

## Soy $\beta$ -Conglycinin (7S Globulin) Reduces Plasma and Liver Cholesterol in Rats Fed Hypercholesterolemic Diet

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**ABSTRACT** The aim of this study was to examine the comparative hypocholesterolemic effect of soybean 7S fraction in rats fed a high-cholesterol diet. Soybean 7S globulin ( $\beta$ -conglycinin) was administered orally once a day to rats, and the effects were measured after 28 days. Wistar rats were divided into four groups: standard diet (STD) (casein alone), hypercholesterolemic (HC) diet (STD plus 1 g/100 g cholesterol and 0.5 g/100 g cholic acid), HC+7S<sup>1</sup> diet (HC diet plus 200 mg of 7S/kg of body weight/day), and HC+7S<sup>2</sup> diet (HC diet plus 300 mg of 7S/kg of body weight/day). Food intake, weight gain, animals' growth, and feeding efficiency ratio were similar among the STD and three HC groups, indicating that these parameters were not affected by treatments. Animals that had received different doses of soybean 7S globulin had lower total cholesterol (TC), triglycerides (TG), and low-density lipoprotein (LDL)/high-density lipoprotein (HDL) ratio in serum and lower levels of hepatic TC and TG than those fed only the HC diet. The atherogenic indexes of HC+7S<sup>1</sup> and HC+7S<sup>2</sup> groups were 40% and 55% lower than that of the HC group, respectively. The results showed that the oral daily administration of  $\beta$ -conglycinin in the diet to HC rats, at between 1.85% and 2.75% of total ingested protein, promotes the reduction of TC, LDL-cholesterol, and TG and an increase in HDL-cholesterol in the plasma, besides a small but significant reduction in cholesterol and TG levels in the liver of the animals as well as a reduced atherogenic index.

**KEY WORDS:** • cholesterol •  $\beta$ -conglycinin • hypercholesterolemic diet • rats • triglycerides

### INTRODUCTION

LEGUMINOUS SEEDS ARE A VALUABLE source of proteins and other compounds that have been shown to have beneficial effects on so-called metabolic-related diseases such as diabetes, hyperlipidemia, hypertension, and its consequences.<sup>1</sup> Although various drugs have been developed for some of these diseases, dietary therapy appears to be essential in addition to medical treatment. Soybean (*Glycine max*) seed-protein rich diets improve the blood lipid profiles in animals and humans, reducing the risk of heart disease.<sup>2</sup> The beneficial effects of soy proteins on traditional cardiovascular risk factors became recognized after the publication of several studies that demonstrated hypocholesterolemic and anti-atherogenic properties of soy.<sup>3</sup> Although the mechanisms behind these effects have been studied extensively, there is still controversy about the key roles of proteins, isoflavones, fibers, and other minor components. The Food and Drug Administration authorized a specific health recommendation to include 25 g of soy protein/day in a diet low in saturated fat and cholesterol.<sup>4</sup> This claim has been supported by scientific findings of the central

role represented by soybean proteins in the events related to prevention of cardiovascular diseases. However, this amount of protein can be impractical for most people.

Soy protein is known to be effective in reducing plasma triglycerides (TG) and cholesterol levels in animals and humans and to affect lipid metabolism.<sup>2,3,5,6</sup> Soy protein isolate (SPI) has been used in most studies, but the identity of component or components of SPI that promote the observed changes remains uncertain. Storage globulins make up the bulk of the protein in soybean seeds, and they comprise two main constituents, differentiated by their sedimentation coefficients: the 11S protein (or glycinin) and the 7S protein (or  $\beta$ -conglycinin). These proteins constitute up to 90% of all the protein in the seed. The 7S fraction has been studied as the only source of dietary protein in various experimental designs, using various animals, such as rats,<sup>7–9</sup> normal and genetically obese mice,<sup>10</sup> and monkeys.<sup>11</sup> Except in monkeys, consumption of  $\beta$ -conglycinin had a positive influence on lipoprotein metabolism and cardiovascular health. A body of evidence has pointed to peptides arising from digestion of this protein as responsible for these actions. Lovati *et al.*<sup>6</sup> have described the *in vitro* effects of small peptides released by digestion of a soy protein concentrate and 7S globulin, devoid of isoflavone components, on low-density lipoprotein (LDL) metabolism in cultured HepG2 cells. Duranti *et al.*<sup>9</sup> showed for the first time that the administration of an oral daily dose of  $\beta$ -conglycinin and/or

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its fractions led to alterations in plasma cholesterol and TG in rats. Kohno *et al.*<sup>12</sup> studied slightly hyperlipidemic human subjects and showed that supplementing the food with  $\beta$ -conglycinin was effective only in decreasing serum TG and visceral fat.

