Soy Protein Affects Serum Insulin and Hepatic SREBP-1 mRNA and Reduces Fatty Liver in Rats¹

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ABSTRACT The consumption of soy protein was shown to reduce blood lipids in humans and other animal species. Furthermore, it was shown that the ingestion of soy protein maintains normal insulinemia. Thus, the purpose of the present study was to determine whether soy protein affects the synthesis of lipids in the liver through sterol-regulatory element binding protein-1 (SREBP-1) due to modulation of insulin levels. We first conducted a short-term study in which rats were fed a diet containing 18 g/100 g soy protein or casein for 10 d. Rats fed soy protein had significantly lower serum insulin concentrations than rats fed casein, and this response was accompanied by an elevation in hepatic SREBP-1 mRNA that was 53% lower than that in rats fed casein at d 10. The increase in SREBP-1 mRNA occurred 30 min after consumption of the casein mean, and increased steadily for the next 2 h. We then conducted a second study to assess the long-term effect of soy protein consumption for 150 d on hepatic SREBP-1 expression. Long-term consumption of soy protein normalized insulin concentrations compared with rats fed casein, which were hyperinsulinemic. Thus, rats fed the soy protein diet had significantly lower expression of SREBP-1 mRNA than rats fed the casein diet. Soy protein intake also reduced the expression of fatty acid synthase (FAS) and malic enzyme, leading to low hepatic lipid deposits of triglycerides and cholesterol, whereas rats fed the casein diet developed fatty liver. These data suggest that soy protein regulates SREBP-1 expression by modulating serum insulin concentration, thus preventing the development of fatty liver. J. Nutr. 134: 522–529, 2004.

KEY WORDS: • soy protein • sterol-regulatory element binding protein-1 • fatty liver • insulin

Several studies in humans (1) and animals (2) have established that the ingestion of a soy protein diet reduces serum cholesterol and triglycerides, particularly in hypercholesterolemic subjects (3) although controversy exists concerning its efficacy (4). Soy protein alone or as a major source of protein in a diet containing adequate levels of energy and other essential nutrients promotes adequate growth in infants (5). The nutritional value of soy protein in young children is essentially equivalent to that of milk protein (6). The nutritional quality of soy protein in nitrogen balance studies in adults showed ~83% of the value of egg protein and an equal value for milk protein (5). However, the molecular mechanism by which soy protein decreases serum lipids has not been fully established. Several possible mechanisms have been proposed (7), including enhancement of bile acid excretion, changes in the insulin:glucagon ratio, thyroid hormone concentrations, and an amino acid composition that causes changes in cholesterol metabolism.

Hepatic lipid metabolism is controlled in part by the transcription factors termed sterol regulatory element binding proteins (SREBPs)³ (8,9). These transcription factors belong to the basic helix-loop-helix-leucine zipper family (10,11). There are 3 isoforms of SREBPs that are membrane-bound to the endoplasmic reticulum and nuclear envelope, i.e., SREBP-1a, SREBP-1c, and SREBP-2. The NH₂-terminal domain of the mature forms of these transcription factors is released after 2 proteolytic cleavages in the Golgi apparatus by 2 specific proteases, SP1 and SP2. The NH₂-terminal of SREBPs travels into the nucleus and binds to the cis-acting element termed the sterol regulatory element in the promoter or enhancer regions of genes involved in cholesterol or fatty acid synthesis. In the liver, SREBP-1c and SREBP-2 are the predominant forms (12). SREBP-2 preferentially stimulates transcription of the genes of cholesterol synthesis, including hydroxymethylglutaryl-CoA (HMG-CoA) reductase and HMG-CoA synthase (13), whereas SREBP-1c stimulates transcription of the genes of fatty acid biosynthesis (14), such as acetyl CoA carboxylase and fatty acid synthase (FAS). SREBP-2 is activated in response to a low cholesterol content of the cell, and SREBP-1c by insulin (15). In both instances, activation is

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² To whom correspondence should be addressed.
³ Abbreviations used: CYP7A1, 7-a hydroxylase; DTT, dithiothreitol; FAS, fatty acid synthase; HF, high fat; HMG-CoA, hydroxymethylglutaryl-CoA; PVDF, polyvinylidene difluoride; SREBP, sterol regulatory element binding protein.

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mediated through the SREBP cleavage-activating protein (16).

