Dietary Soy Protein Reduces Cardiac Lipid Accumulation and the Ceramide Concentration in High-Fat Diet-Fed Rats and ob/ob Mice

Ivan Torre-Villalvazo,4 Fabiola Gonzalez,4 Carlos A. Aguilar-Salinas,5 Armando R. Tovar,4 and Nimbe Torres4*

Abstract

Obesity is an epidemic condition strongly associated with cardiovascular morbidity and mortality. Heart disease secondary to obesity is associated with myocardial steatosis, leading to ceramide synthesis and cell dysfunction in a process known as lipotoxicity. Soy protein has been demonstrated to reduce lipotoxicity in the liver and pancreas in different rodent models of obesity. Thus, our purpose in the present work was to assess the effect of dietary soy protein on cardiac lipid accumulation and ceramide formation during obesity and to evaluate its effect in the following 2 rodent models of obesity: 1) a diet-induced obesity model in Sprague-Dawley rats was produced by feeding rats a control or a high-fat casein or soy protein diet for 180 d; and 2) wild-type and ob/ob mice were fed a casein or soy protein diet for 90 d. Soy protein intake led to lower cholesterol and triglyceride concentrations in the hearts of rats and ob/ob mice in association with a greater PPARα mRNA concentration and a lower level of sterol regulatory element binding protein-1 mRNA than those fed casein. The ceramide concentration was also lower in hearts of rats and ob/ob mice that were fed soy protein in association with lower serine palmitoyl transferase (SPT)-1 mRNA concentrations. These results indicate that dietary soy protein can reduce the heart ceramide concentration by reducing the expression of SPT-1, a key enzyme in the formation of this sphingolipid in the heart of obese rodents, and by reducing lipid accumulation. Thus, soy protein consumption may be considered as a dietary therapeutic approach for lipotoxic cardiomyopathy prevention.


Introduction

Obesity is one of the most serious public health problems of the 21st century and is associated with many diseases, particularly heart disease, type 2 diabetes, certain types of cancer, and increased risk of premature death (1). Obesity has both metabolic and cardiovascular health consequences (2). Central deposition of adipose tissue is strongly associated with elevated cardiovascular morbidity and mortality, including stroke, congestive heart failure, myocardial infarction, and cardiovascular death (3,4). For these reasons, the AHA has reclassified obesity as a major modifiable factor for coronary heart disease (5).

At present, most clinicians attribute the cardiac disturbances that occur in obesity to coronary artery disease or hypertension, because these are established diagnostic categories commonly associated with obesity (6). However, lean genetic mouse models of cardiac-restricted steatosis have demonstrated that myocardial-specific steatosis, independent of systemic obesity, is a direct cause of heart disease (7,8). Cardiomyopathy, however, is not a direct consequence of triglyceride (TG)6 accumulation alone; heart disease develops secondary to fatty acid (FA) entry into deleterious pathways such as that for ceramide synthesis, leading to cell dysfunction and ultimately apoptotic cell death (9,10). These processes are known as lipotoxicity and lipoapoptosis (11).

Emerging evidence of the relationship between cardiac lipid accumulation and heart failure could lead to new interventions aimed at ameliorating myocardial steatosis to prevent lipotoxic heart disease. Soy protein has been demonstrated to reduce lipotoxicity in the liver and pancreas in different rodent models of obesity (12–14). This reduction is exerted through several mechanisms, including reduction of the insulin:glucagon ratio and FA synthesis mediated by sterol regulatory element binding

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3 Supplemental Figure 1 is available with the online posting of this paper at jn.nutrition.org.
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6 Abbreviations used: CAS, casein diet; CAS-HF, casein high-fat diet; DAG, diacylglycerol; FA, fatty acid; SOY, soy protein diet; SOY-HF, soy protein high-fat diet; SPT-1, serine palmitoyl transferase-1; SREBP-1, sterol regulatory element binding protein-1; TG, triglyceride; TNFα, tumor necrosis factor-α.
protein-1 (SREBP-1) and an increase in FA oxidation (15). Therefore, it is important to assess the effect of dietary soy protein on cardiac lipid accumulation and ceramide formation in obesity and to evaluate its effect in genetic and dietary rodent models of obesity.

