Casein and Soy Protein Isolate in Experimental Atherosclerosis: Influence on Hyperlipidemia and Lipoprotein Oxidation

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Key Words
Casein - Soy protein isolate - Lipoprotein oxidation - Atherosclerosis - Hyperlipidemia

Abstract
Background/Aims: Nutrients able to modify the susceptibility of lipoproteins to oxidation and/or reduce the cholesterol levels of blood plasma are important for prevention and/or treatment of atherosclerosis. The influence of animal and vegetable proteins on hypercholesterolemia and atherogenesis has been studied, concerning the mechanisms able to modify the digestion, absorption and bioavailability of lipids. In this study, the influence of casein and soy protein isolate on lipoprotein oxidation and atherosclerosis progression was investigated in cholesterol-fed rabbits. Methods: During 2 months, 20 New Zealand rabbits were fed with diets containing 1% cholesterol and 27% casein or 27% soy protein isolate. Blood samples were collected at baseline, 15, 30, 45 and 60 days of feeding. Results: Casein feeding contributed to increasing cholesterol and triglyceride concentrations, lipoprotein oxidation and the area of atherosclerotic lesions. In contrast, the soy protein isolate reduced, when compared to casein, the concentrations of cholesterol and lipid peroxides of β-VLDL and LDL fractions during the experimental time course, as well as the area of atherosclerotic lesions at the end of the study. Conclusion: Soy protein isolate, in comparison with casein, promoted a decrease of lipid peroxides, cholesterol and triglyceride content of atherogenic lipoproteins (β-VLDL and LDL), which had beneficial effects over atherosclerosis progression in cholesterol-fed rabbits.

Introduction

Clinical and experimental studies have demonstrated that the oxidative modification of low-density lipoprotein (LDL) is essential to the development of atherosclerosis [1–5]. It is known that dietetic and/or pharmacological interventions can modify the susceptibility of lipoproteins to oxidation and consequently the progression of atherosclerosis [6, 7]. Studies with animals and humans have demonstrated that the reduction of serum lipids [8–
decreases the incidence of atherosclerosis. Moreover, it is also known that the diet content of fibers [12], carbohydrates [13] and proteins [14] is important for atherosclerosis development and that diet manipulations can retard or accelerate the progression of this pathology. In the last years, the lipoxidative oxidative stress has stimulated many studies focusing on the role of free radicals in atherosclerosis [15–17]. According to this theory, the elevated generation of free radicals by cells of the vascular wall is involved in the mechanisms responsible for atherogenesis. Important concepts about the role of micronutrients (vitamins and minerals) and antinutritional factors (saponins, flavonoids and isoflavonoids) in the pathogenesis of atherosclerosis have emerged and great attention has been focused on antioxidants and hyalopidemic nutrients [18–20]. It has been demonstrated that casein can modify the digestion, absorption and metabolism of lipids leading to hypercholesterolemia and atherosclerosis [21]. In contrast, soy protein induces a low postprandial insulin/glucagon ratio [22], elevates plasma thyroxine concentrations [23, 24] and enhances apo-B/E receptor activity which contributes to an increase of LDL removal from circulation in rabbits [25] and rats [26]. Recently, it was demonstrated that apo-B/E receptor mRNA levels increased 75% in mononuclear cells of humans consuming soy protein [27]. Moreover, soy protein consumption increased the activities of 7α-hydroxylase in animals and humans, by reducing the enterohypothalamic circulation of cholesterol and bile acids [23, 28, 29]. Together, these actions contribute to the reduction of plasma cholesterol which is an important risk factor in the initiation and progression of atherosclerosis. In this study, we investigated the influence of casein and soy protein isolate supplementation on lipoprotein oxidation and lipid profile during experimental atherosclerosis induced in rabbits by 1% cholesterol-enriched diet.