It is not known whether the consumption of a soy protein diet regulates cholesterol and fatty acid synthesis through SREBPs. Recent evidence exists that rats fed a soy protein diet have reduced SREBP-1 expression (17). Furthermore, we demonstrated that after 1 h of casein diet consumption, serum insulin concentration increases 3.5-fold over the basal levels, whereas ingestion of a soy protein diet increases serum insulin by only 50% (18). Recent evidence indicates that ingestion of a high-isoflavone soy protein diet improves insulin resistance in obese Zucker rats, and it was suggested that part of the beneficial effects are mediated by peroxisome-proliferator-activated receptors (19). On the other hand, it was demonstrated that insulin selectively stimulates SREBP-1 expression in liver (15). Therefore, if consumption of soy protein maintains serum insulin concentration in the low-normal range, we can speculate that soy protein may not stimulate fatty acid synthesis in liver. Thus, the purpose of the present work was to study the short- and long-term effects of the consumption of a soy protein diet on serum insulin, and serum and hepatic lipid concentrations, and its association with the hepatic mRNA concentrations of SREBP-1, FAS, and malic enzyme. Reduction in the expression of lipogenic genes in the liver may prevent the development of steatosis.

**MATERIALS AND METHODS**

**Expt. 1.** To study the time course of the short-term response of hepatic SREBP-1 expression and its association with serum insulin levels in rats fed soy protein or casein diets, the following protocol was used. Male Wistar weanling rats (n = 30), obtained from the Experimental Research Department and Animal Care Facilities at the National Institute of Medical Sciences and Nutrition, Mexico, D.F., were housed in individual metabolic cages at ~22°C with a 12-h light:dark cycle and free access to water. To synchronize food intake, rats were trained to consume the experimental diets during a restricted period of 7 h (0900–1600 h) for 10 d. The diets contained 18 g casein or 18 g soy protein/100 g (Control diet; Table 1). On d 10, 5 rats from each group previously anesthetized with CO2 were killed by decapitation at 0, 30, 60, 90, and 120 min after they started to eat their meal. The liver sample used for RNA preparation was removed rapidly, frozen immediately in liquid nitrogen, and stored at –80°C. Blood samples were obtained and serum was separated and frozen at –20°C to measure serum insulin and glucagon concentrations.

**Expt. 2.** To assess whether the long-term consumption of a soy protein diet might maintain low serum insulin concentrations, thus preventing accumulation of hepatic lipids in association with low SREBP-1 expression, the following protocol was carried out. Additionally, if consumption of a high-fat diet is associated with insulin resistance, obesity and fatty liver, we examined whether the consumption of soy protein diet was able to reduce some of the effects of a diet high in saturated fat. Male Wistar weanling rats (n = 20), obtained from the Experimental Research Department and Animal Care Facilities at the National Institute of Medical Sciences and Nutrition, Mexico, D.F., were housed as in Expt. 1. Rats were divided into two groups: 1) rats fed 18 g soy protein/100 g (control diet), or 2) rats fed 18 g casein/100 g (control diet) from d 21 until d 120 (Table 1). Then, rats were fed for 30 d one of the following 4 experimental diets: 1) soy protein control: 18 g soy protein/100 g; 2) soy protein high fat (HF): 18 g soy protein/100 g + 15 g/100 g lard; 3) casein control: 18 g casein/100 g diet; and 4) casein HF: 18 g casein/100 g + 15 g/100 g lard (Table 1). Rats previously anesthetized with CO2 were killed on d 150 by decapitation between 0800 and 0930 h. The liver samples used for RNA preparation were removed rapidly, frozen immediately in liquid nitrogen, and stored at –80°C. The rest of the liver was divided and used for hepatic lipid quantification, immunoblotting analysis, malic enzyme activity, and histology. Blood samples were obtained and serum was separated and frozen at –80°C. The protocol for the present study was approved by the Animal Ethics Committee of the Instituto Nacional de Ciencias Médicas y Nutrición.