Although several studies with rats have found that the soy 7S fraction had no effect on plasma cholesterol and lipoprotein cholesterol concentrations, or only a modest effect that was similar to that of typical SPI,<sup>10,11,13</sup> few studies have explored the effect of oral administration of purified  $\beta$ -conglycinin on the control of dyslipidemia. We hypothesize that the administration of an oral daily dose of soybean 7S globulin ( $\beta$ -conglycinin) may reduce high levels of cholesterol, non-high-density lipoprotein (HDL)-cholesterol, and TG in the plasma and in the liver. Thus, the study was performed to investigate the effects of a daily dose of  $\beta$ -conglycinin on lipid metabolism of rats subjected to a hypercholesterolemic (HC) diet.

## MATERIALS AND METHODS

### Isolation of 7S globulin

Soybean flour obtained from the local market (60 mesh) was defatted with hexane (1:8 [wt/vol] flour to solvent ratio) by rocking at room temperature for 12 hours, then reextracted in hexane (1:6 [wt/vol]), evaporated to dryness at room temperature, and used for protein extraction. The 7S and 11S globulins were isolated by the method of Nagano *et al.*<sup>14</sup> with some modifications, as reported below. Defatted soybean flour was extracted with water (1:15 [wt/vol] flour to water ratio) adjusted to pH 7.5. The soluble extract was centrifuged at 2,000 g for 30 minutes. The supernatant was treated with anhydrous sodium bisulfite (0.98 g/L), and the pH was adjusted to 6.4. The solution was stirred and stored on an ice bath overnight, followed by centrifugation at 6,500 g for 20 minutes. The insoluble fraction containing 11S globulin was washed, centrifuged again under the same conditions, suspended in distilled water, dialyzed overnight, and lyophilized. The soluble fraction was treated with 0.25 mol/L NaCl and adjusted to pH 5.0 and after 1 hour centrifuged at 9,000 g for 30 minutes. The precipitate (7S globulin) was solubilized in 0.25 mol/L sodium phosphate buffer (pH 7.0) and stored at  $-14^{\circ}\text{C}$ . All steps were performed at  $4^{\circ}\text{C}$ . All chemicals are of analytical purity.

Protein content was determined by the method of Lowry *et al.*<sup>15</sup> using bovine serum albumin (Sigma Chemical Co., St. Louis, MO, USA) as a standard. Electrophoresis of soybean total protein and separate globulins (11S and 7S) was performed as described by Laemmli<sup>16</sup> in 10% polyacrylamide gels with 0.1% sodium dodecyl sulfate in the buffers using a Mini Protean II cell (Bio-Rad, Hercules, CA, USA). About 10  $\mu\text{g}$  of sample was applied to each gel. The electrophoretic run was conducted at 30 mA per slab. The gels were stained in Coomassie Brilliant Blue (R-250) and destained by diffusion in methanol-acetic acid-water (1:1:8 by volume). Marker proteins of known molecular weight were included in the gel: rabbit muscle phosphorylase b,

bovine serum albumin, hen egg white albumin, bovine carbonic anhydrase, soybean trypsin inhibitor, and hen egg white lysozyme. Gels bands were digitized, and densitometry analysis was performed by the alpha Imager version 6.0 Scanner (Alpha Innotech<sup>®</sup>, San Leandro, Ca, USA), to quantify the bands and level of purity. All chemical reagents were from Sigma Chemical Co.