Methods

Animals

Twenty adult male New Zealand rabbits, weighing 2.5–3.0 kg, were purchased from Creix (São Paulo, Brazil), were separated at random in two groups. A group named CAS (n = 10) was fed with a commercial diet (Nutri-coelhos Especial Parina®, São Paulo, Brazil) containing 1% cholesterol (Sigma Co., St. Louis, Mo., USA) and supplemented with 27% casein (Casein 25, Rodoma S/A, São Paulo, Brazil); the other group, named SPI (n = 10) was fed a 1% cholesterol-enriched diet supplemented with 27% soy protein isolate (Samprossoy 90 NB, Ceval Alimentos S/A, São Paulo, Brazil). To prepare the supplemented diets, commercial chow was triturated, homogenized with prehydrated casein or soy protein isolate, and re-pelletized and dried at 50°C for 12 h. Ether-diluted cholesterol was pulverized on the diets, which were maintained under the hood for 12 h for ether to evaporate. The diets were prepared weekly and maintained at −20°C to reduce the oxidative modifications. Food and water were provided ad libitum. The diet composition is shown in table 1. The chemical composition of the commercial nonpurified and supplemented diets were appropriate to maintain the health of the rabbits. Protein, amino acids, total lipid, mineral, water and fiber contents were analyzed by methods indicated by the Association of Official Analytical Chemists (1980) [30] after addition of casein and soy protein isolate. The protocol for the study compiled with NIH guidelines [31]. During the experiment, rabbits were maintained in individual metal cages and allowed free access to food and water. Room temperature was maintained at ~22°C. All procedures in this study compiled with the ethical guidelines of the institution for experiments with animals.

Coefficient of Alimentary Efficacy (CAE)

During the experimental course the animals were monitored daily in relation to chow ingestion and body weight. The CAE was determined by the chow ingested weight/body weight ratio (table 2). Body weight change was determined through difference between body weight at T = 60 and T = 0 days of diet, and chow ingested weight represents the diet consumed during the whole experimental time.

Purification of Lipoproteins

Blood samples were collected, after 12 h fasting, in plastic tubes containing 1.0 mg/ml ethylenediaminetetraacetic acid (EDTA) before and at 15, 30, 45 and 60 days of feeding. Immediately after separation, 1.0 mM phenylmethylsulfonyl fluoride (PMSF), 2.0 mM benzamidine, 2.0 μg/ml aprotinin and 20.0 mM butylated hydroxytoluene (BHT) (Sigma Co.) were added to the plasma. Lipoproteins were purified by sequential ultracentrifugation (40,000 rpm, 4°C, 24 h) [32]. The lipoprotein fractions VLDL/β-VLDL (1.006 < d < 1.019 g/ml), LDL (1.020 < d < 1.063 g/ml) and HDL (1.065 < d < 1.210 g/ml) were extensively dialyzed against 150.0 mM sodium chloride, 1.0 mM EDTA, 10.0 mM Trisma base and 3.0 mM sodium azide at 4°C for 12 h. All samples were maintained at −70°C until analysis.

Concentration of Cholesterol and Triglycerides

Cholesterol and triglyceride concentrations in lipoproteins were determined by enzymatic methods using commercial kits (cholesterol, Cat. No. 115050; triglycerides, Cat. No. 11528, BioSystem, Barcelona, Spain).

Concentration of Lipid Peroxides

Lipid hydroperoxides (LOOH) are the primary products of lipid peroxidation and may be reduced by enzymes or redox metals to their respective hydroxy-derivatives (LOH). In the present study we measured the lipid peroxides (total amount of LOOH + LOH) derived from oxidation of cholesterol esters and triglycerides. The content of lipid peroxides (lipid hydroxy + hydroperoxides) was normalized by the sum of cholesterol plus triglyceride content of each lipoprotein class. Lipid peroxides in lipoproteins were determined by HPLC (LC10 Shimadzu Corp., Tokyo, Japan) using a C8 Inertsil column (GL Sciences, Inc., Japan) and eluted with methanol containing 20.0 mM lithium acetate at a flow rate of 1.0 mL/min through a LC-10AD pump (Shimadzu Corp.) and a diode array detector (SPD-M10A, Shimadzu Corp.) set at 234 nm. Peaks were identified using
Table 1. Diet composition of CAS and SPI groups