Materials and Methods

Animals and experimental protocols. Male Sprague-Dawley rats (4 wk old) were obtained from Harlan and housed in individual cages. Rats were divided into 4 groups (n = 5) and fed 1 of the following experimental diets for 180 d: 1) a control diet containing 30% casein and 5% fat (11% of total energy) (CAS); 2) a control diet containing 30% soy protein and 5% fat (11% of total energy) (SOY); 3) a high-fat diet containing 30% casein and 25% fat (45% of total energy) (CAS-HF); or 4) a high-fat diet containing 30% soy protein and 25% fat (45% of total energy) (SOY-HF). The protein composition was adjusted on the basis of protein purity (90.6% casein, 86% soy protein). The compositions of the experimental diets as well as the isolavone concentration of the soy isolate are presented in Table 1 (16). Male C57BL6/J wild-type mice and leptin-deficient C57BL6/J ob/ob mice (4 wk old) were obtained from Harlan and maintained in groups of 5 mice in microisoincubator cages. Mice were divided into 2 groups (n = 5) and fed the CAS or SOY diet for 90 d. All animals were maintained in the Experimental Research Department of the Institute with a 12-h-light/dark cycle. At the end of the study, animals were killed after overnight food deprivation. Hearts were rapidly excised, frozen in liquid nitrogen, and stored at −70°C. Institutional guidelines for animal care and use were followed. The Institutional Animal Care and Research Advisory Committee of the Instituto Nacional de Ciencias Medicas y Nutricion in Mexico City approved the animal protocol.

Serum biochemistry. Concentrations of glucose, cholesterol, and TG were measured in serum obtained from food-deprived rats and mice using colorimetric kits (glucose GOD FS, cholesterol FS, and TG FS kits, DiaSys Diagnostic Systems). The serum FFA concentration was assayed with a FFA Half Micro test (Roche Applied Science). Serum insulin and leptin were assayed using RIA kits (Linco Research Immunoenzymassays).

Lipid concentration of heart. Total tissue lipids were extracted following a modified Bligh and Dyer (17) protocol as follows: 90 mg of tissue were homogenized in 1.9 mL of chloroform:methanol:1 mol/L NaCl [1:2:0.4 (v:v)] followed by 500 µL chloroform and 500 µL 1 mol/L NaCl. The mixture was then centrifuged at 10,000g for 10 min. The supernatant was removed, and the organic phase containing lipids was dried under a nitrogen stream. Lipids were resolved by TLC in a solvent system containing chloroform:methanol:acetic acid:water [10:4:3:2:1 (v:v)] followed by 500 µL chloroform and 500 µL hydrochloric acid. The organic phase was dried under a nitrogen stream. Lipids were resolved by TLC in a solvent system containing chloroform:methanol:acetic acid:water [10:4:3:2:1 (v:v)]. The phosphorylated products of ceramide were identified by comparison with known standards run on the same plate. The level of ceramide was determined by comparison with a standard curve composed of a known amount of brain-derived ceramide (Sigma-Aldrich). Labeled ceramide 1-phosphate was quantified using a Packard Instant Imager (Packard Instrument). Results are expressed as counts per minute. Data are representative of 3 separate experiments. Plates were also exposed to Kodak XAR film (Eastman Kodak) at −70°C with an intensifying screen.

Real-time PCR. Total RNA was extracted from heart tissue by guanidine isothiocyanate/GCl gradient ultracentrifugation according to Chomczynski and Sacchi (19). Total RNA (300 ng) from each animal was subjected to RT prior to PCR amplification with the Two-Step Master mix (Applied Biosystems). TaqMan fluorogenic probes and oligonucleotide primers [SREBP1, PPARα, tumor necrosis factor-α (TNFa), serine palmitoyl transferase-1 (SPT-1)] were obtained from Applied Biosystems. All samples were analyzed in triplicate. The relative amounts of mRNA were calculated using the Comparative CT method (User Bulletin no. 2, Applied Biosystems). 18S Ribosomal RNA was used as the invariant control.