### Animals and diets

Thirty-six male Wistar rats, weighing  $148 \pm 12.3$  g, were obtained from the Central Laboratory for Animals of São Paulo State University, Botucatu, SP, Brazil. All procedures were performed according to the principles in the *Guide for the Care and Use of Laboratory Animals*.<sup>17</sup> All animals were maintained in conformity with the guidelines of the Laboratory Animals Committee of the University, and the experiments were approved (resolution 19/2007) by the Research Ethics Committee of the School of Pharmaceutical Sciences, São Paulo State University. The animals were acclimatized for a week and fed standard laboratory rat chow (Purina<sup>®</sup>, São Paulo, SP, Brazil). After this period, they were randomly divided into four groups ( $n=9$ ): a standard group (STD) that received a normal diet, following the AIN-93 diet recommendations of the American Institute of Nutrition,<sup>18</sup> and three HC groups—control HC; 7S globulin, 200 mg/kg/day (HC+7S<sup>1</sup>); and 7S globulin, 300 mg/kg/day (HC+7S<sup>2</sup>)—all of which were fed an AIN-93M diet, modified by adding 1 g/100 g cholesterol and 0.5 g/100 g cholic acid,<sup>9,19</sup> as described in Table 1. Animals were individually housed in stainless steel cages, and food and water were given *ad libitum*. The room was maintained at a constant temperature of  $23 \pm 2^{\circ}\text{C}$  and relative humidity of  $60 \pm 5\%$ , with a 12-hour light:dark cycle (6:00 a.m. to 6:00 p.m. being the light period). The soybean 7S protein

TABLE 1. PERCENTAGE COMPOSITION OF THE EXPERIMENTAL DIETS

	Percentage			
	STD	HC	HC + 7S <sup>1</sup>	HC + 7S <sup>2</sup>
Casein <sup>a</sup>	15	15	15	15
Starch	61.07	59.57	59.57	59.57
Sucrose	10	10	10	10
Soybean oil	4	4	4	4
Cellulose powder	5	5	5	5
Mineral mixture <sup>b</sup>	3.5	3.5	3.5	3.5
Vitamin mixture <sup>b</sup>	1	1	1	1
L-Cysteine	0.18	0.18	0.18	0.18
Choline bitartrate	0.25	0.25	0.25	0.25
Cholesterol <sup>c</sup>	0	1	1	1
Cholic acid <sup>a</sup>	0	0.5	0.5	0.5
Treatment				
$\beta$ -Conglycinin (mg/kg/day)	—	—	200.0	300.0

The base diet was of AIN-93M composition.

<sup>a</sup>Sigma-Aldrich Co., St. Louis, MO, USA.

<sup>b</sup>PragSoluções<sup>®</sup> Co., Jaú, SP, Brazil.

<sup>c</sup>Reagen Co., Rio de Janeiro, RJ, Brazil.

STD, standard diet; HC, hypercholesterolemic diet; HC+7S<sup>1</sup> and HC+7S<sup>2</sup>, HC diet+ $\beta$ -conglycinin at 200 mg/kg/day and 300 mg/kg/day, respectively.

was given daily by gavage at 9:00 a.m., in proportion to the individual body of the animal, to groups HC+7S<sup>1</sup> and HC+7S<sup>2</sup> at 200 and 300 mg/kg/day, respectively, dissolved in saline. Group HC received saline only.

Body weight, food intake, fecal excretion, and feeding efficiency were measured every other day for comparative analysis between groups during the 28 days of the experiment. The feeding efficiency coefficient was calculated as the (weight gain/daily intake) × 100. On Day 28 the animals were deprived of food for 12 hours and euthanized by guillotine. Blood was then collected in tubes containing gel separator SST™ II (Vacutainer®, B, Franklin Lakes, NJ, USA) and centrifuged at 1,900 g for 15 minutes. The serum was separated, stored at -24°C, and used for biochemical analysis. Retroperitoneal fat, liver, and heart were isolated, washed in cold saline, weighed, immediately frozen, and stored at -40°C for subsequent comparative analysis.

#### Analysis in the plasma and liver

Plasma total cholesterol (TC), HDL-cholesterol (HDL-C), and TG were assayed by enzymatic colorimetric methods using commercially available reagent kits (Laborlab®, São Paulo, SP, Brazil) in triplicate. The non-HDL-C fraction (LDL-cholesterol [LDL-C] + very-LDL-C) was calculated as the difference between TC and HDL-C. The TC/HDL-C ratio and the atherogenic index [(TC - HDL-C)/HDL-C] were calculated and compared between groups. Hepatic-somatic and visceral fat indexes, respectively, were calculated as follows: (liver weight/body weight) × 100 and (fat visceral weight/body weight) × 100. Liver lipids were extracted by the method of Haung and Høstmark.<sup>20</sup> The liver TC and TG concentrations were measured as describe previously for plasma analysis.

