. These results suggest that BAT from rats fed HF-CAS oxidizes FA to a lesser extent than BAT from rats fed HF-SOY and as a consequence, FA storage and lipid droplet area increased, resembling white adipose tissue morphology. These results may explain in part the higher body weight gain in rats fed HF-CAS.

In conclusion, this work demonstrated that long-term consumption of soy protein is associated with beneficial effects on liver and adipose tissue despite the consumption of a high-fat diet.

Acknowledgments
We greatly appreciate the technical assistance of Maricela Rodriguez for the corporal fat determination and of Jaime Ayala Garcia and Karina Gabriela Cedillo Rivera for histological procedures.

Literature Cited