Statistical analysis. Values are expressed as mean ± SEM. Data were evaluated using 2-way ANOVA followed by Fisher’s protected least significant difference test when the interaction was significant. Differences were considered significant at P < 0.05.

Results

Weight gain, food intake, and serum biochemistry. Rats fed soy protein or casein high-fat diets had 15 and 19% higher final body weights, respectively, than those fed the control diets. However, rats fed SOY or SOY-HF had significantly lower final body weights than rats fed CAS or CAS-HF by 11 and 14%, respectively (Table 2). Daily food intake was lower in the groups fed the high-fat diets. Energy intake did not differ among the different groups.

Histological analysis. To evaluate lipid droplets in cardiomyocytes, frozen heart sections (10 µm) were mounted on albuminized slides, fixed in 10% formalin, dried in propylene glycol, stained with 0.7% Oil red O, and counterstained with hematoxylin QS. Stained slides were mounted with aqueous mounting media and analyzed with a Sony CCS-IRIS digital camera coupled to a Leica microscope at 40× magnification.

TUNEL assay. A total of 100 µg of cardiac tissue was fixed in 10% formalin and embedded in paraffin. Sections (3 µm) were processed using the ApopTag Peroxidase in situ Apoptosis Detection kit (Millipore) following the manufacturer’s protocol.

Heart ceramide. The ceramide concentration in heart was measured using a modification of the diacylglycerol (DAG) kinase method as described by Perry and Hannun (18). Briefly, extracted lipids were resuspended in 20 µL of 7.5% 2-octyl-glucopyranoside and 1 mmol/L diethylenetriaminepentaacetic acid (Sigma-Aldrich) and mixed vigorously to form micelles. Enzyme buffer (70 µL) and 3 µL purified DAG kinase (Sigma-Aldrich) were added. DAG kinase was added in excess to ensure the total conversion of the substrate to the phosphorylated product. A total of 10 µL of 10 mmol/L ATP solution containing 74 kBq of [γ-32P] ATP (GE Healthcare Biosciences) was added to start the reaction. After 30 min at 22°C, the reaction was stopped by extraction of lipids with 1.9 mL chloroform:methanol:water [1:2.0.4 (v:v)] followed by 500 µL chloroform and 500 µL hydrochloric acid. The organic phase was dried under a nitrogen stream. Lipids were resolved by TLC in a solvent system containing chloroform:methanol:acetic acid:water [10:4:3:2:1 (v:v)]. The phosphorylated products of ceramide were identified by comparison with known standards run on the same plate. The level of ceramide was determined by comparison with a standard curve composed of a known amount of brain-derived ceramide (Sigma-Aldrich). Labeled ceramide 1-phosphate was quantified using a Packard Instant Imager (Packard Instrument). Results are expressed as counts per minute. Data are representative of 3 separate experiments. Plates were also exposed to Kodak XAR film (Eastman Kodak) at −70°C with an intensifying screen.