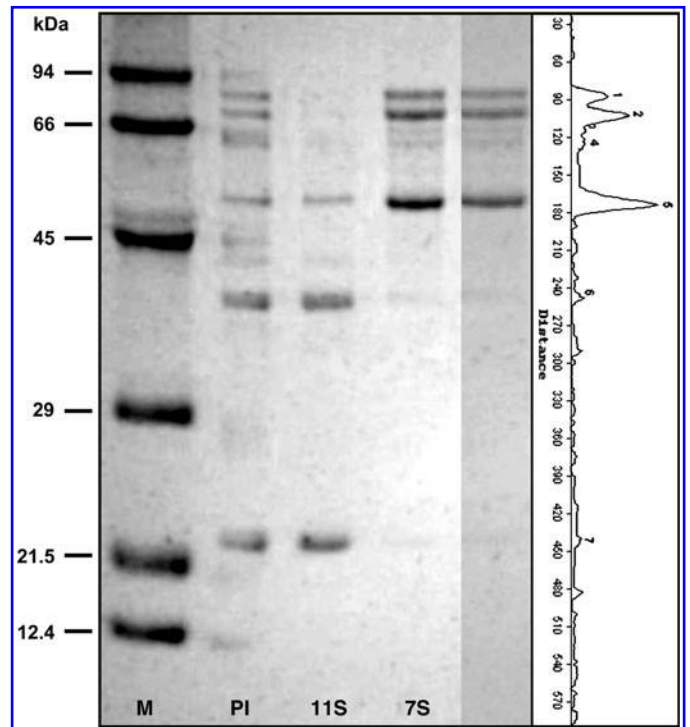
#### Statistical analyses

Data were analyzed by one-way analysis of variance and Student–Newman–Keuls multiple range tests, using Sigma-Stat® version 3.5 (1999) software (Dundas Software, Erkrath, Germany). All values are expressed as mean ± SEM for nine rats per group. A difference was considered statistically significant when  $P < .05$ .

## RESULTS AND DISCUSSION

The  $\beta$ -conglycinin obtained was reprecipitated at the end of the extraction procedure described above, to remove contaminant compounds. This step was important to obtain a sufficiently pure protein for the *in vivo* studies. The electrophoretic pattern of crude soybean protein and its fractions clearly indicated successful fractionation of the soybean 7S protein (Fig. 1). Densitometric analyses of total protein and isolated globulins showed that the glycinin and  $\beta$ -conglycinin were the major fractions of the soybean seed. The latter fraction (7S globulin) comprised 92.2% (wt/wt) protein, made up of 47.4%, 25.1%, and 19.7% as  $\beta$ -,  $\alpha$ -, and  $\alpha'$ -subunits.

Various authors have found variation in the contents of 7S and 11S soybean proteins and slight differences in the



**FIG. 1.** Sodium dodecyl sulfate–polyacrylamide gel electrophoresis under reducing conditions of the glycinin and  $\beta$ -conglycinin isolated: lane M, marker proteins; lane PI, soybean total protein extract; lane 11S, protein glycinin; and lane 7S, protein  $\beta$ -conglycinin. Sample amounts ranged from 5 to 20  $\mu$ g.

composition of  $\beta$ -conglycinin subunits in a lot of cultivars.<sup>9,10,21</sup> This observation indicates that there may be a mechanism in the seed that regulates the synthesis and storage of these proteins, which could be used to increase selectively their concentration in the seed. In the Brazilian soybean cultivar used in this study the  $\beta$ -subunit was encountered in higher proportion than the  $\alpha'$ -subunit in the 7S fraction, but these ratios are variable, as already demonstrated in U.S., Japanese, and Egyptian cultivars.<sup>20</sup>