**Isolation of nuclear extracts.** Nuclear extracts from liver of rats fed only soy protein or casein were obtained by centrifugation in a sucrose gradient as described by Shen et al. (20). Tissues were homogenized with a teflon pestle in lysis buffer (4 g tissue/30 mL) containing 0.3 mol/L sucrose, 5 mmol/L dithiothreitol (DTT), 5 mmol/L MgCl2, 0.05% Triton X-100, and 1 mmol/L leupeptin, was added to the lysis buffer. Tissue homogenates were centrifuged at 800 × g for 10 min at 4°C. The supernatant was discarded and the pellet was resuspended in 20 mL of sucrose buffer containing 2.3 mol/L sucrose, 2 mmol/L MgCl2, and 10 mmol/L Tris/HCl, pH 7.5, with the same cocktail of protease inhibitors. The suspension was layered in Quick seal tubes onto 10 mL of sucrose buffer containing 2.3 mol/L sucrose, 2 mmol/L MgCl2, and 10 mmol/L Tris/HCl, pH 7.5, with the same cocktail of protease inhibitors. The suspension was layered in Quick seal tubes onto 10 mL of sucrose buffer. Tubes were centrifuged at 100,000 × g for 45 min at 4°C. The pellet containing nuclei was resuspended in 500 μL of storage buffer containing 50% glycerol, 2 mmol/L MgCl2, 0.1 mmol/L EDTA, and 50 mmol/L HEPES, pH 7.5. Nuclei were stored at –70°C until analysis. The protein concentration of the nuclear extracts was determined by the Lowry assay (21).

**Immunoblot analysis of SREBP-1 in nuclear extracts.** Nuclear extracts (20 μg) were separated by SDS-PAGE in 8% gels according to Laemmli (22). Before electrophoresis, all samples were boiled for 2 min in the presence of 1% SDS, with 0.2 mol/L DTT. For immunoblotting, proteins in the SDS-PAGE gel were transferred to polyvinylidene difluoride (PVDF) membranes (Boehringer Mannheim) using a Transphor electrophoresis unit (Hoefer Scientific Instruments). The PVDF membranes were blocked with 40 g/L powdered skim milk overnight at room temperature, and immunoblotting was performed using a monoclonal antibody against SREBP-1 overnight at 4°C. The monoclonal antibody anti-SREBP-1 was obtained from the hybridoma of ATCC (CRL-2121). Immunoreactive protein bands were visualized using horseradish peroxidase-labeled goat anti-mouse antibody (1:6000) after the oxidation of luminol as luminescent substrate. Light emission was detected by exposure to autoradiography film (ECL, Amersham Life Science).

**Northern blot analysis.** Total liver RNA was isolated from liver according to Chomczynski and Sacchi (23). The abundance of malic enzyme, HMG-CoA reductase, fatty acid synthase (FAS), and SREBP-1 mRNA was determined by Northern blot analysis. RNA (20 μg) was electrophoresed in a 1% agarose gel containing 18% formaldehyde, transferred to a nylon membrane filter and

**TABLE 1**

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Control diet</th>
<th>High-fat diet</th>
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<tbody>
<tr>
<td>g/kg diet</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Casein1 or soy protein2</td>
<td>18.00</td>
<td>18.00</td>
</tr>
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<td>299.17</td>
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<tr>
<td>Lard</td>
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1 Harlan Teklad, Madison, WI.
2 Supro 710, Protein Technologies, Mexico, D.F.
3 Rogers-Harper, Teklad test diets, Madison WI (41).
4 Vitamin mix, Teklad 40060 (mg/kg diet): p-aminobenzoic acid, 110; ascorbic acid, 991; biotin, 04; vitamin B-12, 30; calcium pantothenate, 66; choline dihydrogen citrate, 3497; folic acid, 2; inositol, 110; menadione, 50; niacin, 99; pyridoxine-HCl, 22; riboflavin, 22; thiamine-HCl, 22; retinyl palmitate, 40; cholecalciferol, 4; dl-a-tocopherol acetate, 242.
PCR products were purified with the high pure PCR product purification kit (Roche) and labeled with Redivue [α-32P] dCTP (110 TBq/mmol) using the Rediprime DNA labeling kit (Amersham). Membranes were prehybridized with rapid-hyb buffer (Amersham) at 65°C for 1 h, and then hybridized with the cDNA probe (53.3 MBq/L) for 2.5 h at 65°C. Membranes were washed once with 2X SSC (1X SSC = 0.15 mol/L sodium chloride, 15 mol/L sodium citrate containing 0.1% SDS) at room temperature for 20 min and then twice for 15 min with 0.1X SSC containing 0.1% SDS at 65°C. Digitization of the images and quantitation of the radioactivity of the bands were done using the Instant Imager (Packard Instrument). Membranes were also exposed to Extrakon film (Kodak) at −70°C with an intensifying screen.