Table 2

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>SOY</th>
<th>CAS</th>
<th>SOY-HF</th>
<th>CAS-HF</th>
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<tbody>
<tr>
<td>Soy protein (86% purity)</td>
<td>348.8</td>
<td>348.8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Casein (90.6% purity)</td>
<td>331.1</td>
<td></td>
<td>331.1</td>
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</tr>
<tr>
<td>Cornstarch</td>
<td>269.7</td>
<td>278.6</td>
<td>189.7</td>
<td>178.6</td>
</tr>
<tr>
<td>Dextrose</td>
<td>269.7</td>
<td>278.6</td>
<td>189.7</td>
<td>178.6</td>
</tr>
<tr>
<td>Corn oil</td>
<td>50</td>
<td>50</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>Lard</td>
<td>200</td>
<td>200</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mineral mixture</td>
<td>50</td>
<td>50</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>Vitamin mixture</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</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, Mexico. Isoflavone analysis (mg/g protein): genistein, 1.38; daidzein, 0.71; glycitein, 0.19.
2 Vitamin-free casein, Harlan Teklad Research Diets, Madison, WI.
4 AIN-93-VX, Harlan Teklad Research Diets, Madison, WI (mg/kg diet): nicotinic acid, 150; calcium pantothenate, 80; pyridoxine-HCl, 35; thiamin HCl, 30; riboflavin, 30; folic acid, 10; biotin, 1; vitamin B12, 0.1; cholic acid, 50; sodium chloride, 30; magnesium oxide, 50; potassium chloride, 100; sodium bicarbonate, 50; dicalcium phosphate, 150; vitamin A palmitate (600,000 i.u./g), 40; cholecalciferol (500,000 i.u./g), 10; phylloquinone, 3.75.
5 Harlan Teklad Research Diets, Madison, WI.
6 NaCl. The mixture was then centrifuged at 10,000 × g and the organic phase was dried under nitrogen stream. Cholesterol and TG concentrations were measured with the colorimetric kits mentioned above.

Statistical analysis. Values are expressed as mean ± SEM. Data were evaluated using 2-way ANOVA followed by Fisher’s protected least significant difference test when the interaction was significant. Differences were considered significant at P < 0.05.
Cardiac lipid accumulation and ceramide concentration. Obesity-induced cardiac dysfunction is mediated in part by lipid overaccumulation in cardiomyocytes (20). In the present study, rats fed SOY or SOY-HF diets had lower TG concentrations in heart than those fed CAS-HF (42 and 63%, respectively) (Table 2). Similarly, rats fed high-fat diets had higher serum glucose, cholesterol, TG, and FFA concentrations than those fed control diets. Rats fed SOY-HF had 67% lower serum TG than those fed CAS-HF (Table 2). Nonetheless, the type of dietary protein did not affect the serum insulin concentration. As expected, the ob/ob mice had an undetectable serum leptin, whereas mice fed CAS had a 1.3-fold higher cardiac ceramide concentration than the respective control groups (Tables 2 and 3). However, the type of dietary protein did not affect the serum insulin concentration. As expected, the ob/ob mice had an undetectable serum leptin, whereas mice fed CAS had a 1.3-fold higher cardiac ceramide concentration than the respective control groups (Tables 2 and 3). However, the type of dietary protein did not affect the serum insulin concentration.  

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The ob/ob mice had significantly higher final body weights and daily food intakes than the wild-type mice (Table 3). The ob/ob mice had higher serum glucose, cholesterol, TG, and FFA concentrations than wild-type mice. Interestingly, the ob/ob mice fed SOY had significantly lower serum glucose, cholesterol, and TG concentrations than those fed CAS (Table 3). Similarly, rats fed high-fat diets had higher serum glucose, cholesterol, TG, and FFA concentrations than those fed control diets. Rats fed SOY-HF had 67% lower serum TG than those fed CAS-HF (Table 2). Nonetheless, ob/ob mice or rats fed high-fat diets had significantly higher serum insulin concentrations than the respective control groups (Tables 2 and 3). However, the type of dietary protein did not affect the serum insulin concentration.  