<table>
<thead>
<tr>
<th>Nutrients, %</th>
<th>CAS²</th>
<th>SPI³</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein</td>
<td>31.30</td>
<td>32.80</td>
</tr>
<tr>
<td>Total lipid</td>
<td>2.00</td>
<td>1.90</td>
</tr>
<tr>
<td>Fiber</td>
<td>26.65</td>
<td>25.21</td>
</tr>
<tr>
<td>Minerals²</td>
<td>7.00</td>
<td>7.00</td>
</tr>
<tr>
<td>NIFEX fraction⁵,⁶</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Humidity⁴</td>
<td>10.50</td>
<td>12.00</td>
</tr>
<tr>
<td>Amino acid⁷</td>
<td>4.83</td>
<td>3.61</td>
</tr>
<tr>
<td>Arginine</td>
<td>7.15</td>
<td>10.57</td>
</tr>
<tr>
<td>Aspartic acid</td>
<td>11.33</td>
<td>7.04</td>
</tr>
<tr>
<td>Cystine</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Glutamic acid</td>
<td>21.58</td>
<td>20.98</td>
</tr>
<tr>
<td>Glycine</td>
<td>4.77</td>
<td>2.82</td>
</tr>
<tr>
<td>Histidine</td>
<td>2.68</td>
<td>2.96</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>5.00</td>
<td>4.69</td>
</tr>
<tr>
<td>Leucine</td>
<td>8.42</td>
<td>8.52</td>
</tr>
<tr>
<td>Lysine</td>
<td>5.88</td>
<td>6.72</td>
</tr>
<tr>
<td>Methionine</td>
<td>1.65</td>
<td>0.97</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>5.49</td>
<td>4.64</td>
</tr>
<tr>
<td>Proline</td>
<td>6.44</td>
<td>9.50</td>
</tr>
<tr>
<td>Serine</td>
<td>4.22</td>
<td>3.98</td>
</tr>
<tr>
<td>Threonine</td>
<td>3.46</td>
<td>3.31</td>
</tr>
<tr>
<td>Tyrosine</td>
<td>2.08</td>
<td>2.75</td>
</tr>
<tr>
<td>Valine</td>
<td>5.70</td>
<td>6.08</td>
</tr>
<tr>
<td>Arginine/lysine</td>
<td>1.22</td>
<td>1.57</td>
</tr>
</tbody>
</table>

¹ Determinations were done in quadruplicate and the results are shown as the mean value; NA, not applicable.
² CAS, nonpurified diet Purina⁸ (Nutri-coelhos Especial Purina, São Paulo, Brazil) supplemented with 1% cholesterol and 27% casein (Casein 25, Rodoma S/A, São Paulo, Brazil).
³ SPI, nonpurified diet Purina⁸ (Nutri-coelhos Especial Purina) supplemented with 1% cholesterol and 27% soy protein isolate (Samprosyn 90 NB, Ceval Alimentos S/A, São Paulo, Brazil).
⁴ The concentrations of protein, lipids, fibers, minerals, and humidity were analyzed after the addition of cholesterol, and either casein or soy protein isolate to the nonpurified diet.
⁵ Fraction consisting of gum, amide, organic acids and other digestible carbohydrates and free of nitrogen, fibers, minerals, vitamins and lipids.
⁶ Amino acids in grams/100 g of protein.
⁷ External standards prepared from their respective photosensitized oxidation products by the procedure previously described [33, 34] and quantified using the package Class-LC10 software (Shimadzu Corp.). The samples were first extracted with methanol/hexane (1:3, v/v). The contents were vortexed by 2 min and centrifuged at 2,500 rpm for 10 min for phase separation. The hexane phase was collected and evaporated with nitrogen. The residue was dissolved with mobile phase, filtered through a 22-μm Millex filter (Millipore, São Paulo, Brazil) and analyzed by HPLC.

Table 2. CAE obtained from CAS and SPI groups

<table>
<thead>
<tr>
<th>Group</th>
<th>Δ weight, kg¹</th>
<th>Ingested diet, kg²</th>
<th>CAE</th>
</tr>
</thead>
<tbody>
<tr>
<td>CAS</td>
<td>0.50</td>
<td>3.83</td>
<td>0.13</td>
</tr>
<tr>
<td>SPI</td>
<td>0.45</td>
<td>4.14</td>
<td>0.11</td>
</tr>
</tbody>
</table>

¹ Δ weight (T = 60 days or T = 0 days) and ingested diet represent chow consumed during the whole experimental time (n = 10).
² Diet ingested during the whole experimental time.

Morphometric Analysis

After the experimental period, the rabbits were anesthetized with an intramuscular injection of 50.0 mg/kg body weight Ketalar® (Parke-Davis, São Paulo, Brazil) and 5.0 mg/kg body weight Rompun® (Bayer SA, São Paulo, Brazil) and sacrificed. Once the aorta had been removed intact from each animal, cleaned of the bulk of adhering fat and immersed in 10% formaldehyde, the vessels were opened longitudinally. These segments were dehydrated in alcohol, cleared in propylene oxide and included in paraffin. Semithin sections of aortic arch (5 μm) were cut (1.0 cm from cardiac ostium), mounted on glass slides, stained with hematoxylin and eosin (HE) and examined by light microscopy (n = 20 sections/animal). Quantitative measurements were made by Optimas® imaging analysis system (BioScan Inc., Edmonds, Wash., USA). Samples were analyzed for the area (in μm²) occupied by linear length of lesions and multiplied by 5 (thickness of each section). The volume (in μm³) occupied by lesions was calculated as the sum of the lesion area for each section multiplied by 5 (thickness of each section). This analysis was based previous studies conducted by Daley et al. [35].