Some studies in animals and human subjects have shown a significant HC effect of dietary soybean protein.<sup>1,11,12</sup> In this study, we examined the effects of different doses of  $\beta$ -conglycinin in rats that had received HC diet. At the end of the 28-day treatment there were no differences in the animals' growth among groups during the experimental period. The data in Table 2 show that food intake, weight gain, and feeding efficiency ratio were similar among the STD and the three HC groups, indicating that the final weight gain and intake were not affected by treatments. Surprisingly, however, the mean fecal excretion of the HC group was 37.55% higher ( $P < .001$ ) than that of the STD group without cholesterol in the diet. There were no significant differences in the relative weight of organs (liver and heart) and visceral fat deposits, as indicated by retroperitoneal adipose tissue weight, among the HC groups, with or without ingestion of  $\beta$ -conglycinin. However, the liver weight of rats in the STD group tended to be smaller than those fed HC diets, as did the hepatosomatic index (Table 3).

TABLE 2. BODY WEIGHT CHANGE, FOOD INTAKE, AND FEEDING EFFICIENCY RATIO OF RATS FED HYPERCHOLESTEROLEMIC DIETS WITH AND WITHOUT DIFFERENT DOSES OF β-CONGLYCININ

	Dietary group				ANOVA P ≤
	STD	HC	HC + 7S <sup>1</sup>	HC + 7S <sup>2</sup>	
Initial weight (g/rat)	195.23 ± 5.03	196.55 ± 4.63	191.12 ± 4.79	195.26 ± 3.64	.96
Final weight (g/rat)	287.77 ± 7.07	295.01 ± 5.43	287.61 ± 5.75	300.64 ± 7.71	.08
Weight gain (g/rat/day)	3.31 ± 0.24	3.51 ± 0.32	3.44 ± 0.22	3.76 ± 0.29	.06
Food intake (g/rat/day)	15.47 ± 0.27	16.74 ± 0.33	16.22 ± 0.32	16.25 ± 0.22	.06
Fecal excretion (g/rat/day)	2.61 ± 0.08 <sup>c</sup>	3.59 ± 0.12 <sup>a</sup>	2.84 ± 0.09 <sup>b</sup>	2.94 ± 0.09 <sup>b</sup>	.05
Feeding efficiency (%)	21.36 ± 1.67	21.01 ± 1.44	21.24 ± 1.26	23.05 ± 1.93	.09

Rats were fed diets for 28 days. Data are mean ± SEM values for nine rats.

<sup>abc</sup>Letters indicate differences between treatments.

ANOVA, analysis of variance.

TABLE 3. TISSUE WEIGHTS OF RATS FED HYPERCHOLESTEROLEMIC DIETS WITH AND WITHOUT DOSES OF β-CONGLYCININ

	Dietary group				ANOVA P ≤
	STD	HC	HC + 7S <sup>1</sup>	HC + 7S <sup>2</sup>	
Liver weight (g/rat)	10.90 ± 0.45 <sup>b</sup>	13.54 ± 0.36 <sup>a</sup>	12.61 ± 0.41 <sup>a</sup>	13.55 ± 0.87 <sup>a</sup>	.05
Hepatosomatic index (%)	3.79 ± 0.12 <sup>b</sup>	4.59 ± 0.14 <sup>a</sup>	4.38 ± 0.10 <sup>a</sup>	4.41 ± 0.20 <sup>a</sup>	.05
Adipose tissue epididymal (g/rat)	5.05 ± 0.36	5.54 ± 0.23	5.22 ± 0.44	5.39 ± 0.46	.52
Epididymal weight/body weight (g/100 g)	1.75 ± 0.11	1.89 ± 0.10	1.81 ± 0.15	1.79 ± 0.13	.50
Heart (g/rat)	1.088 ± 0.040	1.032 ± 0.040	0.998 ± 0.030	0.944 ± 0.030	.08
Heart weight/body weight	0.379 ± 0.010	0.349 ± 0.010	0.347 ± 0.010	0.309 ± 0.020	.07

Rats were fed diets for 28 days. Data are mean ± SEM values for nine rats.

<sup>ab</sup>Letters indicate statistically significant differences between treatments.

The results showed that the diet containing 1% cholesterol and 0.5% cholic acid was sufficient to induce hypercholesterolemia and hypertriglyceridemia when fed to the animals. TG levels in the plasma were significantly higher in the HC group than in the STD group (Table 4).