**Serum biochemistry.** Serum glucose was measured with a Glucose Analyzer II (Beckman). Serum triglycerides and cholesterol concentrations were assayed using an enzymatic/colorimetric SERA-PAK (Fast color, Bayer) kit. Insulin, leptin, and glucagon were measured by RIA (RIA rat insulin, rat leptin and glucagon kits, Linco Research). The sensitivity for the rat insulin assay was 15 pmol/L, and the intra- and interassay CV were both <5%. The sensitivity of glucagon assay was 20 ng/L, and the intra- and interassay CV were <2.12% and <5%, respectively. The sensitivity of leptin assay was 0.2 μg/L, and the intra- and interassay CV were <4.0% and <5.9%, respectively.

**Liver lipids.** Total lipids were extracted according to the method of Folch et al. (24), and triglycerides and cholesterol concentrations were assayed as described above.

**Oil red O staining for lipids in rat liver.** Tissues were sliced (4 μm) and stained in 60% of the oil red O stock solution (0.5 g oil red O in 100 mL isopropanol) for 30 min. Tissues were briefly washed in 60% isopropanol and then rinsed in distilled water for microscopic observation and photography.

**Malic enzyme activity.** Liver (1 g) was homogenized according to Haney et al. (25) in 5 volumes of iced buffer containing 250 mmol/L sucrose, 10 mmol/L Tris HCl, 1 mmol/L mercaptoethanol, pH 7.4, and centrifuged at 100,000 × g, for 1 h at 4°C. The clear supernatants were used to measure enzyme activity. Liver malic enzyme activity was assayed by measuring NADPH formation with an intensifying screen.

**RESULTS**

**Expt. 1. Effect of short-term soy protein consumption**

**Time course response of serum insulin in rats fed soy protein or casein diet.** Serum insulin concentration in food-deprived rats did not differ between groups. The casein diet, as expected, increased insulin concentration rapidly compared with rats fed the soy protein diet. Thus on d 10, rats fed the casein diet had 5.3-, 6.5-, and 6.7-fold higher serum insulin concentration with respect to food-deprived levels 30, 60, and 90 min after food consumption, respectively. Rats fed the soy protein diet had 1.4-, 1.9-, and 1.9-fold higher serum insulin concentration with respect to food-deprived levels at the same time points (P < 0.05). Rats fed the soy protein diet had ~36% less serum insulin than rats fed the casein diet. Two hours after consumption of the respective diets, serum insulin concentrations did not differ between the groups (Fig. 1a).

**Time course response of hepatic SREBP-1 expression and its association with serum insulin levels in rats fed the casein or soy protein diets.** Rats fed the casein diet also had a greater abundance of SREBP-1 mRNA than rats fed soy protein. After 30 min of food consumption, rats fed casein had 3.3-fold higher SREBP-1 mRNA concentration than rats fed soy protein. Thereafter, SREBP-1 mRNA abundance increased, and at the end of the study, rats fed the casein diet had 1.2-fold more mRNA of this transcription factor than rats fed soy protein (Fig. 1b, c). This response clearly shows that there is an association between hepatic SREBP-1 mRNA abundance and

<table>
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<th>Primers (5′-3′)</th>
<th>Predicted size</th>
<th>Position</th>
<th>Species</th>
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<td>HMG-CoA reductase</td>
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<td>LDL receptor</td>
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<td>63-1687</td>
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<td>1009</td>
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<td>U09103</td>
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Rats fed the soy control diet (\(P\) high-fat soy protein diet had greater serum leptin levels than \(P\) concentrations did not differ among the groups (Table 3). Rats fed the Table 3). Feed intakes and serum glucose concentration of the four experimental diets for 30 d, only rats fed soy protein or soy protein HF diets had signifi-
cantly higher weight gain (data not shown). However, after consump-
tion of the four experimental diets for 30 d, only rats fed soy protein HF or casein HF diets had significant weight gains (79 and 103\% respectively) compared with their respective control groups (Table 3). Feed intakes and serum glucose concentra-
tions did not differ among the groups (Table 3). Rats fed the high-fat soy protein diet had greater serum leptin levels than rats fed the soy control diet (\(P < 0.001\)), whereas rats fed the casein control or casein HF diets had serum leptin concentra-
tions that did not differ. The final body weight of all rats studied was significantly correlated with their serum leptin concentrations (Fig. 2).