Statistical Analysis

All samples were run in duplicate. Statistical analysis was done by SAS statistical package (Version 6.03, 1995) developed by the SAS Institute, Inc. (Cary, N.C., USA). Analysis of variance was carried out using ANOVA. Intra-group differences were analyzed by Tukey’s studentized range test. For statistical comparison between the groups, the area under the curves was calculated from basal time through 2 months. Analysis of variance with repeated measurements (MANOVA) was used for inter-group comparison and the differences were measured by Tukey’s studentized range test. MANOVA was carried out after subtracting basal values (T = 0). The significance level adopted was p < 0.05. The correlation analysis was done using the Origin® software.

Results

Lipid Analysis

Figure 1 shows the concentrations of cholesterol and triglycerides in lipoproteins during the experimental course. Both diets induced a sharp hypercholesterolemia mainly associated with β-VLDL and LDL fractions. By analyzing the area under the curves during the experimental time course, lower levels of β-VLDL cholesterol and LDL


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cholesterol were found in the SPI group (1,531.05 ± 460.41 and 479.83 ± 174.32, respectively) as compared to the CAS group (4,103.38 ± 1,252.38 and 648.35 ± 253.14, respectively). No difference was found between groups for HDL cholesterol (CAS 139.31 ± 50.30 and SPI 147.37 ± 49.68). When the area under the curve of the triglyceride profile was analyzed, significant differences for β-VLDL and LDL between CAS (469.97 ± 150.61 and 171.96 ± 55.20, respectively) and SPI (318.86 ± 117.24 and 93.91 ± 29.24) groups were found. HDL triglyceride levels were similar between CAS (103.56 ± 115.97) and SPI (19.46 ± 27.38) groups.

**Lipid Peroxides**

When the areas under the curve of the lipid peroxide/lipid ratio were analyzed, significant differences between CAS and SPI groups were found for all analyzed lipoproteins (fig. 2). These differences demonstrated that the content of lipid peroxides in β-VLDL (0.916 ± 0.317), LDL (0.773 ± 0.298) and HDL (0.632 ± 0.437) in the CAS group was significantly increased in relation to that of β-VLDL (0.309 ± 0.063), LDL (0.157 ± 0.177) and HDL (0.093 ± 0.055) present in the SPI group. The correlation analysis showed that the concentrations of CL-OHH were positively correlated to the content of β-VLDL cholesterol in both groups (CAS: r = 0.864, p < 0.022; SPI: r = 0.964, p < 0.022). A positive correlation was also found between...
Fig. 2. Lipid peroxide/lipid ratio content of β-VLDL (A), LDL (B) and HDL (C) obtained in casein (●) and soy protein isolate (■) groups. The results are mean ± SD obtained from 10 rabbits. For statistical comparison between CAS and SPI groups, the areas under the curves were calculated from basal time to 60 days. Statistical differences were evaluated by Tukey’s test (p < 0.05).

Discussion

Casein, associated or not with cholesterol, has been considered as an atherogenic nutrient for rabbits [36, 37], leading to increased levels of LDL cholesterol and atherosclerotic lesion development [38]. Hypercholesterolemia induced in rabbits fed cholesterol-enriched diets is associated with the formation of β-VLDL that has a higher cholesteryl ester content than the normal VLDL [39]. β-VLDL is formed only in human carriers of type III hyperlipidemia, which is a relatively rare disease. In contrast, hypercholesterolemia in humans is often associated with increased LDL levels in blood plasma. In the present study the lipid profile of the CAS group showed an increase of both β-VLDL and LDL, reflecting the simultaneous supplementation of cholesterol and casein in the diet. Homeostasis of body cholesterol is a complicated interrelation of cholesterol absorption, synthesis, degradation and excretion [40]. In spite of differences in cholesterol metabolism in rabbits and humans, these animals have been amply used in development of hypercholesterolemia and atherosclerosis because rabbits are particularly sensitive to dietary cholesterol. The liver and intestine are the two principal sources of endogenously synthesized cholesterol and normally synthesis is inhibited in cholesterol-fed rabbits, but this is not sufficient to compensate for the massive influx of absorbed dietary cholesterol. Moreover, several studies have demonstrated that excretion of bile acids is not increased in cholesterol-fed rabbits [41]. The overall result is the accumulation of lipids in arteries and other organs and tissues. Although the experimental model of diet-induced atherosclerosis in rabbits has been criticized [42], Anitschkow’s cholesterol-
fed rabbit model [43], combined with different methods of plaque induction, offers a useful animal model for solving problems in nutritional studies related to atherosclerosis research. In spite of differences between atherosclerotic lesion development in rabbits and humans, already described [44, 45], the use of shortened induction times and lower doses of hypercholesterolemic promoter have contributed to make this animal model more suitable for experimental atherosclerosis studies.