Cardiac lipid accumulation and ceramide concentration. Obesity-induced cardiac dysfunction is mediated in part by lipid overaccumulation in cardiomyocytes (20). In the present study, rats fed SOY or SOY-HF diets had lower TG concentrations in heart than those fed CAS-HF (42 and 63%, respectively) (Table 4). Mice fed SOY also had lower TG concentrations in heart compared with those fed CAS (50 and 14%, respectively) (Table 5). Interestingly, the ob/ob mice accumulated more TG in heart compared with wild-type mice and obese rats. The cholesterol concentration in the heart of high-fat–fed rats did not follow the same pattern as TG; the concentration was slightly but significantly greater than in all other groups in the rats fed CAS-HF (Table 4). In ob/ob mice fed CAS, the heart cholesterol concentration was slightly but significantly greater than in those fed SOY; however, it was ~4-fold higher in ob/ob mice than in wild-type mice or obese rats (Table 5). Frozen cardiac sections stained with Oil red O staining had higher lipid depots in the cardiomyocytes of rats fed CAS-HF compared with those fed SOY-HF and the control groups (Supplemental Fig. 1A). Because heart of ob/ob mice contained significantly higher concentrations of TG and cholesterol than those of wild-type mice, independent of the protein source, this result was confirmed with the Oil red O staining (Supplemental Fig. 1B). These data indicate that cardiac lipid accumulation strongly depends on genetic background and the amount of fat in the diet. It has been proposed that excess intracellular TG can serve as a substrate for de novo ceramide synthesis, leading to insulin resistance and ultimately apoptotic cell death (21). Rats fed CAS-HF had a significantly higher heart ceramide concentration than the other groups (Table 4; Supplemental Fig. 1C). The ob/ob mice fed CAS had a 1.3-fold higher cardiac ceramide concentration than the ob/ob mice fed SOY (Table 5; Supplemental Fig. 1D). The results of this study show that consumption of casein as part of a high-fat diet or an obese background can increase the ceramide concentration of the heart. Interestingly, despite the increase in the cardiac ceramide concentration of mice and rats fed casein diets, we did not observe apoptotic cardiomyocytes by the TUNEL assay (data not shown).
Heart SPT-1 and TNFα mRNA. The sources of cardiac ceramide are uptake from the circulation and endogenous synthesis through a series of reactions that are initiated by SPT-1, which catalyzes the condensation of palmitate with serine (22). To assess the relative participation of endogenous biosynthesis on the ceramide pool in the heart, we measured SPT-1 mRNA relative abundance. Rats fed SOY-HF had a 26% lower SPT-1 mRNA concentration compared with those fed CAS-HF (Table 4). The ob/ob mice fed casein had significantly more SPT-1 mRNA than those fed soy protein and the control groups (Table 5). Our results suggest that at least in rats fed CAS-HF and ob/ob mice fed CAS, endogenous synthesis may make an important contribution to the heart ceramide concentration. Furthermore, cellular ceramide accumulation is known to enhance the inflammatory response mediated by TNFα, which, in turn, increases ceramide synthesis and cellular damage (23,24). TNFα has been shown to be produced by cardiomyocytes in obese rodents and humans (25). Thus, we measured TNFα mRNA expression in the heart as an indicator of cardiomyocyte inflammatory stress associated with ceramide accumulation. Even though rats fed SOY had more TNFα mRNA than those fed CAS, the group fed the CAS-HF diet had 3.1-fold more cardiac TNFα mRNA than those fed the CAS diet. Addition of soy protein to the high-fat diet prevented this accumulation. Even though rats fed SOY had more TNFα mRNA than those fed CAS, the group fed the CAS-HF diet had 3.1-fold more cardiac TNFα mRNA than those fed the CAS diet. Moreover, cellular ceramide accumulation is known to enhance the inflammatory response mediated by TNFα, which, in turn, increases ceramide synthesis and cellular damage (23,24). 

Heart PPARα and SREBP-1 mRNA. Due to its large work output, the heart has a major energy requirement that is supplied primarily by FA oxidation. In the healthy heart, myocardial energy demand and energy substrate supply are tightly coordinated processes (26). However, in obesity, the FA supply overcomes mitochondrial FA oxidation and lipid excesses are stored as TG (27). To gain insight into the regulation of FA oxidation and esterification, we measured PPARα and SREBP-1 mRNA levels in the heart. The PPARα mRNA concentration was higher in rats fed SOY and SOY-HF diets than in those fed casein (Table 4). The PPARα mRNA concentration was higher in wild-type mice and ob/ob mice fed SOY than in those fed CAS (Table 4). The SREBP-1 mRNA concentration was higher in rats fed CAS-HF than in those fed SOY-HF or the control groups (Table 4). Similarly, the ob/ob mice fed CAS also had higher SREBP-1 gene expression than those fed SOY or the wild-type mice (Table 5).