Plasma TC and TG concentrations increased significantly after 28 days in rats fed on the HC diet in relation to those given the STD diet and showed increases of 142.5% and 91.7%, respectively ( $P < .001$ ). On the other hand, administration of the two different concentrations of β-conglycinin decreased the levels of TC and TG of the rats,

relative to those fed the HC diet (Table 4). The HC+7S<sup>2</sup> group showed greater reductions ( $P < .05$ ) than the HC+7S<sup>1</sup> group. As can be observed in Table 4, plasma TC and TG of the animals in the HC+7S<sup>1</sup> and HC+7S<sup>2</sup> groups were 11% and 25.2% ( $P > .05$ ) and 23% and 34.2% ( $P > .001$ ) lower, respectively, than in the HC group. The increase in the daily intake of β-conglycinin led to a decrease of 13.5% in the plasma cholesterol. HDL-C plasma concentrations were reduced in the HC group ( $P < .05$ ) relative to the STD group, while the rats fed β-conglycinin tended to have higher levels than the HC rats, so that the HC+7S<sup>1</sup> and

TABLE 4. EFFECT OF DIETS ON PLASMA LIPID PROFILES AND HEPATIC LIPID CONTENTS IN RATS FED HYPERCHOLESTEROLEMIC DIETS WITH AND WITHOUT DIFFERENT DOSES OF β-CONGLYCININ

	Dietary group			
	STD	HC	HC + 7S <sup>1</sup>	HC + 7S <sup>2</sup>
Plasma				
TC (mmol/L)	1.6 ± 0.05	3.88 ± 0.22 <sup>a</sup>	3.45 ± 0.09 <sup>b</sup>	2.99 ± 0.10 <sup>c</sup>
HDL-C (mmol/L)	0.79 ± 0.03 <sup>a</sup>	0.45 ± 0.02 <sup>c</sup>	0.59 ± 0.02 <sup>bc</sup>	0.70 ± 0.05 <sup>ab</sup>
Non-HDL-C (mmol/L)	0.81 ± 0.08	3.43 ± 0.12 <sup>a</sup>	2.86 ± 0.14 <sup>b</sup>	2.29 ± 0.10 <sup>c</sup>
TG (mmol/L)	0.48 ± 0.02	0.92 ± 0.05 <sup>a</sup>	0.69 ± 0.05 <sup>b</sup>	0.60 ± 0.04 <sup>b</sup>
Liver				
TC (μmol/g)	7.1 ± 0.33	58.43 ± 0.85 <sup>a</sup>	52.04 ± 0.90 <sup>b</sup>	46.22 ± 0.65 <sup>c</sup>
TG (μmol/g)	15.60 ± 0.54	56.97 ± 2.15 <sup>a</sup>	52.31 ± 3.30 <sup>ab</sup>	48.52 ± 2.80 <sup>b</sup>

Rats were fed diets for 28 days. Data are mean ± SEM values for nine rats.

<sup>abc</sup>Letters indicate statistically significant differences between treatments.

HDL-C, high-density lipoprotein cholesterol; non-HDL-C, difference between TC and HDL-C; TC, total cholesterol; TG, triglycerides.

HC+7S<sup>2</sup> groups had 32.8% and 56.9%, respectively, more of this lipoprotein in the plasma than the HC group. In addition, the levels of non-HDL-C were higher in the HC group than in the STD group ( $P < .001$ ), while this fraction showed values 8% ( $P > .05$ ) and 24.8% ( $P < .001$ ) lower for HC+7S<sup>1</sup> and HC+7S<sup>2</sup> groups, respectively, than for the HC group. The HC+7S<sup>2</sup> group showed a reduction of 18.3% ( $P < .001$ ) relative to the HC+7S<sup>1</sup> group ( $P < .05$ ), but even so none of the groups reached the level of the STD group (Table 4).