The percentage of soy protein which avoids or neutralizes the hypercholesterolemic effect of casein or cholesterol is not well defined yet. Carroll et al. [46] studied the proportions of vegetal to animal dietary proteins able to modify the serum cholesterol levels in humans and verified that more than 30% of animal protein in the diet abolished the cholesterol-lowering benefit of soy protein. The soy protein isolate:cholesterol (27:1, w/w) ratio used in this study was able to modify the content of cholesterol and triglycerides in β-VLDL and LDL during the experimental time, in comparison to the same casein:cholesterol ratio. This suggests an effective hypercholesterolemic action of soy protein isolate, even in the presence of high cholesterol consumption. It has been demonstrated that casein and soy protein isolate can also modify the initiation and progression of atherosclerosis by diverse mechanisms. Either casein or soy protein isolate modify the absorption and metabolism of lipids [36]. One important mechanism that contributes to casein hyperlipidemic action is its effect of increasing cholesterol and bile salts (re)absorption from gut [46]. The increase in the influx of cholesterol and bile acids from the intestine to the liver promotes an increase in the hepatic amount of both components. The liver responds by an inhibition of the cholesterol biosynthetic and catabolic pathways and a reduction of the number of B/E receptors [23]. In fact, it has been demonstrated that casein-fed rabbits and rats showed decreased HMG-CoA reductase activity, as well as down-regulation of 7α-hydroxylase and of apo-B/E receptor leading to decreased cholesterol catabolism and delayed removal of lipoproteins from blood plasma [47]. Moreover, casein increases the production of cholesterol ester-rich VLDL by the liver [48] and, by reducing the activity of LDL receptor [47], stimulates further conversion of VLDL to LDL [48]. In contrast, soy protein promotes a reduction of intestinal (re)absorption of bile acids and cholesterol in animals [38], leading to increased activity of 7α-hydroxylase [28] and apo-B/E receptor [25], which has an important hypercholesterolemic effect. Both casein and soy protein isolate affect the transfer of apolipoproteins between lipoproteins [36]. Casein reduces the transfer of apolipoproteins E and C from VLDL to HDL, resulting in accumulation of these proteins on VLDL and IDL particles and, finally, LDL [36]. In contrast, soy protein isolate stimulates the transfer of these apolipoproteins from VLDL to HDL [36]. Moreover, casein feeding stimulates the synthesis of apo B, increasing VLDL production by the liver and, consequently, the LDL levels [8]. All these effects may contribute to the increase in β-VLDL and LDL particles observed in the present study. According to Vahouny et al. [23], the antiatherogenic effect attributed to soy protein can be associated to its high arginine/lysine ratio, in contrast to that of casein. It is well described that the content of arginine in soybean protein is twice that of casein [49]. The amino acid analysis of casein and soy protein isolate preparations used in the present study (table 1) confirmed these previous observations, suggesting that this ratio may be related to the differences of lipid profile observed between CAS and SPI groups. It is known that supplementation with arginine and glycine promotes a decrease of cholesterol levels [23]. In contrast, casein contains a high amount of lysine and branched-chain amino acids, which may contribute to enhance serum cholesterol levels [22]. Moreover, it is suggested that increased lysine incorporation into LDL apo B could contribute to stimulate hypercholesterolemia in rabbits [50]. The cholesterol concentration in plasma and lipoproteins is affected by the insulin/glucagon ratio and the release of these hormones is modified by dietary amino acids. Arginine stimulates the secretion of glucagon and lysine is associated with increased serum insulin concentration [22]. Moreover, casein promotes a reduction of thyroid hormones in blood plasma [47]. As it is well known that a decrease of thyroid hormones contributes to modify lipid metabolism, leading to hypercholesterolemia and atherosclerosis development [24, 51], this may represent another hyperlipidemic effect of casein. Several studies suggest that low concentrations of methionine can be associated with the soy hypolipidemic action [52, 53]. Our results indicate that the methionine concentration of soy protein isolate is in agreement with previously reported data [53]. The reduced methionine levels of SPI, compared with CAS, could be associated with the hypocholesterolemic effect promoted by soy protein observed here. In parallel, the amino acid composition of proteins may also influence the rate of fatty acid desaturation [53]. Essential amino acids seem to be important for this process as protein depletion promotes significant reduction of Δ6-desaturase activity [53]. Soy protein, in comparison to casein, reduces the desaturation of linoleic acid to arachidonic acid and, hence, causes modification of eicosa-
noid production associated with maintenance of vascular
tonus [53, 54]. Soy and casein protein feeding can modify
the fatty acid composition of membranes and possibly
some cellular functions related with atherosclerosis de-
velopment. Casein feeding stimulates VCAM-1 and ICAM-1
expression in endothelial cells [47] and modifies gene
expression of proteins involved in cholesterol homeosta-
sis [47].