**Discussion**

Cardiovascular disease is one of the leading causes of mortality and morbidity in individuals with type 2 diabetes and metabolic syndrome (2). As the global incidence of obesity and diabetes increases, it is anticipated that the burden of cardiovascular disease will continue to rise (28). Ceramide is now recognized as an important lipid-derived metabolite associated with cardiac dysfunction in obesity (29). Thus, new strategies aimed at reducing cardiac lipid accumulation may be a primary approach to prevent lipotoxic heart disease.

Soy protein is known to reduce lipid deposition in the liver and pancreas of obese rats, hence preventing lipotoxicity (12–14), but the effect of dietary soy protein on cardiac lipid deposition is not known. In the present study, soy protein intake

### Table 4

<table>
<thead>
<tr>
<th></th>
<th>CAS</th>
<th>SOY</th>
<th>CAS-HF</th>
<th>SOY-HF</th>
<th>Protein</th>
<th>Fat</th>
<th>Protein × fat</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cholesterol, mmol/g wet tissue</td>
<td>0.004 ± 0.001&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.004 ± 0.001&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.005 ± 0.001&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.004 ± 0.001&lt;sup&gt;b&lt;/sup&gt;</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>TG, mmol/g wet tissue</td>
<td>0.004 ± 0.001&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.002 ± 0.001&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.008 ± 0.001&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.003 ± 0.001&lt;sup&gt;b&lt;/sup&gt;</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Ceramide, relative density units</td>
<td>106 ± 3&lt;sup&gt;b&lt;/sup&gt;</td>
<td>98 ± 4&lt;sup&gt;b&lt;/sup&gt;</td>
<td>175 ± 29&lt;sup&gt;a&lt;/sup&gt;</td>
<td>124 ± 16&lt;sup&gt;b&lt;/sup&gt;</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
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<tr>
<td>SREBP1 mRNA, relative units</td>
<td>0.99 ± 0.04</td>
<td>0.99 ± 0.09</td>
<td>1.46 ± 0.06</td>
<td>1.22 ± 0.02</td>
<td>NS&lt;sup&gt;2&lt;/sup&gt;</td>
<td>0.001</td>
<td>NS</td>
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<tr>
<td>PPARα mRNA, relative units</td>
<td>1.20 ± 0.10&lt;sup&gt;c&lt;/sup&gt;</td>
<td>2.37 ± 0.21&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.05 ± 0.12&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1.60 ± 0.09&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.002</td>
<td>0.01</td>
<td>&lt;0.05</td>
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<tr>
<td>TNFα mRNA, relative units</td>
<td>1.09 ± 0.07&lt;sup&gt;c&lt;/sup&gt;</td>
<td>2.14 ± 0.07&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4.51 ± 0.03&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.51 ± 0.26&lt;sup&gt;b&lt;/sup&gt;</td>
<td>NS</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
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<tr>
<td>SPT1 mRNA, relative units</td>
<td>0.94 ± 0.04&lt;sup&gt;d&lt;/sup&gt;</td>
<td>1.40 ± 0.08&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.94 ± 0.06&lt;sup&gt;d&lt;/sup&gt;</td>
<td>1.43 ± 0.10&lt;sup&gt;b&lt;/sup&gt;</td>
<td>NS</td>
<td>&lt;0.001</td>
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<sup>1</sup> Values are means ± SEM, n = 5. Means in a row with superscripts without a common letter differ, P < 0.05.

<sup>2</sup> NS, P = 0.05.