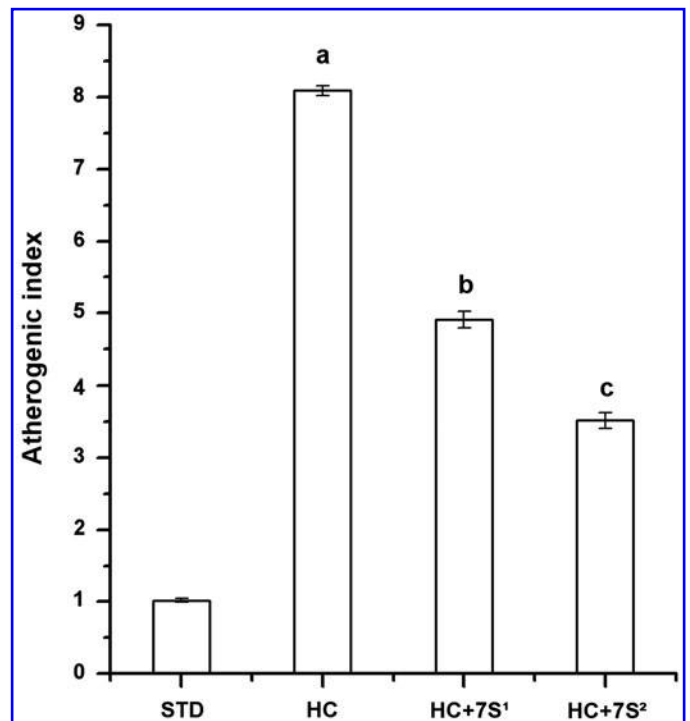
Much effort has done into researching the role of soy protein, in the form of SPI, concentrate, and isolated fractions in the metabolism of cholesterol and TG; also, the beneficial effects of all these proteins on plasma lipid and lipoproteins have been well documented in both the presence or absence of isoflavones.<sup>3,7,8,22-24</sup> In our study the isoflavones were not determined in the  $\beta$ -conglycinin fraction; however, McVeigh *et al.*<sup>25</sup> and Adams *et al.*<sup>26</sup> showed in humans and monkeys that a soy protein diet low in isoflavones produces the same effect on cholesterol levels as a soy protein diet high in isoflavones. These products may be vastly different when comparing studies and/or distinct sources. Thus, dietary replacement of casein with alcohol- or water-washed soy protein in HC hamsters lowered TG and LDL-C plasma levels; however, only the hamsters given alcohol-washed soy protein accumulated less aortic cholesterol.<sup>27</sup> It can also be observed that in the vast majority of these studies the authors used these proteins as the only dietary protein source and employed various animal models, similar to those in experiments of nutritional evaluation.<sup>5,8,10,11,27</sup> In our study, however, the isolated  $\beta$ -conglycinin was given in a *single* oral daily dose, after an extensive process of isolation (Fig. 1). The fact that rats fed  $\beta$ -conglycinin exhibited marked alterations of their lipid metabolism confirms that the feeding period was long enough to adequately study the effects of this protein on the lipid metabolism.

Serum biochemical parameters have been influenced in different ways when 7S soy protein was used as the only source of protein in rat diets. Fukui *et al.*<sup>7</sup> and Aoyama *et al.*<sup>8</sup> observed a significant reduction in TG in the plasma of rats, but the former did not observe any change in the level of TC, while the latter recorded a significant reduction. Moriyama *et al.*<sup>10</sup> showed in normal and genetically obese mice that ingestion of  $\beta$ -conglycinin lowered the rate of fatty acid synthetic activity and increased that of  $\beta$ -oxidation enzymes in the liver; furthermore, the fecal excretion of TG was increased with respect to the controls. It is also important to note that  $\beta$ -conglycinin offered as the only protein source in the diet had the effect of reducing the mass of the liver, relative to those animals fed on casein.<sup>7,8,10</sup> However,  $\beta$ -conglycinin, administered daily by gavage, did not alter the rat liver weight (Table 3) in our study. In addition, this way of administration of the protein promoted significant reductions in the levels of TC and TG in HC rats. Our observations of the lowering effect by 7S soybean protein in TG levels are supported by the results of Aoyama *et al.*,<sup>8</sup> Duranti *et al.*,<sup>9</sup> and Moriyama *et al.*,<sup>10</sup> on the other

hand, the similar effects of this protein on plasma TG levels observed in different feeding protocols, as a single source of protein or a daily dose and in different animals, strengthens the suggestion by Aoyama *et al.*<sup>8</sup> that the effect might be caused by  $\beta$ -conglycinin itself and not by the experimental model.