It has been demonstrated that polyunsaturated fatty
acids of mammalian tissues and body fluids undergo lipid
peroxidation [54]. Lipid peroxides have attracted much
attention due to their deleterious effects related to aging
and atherosclerosis. Our data suggest that casein and soy
protein isolate feeding has different effects on lipid perox-
idation. Soy protein isolate was more effective in prevent-
ing the formation of lipid peroxides when compared with
casein. This suggests that soy protein isolate, even in asso-
ciation with cholesterol, was able to protect plasma lipo-
proteins against oxidation at the beginning of the hyper-
cholesterolemia (Fig. 2). When the lipid peroxide content
was adjusted in relation to the lipid concentration, the dif-
fences between CAS and SPI groups were confirmed.
Although the endogenous content of lipid- and water-solu-
ble antioxidants seems to protect lipoproteins against oxi-
dation at baseline, our results showed that the content of
oxidized lipoproteins of blood plasma increased in the
course of hypercholesterolemia. This finding suggests that
the oxidizable lipids present in lipoproteins were targets
for free radicals generated in vivo, in spite of antioxidants
present in lipoprotein particles and in blood plasma.
Thus, the increase of lipid peroxides in parallel to the lipid
increment indicates that an oxidative stress is occurring in
this situation of hyperlipidemia. Recent studies have
demonstrated that other soy components besides protein
(i.e., phenolic acids, isolavones, trypsin inhibitor, sapon-
ins, phytic acid and fibers) can contribute to the hypocho-
lesterolemic and antioxidant action attributed to soybean
[55–57]. However, the soy protein isolate used in this
study was obtained through hydro-alcoholic conditions
which remove fibers. Most isolavones, phenolic acids and
saponins besides inactivating trypsin inhibitor by high
temperature used during manufacturing. Therefore, our
results demonstrate that the hypolipidemic effect ob-
served in the SPI group may be attributed to an action of
soy protein by itself. Thus, the isolavone-free soy protein
used in our study was able to modify cholesterolemia and
lipid peroxide formation, suggesting that the protective
effects observed here may be attributed to an intrinsic
action of the soy protein, as previously indicated [58].
This could contribute to the protective effect of this

nutrient against atherosclerosis progression as observed
here in rabbits. Possibly, the direct actions of casein and
soy protein isolate on cholesterol homeostasis influence
the clearance rate of lipoproteins and their contact with
oxidant species generated in the blood circulation. There-
fore, our results indicate that soy protein decreases chole-
sterol concentration and lipid peroxidation in cholesterol-
ized rabbits. Considering the differences of lipid metabo-
lism between humans and rabbits, it is not possible to
directly extrapolate our conclusions to humans. However,
our results give support for further studies to investigate
the beneficial effects of soy protein isolate in human ath-
erosclerosis progression.

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References

Announcement

Trace Element Meeting
Quebec City, Canada, September 16-20, 2001

ISTERH, the International Society for Trace Element Research in Humans, announces its sixth triannual meeting, to take place in Quebec City, Canada, September 16-20, 2001. An exciting scientific and social program is planned. The officers and board of ISTERH urge all ISTERH members and non-members interested in trace elements to attend and submit abstracts. Most abstracts will be accepted for either platform or poster presentation. For information on how to register and/or submit abstracts, contact:

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