**Serum lipids.** Rats fed casein or casein HF diets had higher serum triglycerides than rats fed the soy protein or soy protein HF diets (\(P < 0.0001\)) (Table 3). Thus, the reduction in serum triglycerides in rats fed the soy protein or soy protein HF diets was ~68\% compared with rats fed the casein or casein HF diets, respectively. The fat content in the soy protein diets did not alter the capacity of this protein to reduce serum cholesterol levels compared with the respective groups fed the control or HF casein diets (Table 3). Rats fed the soy or soy HF diets had serum cholesterol that was 21 and 24\% lower compared with rats fed the casein and casein HF diets, respectively (\(P < 0.01\)).

**Hepatic lipids.** Hepatic triglycerides and cholesterol were also reduced by consumption of the soy protein diets compared with rats fed the casein diets (Table 3). Liver triglycerides were dramatically reduced in rats fed soy protein or soy protein HF diets (72 and 77\%) compared with rats fed the casein or casein HF diets, respectively (\(P < 0.001\)). Rats fed the soy protein or soy protein HF diets had a liver cholesterol content that was ~57\% lower than that in rats fed the casein or casein HF diets.

**Serum insulin and glucagon.** Rats fed the casein or casein HF diets for 30 d developed hyperinsulinemia. Rats fed the soy protein or soy protein HF diets had 65 and 45\% lower serum insulin concentrations than rats fed the casein or casein HF diets (\(P < 0.001\)) (Table 3). Interestingly, serum glucagon had a similar response, i.e., rats fed the casein or casein HF diets had the highest concentrations of this hormone. Serum glucagon concentrations were 44 and 39\% lower in rats fed the soy protein or soy protein HF diets, respectively, than in rats fed the casein or casein HF diets (\(P < 0.001\)) (Table 3). Thus, the increase in insulin in rats fed the casein or casein HF diets could trigger the lipogenesis. The insulin:glucagon ratio tended to be higher (\(P = 0.08\)) in rats fed the casein diets than in rats fed the soy protein diets (Table 3).

**mRNA concentrations of hepatic genes of lipid metabolism.** Rats fed the casein or casein HF diets had higher SREBP-1 mRNA concentrations than rats fed the soy protein or soy protein HF diets (Figs. 4, 5). The increment in SREBP-1 was associated with an increase in FAS and malic enzyme mRNA in rats fed casein, whereas a low abundance of SREBP-1, as observed in rats fed the soy protein diets, was accompanied by an extremely low abundance of FAS and malic enzyme mRNAs. On the other hand, there were greater increases in HMG-CoA reductase and LDL receptor mRNA concentration in rats fed the soy protein diets than in rats fed the casein diets. Because cholesterol is eliminated from the body primarily as bile acids, and the limiting step for the synthesis of bile acids is catalyzed by the enzyme 7-\(\alpha\)-hydroxylase (CYP7A1), mRNA concentration of this enzyme was measured in control rats fed the casein or soy protein diet. Rats fed the soy protein diets had significantly higher CYP7A1 mRNA expression than rats fed the casein diets (Figs. 4, 5).

**SREBP-1 in hepatic nuclear extracts.** SREBP-1 protein concentration in liver nuclei of rats fed the soy protein diets was significantly lower than in rats fed the casein diets (Fig. 6). The increase in mRNA abundance of this transcription factor was associated with an increase in the proteolytic cleav-

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**FIGURE 1** Serum insulin concentration (a), relative mRNA abundance (b), and hepatic SREBP-1 expression (c) in rats fed casein or soy protein 0, 30, 60, 90, and 120 min after they started to eat their meal. Values are means ± SEM, \(n = 5\). *Different from the soy group at that time, \(P < 0.05\)."
specific mRNA abundance. Rats fed the casein or casein HF enzyme followed a pattern similar to that observed for its vates lipogenic genes in the liver.

The age of the mature form of SREBP-1, which functionally activates lipogenic genes in the liver.

**Hepatic malic enzyme activity.** The activity of malic enzyme followed a pattern similar to that observed for its specific mRNA abundance. Rats fed the casein or casein HF diets had the greatest malic enzyme activities, whereas rats fed the soy protein or soy protein HF diets had 68 and 88% lower enzyme activities, respectively, than rats fed the casein or casein HF diets ($P < 0.001$) (Fig. 7).