### Table 5

<table>
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<th>Wild-type</th>
<th>ob/ob</th>
<th>P-value</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Protein</td>
<td>Genotype</td>
<td>Protein × genotype</td>
</tr>
<tr>
<td>Cholesterol, mmol/g wet tissue</td>
<td>0.007 ± 0.001&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.006 ± 0.001&lt;sup&gt;c&lt;/sup&gt;</td>
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<tr>
<td>TG, mmol/g wet tissue</td>
<td>0.002 ± 0.001&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.001 ± 0.001&lt;sup&gt;d&lt;/sup&gt;</td>
<td>&lt;0.001</td>
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<tr>
<td>Ceramide, relative density units</td>
<td>61 ± 3&lt;sup&gt;b&lt;/sup&gt;</td>
<td>62 ± 3&lt;sup&gt;b&lt;/sup&gt;</td>
<td>162 ± 4&lt;sup&gt;d&lt;/sup&gt;</td>
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<td>SREBP1 mRNA, relative units</td>
<td>1.03 ± 0.02&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.95 ± 0.06&lt;sup&gt;g&lt;/sup&gt;</td>
<td>1.46 ± 0.11&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>PPARα mRNA, relative units</td>
<td>1.13 ± 0.07&lt;sup&gt;c&lt;/sup&gt;</td>
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<tr>
<td>TNFα mRNA, relative units</td>
<td>0.94 ± 0.03</td>
<td>0.98 ± 0.09</td>
<td>3.18 ± 0.47</td>
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<td>SPT1 mRNA, relative units</td>
<td>1.12 ± 0.06&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1.17 ± 0.05&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.41 ± 0.35&lt;sup&gt;a&lt;/sup&gt;</td>
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<sup>1</sup> Values are means ± SEM, n = 5. Means in a row with superscripts without a common letter differ, P < 0.05.

<sup>2</sup> NS, P = 0.05.
led to a significant decrease in cholesterol and TG concentrations in heart of rats with diet-induced obesity and in ob/ob mice associated with less ceramide accumulation. Ceramide synthesis is dependent on the availability of long-chain SFA, which participate in the initial reaction involving the condensation of palmitoyl-CoA and serine, catalyzed by the enzyme SPT-1 (30). The availability of palmitoyl-CoA strongly influences the rate of this reaction, which ultimately leads to ceramide formation (22). In the present study, the increase in the availability of FA in the heart of rats fed CAS-HF and ob/ob mice fed casein may promote ceramide synthesis. Interestingly, the lower cardiac lipid concentration of obese rats and ob/ob mice fed soy protein was associated with lower ceramide concentration. These results suggest that soy protein may prevent ceramide formation in the heart in part by reducing substrate availability.

Ceramide concentration is elevated in skeletal muscle, liver, and heart from insulin-resistant rodents or humans (16,31). When added to cultured cells, ceramide analogs inhibit insulin-stimulated glucose uptake, glucose transporter-4 translocation, and RAC-alpha serine/threonine-protein kinase phosphorylation and activation (32,33). In Zucker diabetic fatty rats, impairment of contractile function of the heart, cardiomyocyte apoptosis, and myocardial fibrosis are associated with TG accumulation and ceramide synthesis (11). Moreover, in a rodent model of dilated lipotoxic cardiomyopathy, inhibition of SPT-1 reduces ceramide concentration and is associated with improved cardiac function and survival (9). In the present study, the lower cardiac SPT-1 mRNA in rats fed SOY-HF or in ob/ob mice fed soy protein implies that soy protein consumption may reduce de novo ceramide synthesis and prevent lipotoxic cellular damage. Surprisingly, we did not observe cardiomyocyte apoptosis in rats fed CAS-HF or in ob/ob mice despite the increase in cardiac ceramide concentration. The ceramide concentration was probably not high enough to trigger apoptosis and a more prolonged study may be necessary to observe apoptotic cardiomyocytes.

Ceramide has been identified as an important mediator in the TNFα signaling pathway (23). TNFα stimulates de novo ceramide synthesis that, in turn, can induce TNFα expression, as was demonstrated in different cell types (34). In the present study, the reduction in SPT-1 mRNA concentration in the heart of rats fed soy protein was associated with lower TNFα expression. However, the reduction in SPT-1 mRNA in the heart of ob/ob mice fed soy protein was not associated with changes in TNFα expression, possibly due to the overaccumulation of lipids in heart of the ob/ob mice (Table 5).