The atherogenic index, an indicator of a predisposition for heart problems, showed that animals treated with soybean 7S protein, in the HC+7S<sup>1</sup> and HC+7S<sup>2</sup> groups, had 40.2% ( $P < .001$ ) and 56.4% ( $P < .001$ ) lower risks, respectively, than the HC group (Fig. 2). There was a pronounced increase in cholesterol and TG in the liver of the animals fed the HC diet (Table 4). The values for the rats in the HC group were 7.5 and four times higher, respectively, than for the STD group. The groups that received 7S soybean protein showed reductions in these values of up to 21% and 13% for TC and TG, respectively. When the TC/HDL-C ratio was compared (Table 4), the HC+7S<sup>1</sup> and HC+7S<sup>2</sup> groups showed values 32.2% and 50.5% lower, respectively, than the HC group. Thus, the HC+7S<sup>2</sup> group showed a ratio 26.9% lower than that of the HC+7S<sup>1</sup> group. The value for the HC+7S<sup>2</sup> group could represent a protection from atherosclerosis, as confirmed by the atherogenic index. Data indicate that the 7S protein decreased the predisposition to coronary problems, as already reported by various authors.

Accumulating evidence suggests that concentrations of triacylglycerols in the liver and plasma depend mainly on the rate of hepatic lipogenesis.<sup>3,10,23,24,28</sup> Sawashita<sup>29</sup>



**FIG. 2.** Atherogenic index ( $[(TC - HDL-C)/HDL-C]$ ) of rats fed HC diets with and without doses of  $\beta$ -conglycinin. Data are mean  $\pm$  SEM values for nine rats. <sup>abc</sup>Different letters indicate statistically significant differences between means.

showed a significant antithrombotic effect of whole soy powder, and this was due to an inhibition of atherogenesis. The author considered that  $\beta$ -conglycinin and isoflavones could be involved in the mechanism of these anti-atherogenic effects. On the other hand, Adams *et al.*<sup>13</sup> showed that diets containing various soy protein fractions reduced atherosclerosis in mice, compared with a diet containing casein/lactalbumin, and that the diet containing  $\beta$ -conglycinin (7S globulin) reduced the amount of aortic cholesteryl esters, affording greater protection against cardiovascular diseases than the isoflavone-containing SPI diet. The authors concluded that the effect of  $\beta$ -conglycinin rich-diets does not depend on LDL receptors or effects on plasma lipoprotein and liver lipogenic enzyme activities, contrary to what some authors have suggested. Although these  $\beta$ -conglycinin effects are still unexplained, Lovati *et al.*<sup>6</sup> and Duranti *et al.*<sup>8</sup> suggested that they could be associated with peptides arising from the digestion of  $\beta$ -conglycinin that, after absorption and transport to the liver, could modulate the homeostasis of the cholesterol. Other studies indicate that the plasma cholesterol reduction might be a consequence of an interaction between peptides and cholesterol or its metabolites, neutral sterols and bile salts, which promotes its excretion.<sup>8,30</sup>

Despite many studies in animals and *in vitro* models,<sup>1,8,9,11,13</sup> the mechanisms of lowering cholesterol by soy protein is still far from being elucidated, and although many advances have been made controversy remains.<sup>1,9</sup> The results of the present study, in our opinion, are extremely intriguing because they show that the HC effect of the soybean protein is related to the  $\beta$ -conglycinin fraction, which suggests that biologically active peptides produced from  $\beta$ -conglycinin would be capable of modulating cholesterol and TG homeostasis.

In conclusion, the results showed that the oral daily administration of  $\beta$ -conglycinin in the diet to HC rats, at between 1.85% and 2.75% of total ingested protein, has promoted the reduction of TC, LDL-C, and TG and an increase in HDL-C in the plasma, besides a small but significant reduction in cholesterol and TG levels in the liver of the animals, as well as reducing the atherogenic index. Studies in this laboratory are in progress to investigate the effects of  $\beta$ -conglycinin alone and/or combined with fibrates and statins.

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## AUTHOR DISCLOSURE STATEMENT

No competing financial interests exist.

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