**DISCUSSION**

The results of this study demonstrated that short- and long-term ingestion of a soy protein diet maintained normal serum insulin concentrations compared with rats fed a casein diet. This in turn was accompanied by a low expression of SREBP-1 mRNA in liver and by decreasing expression of the lipogenic enzymes, FAS and malic enzyme. In contrast, short- and long-term consumption of a casein diet increased serum insulin concentrations compared with rats fed a casein diet. This in turn was accompanied by a low expression of lipogenic enzymes in rats fed a casein diet long term increased liver lipid depots, mainly triglycerides, whereas a soy protein diet dramatically reduced the accumulation of hepatic lipids.

Insulin has been shown to play a key role in the regulation of the expression of SREBP-1 (27). Studies with obese mice or with a transgenic mouse model that resembles generalized lipodystrophy in humans (28) showed that elevated SREBP-1c levels are associated with hyperinsulinemia (29), whereas the disruption of SREBP-1 in mice reduces the expression of lipogenic enzymes, as well as the triglycerides content in liver (30). Interestingly, our data showed that SREBP-1 mRNA expression increased rapidly after the short-term consumption of a casein or soy protein diet (Fig. 1b). The casein diet stimulated postprandial serum insulin levels to a greater extent

![FIGURE 2](image_url) Correlation between final body weight and serum leptin concentration in rats fed a soy protein or casein diet for 130 d.

![FIGURE 3](image_url) Lipid depots after staining with oil red O in liver of rats fed casein (A), soy (B), casein HF (C), and soy HF (D) for 139 d.

### TABLE 3

<table>
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<th>Type of</th>
<th>Fat content</th>
<th>Protein content</th>
<th>ANOVA (P-values)</th>
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<tr>
<td>Casein</td>
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<td>Soy HF</td>
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<td>Weight gain, d 121–150, g</td>
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<td>Food intake, d 121–150, g/d</td>
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<td>Glucose, mmol/L</td>
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<td>8.5 ± 0.3</td>
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<td>Leptin, μg/L</td>
<td>14.7 ± 0.34ab</td>
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<td>Triglycerides, mmol/L</td>
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<td>Cholesterol, mmol/L</td>
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1 Values are means ± SEM, $n = 5$. Means in a row without a common letter differ, $P < 0.05$. 

| FIGURE 2 | Correlation between final body weight and serum leptin concentration in rats fed a soy protein or casein diet for 130 d. | 
| FIGURE 3 | Lipid depots after staining with oil red O in liver of rats fed casein (A), soy (B), casein HF (C), and soy HF (D) for 139 d. |
than did soy protein, leading to a higher expression of SREBP-1 mRNA. It is important to emphasize that the modulation of serum insulin and SREBP-1 abundance depended on the type of protein consumed in the short-term study (Expt. 1) because the diets differed only in the type of protein.

On the other hand, we observed that rats fed the casein HF diet had less induction of lipogenic genes than control rats fed casein even though both groups had high serum insulin concentrations, and also high serum and hepatic triglycerides. Our results agree with previous findings showing that consumption of a high-fat diet reduces lipogenesis (31). Furthermore, there is evidence that not only PUFA, but also SFA decrease the rate of fatty acid synthesis in Caco-2 cells; this repression is accompanied by a decrease in the expression of SREBP-1 and FAS (32). Therefore, the long-term consumption of a HF diet reduces lipogenesis, although it does not prevent the accumulation of lipids of dietary origin.

The accumulation of triglycerides in nonadipose cells is associated with lipotoxicity (33). In genetically obese Zucker diabetic fatty rats, there is an increase of triglycerides in skeletal muscle, heart, pancreatic β-cells, and liver (34). The excessive energy intake by these rats due to the absence of the leptin receptor does not allow nonadipose tissue to oxidize the fatty acid excess through the stimulation of PPAR-α by leptin (35). High energy intake results in elevated serum insulin levels that induce SREBP-1 expression, leading to an accelerated lipogenesis in nonadipose tissues (15,27). The consequence of all these alterations is the development of the metabolic syndrome X (36). In our study, we observed that long-term consumption of a soy protein diet prevented an increase in lipid deposits, mainly triglycerides, in the liver. Thus, ingestion of this protein might slow the progress of insulin resistance, whereas consumption of casein can cause fatty liver, hypertriglyceridemia, and hyperinsulinemia, factors that may lead to the development of insulin resistance and diabetes.