In islets and heart of Zucker diabetic fatty rats, the overaccumulation of TG is the result of an increase in esterification and impaired oxidation of FFA due to the overexpression of enzymes of FA esterification coupled with underexpression of enzymes of FA β-oxidation (11,35). The expression of genes involved in cardiac lipid metabolism is controlled mainly by PPARα and PPARδ (36). It has been demonstrated that alterations in the expression and activity of PPARα during obesity is a key step in metabolic syndrome initiation (37,38). The identification of the insulin-sensitizing thiazolidinediones and the lipid-lowering fibrates as synthetic PPARγ and PPARα ligands, respectively, has provided opportunities for the identification of novel compounds for the treatment of metabolic syndrome (39). In this respect, administration of troglitazone, a thiazolidinedione, considerably reduces the TG concentration in islets and heart of Zucker diabetic fatty rats, preventing β cell and cardiomyocyte dysfunction (40). In the present study, soy protein intake led to an increase of PPARα mRNA concentration in heart of diet-induced obese rats and ob/ob mice in association with low fat accumulation in cardiomyocytes. This result indicates that soy protein can enhance the expression of this nuclear receptor in the heart, even when fed a high-fat diet or with an obese genetic predisposition. It has been shown that soy protein-associated isoflavones can bind and activate PPARα in liver of Zucker diabetic fatty rats, increasing PPARα-regulated gene expression and enhancing lipid metabolism (41–43). Thus, dietary soy protein can activate PPARα in the heart to increase lipid oxidation and prevent fat deposition and lipotoxicity.

Evidence derived from human and rodent studies indicates that lipotoxicity is produced by an imbalance between de novo lipogenesis and FA oxidation (44). In the heart, lipotoxicity occurs when FA entry into cardiomyocytes exceeds mitochondrial oxidative capacity. In fact, target overexpression of genes that are involved in lipid delivery and synthesis in the myocardium disrupts the balance between lipid import and export and leads to severe myocardial steatosis and heart failure (8,45). The transcription factor SREBP-1 regulates lipogenic gene expression in response to insulin signaling (46) and several lines of evidence implicate SREBP-1 in lipid metabolism abnormalities during obesity and type 2 diabetes (47,48). SREBP-1 has also been shown to be active even in the presence of insulin resistance, producing greater hepatic lipid synthesis and TG esterification, which leads to dyslipidemia and hepatic steatosis (49). On the other hand, SREBP-1 could be regulated by the type of protein. In obese rats, dietary soy protein is able to reduce the increase in hepatic SREBP-1 expression by reducing the insulin:glucagon ratio (14,50). In the present study, cardiac SREBP-1 mRNA was higher in rats fed CAS-HF and ob/ob mice fed CAS compared with their respective controls and soy protein intake led to a significant decrease of its mRNA concentration in both species. These results suggest that dietary soy protein may prevent cardiac lipid accumulation in part by reducing SREBP-1 gene expression and, as a consequence, lipid uptake and esterification. Thus, reduction in TG formation may increase the intracellular pool of FA available for entry into the mitochondria for oxidation (51,52) and protect the heart from ceramide synthesis and lipotoxicity.

Soy protein intake is one of the most recommended vegetal proteins for reducing cardiovascular risk (53–55). This study reveals a protective effect of dietary soy protein in lipid accumulation and ceramide synthesis in the heart of rats fed a high-fat diet and in ob/ob mice. Therefore, soy protein consumption may be considered as a dietary therapeutic approach for lipotoxic cardiomyopathy prevention.

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Literature Cited
3. Poirier P, Giles TD, Bray GA, Hong Y, Stern JS, Pi-Sunyer FX, Eckel RH. Obesity and cardiovascular disease: pathophysiology, evaluation, and effect of weight loss: an update of the 1997 American Heart Association Scientific Statement on Obesity and Heart Disease from the