It has been shown that during peripheral insulin resistance, there is a concomitant increase in serum insulin and glucagon (37). In rats fed the casein or casein HF diet, we observed that the insulin:glucagon ratio tended to be higher than in rats fed the soy protein diets. Thus, our results may support the hypothesis that long-term consumption of a casein diet allows the development of insulin resistance independently of the fat content of the diet. We are conducting studies with euglycemic clamps to test this hypothesis.

At present, it is unclear why casein stimulates the increase in serum insulin, whereas soy protein maintains normal levels of this hormone. There is evidence that soy protein decreases insulin secretion and increases hepatic insulin extraction (38). It is assumed that the amino acid pattern of each protein can stimulate the pancreatic release of insulin. A mechanism involving glutamine oxidation in β-cells by glutamate dehydrogenase was proposed to demonstrate how a specific dietary amino acid pattern can modulate insulin secretion (39); however, more studies at the molecular level are required to understand the basis for the stimulation of insulin secretion by amino acids. We are currently studying the differences in the pattern of gene expression in pancreatic β-cells; this may help explain the role of soy protein in preventing the elevation of serum insulin concentration.

![FIGURE 4](https://example.com/figure4.png) Northern blot analysis of hepatic genes of fatty acid and cholesterol biosynthesis in rats fed a soy protein or casein diet for 130 d. Blots were exposed to an imaging analyzer and also to Kodak film with an intensifying screen.

![FIGURE 5](https://example.com/figure5.png) Electronic autoradiography quantitation of mRNA of hepatic genes involved in fatty acid and cholesterol metabolism of rats fed 18 g/100 g casein or soy protein diets containing adequate or high fat. Values are means ± SEM, n = 5. Means without a common letter differ, P < 0.05.
On the other hand, the mechanisms by which the ingestion of soy protein reduces serum and hepatic cholesterol are not clear. Our results showed that there is a greater abundance of LDL receptors and HMG-CoA reductase mRNA in rats fed the soy protein diet than in those fed the casein diet. Furthermore, we observed that low hepatic cholesterol concentrations were associated with a greater abundance of LDL receptors and HMG-CoA reductase mRNA. Thus, there is an induction or a repression of genes involved in the synthesis and utilization of cholesterol, depending of the intracellular cholesterol concentration as previously described (9). The reduction in serum cholesterol is associated with a stimulation of bile acid synthesis, possibly through the induction of the enzyme CYP7A1, the rate-limiting step in the biosynthesis of bile acids (40). In addition, the low serum cholesterol concentrations observed in rats fed the soy protein diet might be due to the reduction of lipogenesis. Thus, the reduced biosynthesis of fatty acids in turn will reduce the production of VLDL particles, thus limiting the formation of LDL particles and resulting in low serum triglycerides and cholesterol concentrations. At the present time, we are studying the effect of soy protein on the synthesis and enterohepatic transport of bile acids.

Our results indicate that the regulation of hepatic lipid synthesis and deposition, mainly triglycerides, is associated with the type of dietary protein consumed. This regulation likely occurs indirectly via insulin through SREBP-1, and this will depend of the type of protein consumed. Nonetheless, more studies are warranted to determine whether the reduction in cholesterol concentration is a direct or an indirect effect of the soy protein.

LITERATURE CITED


FIGURE 6 Immunoblot analysis of SREBP-1 in nuclear extracts from livers of rats fed soy protein or casein diet containing adequate (C) or high fat (HF). Panel A shows a representative Western blot. Nuclear extracts (20 μg of protein) were subjected to immunoblotting with polyclonal antibody against SREBP-1. Immunoreactive protein bands were visualized using horseradish peroxidase-labeled goat anti-mouse antibody (1:8000) after the oxidation of luminol as luminescent substrate. Panel B shows the densitometric analysis of Western blots of nuclear SREBP-1 (nSREBP-1) from rats fed the casein or soy protein diets containing adequate or high fat. Values are means ± SEM, n = 5. Means without a common letter differ, P < 0.05.

FIGURE 7 Liver malic enzyme activity in rats fed casein or soy protein diet containing adequate or high fat. Values are means ± SEM, n = 5. Means without a common letter differ, P < 0.05.
induced by cholesterol deprivation and insulin elevation. Genes Dev. 15: 1206–1216.


